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# The Use of Near-Infrared (NIR) Reflectance Spectroscopy to Predict Mohair Quality in Greasy Fleece Samples of Angora Goats

Daniel Allain, Sylvie Brenot, Gerard Auvinet, Berengere Pena-Arnaud, Pierer Martin

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# Advances in Fibre Production Science in South American Camelids and other Fibre Animals

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Martina Gerken, Carlo Renieri, Daniel Allain, Hugh Galbraith, Juan Pablo Gutiérrez, Lisa McKenna, Roman Niznikowski, Maria Wurzinger (eds.)

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## **Preface**

Animal fibres from South American camelids and other fibre or wool bearing species provide important products for use by the human population. The contemporary context includes the competition with petrocarbon-based artificial fibres and concern about excessive persistence of these in the natural environment. Animal fibres present highly valuable characteristics for sustainable production and processing as they are both natural and renewable. On the other hand, their use is recognised to depend on availability of appropriate quality and quantity, the production of which is underpinned by a range of sciences and processes which support development to meet market requirements.

Such support includes the efforts of the Animal Fibre Working Group (AFWG) of the European Federation of Animal Sciences (EAAP) which was instituted in 2007 and tasked with creating a network for investigation and dissemination of information in Europe and internationally. One task has been the organisation of scientific meetings, and continuing the tradition of previous European Symposia on South American camelids. These include the recent 5<sup>th</sup> Meeting in Sevilla (Spain: 2010) and 6<sup>th</sup> Meeting at EAAP, Nantes (France: 2013). References to these and other meetings, workshops and publications may be found on the AFWG website: <http://www.eaap.org/presentation/scientific-structure/commissions-working-groups/animal-fiber-working-group/>.

The present publication derives from the 7<sup>th</sup> European Symposium on South American Camelids and 3<sup>rd</sup> European Meeting on Fibre Animals (<http://www.sympcam.org/>). This meeting was held in the conference facility of the Domus Pacis Hotel, Assisi, Italy, on 12 to 14 June 2017. It was organised by Prof Dr Carlo Renieri and his colleagues Dr Attilio De Cosmo, Dr Francesco Fantuz, Dr Antonietta La Terza, Prof Alessandro Valbonesi (University of Camerino), Dr Marco Antonini (ENEA), and Maurizio Gubbiotti (University Marconi, Roma) with support from the scientific board comprising AFWG colleagues. We wish to thank Dario Pediconi, Cristina Nocelli, Irene Pazzaglia, Stefano Pallotti (University of Camerino) who helped us during the symposium. We also thank all participants who readily agreed to chair sessions or to participate in the Round Table.

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Individual papers and abstracts, where full papers were not available, were printed from the manuscripts supplied by the authors. The assistance of Marvin Heuduck (Göttingen University) and the editors of Göttingen University Press in the editorial process is acknowledged.

*Martina Gerken (Göttingen University), Carlo Renieri (University of Camerino, Italy), Daniel Allain (INRA, France), Hugh Galbraith (University of Aberdeen, UK), Juan Pablo Gutiérrez (Complutense University of Madrid, Spain), Lisa McKenna (Göttingen University), Roman Niznikowski (University of Life Sciences, Warsaw, Poland), and Maria Wurzingger (University of Natural Resources and Life Sciences (BOKU), Vienna, Austria)*

The editors

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# **Sustainable Development, Climate Change and Biodiversity**





# Sustainable Development of Livestock Production: What and how can Research Contribute?

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**Abstract.** World-wide livestock constitutes a substantial component of agricultural production by contributing to the agricultural GDP of many countries. Livestock contributes to food and nutrition security through the provision of animal-sourced foods. It is a source of income and capital assets, as well as a source of draught power and manure for farming. In pastoral societies, livestock is the prime resource and the largest non-land asset they own. Smallholder farmers contribute a large share to the agricultural production throughout the developing world. Poor households especially depend on and keep livestock. At the same time, we can observe an increasing demand, particular in developing countries, for animal-source food. This so-called livestock revolution is often seen as an opportunity for livestock owners to improve household incomes, secure food for their families and escape poverty. But simultaneously livestock keepers are confronted with many challenges such as climate change, volatile prices and new market demands. There is increasing consensus that change is accelerating and becoming less predictable, as global interconnections lead to events having consequences beyond their immediate context. Research, innovation and (higher) education can play an important role in addressing many of the challenges livestock keepers are facing. But this requires a paradigm shift towards more inter- and transdisciplinary research. Beyond the engagement of the relevant disciplines, it is now increasingly acknowledged that the understanding of and solutions to societal challenges may be better achieved by involving academic and non-academic partners in all stages of the research process. Collaboration of different stakeholders can lead to new thinking and might bring new solutions – not only technical, but also institutional ones. In recent years, innovation platforms have gained some attention as it gives different actors along a certain value chain the opportunity to interact on a regular basis. These platforms provide the necessary room to discuss current and relevant topics. For researchers it will remain difficult to balance their efforts between basic and more applied research – both equally important for the sustainable development of the livestock sector. By supporting resource-poor and often marginalized livestock keepers, research can contribute to achieving the SDG-Sustainable Development Goals endorsed by the United Nations.

**Resumen.** La ganadería a nivel mundial constituye un componente importante de la producción agrícola mediante su contribución al PBI del sector agrícola en muchos países. El sector ganadero contribuye a la alimentación y seguridad alimentaria a través de la provisión de alimentos de origen animal. También, la ganadería es fuente de ingresos y de

bienes de capital, así como aporta con fuerza de tracción y guano para la agricultura. En sociedades pastoriles, la ganadería es el recurso principal y el bien de capital, sin considerar a la tierra, más grande. Los agricultores pequeños contribuyen en gran escala a la producción agrícola en los países en desarrollo. Las familias pobres especialmente crían y dependen del ganado. Al mismo tiempo, nosotros podemos observar un incremento de la demanda, particularmente en países en desarrollo, de alimentos de origen animal. La llamada “Revolución ganadera” es vista como una oportunidad para los propietarios de ganado para mejorar sus ingresos familiares, asegurar la alimentación de sus familias, y escapar de la pobreza. Pero simultáneamente los criadores de ganado se enfrentan a muchos retos como el cambio climático, precios volátiles y nuevas demandas del mercado. Existe un creciente consenso que el cambio se está acelerando y es menos predecible, así como las interconexiones internacionales nos están llevando a eventos con consecuencias más allá del contexto inmediato. La investigación, la innovación y la educación (superior) jugaran un rol importante en encontrar soluciones a varios de los cambios que los criadores de ganado están enfrentando. Pero, se requerirá un cambio de paradigma hacia una mayor investigación inter y trans-disciplinaria. Más allá del involucramiento de las disciplinas relevantes, cada vez se está reconociendo que el entendimiento y el planteamiento de soluciones a los retos sociales podrán ser mejor logrados con el involucramiento del sector académico y no académico en todas las fases del proceso de la investigación. En años recientes, las plataformas de innovación han recibido mucha atención debido a que los diferentes actores de una cierta cadena de valor tienen la oportunidad de interactuar de manera regular. Estas plataformas proveen el espacio necesario para discutir temas actuales y relevantes. Para los investigadores persistirá la dificultad de mantener un balance entre sus esfuerzos de realizar investigación básica versus la investigación aplicada- ambos son importantes para el desarrollo sostenible del sector ganadero. Mediante el apoyo a los ganaderos de recursos pobres y marginalizados, la investigación puede contribuir al logro de los Objetivos de Desarrollo Sostenible (ODS) endosado por las Naciones Unidas.

**Keywords:** sustainable production, multi-actor, livestock

## Introduction

In 2015, the Sustainable Development Goals (SDG) were adopted by the United Nations member states. This agenda calls for action by all countries to improve the livelihoods of all people globally. By addressing challenges in the livestock sector, contribution to SDG1 (no poverty), SDG2 (no hunger), SDG3 (good health and wellbeing), SDG12 (responsible consumption and production) and SDG17 (partnerships for the goals) can be achieved as the livestock sector plays an important role in many economies of low to middle income countries. The livelihoods of millions of rural households depend partly or totally on income from livestock products (Banerjee et al., 2015, IFPRI, 2016). In Peru the area of alpaca production overlaps with high incidences of rural poverty. The figures from 2017 indicate that 34 % of all alpaca farmers are considered as poor and 12 % as extreme poor (MINAGRI, 2017).

Income growth in low- and middle-income countries increases consumption of products of animal origin, contributing to food security, wellbeing and human health (FAO, 2017). This so-called Livestock Revolution is often referred to as a possible pathway out of poverty for livestock keepers. Global growth of meat and milk consumption is projected to be 1.6 and 1.3 percent per year, respectively, in the 2007–2030 period (FAO, 2015). In India, per capita demand for milk is expected to increase by 57 percent between 2007 and 2030 (FAO, 2011) and milk consumption is likely to triple by 2050 in sub-Saharan Africa, mostly led by East Africa (Herrero et al, 2014). Although animal fiber has a relatively low share on the global fiber market, it is very well positioned in the high-quality fiber sector. Since 2001 in Peru the alpaca fiber production increased annually by 1.79 %, which is mainly a result of an increase in number of animals. In 2015 the Peruvian fiber production reached 4,478 t, of which 90 % was for export market and 10 % for the national market (mainly for handicraft). Main destinations are Italy, China, South Korea and Taiwan. In 2016 the export volume of tops was 51 million US \$ (Minagri, 2017).

At the same time, livestock production is facing many challenges such as competition of resources like water and land and the negative impact of climate change and disease outbreaks. Climate change increases stress for livestock (HLPE, 2016) and could cause a 10 to 25 percent decrease in milk production (IPCC, 2014), which is an important source of nutrition for children (Marquis et al., 1997). An estimated 1.96 billion people rely on livestock to supply part, or their entire daily needs (Anderson, 2003). Currently, societies are confronted with multiple trends that exert pressure on socio-ecological systems such as population dynamics and urbanization (UN 2011; Dlugosz 2011), globalization (Guillén 2001), climate change and natural resource depletion or degradation (IPCC 2017; Prior et al. 2012). These changes make the need for livestock professionals, able to respond to changing needs and demands of the sector stakeholders, apparent. The necessary transformation to a sustainable society will require guidance by responsible actors who have a deep and contextualized understanding of current trends and challenges in socio-ecological systems (Webster 2007). Socio-ecological systems have social, economic and ecological dimensions that characterize a multi-level complex adaptive system (Smit and Smithers 1994; Tiftonell 2014). Complementing their knowledge, future decision makers in such systems will need particular capabilities and skills to facilitate societal innovation (WBGU 2012; Brown et al., 2000). A key skill, according to Fortuin and Bush (2010), will be the ability to cross boundaries between disciplinary knowledge, cultures and theory and practice. Indeed, the discussion on how we can facilitate sustainability learning in higher education has gained momentum in the past several years (Tàbara and Pahl-Wostl 2007; Shephard 2008; UNESCO 2005; Wright and Horst 2013). As universities educate future decision makers, they have a key responsibility for the transformation towards sustainable socio-ecological systems (Shephard 2008; Gadotti 2008; Ciurana and Filho 2006; Moore 2005; Stephens et al. 2008; Mochizuki and Fadeeva 2010).

In turn, Gadotti (2008) argues that education today is part of the causes of unsustainable lifestyles. Krizek et al. (2012) and Beringer (2007) underline that the institution university with all its components, including curricula, has to undergo a sustainability transition.

This paper presents two different case studies in which scientists aim to engage in a dialogue with different stakeholders in the sector of South American camelid production in Peru and sheep production in Argentina. Based on these two case studies some general conclusions will be drawn.

### **Description of case studies**

The case studies presented in this paper differ in their research scope, number and type of stakeholders involved and scale of impact.

#### *Case study 1 – Development of a breeding program for llamas in Peru*

From 2011 – 2013 the 1st project phase “Strengthening llama production in the Central Andes of Peru” and from 2014-2017 the 2nd project phase “Design and implementation of community-based breeding strategies for llamas in the Peruvian Andes” were jointly implemented by Universidad Nacional Agraria La Molina in Lima and BOKU-University of Natural Resources and Life Sciences, Vienna. This project was designed using a transdisciplinary research approach. Participatory, action research methodology was used to ensure the interaction and communication between the research team and the livestock keepers. At the beginning of the project the main goal was to characterize the llama production systems and llama populations in three locations of the Peruvian Central Andes and to develop and to use this information for the joint implementation with the livestock keepers a breeding program for a sustainable use of the local llama populations (Wolfinger, 2012; Radolf, 2014). In the second phase different intervention strategies in the herd management accompanying the breeding program were tested together with livestock keepers. Further details of this research are reported by Apaza et al. 2015, Gómez et al. 2015, Pizarro et al. 2015, Gutierrez et al. 2017 and Wurzinger & Gutierrez 2017.

Llama farmers were in the spotlight of the research. In individual meetings and workshops they could express their needs and ideas. Based on this information, training sessions with livestock keepers were held. Mutual learning for both groups, scientists and livestock keepers, was an essential component of the project. Masters students got the opportunity to learn this new research approach, which puts the farmers in the center of all activities. Other stakeholders like representatives of the Ministry of Agriculture and persons from an NGO were also invited to participate as the project evolved. The idea was that both, the ministry and the NGO could take over responsibilities in the long run and support the local farming community. Keeping the constant contact with livestock keepers in very re-

mote areas posed a challenge for the research team. Time consuming and expensive travels over a long period are difficult to maintain. A field assistant, who frequently travelled could ensure a constant interaction with all people involved. Bridging the scientific excellence (e.g., publications in well-recognized journals) with local, tailor-made solutions for smallholder farmers was a challenge for the researchers.

### *Case study 2 – Transformation of higher education in the area of animal science in Peru and Argentina*

The EU-funded project “EDULIVE-Transforming higher education to strengthen links between universities and the livestock sector in Argentina and Peru” addresses the integration of universities within society at large using the concept of knowledge triangle. According to this concept, “the contribution of higher education to jobs and growth, and its international attractiveness, can be enhanced through close, effective links between education, research, and innovation (...)” (EU, 2017). Applying this to the livestock sector, it seems vital to strengthen the cooperation between universities for animal science and the relevant livestock sector stakeholders, promoting the offer of demand-driven higher education and with it the ability to react to current needs and expectations of the sector. Further details under: <http://edulive-international.eu/>

The aim of the project is to make the academic offer more demand-oriented and addresses topics of relevance for the different stakeholders. In Peru two different value chains, alpaca fibre and dairy value chain, and in Argentina the sheep (wool and meat) value chain were taken as an example. In each country, two public universities, the national research organisations, farmers’ organisations, NGOs and private companies participated in the project. A series of workshops with universities and all stakeholders were carried out to get a better understanding of the different needs, but also to identify mechanisms and possible areas of interest for future cooperation. Internships for students for a period between 2 weeks and 3 months (depending on the requisites of the universities) were implemented. The aim of these internships was to offer students the opportunity to test their knowledge in a real world setting, but also to provide the stakeholders with insights of the capacities of possible future employees and give universities an immediate feedback loop on their curriculum.

All universities revised their policies for internships and made adjustments in their curricula. The idea is that not only the students, but also the university and the receiving institution can benefit from the internship of students. Therefore, an accompanying, well-documented process is launched in the universities. Nevertheless, it remains a challenge for universities to address the large variation of demands coming from the livestock sector and reflect these in their training programs.

## Conclusions

Addressing the demands from the livestock sector in research and higher education is an important and relevant topic. Research has the responsibility, especially as it often receives public funding, to identify possible solutions for the different stakeholders. But it remains a challenge for many researchers to engage in a dialogue with farmers as it requires specific skills, open-mindedness, resources and time. Therefore, it is imperative that universities constantly get feedback from the representatives of the sector to improve and up-date their training programs on a regular basis. This can ensure that graduates are equipped with the necessary skills and knowledge to further develop their working environments and contribute to a sustainable development of the livestock production.

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# Animal Fibre Production in Europe: Biology, Species, Breeds and Contemporary Utilisation

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**Abstract.** Animal fibre “wool” of the pelage is a keratin protein-based product formed in specialised hair follicles located in the skin of a range of animal species and breeds farmed in Europe. It is described as a natural, renewable and biodegradable product of long-standing historical value. Its use in recent decades has been affected by issues such as political changes, poor economic returns and from competition with petro-carbon-based artificial fibres. Such artificial fibres from clothes are typically poorly degraded and are now reported to wash into, and pollute, the world’s oceans and the food chain. Ecologically-sensitive methods for wool production and processing and its inherent degradability have recognised importance. Primary hair follicles typically, although with some exceptions, produce outer fibres of greater diameter, than the underlying and more valuable finer fibres from secondary follicles. Aspects of biology important for production and properties of fibres are recognised. The coarse and fine fibre products require separation after harvesting. This report concerns the animal species, goats (cashmere: Angora) and South American camelids (alpaca: llama), which are present in limited numbers and supply relatively small, and frequently niche, markets in Europe. Angora wool from rabbits supplies an established but reducing market. These species of animals generally produce fibres of fine quality. The multi-million populations of different breeds and cross breeds of sheep, with meat and milk as primary products, are also reviewed. These produce wool of a wide range of qualities much of which is exported overseas particularly to China. The fibres from all species have properties, such as colour, medullation, tensile strength, diameter (fineness) and staple length. These properties determine end-use from small-diameter superfine garments to the coarser fibres utilised in carpets and furniture upholstery. Poor quality wool may be used in geotextiles, or disposed of in landfill, or incinerated.

Numbers of these animals, systems of husbandry, economics, social contribution, sustainability and arrangements for collection and marketing of fibre are described in different countries in Europe. Sources include statistical reports and case studies provided by members of the European Federation of Animal Sciences (EAAP), Animal Fibres Working Group (AFWG). Reference is made to international and European initiatives for the promotion of wool such as The Campaign for Wool and The Wool Group. Note that all websites were accessed on 16 April 2018.

**Resumen.** La estructura de la fibra de lana de origen animal presenta una base proteínica de queratina que se forma en folículos capilares especializados localizados en la piel de varias especies y razas de animales criadas en Europa. Se describe como un producto natural, renovable y biodegradable de valor histórico muy antiguo. Su uso en las últimas décadas se ha visto afectado por cuestiones como cambios políticos, bajos rendimientos económicos y la competencia con fibras sintéticas derivadas del carbón y del petróleo. Este tipo de fibras presentes en la ropa presenta una muy pobre degradación natural, y recientemente se ha descrito que acaban contaminando los océanos del mundo afectando a la cadena alimenticia. Por ello se ha reconocido la importancia de los métodos ecológicamente sensibles para la producción y el procesamiento de lana. Aunque con algunas excepciones, los folículos capilares primarios producen típicamente fibras externas de mayor diámetro que las fibras más finas subyacentes más valiosas de los folículos secundarios. Existen aspectos biológicos de reconocida importancia en la producción y las propiedades de las fibras. Se precisa la separación de las fibras finas de las gruesas a partir del vellón esquilado. Este trabajo se centra en especies de animales domésticos, como cabras (cashmere y angora) y camélidos sudamericanos (alpacas y llamas), con números pequeños en Europa y que abastecen pequeños mercados, que normalmente constituyen nichos de mercado. La lana de conejos de angora abastece mercados establecidos pero en reducción. Estas especies de animales generalmente producen fibras de buena calidad. También se revisa la producción de lana de multitud de millones de ovejas de diferentes razas y cruces destinadas a la producción de carne y leche. Estas ovejas producen lana de una amplia gama de calidades, muchas de las cuales se exportan al extranjero, especialmente a China. Las fibras de todas las especies tienen propiedades, como el color, la medulación, la resistencia a la tracción, el diámetro (finura) y la longitud de la fibra. Estas propiedades determinan el uso final desde las prendas superfina de pequeño diámetro a las fibras más gruesas utilizadas en alfombras y tapicería de muebles. La lana de baja calidad se puede usar en geotextiles o desecharse en vertederos o incinerarse. Se presentan las poblaciones de animales, los sistemas de cría, la economía, la contribución social, la sostenibilidad y la organización para la recolección y comercialización de fibra en distintos países de Europa. La información se obtuvo de informes estadísticos y estudios de casos proporcionados por miembros de la Federación Europea de Ciencias Animales (EAAP) y el Grupo de Trabajo de Animales de Fibra (AFWG). También se hace referencia a la participación del grupo en iniciativas internacionales y europeas para la promoción de la lana, como La Campaña por la Lana (The Campaign for Wool) y El Grupo de Lana (The Wool Group). Téngase en cuenta que el último acceso a las Web con información fue el 16 de abril de 2018.

**Keywords:** animal fibre production, fibre markets

## Introduction

Natural fibres such as wool from animals have a long history of high economic value and effective utilisation but have been affected in recent decades by competition from artificial synthetics and those based on plant-derived materials (Galbraith, 2010a). Wool fibres contain polymers of keratins and other proteins

located in the cytoskeleton of cells. Plant fibres contain cellulose-based polymers while artificial fibres derived from petrocarbons are polymers of simpler organic molecules and include polyester, nylon, and polypropylene.

However, one feature of the latter is their limited degradability and which, along with certain plastics and “microfibres” shed from clothes, have been linked with pollution in land and water (Brown et al., 2011). One consequence of this may be a greater focus on better utilisation of natural fibres which, while retaining properties of durability in use and potential for recycling, have ultimately greater degradability and lesser ecological persistence.

It is also important to be aware of the impact of wool production and processing on the environment and to note developments in eco-labelling and application of life cycle assessment (IWTO, 2018). In this context, the availability of scouring resources, such as provided by the Nejdek Wool Combing (2018) facility in the Czech Republic is recognised as important in Europe along with a focus on maximising ecological outcomes (Chaupin, 2018). Ketterle (2018) has commented on the objective of deregulating the EU-Regulation 1069/2009 which classifies greasy wool as a Category 3 animal by-product.

IWTO (2018) has summarised the wool industry supply chain including production, processing, product manufacturing, recycling and biodegradation. Considering collectively all fibres, these have many uses which depend on their physical and compositional properties. For animal fibres, their properties derive from biology of production in follicles in the skin of animals and are dependent on species and breeds and mechanisms of production within the animal. Such biological mechanisms in animals has been the subject of extensive study for considerable time (eg Ryder, 1958). Interest has been maintained in Europe by the EAAP AFWG and the sharing of developments in knowledge and practices in production such as described in the current Symposium (Sympcam, 2017).

## **Basic biology and husbandry**

In considering basic biology (Galbraith, 2010b, 2010c), the fibre of major interest is the “wool” covering the body of the animal and referred to as the pelage or fleece coat. Wool fibre is produced in hair follicles embedded in the skin, uniquely of mammals. There are two main types of follicles, primary and secondary and a range of subtypes, the hair fibre products of which vary in property according to physical characteristics.

Primary hair follicles have accessory structures of apocrine sweat glands, sebaceous gland and arrector pilae muscle and produce fibres which may be coarse and function as protective guard hair in overlying fibres of the secondary follicle. The latter is typically of finer diameter, has a role in thermoregulation by providing

insulation (Gerken, 2010), and on harvesting, has greater economic value. Secondary follicles differ in having only a sebaceous gland. The products of glands contribute to environmental protection of the fleece, but require removal in processing of harvested fibre. Lanolin from sebaceous glands of sheep has important commercial value in treatment of human skin conditions.

The fibre products of the (coarser) primary and (finer) secondary follicles in double-coated animals require to be separated as the end-uses, generally determined by diameter, differ.

### **Fibre production in follicles**

Hair fibre is produced by division of keratinocyte (epidermal) cells which line the base of the follicle in contact with underlying dermal tissue (Galbraith, 2010b, 2010c). These cells divide and migrate towards, and beyond, the skin surface. The rate of proliferation of these cells also determines the rate of growth of fibre (cortex and cuticle), and fleece, and determines staple length. As the cells migrate they deposit a range of proteins in the internal skeleton and other proteins and lipids which are important in adhesion between cells and which contribute to properties of softness, flexibility, moisture absorption and tensile strength (Lyons, 2009). In addition, consistency of husbandry and nutrition of animals has implications for uniformity of chemical and physical composition along the length of fibres (Galbraith, 2000).

The number of epidermal cells across each fibre contributes to its diameter. Small diameters are typically associated with secondary fibres, finer products and greater economic value. Certain fibres have medullation which is shown by a poorly formed or hollow central shaft which contributes to insulative properties. The pattern of growth of fibres is typically cyclical in nature with an anagen growth phase followed by cessation of growth in catagen and a telogen resting phase before appearance of renewed anagen and regeneration of the follicle. The timing of the following shedding or moulting of the hair is important in harvesting of fibre, particularly if a combing, rather than shearing, method is used.

The hair growth cycle may be influenced by photoperiod, such as seasonally-produced cashmere production in goats with short anagen (months), or where largely uninfluenced by external light stimuli, and with endogenous genetic control determining long anagen (in years) and typical of wool production in Merino sheep (Allain and Renieri, 2010).

Seasonal producers of wool, if unshorn, may also shed fleeces usually in summer, whereas other species and breeds which do not, or only partially, shed and require shearing to remove the fleece. Such removal of fleece provides a harvest of wool as well as improving the physical status of the animal in hot environments.

Where wool has little or no commercial value, and does not shed, for example in sheep, shearing is still required on grounds of animal welfare. There is, in addition, interest in genetic selection for natural shedding traits, such as in the Romane sheep breed (Allain et al. 2011) in France and Exlana sheep in the UK (<https://www.exlana.co.uk/exlana>) to avoid the need for physical removal of wool with related financial and environmental costs. Breeding and selection indices for the most valuable product of follicles, include fineness (smallness of diameter), also frequently associated with juvenile animals, high numbers of follicles and long anagen fibre growth phase Allain and Renieri (2010). Other properties of commercial interest include colour, derived from pigmentation-containing vesicles in cells in the hair fibres, light reflection giving sheen and lustre, medullation and crimp (bending of fibres) frequently associated with fineness and spinning capacity. A smooth covering of cuticle cells on the outer surface of fibres is recognised to contribute to softness. IWTO (2018) has summarised cellular structure in wool fibre in relation to properties of value in end-use. The quality of the fleece is reduced by contaminating material and matting of the wool.

### **Summary of processing and typical end-uses for animal fibres**

Following harvesting by combing or shearing, fibres are cleaned and processed to form the major “worsted” or “woollen” yarns. (British Wool Marketing Board (BWMB), 2018). The worsted system involves fibres of longer staple length which are combed to lay end-to-end in parallel with much of the natural crimp of wool removed and which forms a smooth, variably tight, yarn when spun. Following weaving, these typically make lighter weight and finer fabrics used in suitings, outerwear, other garments where a smooth finish is preferred and upholstery and carpeting.

The woollen system processes wool fibres of shorter staple length. These are carded to lie together for processing into yarn and used, for example, in knitting or weaving into fabrics. Woollen fabrics are typically softer and fluffier than worsted and are used for jackets, coats, rugs and blankets where a bulkier and more textured finish is required. The end use of animal fibres is determined by their physical properties and the preferences of human consumers either in clothing (typically smallest diameters and highest monetary value for knitwear and suiting), in domestic “home” environment (typically coarser and hard wearing for upholstery, carpets, insulation) and similarly externally (materials in transport vehicles (carpets, upholstery); horticulture (plant beddings; fertiliser) or if inadequate quality, unused and deposited in land fill or incinerated.

### **Species and breeds of importance**

The species of major interest in production of fibre (“wool”) in Europe are goats, South American Camelids, rabbits and sheep.

### *Cashmere-bearing goats*

These animals (*Capra hircus*) have seasonal production and are double-coated with both primary and secondary follicles. The latter follicles produce the fibres of small diameter (12-18  $\mu\text{m}$ ) and with annual yields per European goat, typically of 50-150 g. This is in contrast with yields of cashmere up to 1kg in Chinese breed types. Harvesting may be by combing, or shearing. The raw fibre is processed which includes separating from coarse guard hairs and contaminating material and cleaning to produce “pure” cashmere. World production is of the order of 6,500 tonnes predominantly from China and Mongolia (Common Fund for Commodities, 2009).

With respect to Europe, there was considerable interest in the 1980's and 1990's (Ibraheem et al. 1992; Galbraith, 2006) and one analysis (Russel, 1998), concluded that “cashmere production would constitute a viable alternative to traditional forms of livestock farming throughout the EU”. However, the 2017 perspective suggests limited success in Europe due to inadequacies in numbers of animals and yields and quantities of cashmere. Utilisation largely derives from smaller scale production and manufacture such as that described for Chianti Cashmere (Kravis, 2018) in Italy: <http://www.chianticashmere.com/en/>) and Bowmont™ (2018) in the UK.

As regards end uses, cashmere is a fibre combining light weight and softness with insulative properties. It can be dyed and spun into yarns and utilised in high value knitware such as sweaters, hats, gloves and socks, or woven into fabrics and used to produce garments such as outer coats, jackets, trousers and scarves. Larger scale fabric and garment producers in, for example, Scotland and Italy utilise cashmere fibre imported from outwith Europe (eg. <https://www.loropiana.com/en/>).

### *Angora goats*

The other major fibre of goats is mohair produced by the Angora genotype (*Capra hircus angorensis*). These animals have predominantly secondary follicles generating fibres in the range in diameter of 22-35  $\mu\text{m}$  and 2-5 kg annual yield. Contaminating primary follicle products require to be separated to ensure homogeneity of fibres. Fibres from young animals have smallest diameters and are softest in handle.

World production total up to approximately 5,000 t/year (Common Fund for Commodities, 2009). Similarly to cashmere, attempts to expand production in Europe in the 1980's and 1990's have met with limited success. Examples of contemporary production in European countries include the UK where 2.8 tonnes of unprocessed mohair were recently auctioned in South Africa by British Mohair Marketing Ltd (2016). Highest monetary values of kid and adult fleeces respectively, reflecting differences in diameter, and after deduction of post-farm costs, were (£ / kg) 10.31 and 9.08. Data for France indicate a population of 5,000 goats in 140 flocks and producing typically 5.00 kg/animal giving an annual production of 25 tonnes (<http://en.france-genetique-elevage.org/Conservation->

breeds, 405.html). A selection scheme comprising 2,500 goats is described on 35 farms belonging to Capgènes-section Angora (<http://www.capgenes.com/les-races-caprines/race-angora-francaise/>).

Typical properties of mohair fibres include durability, flame- and crease-resistance and significant moisture- and dye-absorbing capability, the latter contributing to dense colors in processed products. Its smooth surface, arising from relatively flat cuticle scales gives a lustrous appearance. Mohair is used to manufacture many products. These include knitting yarn for hand or machine knitwear, socks and sweaters and fabric for lightweight suitings, scarves, blankets and upholstery velours. Fine mohair from younger animals is used in clothing, while thicker and coarser fibres from older animals are used in rugs and carpets and heavy fabrics for jackets and coats.

### *South American Camelids (SAC)*

The main interest is alpaca (*Lama pacos*) with lesser focus on llama (*Lama glama*) and with little or no production of guanaco (*Lama guanicoe*) and vicuña (*Vicugna vicugna*) fibre in Europe.

Alpaca are predominantly single-coated and produce “alpaca” fibre from secondary follicles with typical diameters of 18-30  $\mu\text{m}$  and yields of raw fibre of 1.5-5.5 kg (Antonini, 2010). The main producing country is Peru with an estimated population of 3,685 m yielding 6,500 tons. The fibre of cria, (young animals) has the smallest diameter and is considered lighter, warmer and softer than cashmere (Common Fund for Commodities, 2009). Alpaca fibre is partially medullated which contributes to insulative properties and is produced in a range of natural colours. The alpaca SAC are typically sheared once per year. Two different phenotypes are described in alpaca, identified as Suri and Huacaya which differ for the type of the fleece (Presciuttini et al., 2010). The biological basis for the differences between Huacaya and Suri has been the subject of recent genetic research (Presciuttini et al., 2010). There are about 45,000 registered alpaca in the UK where individual pregnant female alpaca are described as having a price tag of £ 3,000 to £ 15,000 (Inca Alpaca, 2018).

Alpaca fibre is used primarily for knitware in addition to woven products for clothing, including those for outdoor sports, and accessories such as shawls, rugs and duvets. Blends of alpaca with wool, cotton and silk are also made and used in both woven cloth and knitware. (Common Fund for Commodities, 2009). In the UK, with an estimated processing quantity of 35-40 tons of raw fibre in 2016, many farmers process their own alpaca fibre and sell directly to the public, yarns and products ranging from insoles for boots and wellingtons with coarser fibre, to babywear and exclusive luxury fashion items with the finest fibres (British Alpaca Fleece Buyer, 2018).

CBI (2016) provides a useful overview on many aspects of utilisation of alpaca products in Europe and of assistance to exporters from developing countries.

For llama, typical diameters of the undercoat are 20-30  $\mu\text{m}$  with annual yields of 1.5-2.0 kg in a range of colours. Two main breed types, “Chaku” and “Q’aras” are described by Antonini, (2010). The British Llama Society (2018) describes the limited production of llama fibre and identifies three subspecies: tapada, lanuda and ccara.

Uses of llama fibre include fine yarns made from the separated undercoat which are very soft and characteristically lighter than wool of sheep. Typical knitware products include cardigans, jumpers, scarves, gloves and socks. Coarser fibres from the outer coat are used to make rugs and tapestries. Llama fibre may be combined with other fibres to make blends with variable properties.

In terms of populations for South American camelids in Europe, data appear variable. For example, Bonavia (2008) provides, what appear as underestimate, at least for alpaca, of presence in certain countries which give totals of 7,000 llamas, 2,000 alpacas, and 1,000 vicuñas. Estimates were for France, 2-3,000 alpacas and llamas; England, 2-3,000 alpacas and llamas (contrasting with the values given earlier of 45,000); Germany, 2,500 alpacas and llamas and Italy 150 alpacas. Numbers of alpacas and llamas in Austria of up to 6,000 were recently described (Trah and Wittek, 2013). Following shearing, fleeces and fibre of these animals are mostly processed at home or given to small companies for processing (M. Wurzinger, personal communication).

The Llama & Alpaca Registries Europe (LAREU: <http://www.lareu.org/> ) (Kiesling, 2018) provides an online registration service, with database comprising in excess of 19,000 SAC, for alpaca and llama breeders and owners in Europe.

### *Rabbit angora*

The third species of interest comprises angora-producing rabbits (*Oryctolagus cuniculus*). Angora wool comprises very fine medullated fibres with diameters in the range 14-16  $\mu\text{m}$  and flat cuticle scales (Rougeot and Thebault, 1984). Current world production approximates to 3,000 tons with 90 % in China which farms 50 million rabbits (Common Fund for Commodities, 2009). European production approximates to 10 tons, with 3.5 tons in France in 2015, produced by 2,500 rabbits and which has seen major decreases in production since the 1960s. Other European countries which produce angora wool include Hungary and Czech Republic.

There are two main breed types which are both double-coated with an outercoat of coarse fibres and a fine down undercoat. The fleece is harvested three or four times each year. The French type characteristically produces an annual yield of 1.5 kg. While expressing natural moulting, the fleece is typically harvested following feeding of a forage (leucaena) (Allain and Renieri, 2010) containing the chemical anti-nutrient mimosine. This produces a break in the connection of the fibre to the follicle and results in shedding of the fleece. This fleece may be

collected by plucking. The fleece from these animals produces yarn with a “fluffing” effect.

The German type has a fleece of coarse hair and fine down, producing an annual yield of 1.5-2.0 kg. The fleece does not moult and removal is by shearing. The yarn produced is well suited for warm and thermal clothes particularly for people suffering from arthritis or allergies to sheep wool. The price (2015) of Chinese Angora wool imports was 30 €/kg and 60 €/kg for French-produced. The French and EU Angora industry is described as a niche market vertically controlled by farmer/breeder organisations with direct marketing of final products by farmers.

Angora wool is soft to the touch, has good absorption of water and absorbs dyes effectively. Its uses are in luxury products in knitted clothes, such as pullovers, scarves, socks and gloves. It is frequently blended with up to 20 % of sheep wool. One negative feature of the Angora rabbit husbandry, particularly large scale, is the perception of poor welfare conditions. This has resulted in retail outlets declining to sell angora wool garments with associated reductions in production of angora fibre in Europe.

### *Sheep wool*

The animal fibre produced in largest quantities internationally and in Europe is the wool of sheep (*Ovis aries*). The world sheep population is given as 1,163,045 million (British Wool Marketing Board, 2018). The major producing country (with numbers of sheep (x103) and clean raw wool, including skin wool from abattoirs, (tonnes)) is China (162,062: 276,803) with the largest production in Europe in the United Kingdom (33,743: 20,158). Numbers for Europe (FAO, 2016) are 131,059,072. Different sources vary in the values reported. A selection of published data from Eurostat and FAO is shown in Table 1. The greatest numbers of sheep are seen in the order of the United Kingdom, Spain, Romania, Greece, France and Italy. The data for wool production appear only approximately consistent in respect of sheep numbers and similarly for monetary values of raw wool. The European Parliament Briefing (2017) indicated that raw wool production was valued at € 159 million in 2015 in the EU and referred to the value in the United Kingdom as more than € 40 million which is not consistent with the value recorded in Table 1 from the same source. Reference is made to the value of raw wool, as a percentage, of national value of animal products in the EU as averaging at 0.2 % with 2.2 % and 1.7 % for Romania and Slovenia respectively.

### **Uses of wool and contemporary statistics**

As for other fibres, the use of sheep wool is determined by a range of properties such as fineness and includes suits and jackets, shirts, knitwear, ties, plaids and blankets, Japanese futons, rugs and carpets. IWTO (2018) summarizes uses in

categories of apparel, smart textiles, sports, manufacturing, medical, architecture, protective apparel, aviation and protection in industry.

Wool is generally recognized a secondary product of meat and milk production by sheep. EU-Regulation 1069/2009 classifies it as a category 3 animal by-product. Current statistics describe dairy and non-dairy ewe populations in European geographical locations (Eurostat 2014). The “Northern Group” countries (Germany, Ireland, Hungary, Netherlands, Sweden and the United Kingdom) are described as having 35,000 dairy ewes (0.2 % of total) against a population of non-dairy animals of 15.374 million. In contrast “Southern Group” countries (Bulgaria, Spain, Greece, France, Croatia, Italy, Portugal and Romania) are recorded as having 24.525m dairy (59.7 % of total) and 16.559 million non-dairy animals.

**Table 1:** Estimates of sheep populations, greasy wool production and monetary value of raw wool for a range of European countries according to statistical sources.

Countries	Sheep	Greasy Wool	Raw wool value
	(million heads) 2016*	(tonnes x10 <sup>3</sup> ). 2011**	(million Euro) 2016***
France	7.2	14	3.4
Germany	1.6	3****	2.75
Greece	8.8	8	2.08
Hungary	1.2	4	4.22
Ireland	3.4	14	5.01
Italy	7.3	9	9.41
Netherlands	1.0	3	4.68
Portugal	2.1	6	8.17
Romania	9.9	18	44.65
Spain	16.0	22	16.46
UK	23.8	67	30.76
Total	82.3	168	131.59

\* Eurostat, 2017a; \*\* FAO, 2014; \*\*\* Eurostat, 2017b; \*\*\*\* corrected.

Another important factor is the reduction in sheep populations in recent decades, for example, 11.4 million animals between 2005 and 2014 in the 14 countries with more than 0.5 million sheep (Eurostat 2015). The major decreases are in sheep meat-producing countries such as Spain, (31 %), Portugal (30 %), Ireland (22 %) Germany (21 %), France (18 %). Reductions in the United Kingdom, with 28 % of EU-28 production, are reported as 3 %.

Niznikowski and Strzelec (2012) describe reductions in populations of sheep, from 55 to 35 million head, and annual production of wool from 60,000 to 30,000 tonnes in countries in Central and Eastern European between 1990 and 2010.

These followed the change of the political system in 1990 and removal of subsidies for wool. The largest reduction was observed in Poland (94 %) with lesser reductions in Hungary (41 %) and Romania (42 %). Sheep populations in these countries in 2010 are given as 0.261 m, 1.223 m and 9.141 m respectively (FAO, 2018). Contemporary utilisation includes a focus on agro-tourism and production of artistic handicrafts and different types of thermal insulation.

### **Case studies in Europe**

In addition to evaluation of published statistics, members of AFWG provided a summary of contemporary production and utilisation of animal fibre in certain European countries.

#### *United Kingdom*

For example, in the UK which has the largest population of sheep and production of wool, the British Wool Marketing Board (2018) has a central role in collecting, grading, promoting and selling British Wool. More than 70 breeds of sheep are recognised by the Board. The breeds are divided into groups: Fine, Medium, Cross, Lustre, Hill, Mountain and Naturally Coloured. Clean raw wool production in the UK is estimated at 21,600 tons from a sheep population of 33 m.

Historically, as in other countries, wool has had major economic importance, such as exemplified by the wealth of the family of William Shakespeare in the 17th century. As indicated above, meat is recognised as the primary product, with wool sometimes a nuisance “by-product”. There are many specialist wool producers and breed organisations in the UK, which include the Shetland Sheep Society (2018) and Bowmont™ (2018) (Superfine Merino and Cashmere). Additional interest is maintained in fine wool merino which serves a niche market for worsted processing and fine suiting. Reference is made to the weaving and finishing of high quality wool in Yorkshire and supplying Hirsch Tailoring (2018).

Sheep wool production maintains importance in the UK, and Scotland as the base of the present author. There is considerable interest in cross breeds of sheep with a variety of genetics contributing to meat production, reproductive prolificacy, milk production and resilience under intensive husbandry conditions. Moreno Martinez et al. (2012), describe the considerable variation in mean values for diameter of individual wool fibres from individual cross breed animals, among other traditional pure breeds, with raw wool yield of 2-4 kg. There was variation in the presence of crimp and lustre in fleeces of sheep, indicating a heterogeneous product in collected wool in the same flock.

These higher-yielding genotypes contrast with the breeds such as the lower-producing Backface reared under more demanding extensive environmental conditions and contributing to the rural economy such as in the west coast of Scotland. As in other countries, economic returns from wool are small and do not always

cover the cost of wool removal. Interestingly, strong, coarse Blackface wool finds a market in the Italian mattress trade (<http://www.scottish-blackface.co.uk/blackface-sheep-information.cfm>). An example of economic returns (data from 16 August 2016 and average clip value guide), after all costs, and excluding tax, was for breeds as follows (£/kg), Fine, 0.93: Medium, 1.00: Cross, 0.92: Lustre, 4.13 (Blue Faced Leicester): Hill, 0.78-1.18: Mountain, 0.4-0.6). (<http://www.britishwool.org.uk/page/wool-marketing/price-indicator.php>).

### *Italy*

Selected information for sheep includes reference to fourteen breeds which are recognized in the national stud book (Carlo Renieri, personal communication). Of these, three are the wool breeds, Gentile di Puglia (6,800: 394 rams and 6,406 ewes), Sopravissana (10,001: 592 rams and 9,409 ewes) and Merinizzata Italiana (New breed 44,841: 2,073 rams and 42,138 ewes) (Assonapa, 2014).

The population of sheep is described as approximately 8 million which are mainly milk breeds. Total wool production is approximately 9,000 tons raw wool of which 250 tons are derived from the Merino breed type. Approximately 90 % is exported in the international market (similar to Germany) with approximately 10 % processed in Italy.

Official arrangements for collection of wool include the established centre at Biella The Wool Company (2018) and more recent developments in the Abruzzo Region for collection of wool in the Gran Sasso and Monti della Laga National Park. Additional centres are in the process of formalization in Puglia Region (Alta Murgia National Park), Sicily Region and Umbria Region.

Thirty three breeds of sheep are described by Biella The Wool Company which in addition to providing a service in processing of wool is a source of expertise and information in the wool supply chain (Thomson, 2018). Gubbiotti et al (2012) have described the structural characteristics of fleece from a range of Italian meat and dairy breeds of sheep. As in other European countries, regions of Italy have had a long tradition of financial wealth based on the wool trade. One important example is the Wool Guild (Arte della lana) of Florence and relationship with the Medici banking family up to the 16th century. Interestingly, Munro (2012) refers to the importance of imported English wool in the success of the Guild. Currently, Italy has been recognised as second in the world, for manufacture and third for export, of textiles internationally (Greta and Lewandowski, 2010).

### *Germany*

Details for Germany include information provided by Martina Gerken (personal communication). The sheep population is estimated at 1.579 m (Statistisches Bundesamt, 2017). The population is made up of mainly meat-producing breeds and is more limited for milk at 12,300 head. 51 breeds of sheep are registered.

These are categorized as follows: (numbers in parenthesis: Schafzuchtverbände Niedersachsen (2016), as merino (3), meat (9), landrace (22), mountain (9) and hair (5). It is notable that in common with certain Eastern European countries, (Niznikowski and Srezlec 2012), production from fine-wool Merino maintained in East Germany up to reunification in 1991, is no longer economically viable. Subsidies are available according to regional location, for certain endangered and mainly landrace breeds producing course wool fibre. There is interest in preserving rare indigenous sheep breeds (Ketterle, 2018).

Production of greasy/raw wool is currently estimated at 3,000 tons. This is valued at 0.5 to 1.0 €/kg, and at 50 % yield, provides an estimated 1,500 tons of pure wool. The revenue for wool is barely adequate to cover the cost of shearing. Approximately 10 % of the greasy wool product (300-400 tons/year) is processed in Germany, with the remaining 90 % exported to Asian countries, particularly China. The end-use of such wool, typically with diameters more than 28  $\mu\text{m}$ , includes carpets, socks and “Regia Wool™ for hand knitting. Additional uses are insulation material (due to low flammability) for roofs and walls of houses, filling and padding material such as in automobiles and airplanes, and geotextiles including pads made from wool infiltrated with plant seeds for greening of land endangered by soil erosion. Wool is also frequently combined with synthetic fibres in the textile industry.

### *France*

Information for France includes data and statistics compiled by Daniel Allain (personal communication) and sourced from Dispositif INOSYS Réseaux d'élevage – Institut de l'Élevage/Chambres d'Agriculture, France. Totals for populations of sheep (million) and annual wool production (tonnes) for different breed types are reported as follows, for dairy (1.47: 1,537), meat (1.253: 3,901), “hardy” (2.150: 3,642) and conservation (0.089: 248). Overall population and annual wool production calculates to 4.962 m and 9,330 tons respectively. Similarly, data for mean values for different fineness of wool and approximate population (million) are reported as follows: fine merinos (21  $\mu\text{m}$ : 0.78), medium merinos (25  $\mu\text{m}$ ; 0.351), fine medium (28.6  $\mu\text{m}$ , 4.117), medium crossed (32.1  $\mu\text{m}$ ; 1.571), coarse (37.0  $\mu\text{m}$ ; 0.028), kempy (35.6  $\mu\text{m}$ ; 2.307) and pigmented (29.2  $\mu\text{m}$ ; 0.173).

France benefits from a national genetic improvement program involving 4.1 million ewes of high genetic potential (France Génétique Elevage, 2018a). For selection of meat breeds, 10 specialised breeds and a range of hardy meat breeds adapted to adverse environments are included. Among the characteristics tested is genotyping for resistance to scrapie. On-farm performance testing of rams is conducted on 1100 farms with rigorous further selection on test stations. This genetic approach has been successful for both pure breed and cross breed selection

schemes. A similar approach is taken to selection programmes for milk production (France Génétique Elevage, 2018b).

Additional study on genetic diversity has been investigated, for example, by Leroy et al. (2015) in 51 populations of 49 breeds of sheep reared in France, where 57 breeds are officially recognised. The analysis utilised 21 microsatellite markers and concluded that the Merino genetic influences included predominantly until the end of the 19th Century to improve wool production, was weaker than that related to the UK breeds which replaced them with a view to improving meat production. In current practice, costs of shearing are given as 1.5-2.0 €/sheep which, similar to other countries, may not be covered by the value of fleece produced. As indicated above, there is interest in genotypes which shed wool and do not require active shearing (Allain et al., 2011).

### *Spain*

Selected data for Spain include those provided by J P Gutiérrez (personal communication). Spain is well recognised to have the second largest population of sheep in Europe, composed of 80 % meat breeds and the remainder dairy. In common with other countries, populations of sheep, particularly meat breeds have been reducing in recent decades. For example, the breeding population of 11.3 m ewes (Eurostat, 2017a) has been reduced from approximately 18m in 2002. These breeding animals contribute to a total population of 18.55m sheared which produced 22,000 tons of wool. This was composed (tons), for different qualities as follows: fine (4,179) extra fine (12,784), course (4,792) and black wool (174) respectively. Given the poorer economic returns for wool production, there has been recent strategic focus on using genetic selection to improve meat production from Spanish Merino sheep (Valera et al., 2009). These workers estimated heritabilities and phenotypic and genetic correlations for wool characteristics (greasy fleece weight; fibre diameter: staple length; crimp frequency) following decades of selection. They produced simulations of different selection strategies and concluded that wool quality could be improved at the same time as growth rate and meat production.

There are 43 officially recognized ovine breeds in Spain, which have developed from long term artificial selection for production traits of wool, meat and milk (Manunza et al., 2016). These workers have reported on genotype analysis on 11 of these breeds and showed differentiations in populations in certain breeds while others were shown to have a similar genetic background. The work served to highlight the complications of explaining practical outputs of artificial selection in terms of identifiable “signatures” at the level of the genome.

*Austria*

Selected information was provided by Maria Wurzinger (personal communication). Information relating to Austria indicates a population of 14,955 sheep farmers and 349,087 sheep for which there are 30 recognised pure-breeds (BMLFUW 2015). The economic value of these animals is recognised as limited.

The wool crop in 2014 was slightly in excess of 1,000 tons produced mainly in Styria (284 tons), Upper Austria (212 tons) and Tyrol (187.5 tons). The wool crop is either sold to trading companies or processed by farmers and sold directly to end-consumers.

Sheep breeder associations organize collection of wool, for example, for selling to the industry, mainly as insulating material for house constructions (Styrian), processing to produce organic fertilizers for crop production (Upper Austrian), or for use in out-door textiles (Tyrolean).

**Promotion of wool**

Reference is made in the above text to the long history of utilisation of wool in the textile trade, a tradition continued in the current international Campaign for Wool (2018). The patron of the Campaign is Prince Charles, The Prince of Wales. The Campaign seeks to promote the use of wool as a natural, renewable and biodegradable resource and supports the aims of environmental responsibility and sustainability in the development of a commercially viable wool industry. The Campaign is active in Europe and supports events held in different countries including those recently in France, Germany, Italy, Netherlands and the United Kingdom. Good welfare in wool sheep production is recognised in the IWTO Guidelines for ethical wool sheep production (IWTO, 2018). The European Wool Group (2018) is also recognised as acting to improve the value of European-produced wool.

**Conclusions**

Animal fibre continues to be produced in Europe in variable quantities by animal species such as cashmere and Angora (mohair) goats and South American camelids, particularly alpaca. Markets for these tend to be small scale, local and niche. Production of rabbit angora wool continues although has been in decline in recent years following adverse consumer response. Fibres from these animals tend to be finer and of higher value than much of the product from sheep which are present in considerably greater numbers throughout the continent. The main product from the many breeds of sheep is either meat or milk with fibre generally a by-product. There is some production from fine wool sheep in certain countries which does contribute to uses such as higher value apparel and knitware. Sheep wool of lesser quality has value such as in carpets and upholstery and insulation products and geotextiles. Competition with fibres from plant sources and

petrochemicals is recognised, along with problems with the environmental persistence of the latter.

Some reference is made to the science underpinning fibre production by animals ranging from hair follicle biology, thermoregulation and quantitative and molecular genetics. This includes studies on the natural shedding of wool and avoiding shearing which is often uneconomic. National breeding programmes are documented along with initiatives designed to promote the utilisation of wool in Europe and internationally.

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# Effect of Technological Alternatives in the Mitigation of Climate Change in the Aging of Alpacas above 4,000 msnm Puno-Peru

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**Abstract.** The objective of the study was to evaluate the effect of technological alternatives in the mitigation of climate change in the breeding of alpacas over 4,000 meters above sea level. The validation study was conducted in 06 farming communities of two agroecological zones of the Puno region. The variables under study were weight at birth, weight at weaning, weight of fleece, pregnancy and mortality percentage, during 2014 in which no technological alternatives were applied, while in 2015 and 2016 this set was applied of alternatives. The data were analyzed in a completely randomized design using the SAS statistical software version 9.4. The results show that the birth weights were of 6.01, 6.254 and 6.83 kg for the years 2014, 2015 and 2016 respectively ( $p < 0.05$ ). The weaning weights were of 29.02, 29.73 and 30.99 kg for the years 2014, 2015 and 2016 respectively ( $p < 0.05$ ). The fleece weights were 2.28, 2.35 and 2.75 kg for the years 2014, 2015 and 2016 respectively ( $p < 0.05$ ). The pregnancy percentages were of 68.33, 72.84 and 83.77 % in the years 2014, 2015 and 2016 respectively. The mortality percentages were 10.39, 5.11 and 2.44 % for the years 2014, 2015 and 2016 respectively ( $p < 0.05$ ). The set of technological alternatives applied during the years 2015 and 2016 had a significant effect on the birth weight, weaning weight, fleece weight, pregnancy percentage and mortality of the alpacas.

**Resumen.** El objetivo del estudio fue evaluar el efecto de las alternativas tecnológicas en la mitigación del cambio climático en la crianza de alpacas sobre los 4,000 msnm. El estudio de validación se realizó en 06 comunidades campesinas de dos zonas agroecológicas de la región Puno. Las variables en estudio fueron el peso al nacimiento, peso al destete, peso de vellón, porcentaje de preñez y mortalidad, durante el año 2014 en el que no se aplicaron alternativas tecnológicas, en tanto que en los años 2015 y 2016 se aplicaron este conjunto de alternativas. Los datos se analizaron en un diseño completamente al azar mediante el programa estadístico SAS versión 9.4. Los resultados muestran que los pesos al nacimiento fueron de 6.01, 6.254 y 6.83 kg para los años 2014, 2015 y 2016 respectivamente ( $p < 0.05$ ). Los pesos al destete fueron de 29.02, 29.73 y 30.99 kg para los años 2014, 2015 y 2016 respectivamente ( $p < 0.05$ ). Los pesos vellones fueron de 2.28, 2.35 y 2.75 kg para los años 2014, 2015 y 2016 respectivamente ( $p < 0.05$ ). Los porcentajes de preñez fueron de 68.33, 72.84 y 83.77 % en los años 2014, 2015 y 2016 respectivamente. Los porcentajes de mortalidad fueron 10.39, 5.11 y 2.44 % para los años 2014, 2015 y 2016 respectivamente ( $p < 0.05$ ). El conjunto de las alternativas tecnológicas aplicadas durante los años 2015 y

2016 tuvieron un efecto significativo sobre el peso al nacimiento, peso al destete, peso vellón, porcentaje de preñez y mortalidad de las alpacas.

**Keywords:** alpaca, technological alternatives, climate change

## **Introduction**

The CSA have played a fundamental role in the development of Andean societies from the old hunter communities to the current peasant communities (Mengoni, 2008). Before colonization domestic camelids were widely distributed from the altitudes of the Andes to sea level. During the colonization they suffered the uncontrolled sacrifice and were displaced by the domestic animals introduced by the Europeans. This fact remains a clear example of ecological imperialism (Crosby, 1986). As a consequence, both domestic and wild CSAs suffered a severe reduction in number and their geographic distribution was drastically affected, being reduced to the altitudes of the Andean highlands (Wheeler et al., 1995). The CSA have the advantage of resisting adverse environments such as the one existing in the Andean highlands. It is estimated that there are about seven million CSAs in the Andean countries: Argentina, Bolivia, Chile, Colombia, Ecuador, Paraguay and Peru (Fernández Baca, 2005, Raggi, 2005). Of these CSA, 51 % are in Peru and 34 % in Bolivia. Only in Peru are the four species of CSA, being this country which houses the largest population of alpacas and vicuñas. The largest population of llamas is found in Bolivia and guanacos in Argentina. Interest in llamas and alpacas has increased in recent years in other countries including the United States, Canada, Australia, New Zealand and some European countries such as the United Kingdom, Germany, Italy and France (Brown, 2000; Sharpe et al., 2009). The objective of the study was to evaluate the effect of technological alternatives in the mitigation of climate change in the breeding of alpacas over 4,000 meters above sea level.

## **Materials and Methods**

The study was conducted in eight rural communities of the department of Puno located above 4,000 meters above sea level in the agro-ecological zones of dry puna and humid puna. The data for the analysis comes from the records of calving, weaning, shearing, controlled enumeration and health, these were analyzed in a completely randomized design using the statistical software SAS version 9.4. The Duncan test was used for multiple comparisons with a level of significance of  $\alpha = 0.05$ .

## Results and Discussion

Table 1 shows the productive indices of three consecutive years, taking the year 2014 as a reference, as the year in which the technologies were not applied. The results show that the birth weights were of 6.01, 6.254 and 6.83 kg for the years 2014, 2015 and 2016 respectively ( $p < 0.05$ ). The weaning weights were of 29.02, 29.73 and 30.99 kg for the years 2014, 2015 and 2016 respectively ( $p < 0.05$ ). The fleece weights were 2.28, 2.35 and 2.75 kg for the years 2014, 2015 and 2016 respectively ( $p < 0.05$ ). The pregnancy percentages were of 68.33, 72.84 and 83.77 % in the years 2014, 2015 and 2016 respectively. The mortality percentages were 10.39, 5.11 and 2.44 % for the years 2014, 2015 and 2016 respectively ( $p < 0.05$ ). It is possible to observe that the effect of the application of technologies allowed to increase the weight at birth, weight at weaning, weight of fleece, also allowed to increase the pregnancy rate and decrease the percentage of mortality of the alpacas.

**Table 1:** Alpacas' productive indices according to production year

Year of Production	Weight at birth kg	Weight at weaning kg	Weight of fleece kg	Pregnancy %	Mortality %
2014	6.01 ± 1.36 <sup>c</sup>	29.02 ± 6.67 <sup>b</sup>	2.28 ± 0.62 <sup>b</sup>	68.33	10.39
2015	6.25 ± 1.27 <sup>b</sup>	29.73 ± 5.98 <sup>a</sup>	2.35 ± 0.65 <sup>b</sup>	72.84	5.11
2016	6.83 ± 1.21 <sup>a</sup>	30.99 ± 6.12 <sup>a</sup>	2.75 ± 0.59 <sup>a</sup>	83.77	2.44

<sup>abc</sup> Significant differences between means within column,  $p < 0.05$

## Conclusion

The application of technologies contributes to mitigate the effects of climate change at the level of peasant communities above 4,000 msnm, we observe the increase in weight at birth, weight at weaning, weight of fleece, pregnancy percentage and decrease in percentage of mortality of the alpacas.

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# Collection of Diversity – Preserving Rare Indigenous Sheep Breeds in Germany

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**Abstract.** The aim of the project is to preserve the following rare indigenous sheep breeds: Alpine Stonesheep, Black Mountain Sheep, Brown Mountain Sheep, Carniolan Stone Sheep, Coburger Foxsheep, German Karakul Sheep, Forest Sheep, Pomeranian Coursewool Sheep, Spectacle Sheep, Vallachian Sheep and White Mountain Sheep by using their unique wool. Wool quality becomes a very important breeding aspect in order to offer high quality products from the region for the region directly to the customer. One major challenge is to preserve the “wool infrastructure” in Europe, e.g. scouring plants and spinning mills and to modify the EU- regulation 1069/2009 which classifies greasy wool as an animal-by-product of category 3.

**Resumen.** El objetivo del proyecto es la conservación de las siguientes razas autóctonas locales: la raza ovina Alpine Stonesheep, la raza ovina Black Mountain, la raza ovina Brown Mountain, la raza ovina Carniolan Stone, la raza ovina Coburger Foxsheep, la raza ovina Karakul alemana, la raza ovina Forest, la raza ovina Pomeranian Coursewool, la raza ovina Spectacle, la raza ovina Vallachian y la raza ovina White Montain mediante el aprovechamiento de su lana exclusiva. La calidad de la lana se convierte en un aspecto muy importante de mejora para ofrecer productos de alta calidad de la región directamente al cliente. Un desafío importante es preservar la "infraestructura de lana" en Europa, por ejemplo, limpiando plantas e hilanderías y modificando el reglamento de la UE 1069/2009, que actualmente clasifica la lana grasa como un producto derivado del animal de categoría 3.

**Keywords:** conservation, sheep, rare breeds

Many people know that wild animal and plant species are in danger of extinction but only a few are aware of the fact that a similar situation exists with regard to domesticated plant and animal species. A small number of high performance plant and animal strains produce the foodstuffs for the world's population – yet at the same time, a breed of farm animal disappears every two weeks. This means, a breed that successfully adapted to its climate and habitat, which has a genetic heritage and has become a cultural asset, is lost. Many people know about the “Red List” from the realm of wild plants and wild animals. “Red Lists” are registers of

extinct, missing and endangered species of animals, plants and fungi, plant communities as well as biotope types and biotope complexes. They are scientific, expert assessments outlining the current threat for a particular reference area, and evaluate the threat on the basis of population size and population development. Also in the agricultural context, a significant narrowing of genetic variety has already occurred and is still continuing.

In Germany, the Merinolandschaf and a few meat breeds numerically outnumber the other sheep breeds. On the other hand, there are more than 22 different sheep breeds on the Red List in Germany, mostly colored landrace breeds. Innovative ideas are required to utilize the products of these endangered breeds to ensure their long-term conservation.

In former times, farm animals were incorporated in farming in a variety of ways. In the case of cattle, not only their milk and meat supply was appreciated but cattle were also required to pull wagons and ploughs. Sheep were kept for the production of meat, milk and wool. Industrialization and mechanization in agriculture resulted in specialization, which in turn resulted in a departure from multiple-purpose breeds to one-purpose or dual-purpose breeds. In addition to this, the wool price in Europe declined more and more, resulting in wool playing a minor role in sheep husbandry if at all.

Our old and domestic sheep breeds are the result of centuries of breeding. They should therefore be considered a cultural asset worthy of conservation similar to heritage buildings, work of art or an old tree. The “other” performance capability of the old long-established sheep breeds is often underestimated and is even disregarded. They still have valuable characteristics such as a strong physique, frugality, longevity, high fertility and strong mothering abilities. These breeds are resistant to diseases and have adapted to their environment. Modern animal husbandry has led repeatedly to a dead end as a result of its performance breeding goals.

Our project is called “Collection of Diversity – Preserving Rare Indigenous Sheep Breeds” and was initiated in order to work especially with the marketing of particular endangered indigenous sheep breeds and their wool. We always aim to make the sheep breed and their typical wool attractive for both the breeder and the consumer. Furthermore we strive for environmentally friendly and local processing in the manufacturing process. All our woollen products are manufactured in rather small establishments, mostly family run businesses, in Germany, Austria and Northern Italy. Our current product range includes woollen products of ten different colored sheep breeds on the endangered list.

These breeds are:

- Alpines Steinschaf (“Alpine Stone Sheep”)
- Braunes Bergschaf (“Brown Mountain Sheep”)
- Brillenschaf (“Spectacles Sheep”)
- Deutsches Karakulschaf (German Karakul Sheep)
- Coburger Fuchsschaf (“Coburg Fox Sheep”)

- Krainer Steinschaf (“Carniola Stone Sheep”)
- Rauhwolliges Pommersches Landschaf (“Pomeranian Coarsewool Sheep”)
- Schwarzes Bergschaf (“Black Mountain Sheep”)
- Walachenschaf (“Valachian Sheep”)
- Waldschaf (“Forest Sheep”)

Every single one of these endangered colored sheep breeds has a very special wool, typical of the particular breed.



**Figure 1:** A group of Coburger Fuchsschaf at Arkfarm Ketterle. Photo: Arkfarm Ketterle

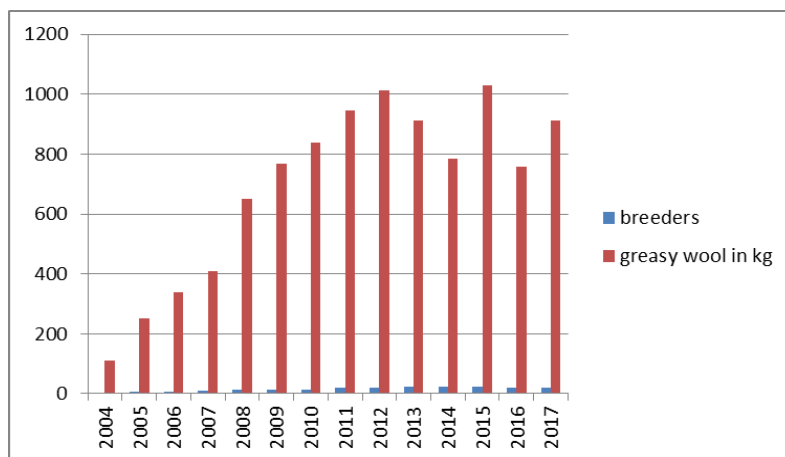
The Coburger Fuchsschaf (“Coburg Fox Sheep”), for instance, plays a major role in the conservation of the countryside (Fig. 1). It is widespread throughout Germany and, above all, ideally adjusted to habitats in low mountain ranges. Its beautiful and unique wool is a distinctive mark of this old but endangered country breed. The wool of the mature animals has a golden hue; this is also referred to as “the Golden Fleece”. However, the fleece of the newborn lambs is reddish brown and brightens as they grow older. The variable tinges and the very good quality, in particular, contribute towards the popularity of its wool. The wool of this breed has a micron count of 33 and 36 (C-D assortment) and is well suited for spinning, knitting, weaving and felting. Furthermore, the wool also absorbs dye well during the dyeing process. The annual wool quantity of the ewes proves to be between 3,5 to 4,5 kg and, in the case of rams, around 5 kg. Due to the fact that the wool is colored it is not in demand with the wool industry and is therefore not used for industrial production in great quantities. As a result, the breeders were not in a position to sell the exceptional wool of their animals in the market place. This ultimate-

ly forced the breeders to dispose of their wool (burning, ploughing under and discarding) instead of selling it at a profit. Due to that there was no necessity to grow the typical “Golden Fleece Wool” of this breed. So it is important that breeders must be able to have the opportunity to sell their wool for a profit.



**Figure 2:** A mother with their Alpines Steinschaf lambs in different colours at Arkfarm Ketterle. Photo: Arkfarm Ketterle

Another breed in our project is the Alpine Steinschaf (Fig. 2). The Alpines Steinschaf, a breed that is mostly found in the Eastern Alps of Bavaria/Germany and Salzburg/Austria and is listed on the Red List in Germany as “extremely endangered”. In 2004 there was a total stock of 123 sheep in flock books. Now, in 2016 there are 680 sheep in flock books. In 2004 the cross-border wool project of the Alpines Steinschaf began. Greasy wool is collected once a year in April at the annual breeders meeting. A big problem within this wool project is the small amounts of greasy wool. In 2004 we started the wool project with only 111kg greasy wool and two breeders. In 2017 913 kg greasy wool was collected from 19 different sheep breeders (Fig.3).



**Figure 3:** Evolution of breeders and greasy wool (kg) from the start of the project 2004.

Since 2012 there was a decrease of greasy wool due to the EU-regulation 1069/2009 concerning the animal by-products came into effect on 1st March 2011. In this regulation greasy wool is classified as an animal by-product of category 3. In conclusion we are no longer authorized to collect greasy wool in Germany from Austrian breeders nor are German breeders authorized to collect greasy wool in Austria from Austrian breeders. So far there exists no registration form that will allow us collecting and transporting greasy wool from more than one EU-country. This is just permitted for professional wool merchants and wool trading organizations but not for small breeder organizations or NGO's.

The wool of the Alpine Steinschaf is coarse wool and it plays an important role in their survival in the high mountains. It's beautiful and unique wool is a special feature of this old breed. The Alpines Steinschaf have a double dual coated fleece with pithy, long coarse hair and fine wavy and short under coat. The woolly undercoat has a micron count of 38 to 48, an indication of the great diversity in this breed. Every wool color from white to black to brown as well as brindled is found. A ewe annually produces between 3 to 3.5 kg of wool and rams about 4 kg. Breeders have been unable to sell the wool of this breed for a profit because it was colored and too coarse. So far there was no use for the wool. Only some felters or hand spinners used the wool for handcrafts.

Another breed in our project is the Karakul. The Karakul is one of the oldest breeds of domestic animals in the world (Fig. 4). In 1900 the Karakul first came to Germany at the suggestion of a fur trade house in Leipzig. Since 1928 the Karakul is pure bred in Germany without substantial addition of homologous transfusion. The Karakul is a fat-tailed breed.



**Figure 4:** A herd of German Karakul (Deutsches Karakul) at Arkfarm Gerstner. Photo: Arkfarm Ketterle

Their wool is coarse and carpet-like; their fleece is also strong, long-stapled and lightweight. It is also high yielding and double coated, with excellent felting qualities. The Karakuls are the only fur bearing sheep known. The lambs are born with a lustrous coat of fur instead of wool covering their bodies known as “Persian Lamb”. The lack of profitability of the breed as a result of the decline of fur prices meant that the Karakul almost extinct. In 1985 there were 1216 sheep in flock books and 125 breeders. In 2016 only 250 sheep in flock books and 6 breeders are left. For this reason and to save this rare sheep breed from extinction we started a wool project with 200 kg greasy wool and 2 breeders in 2016. The most difficulties we had in finding enterprises that are prepared and able to take in small amounts of wool and be able to produce high quality products of this really special wool.

The following example illustrates how innovative marketing concepts can preserve a sheep breed that is close to extinction: The low wool price – selling the raw wool directly after shearing does not even cover the cost of the shearing. This, together with the low demand for smaller quantities of wool, two breeders of the Alpines Steinschaf started to have their wool processed together in November 2004. In 2005, at the annual meeting of the breeders of Alpines Steinschaf, they showcased the products made of their wool to attract more breeders for a joint marketing of these unique woolen products. Here, the idea to launch a wool project for the Alpines Steinschaf was born. With this founding, the cornerstone for a successful marketing of exclusive, first-class woolen products of a particular region was laid. At the annual meeting of the Alpines Steinschaf breeders in May, the wool is jointly collected and sorted. The pre-sorted greasy wool currently fetches 2 € per kilogram in the form of a wool voucher. The aim is to further increase the price so that it will be worthwhile for the breeders to produce wool again, which means to breed Alpine Steinschafe with their typical wool. It was decided that

quality criteria for the wool should be incorporated in the breeding objectives. Collecting the wool at an annual meeting of breeders is also aimed at reducing transport costs as far as possible. The wool is, ideally suited for the production of felting, and knitting materials. That is why typical regional products are being manufactured from the different types of wool according to their structure and history of former production. At the same time our own logo was designed for the different woolen products. This is aimed at securing the brand recognition of these unique products and to further raise the awareness level. This is also the sign of quality, guaranteeing that all products with this special logo have been made exclusively from the wool of this breed. In this manner, the customer can ensure the survival of this rare indigenous sheep breed by buying products displaying its logo (Fig. 5).



**Figure 5:** Logo on wool products from rare indigenous sheep breeds.

The current product range of the Alpines Steinschaf comprises, for example, carded wool, knitting wool, woolen socks, gloves and mittens, woolen beanies, jerseys, knitted sweaters, cardigans, jackets, coats, blankets, various felted bags, insoles, slippers, cushions and woven carpets. Every year more products are added.

The goals of the wool project called “Collection of Diversity – Preserving Rare Indigenous Sheep Breeds” are:

- The acquisition of pre-sorted raw wool at an appropriate price;
- Ecologically friendly processing and production of high quality woolen products;
- Transparent processing in small and mid-sized plants and businesses;
- Manufacturing in the region, thus supporting the regional economic potential;
- Manufacturing under socially acceptable working conditions;
- Supporting sustainable agriculture and conservation of the countryside by means of sheep husbandry;
- Promoting an old sheep breed on the brink of extinction thus supporting genetic variety and preserving a cultural asset.

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AG Coburger Fuchsschafe ([www.agfuchsschaf.de](http://www.agfuchsschaf.de))

Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz  
(<http://www.bmelv.de>)

Bundesamt für Naturschutz (<http://www.bfn.de>)

Bundesamt für Naturschutz (<http://www.bfn.de>)

Gesellschaft zur Erhaltung alter und gefährdeter Haustierrassen e.V. ([www.g-e-h.de](http://www.g-e-h.de))

## Breeding and Genetics





# Advances in Llama (*Llama glama*) Coat Color Genetics

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**Abstract.** Domestic species present a wide phenotypic diversity. As their genomic sequences become available, finding the connection between the genotype and the observed phenotype is a goal possible to achieve. In several species, the genes and mutations responsible for the different coat colors and patterns have already been identified. Color is a Mendelian trait, with little or no environmental influence. Moreover, the biochemical pathway of pigment synthesis is well-known and highly conserved among mammals. Thus, in species such as llama whose complete genome has not yet been sequenced, the study of candidate genes results a valid alternative to identify the genetic variation responsible for different phenotypes. The objective of this work is to present the recent advances in the knowledge of the genes that control the coat color in llamas and compare our results with those published for alpaca.

**Resumen.** Las especies domésticas presentan una amplia diversidad fenotípica. A medida que sus secuencias genómicas están disponibles, encontrar la conexión entre el genotipo y el fenotipo observado es una meta posible de lograr. En varias especies, ya se han identificado los genes y mutaciones responsables de los diferentes colores de capa. El color es un carácter mendeliano, con poca o ninguna influencia ambiental. Además, la ruta bioquímica de la síntesis de la melanina es bien conocida y altamente conservada entre los mamíferos. Por lo tanto, en especies como la llama, cuyo genoma completo aún no ha sido secuenciado, el estudio de genes candidatos resulta una alternativa válida para identificar la variación genética responsable de diferentes fenotipos. El objetivo de este trabajo es presentar los recientes avances en el conocimiento de los genes que controlan el color de capa en llamas y comparar nuestros resultados con los publicados para la alpaca.

**Keywords:** llamas, coat color, MC1R, ASIP, polymorphisms

Melanogenesis is the process of forming melanin, a pigment that gives color to the skin, hair and eyes. The melanin synthesis is produced in the melanosome, a specialized organelle inside the melanocyte. The two types of pigments that are

responsible for color are eumelanin (dark brown/black) and pheomelanin (red/yellow). The ratio between both pigments is controlled by the interaction between the melanocortin receptor (MC1R) and its antagonist, the agouti signaling protein (ASIP). The binding of the  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH), which is the natural ligand of the MC1R, causes the exclusive production of eumelanin, whereas Mc1r inhibition by ASIP leads to the synthesis of pheomelanin (Lu et al., 1994). Polymorphisms in the genes encoding these two proteins have been associated with coat color variation in mammals and birds (Kijas et al., 1998; Andersson et al 2003, Schmutz et al., 2003; 2003; Kerns et al., 2004).

In many species, dominant MC1R alleles (E) produce a uniform black color, while recessive alleles (e) produce red-yellow pigment. Conversely, dominant alleles of the Agouti locus produce a yellow coat whereas the recessive allele in homozygosis is associated with a black coat. In addition to mutations in the genes that control the type of pigment produced, mutations in other genes related to melanogenesis also affect the coat color. For example, genes that affect correct migration and differentiation of the melanocytes, such as KIT or MITF are responsible for unpigmented or spotted phenotypes in most species (Brooks and Bailey, 2005; Pielberg et al., 2002; Cooper et al., 2005). Llamas and alpacas are characterized by a wide variety of coat colors and patterns, although molecular basis of coat color determination is not yet fully understood in camelids. However, in recent years some advances have been made in the knowledge of the genes that control the pigmentation in these species. Molecular genetics studies in alpacas have identified mutations in the MC1R and ASIP genes that are associated to coat color (Powell et al., 2008; Feeley and Munyard 2009; Chandramohan et al., 2011; Feeley et al., 2011; Chandramohan et al., 2013) Much more recently, the first work characterizing these genes and their relation to the color in llamas has been published (Daverio et al., 2016).

The goal of this work is to present the progress made so far in llama coat color genetics, and discuss it with regard to the alpaca findings. To understand how color variation occurs, it is necessary to first understand how the eumelanic and phaeomelanic coats are produced. For these, the coding region of MC1R and ASIP were sequenced in animals with different phenotypes: 1) Eumelanic (animals that have black coat with their variants or dark brown coat) 2) pheomelanic (animals with reddish brown coat) 3) mixed (pheomelanic coat with black face and trims) 4) white (non-albino), representing absence of pigmentation in the coat. Moreover, samples of the llama wild ancestor, the guanaco (*Lama guanicoe*), were also sequenced. Screening for genetic variation in 84 llamas showed 13 Single nucleotide polymorphism (SNPs) in the MC1R gene, 10 of which were non-synonymous. Two of them, c.205C > A and c.638G>A, are novel SNPs that had not been previously described in alpacas. The combination of three polymorphisms, c.259G> A, c.376G> A and c.383T> C, defined three main haplotypes (or alleles) for MC1R: MC1R\*1 (c.259A/c.376A/c.383T), MC1R\*2(c.259G/c.376G/c.383C) and MC1R\*3 (c.259G/c.376G/c.383T). The guanaco sequences showed no variation,

and were identical to MC1R3. Allele frequencies were similar in reddish and black animals. However, MC1R\*1 showed a significant association with pigmented coat ( $P < 0.0001$ ). This allele was not found in any of the 29 white llamas analyzed. In this last phenotypic group, MC1R\*2 was found at frequency significantly higher than in the other groups. Moreover, the wild genotype (MC1R\*3/MC1R\*3) was observed in ten individuals, four “black face” and six white animals, suggesting the influence of other genes in the production of those phenotypes.

MC1R mutations responsible for coat color in llamas appear to be different from those reported in alpacas. Feeley and Munyard (2009) found that alpacas with haplotype combination c.82A/c.126T/c.901C (E/E or E/e) in MC1R are able to produce eumelanin whereas animals which have the combination c.82G/c.126C/c.901T (e/e), only express pheomelanin. We found that SNP c.901C > T, c.82A > G and c.126T > C appeared in very low frequency, being almost fixed for the ‘eumelanic’ combination proposed by those authors in the alpaca. Different results were found by Chandramoan et al. (2011) for alpaca: animals homozygous for A82/A259/A376/C901 expressed black phenotypes whereas white animals were homozygous for the combination of 82/G259/G376/T901. In llamas, the c.259A/c.376A/c.383T haplotype is associated to a pigmented coat, but not exclusively eumelanic and c.259G/c.376G/c.383C (MC1R\*2) is found in high frequency in white animals. Moreover, almost half of the white llamas were homozygous MC1R\*2/ MC1R\*2, a genotype not observed in any of the llamas with pigmented coat. In principle, we did not expect any particular genotype at the MC1R in white animals since white is caused in other species by mutations located in other genes, which are dominant and epistatic to MC1R. The c.383T>C substitution in the MC1R\*2 produces a p.M128T change in the protein, in a site highly conserved in mammals. Functional studies in humans confirmed that this variant, found in malignant melanoma, shows marked loss of function and reduced agonist binding affinity. Nevertheless, for the moment we have no direct experimental evidence of the impact of this substitution on the llama protein. As MC1R alleles were not specifically associated with pheomelanic/eumelanic phenotypes in llamas, the complete coding region of ASIP was also analyzed in the same animals. Two polymorphisms were found within exon 4, a 57 bp deletion (c.325\_381del) and a nonsynonymous SNP (c.292C > T). Both polymorphisms are predicted to have a deleterious effect on the protein (Feeley et al., 2011)

All llamas with a pheomelanic coat carried at least one copy of the non-deleted (wild type) ASIP allele. Instead, 17 from 19 black llamas were homozygous for the deletion, homozygous for the T variant of c.292C > T or heterozygous for the combination of both. This is consistent with a recessive inheritance mode for black color proposed for llamas (Frank et al, 2006). The same two variants have been previously associated to black color in alpacas (Feeley et al., 2011; Chandramohan et al., 2013). Moreover, a third substitution c.353G>A associated with this phenotype has been described but has not been yet identified in llamas. Although we have now molecular evidence of how eumelanic and pheomelanic phenotypes can

be produced, some questions remain to be answered. Segregation analysis has shown that the white phenotype is dominant to pigmented phenotypes in llamas and alpacas (Frank et al, 2006). So, why are MC1R\*2/MC1R\*2 llamas white? Why they are not pheomelanic? Why llamas with wild genotype MC1R \*3/MC1R\*3 can also have a white coat? Non albino-white phenotypes are caused in most species by mutations in MITF and KIT genes. On these bases, these genes were afterward sequenced. To this objective, we obtained total RNA from skin biopsies and the complete cDNA sequence was determined in 23 llama samples. Neither polymorphisms in the coding region nor splicing variants associated with coat color were found. Therefore, to further understand how white phenotype in llama is produced studies on the expression of KIT, MITF and some other genes involved in melanogenesis are currently underway within our group.

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# Characterization and Expression Analysis of SLC7A11 in Llamas

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**Abstract.** The llama (*Lama glama*) is a South American camelid which is gaining worldwide recognition for its fiber. Coat color is one of its most cherished and commercially important characteristics; however, there is very little information about the molecular mechanisms that control pigmentation in llamas. SLC7A11 (solute carrier family 7 number 11) encodes the light chain of cystine/glutamate exchanger, xCT, which has a major role in pheomelanin synthesis. Reports on these issues indicate for instance, that in mouse a mutation in SLC7A11 gene produce a diluted coat color due to a reduction in pheomelanin production (Chintala et al. (2005). Furthermore, differences in the skin expression levels of SLC7A11 have been observed in brown and white alpacas (Tian et al., 2015). The aim of this study is to describe SLC7A11 coding region and to analyze its variation and expression in llamas of different coat color. For this purpose, skin biopsies from 13 animals were collected, the RNA extracted and total cDNA obtained. cDNA was used for PCR amplification and sequencing of the full coding region. In addition, expression levels were analyzed by Real Time PCR. Coding region of SLC7A11 consisted of 1,512 bp that encoded a 503-amino acid protein. Protein sequence analysis showed 12 transmembrane helix regions with cytoplasmic N-terminal and C-terminal residues. Analysis with BLASTP showed 91-99 % identity to other mammals as well as a highly conserved amino acid permease domain. Seven SNPs were observed in the llama DNA sequences, 6 synonymous and 1 non synonymous. Finally, preliminary results indicated that expression levels of SLC7A11 in undiluted phenotypes differ significantly from those in diluted and white, but no differentiation was found between diluted and white phenotypes.

**Resumen.** La llama (*Lama glama*) es un camélido sudamericano que está empezando a ganar reconocimiento mundial por su fibra. El color de la fibra es una de las características de mayor valor comercial, sin embargo, hay muy pocos estudios sobre los mecanismos moleculares que controlan la pigmentación en llamas. El gen SLC7A11 (transportador soluble de la familia 7, número 11) codifica la cadena liviana del intercambiador de cistina/glutamato, xCT, y cumple un rol importante en la síntesis de feomelanina. Chintala et

al. (2005) encontraron que una mutación este gen es responsable del fenotipo subtile gray (sut) en ratón. Los mutantes sut muestran un color de capa diluido que se debe a una reducción de la síntesis de feomelanina. Además, alpacas marrones y blancas presentan diferencias en los niveles de expresión de SLC7A11 (Tian et al., 2015). El propósito de este trabajo es describir la región codificante de SLC7A11 y analizar su variación y expresión en llamas con distinto fenotipo de color Para ello se tomaron biopsias de piel, se extrajo el ARN y se obtuvo el cADN total. Este último fue empleado para reacciones de PCR y posterior secuenciación de la región codificante completa. Los niveles de expresión se analizaron mediante PCR en tiempo real. La región codificante de SLC7A11 está compuesta por 1512 pb y codifica una proteína de 503 aminoácidos. El análisis de la secuencia de la proteína muestra 12 regiones de tipo hélice transmembrana con los residuos C-terminal y N-terminal de orientación citoplasmática. Mediante el análisis con BLASTP se observó entre 91-99 % de identidad con otros mamíferos y la presencia de un dominio aminoácido permeasa altamente conservado. Se identificaron siete SNPs en las secuencias de ADN de llamas, 6 sinónimos y 1 no sinónimo. Finalmente, de acuerdo a resultados preliminares de expresión, los niveles de SLC7A11 en fenotipos no diluidos fueron significativamente diferentes de los hallados en fenotipos diluidos y blancos.

**Keywords:** llamas, coat color, SLC7A11

## Introduction

The fiber of llama (*Lama glama*) is increasingly appreciated by the textile industry. Its cost depends primarily on the diameter and the color of the fiber. However, there is little knowledge about the molecular basis of coat color determination, which makes it very difficult for breeders to obtain the expected color phenotypes or reduce those of less commercial value.

In mammals, the basic coat colors are defined by the relationship between two pigments: eumelanin (from black to brown) and pheomelanin (from red to yellow). Eumelanin/pheomelanin ratio is regulated mainly by the ligand-receptor system of the agouti signaling protein (ASIP) and the melanocortin 1-receptor (MC1R). The binding of alpha-melanocyte stimulating hormone ( $\alpha$ -MSH) to MC1R leads to eumelanin synthesis while binding of ASIP inhibits signal transduction, causing the melanocytes to produce pheomelanin (Lu et al., 1994). However, the final color phenotype will also depend on the expression and interaction of many other genes involved in processes such as the development and differentiation of melanocytes, melanosome formation and pigment synthesis, pigment transport and transference to tissues and survival of melanocyte stem cells. Mutations affecting genes involved in any of these processes can disrupt the normal pigmentation pathway producing diluted and white phenotypes.

The different coat color phenotypes in llamas were described by Frank (2001) and Frank et al. (2006). Based on these descriptions and performing classical crossbreeding analysis, those authors studied the segregation of color phenotypes and postulated that pigmented phenotypes are segregated by the Agouti locus.

Nevertheless, the molecular basis of coat color determination in these species has not yet been established. Recently, we have sequenced the coding region of MC1R and ASIP in llamas and studied the association between polymorphisms in these genes and coat color variation (Daverio et al., 2016). An interesting finding in that work was the detection of association between MC1R\*2 haplotype and white coat. However, white llamas were also homozygous for haplotype MC1R\*3, which was found in colored phenotypes as well. This suggests that other genes might be involved in the production of white phenotypes. In most species, mutations in MITF and KIT genes are responsible of white phenotype (Haase et al., 2007; Pielberg et al., 2002), but we have studied the complete coding region of these genes in llamas and found no differences between white and colored phenotypes (Anello et al., 2015, 2016).

Extreme dilution of pheomelanin has been proposed as a possible mechanism for white or cream phenotypes in other species. Sponenberg and Rothschild, (2001) described in dogs an Intense (I) locus that is thought to dilute only pheomelanin. However, I locus has not been characterized yet. Several genes have been involved in color dilution in different mammal species; one of them is SLC7A11 (solute carrier family 7 number 11). This gene encodes the light chain of cystine/glutamate exchanger, xCT, which has a major role in pheomelanin synthesis. Cystine is a precursor for pheomelanin synthesis but it is not necessary for the synthesis of eumelanin. Therefore, mutations in SLC7A11 are supposed to produce only pheomelanin dilution, without affecting eumelanin. There are only a few reports about this gene and even less that analyses its relationship with pigmentation. One of them is the one carried out by Chintala et al. (2005). They found that a mutation in SLC7A11 gene was responsible for the subtle gray (sut) phenotype in mouse; sut mutants show a diluted coat, due to a reduction in pheomelanin production. In another work, Tian et al. (2015) studied brown and white alpacas and found that there were differences in the expression levels of SLC7A11 between these two phenotypes.

Based on the above information, the aim of the present study is to describe SLC7A11 coding region and to analyze its variation and expression in llamas of different coat color phenotype.

## Materials and Methods

Skin biopsies from llamas with white (n=6) and pheomelanic (n=7) phenotypes were collected by disposable biopsy punch (3 mm diameter), following the Argentinean Ethical Guidelines for Biomedical investigation in Animals from Laboratory, Farm or obtained from Nature (Resolution D N° 1047/05 from CONICET, Argentina). Biopsies were conserved in RNAlater (SIGMA, Germany) until their further extraction. Total RNA was extracted by homogenization in TRIzol® and addition of chloroform to separate phases. The aqueous phase was used for the alcoholic precipitation of RNA. Then, it was washed and resuspended

in RNase free water. Reverse transcription to obtain cDNA was performed in a 20  $\mu$ l reaction volume, using 1  $\mu$ g/ $\mu$ l RNA, RevertAid Reverse Transcriptase (Thermo Fisher Scientific) and random primers (Biodynamics), following the manufacturer's instructions.

PCR primers were designed over conserved regions from other mammal sequences available in GenBank, to fully cover SLC7a11 coding region and flanking 5' and 3' UTRs. Amplification reactions were carried out in 25  $\mu$ l PCR mix containing 1X PCR Buffer (Invitrogen, Carlsbad, CA, USA), 2 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 60  $\mu$ g BSA, 1 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.5  $\mu$ M of each primer and 50 ng of cDNA. The cycling profile consisted of an initial denaturation step at 94 °C for 3 min, 30 cycles of 40 sec at 94 °C, 50 sec at 55–57 °C, 40 sec at 72 °C and a 5-min final extension at 72 °C. PCR products were checked on a 1 % agarose gel stained with GelRed™, purified by PEG precipitation and sequenced. Sequences obtained were aligned and analyzed using Geneious (v.6.1.8, Biomatters). Additionally, comparison with the alpaca sequence available at GenBank (KM095134) was carried out. Protein sequence analysis was performed with TMHMM server (<http://www.cbs.dtu.dk/services/TMHMM>) for the prediction of transmembrane regions, Pfam (<http://pfam.xfam.org>) for the identification of conserved domains and BLASTP ([blast.ncbi.nlm.nih.gov](http://blast.ncbi.nlm.nih.gov)) for protein homology.

For the expression analysis the samples were divided into three color groups: undiluted pheomelanic (n=3), diluted pheomelanic (n=3) and white (n=6). Quantitative Real Time PCR was carried out using specific primers designed over the llama SLC7A11 coding region; 18S gene was used as endogenous control. Amplification reaction consisted of 20  $\mu$ l, including 4  $\mu$ l of HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) (Solis Biodyne), 0.5 mM of each primer and 1 ng cDNA. The cycling parameters were: 15 min at 95 °C, 40 cycles of 15 sec at 95 °C, 20 sec at 60 °C, 20 sec at 72 °C, and a final gradient from 95 °C to 72 °C. Every sample was analyzed in triplicate on a RotorGene Q (Qiagen) equipment. Quantification of SLC7A11 transcript abundance was performed using the comparative threshold cycle (CT) method established by Livak and Schmittgen (2001). Finally, ANOVA analysis of variance was used to assess if differences in expression were significant.

## Results

Complete coding region of the llama SLC7A11 gene consists of 1,512 bp divided into 12 exons. The protein encoded has 503 amino acids and, when aligned with other mammal species, it presented high identity with its homologues: from 99 % with *Vicugna pacos* and *Camelus ferus* to 91 % with *Mus musculus*. The protein displays one highly conserved functional domain Amino Acid Permease\_2 located at amino acids 72-461, and 12 transmembrane helix regions with cytoplasmic N-terminal and C-terminal residues.

We detected 3 positions in the llama *SLC7A11* gene that differ from the ones present in the alpaca sequence: c.15 T>C, c.18 G >T and c.445 C> T. The first two are located in exon 1 and represent synonymous substitutions, while the third one is located in exon 3 and it produces a change from His to Tyr .

Seven SNPs were found along the llama sequences, of which 6 are synonymous changes and one is a non-synonymous substitution. Table 1 shows the extension of each exon in the coding sequence (CDS) and the location of the polymorphisms.

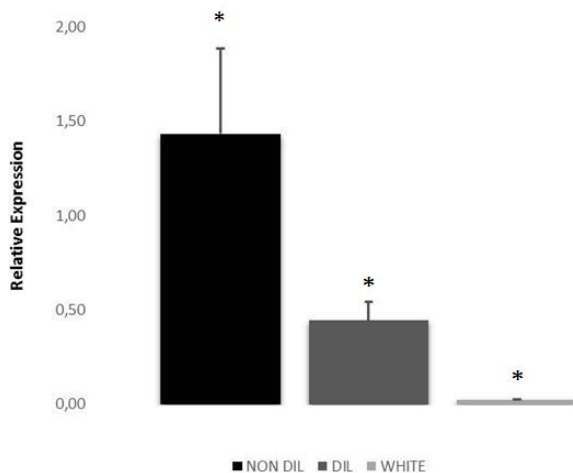
**Table 1:** *SLC7A11* exons and polymorphisms in llama

Exon	Extension in CDS	Polymorphisms	Change in protein
Exon 1	1 - 277	-	-
Exon 2	278 - 403	c.298 T>C    c.381 C>T	no            no
Exon 3	404 - 520	c.418 G>T	p.140 A>S
Exon 4	521 - 646	c.522 T>C	no
Exon 5	647 - 746	-	-
Exon 6	747 - 791	-	-
Exon 7	792 - 915	c.837 T>C	no
Exon 8	916 - 1019	c.984 T>C	no
Exon 9	1020 - 1116	-	-
Exon 10	1117 - 1266	-	-
Exon 11	1267 - 1444	c.1380 G>T	no
Exon 12	1445 - 1512	-	-

We also studied the relative expression of *SLC7A11* in the skin of undiluted, diluted and white phenotypes (Figure 1). *SLC7A11* expression levels in the non-diluted group differ significantly from those in diluted and white ( $p<0.05$ ) animals. Although expression level was consistently lower in white llamas than in the diluted ones, the difference was not significant.

## Discussion

There are very few studies addressing *SLC7A11* gene variation and its role in mammal's pigmentation. Here, we described the coding region of this gene in llamas and found results that support previous information (Tian et al., 2015). *SLC7A11* codes for a highly conserved protein with an AA\_permease\_2 domain, which presents 12 transmembrane helices and it has the function of transport of amino acids. Variation observed in the llama *SLC7A11* gene was mainly due to synonymous substitutions and the unique non-synonymous SNP found does not seem to be associated to color dilution.



**Figure 1:** Relative expression of SLC7A11 in llamas.

SLC7A11 expression levels in undiluted llamas differ significantly from those in diluted and white. This is consistent with the protein function: it allows cystine to enter the cell and cysteine is necessary for the pheomelanin synthesis. Therefore, less expression of SLC7a11 should be translated into a reduction in pheomelanin synthesis. However, though it is evident that level of the diluted group is in between the others two, mRNA expression levels between diluted and white were not significantly different. This could be due to a small difference that would need a larger sample to be detectable. Moreover, as pheomelanin coats can vary from dark red to very light brown, the results obtained may depend on the manner in which the animals from the diluted group were chosen. In other words, at the lower end of the dilution range, expression values close to those of the group of white animals would be expected, while at the other end values should be more similar to those of animals with undiluted coats. This discussion raised the question if white coat could be an extreme dilution of pheomelanin, There is not enough information to answer this question yet, but considering the results from this work and previous findings from other genes (Daverio et al., 2016, Anello et al. 2015, Anello et al., 2016) it is likely that more than one genetic mechanism is responsible for white color in llamas.

Further research is needed to understand how SLC7a11 expression is controlled. We cannot rule out regulatory mutations affecting SLC7A11, but it is also possible that another gene, located upstream in the pigmentation pathway is down-regulating the expression of SLC7A11 in white and diluted llamas.

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# PCR-RFLP Method for Testing ASIP EXON 4 Mutations in Llamas

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**Abstract.** The basic coat colors of mammals are determined by the relative proportion of two types of pigments, eumelanin and pheomelanin. The agouti signaling protein (ASIP) plays a crucial role in melanogenesis, by increasing the production of pheomelanin and decreasing the eumelanin synthesis by blocking the signaling pathway of the melanocortin 1 receptor (MC1R). In several species, loss of function mutations of the ASIP gene is responsible for the black coat color. The polymorphisms of ASIP exon 4, c.325\_381del and c.292C> T, have been previously associated with eumelanic phenotypes in llamas (Daverio et al., 2016) and also in alpacas (Chandramohan et al., 2013). The objective of this work was to develop an alternative method to DNA sequencing for genotyping both polymorphisms. Fifty one llama DNA samples, including controls of known sequence were analyzed. PCR products spanning ASIP exon 4 were digested with the enzyme Bvel, that recognizes the c.292C>T mutation, followed by electrophoresis in 8% polyacrylamide gels to simultaneously detect the SNP and the deletion. Analysis of the band patterns presented complete concordance between DNA sequencing and PCR-RFLP genotypes. Genotyping results from the samples showed that all dark llamas (n=13) were homozygous for the deletion, homozygous for the c.292C> T polymorphism or heterozygous for both, while none of these combinations were observed in the pheomelanic animals here analyzed (n=20). The results of this work support the findings of previous studies and also show the usefulness of the PCR-RFLP technique as a relatively fast, simple and cost-effective method to determine the ASIP exon 4 variants in llamas.

**Resumen.** Los colores de capa básicos de los mamíferos están determinados por la proporción relativa de dos tipos de pigmentos, eumelanina y feomelanina. La proteína de señalización agouti (ASIP) juega un rol crucial en la melanogénesis, incrementando la producción de feomelanina y disminuyendo la síntesis de eumelanina por bloqueo de la vía de señalización del receptor 1 de melanocortina (MC1R). En varias especies, mutaciones con pérdidas de función del gen ASIP son responsables del color de capa negro. Los polimorfismos del exón 4 de ASIP, c.325\_381del y c.292C> T, han sido asociados previamente con fenotipos eumelánicos en llamas (Daverio et al., 2016) y también en alpacas (Chandramohan et al., 2013). El objetivo de este trabajo fue desarrollar un método alternativo a la

secuenciación de ADN para genotipar ambos polimorfismos. Se analizaron 51 muestras de ADN de llamas, incluyendo controles de secuencia conocida. Los productos de PCR que abarcan el exón 4 de ASIP fueron digeridos con la enzima Bvel, que reconoce la mutación c.292C> T, seguida por electroforesis en geles de poliacrilamida al 8 % para detectar simultáneamente el SNP y la delección. El análisis de los patrones de banda presentó concordancia completa entre los genotipos de secuenciación y los de PCR-RFLP. Los resultados de genotipado de las muestras mostraron que todas las llamas oscuras (n=13) eran homocigotas para la delección, homocigotas para el polimorfismo c.292C> T o heterocigotas para ambos, mientras que ninguna de esas combinaciones se observó en los animales feomelánicos aquí analizados (n=20). Los resultados de este trabajo apoyan los hallazgos de estudios previos y también muestran la utilidad de la técnica de PCR-RFLP como un método relativamente rápido, sencillo y de bajo costo para determinar las variantes del exón 4 de ASIP en llamas.

**Keywords:** llamas, PCR-RFLP, ASIP-genotyping, eumelanic

## Introduction

Coat color in animals has important functions in camouflage, protection against UV radiation and communication, among others. Compared to the wild species, domestic animals present a fascinating diversity of colors product of artificial selection (Reissmann & Ludwig, 2013). During the last decade, molecular techniques have allowed the identification of genes and alleles underlying this variation in a large number of species. The color of the coat basically depends on the proportions of the pigments eumelanin (black or dark brown) and pheomelanin (reddish-yellow). The production of these pigments is mainly controlled by the melanocortin-1 receptor (MC1R) and agouti signaling peptide (ASIP) (Bultman et al., 1992). MC1R interaction with its antagonist ASIP produces pheomelanin pigments by blocking the interaction with its natural ligand alpha-MSH (Lu et al., 1994). Dominant black is usually produced by variations in the coding sequence of the MC1R gene (Klungland et al., 1995; Marklund et al., 1996; Kijas et al., 1998). Gain of function mutations that produce constitutive activation of MC1R lead to the synthesis of eumelanin and a dark coat. On the other hand, in species where the black color is recessive, non-functional alleles of the ASIP gene are responsible for the dark coloration. Loss-of-function ASIP mutations have been described in species such as dog (Kerns et al., 2004), horse (Rieder et al., 2001), sheep (Royo et al., 2008). In alpaca, three mutations in ASIP exon 4 c.325\_381del, c.292C>T and c.353G>A have been also associated to black coat (Feeley et al., 2011; Chandramohan et al., 2013). In llama, the mutations c.325\_381del and c.292C>T have been also showed association with black color but the c.353G>A has not been identified so far (Daverio et al., 2016).

In Argentina, the llama is the most abundant domestic camelid and exhibits a wide variety of coat colors that meet different commercial interests. Although the

value of camelids fiber greatly depends on its diameter, the color is also important for the textile industry (Frank et al., 2006, Mueller et al., 2010). In this context, black, reddish brown and golden colors are usually some of the most required (Frank et al., 2006). However, the color of offspring based only on the parents' phenotype is hard to predict in camelids. Thus, the use of molecular tests to determine the genotype of the breeding animals could be useful in selection programs. Nevertheless, if the number of animals is high, sequencing may be expensive and additionally it requires specialized equipment, not always available in small laboratories. The main objective of this work was to develop a simple technique based on Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) to detect c.325\_381del and c.292C>T polymorphisms, as an alternative to DNA sequencing.

### Materials and Methods

Thirty three blood llama samples (20 pheomelanic and 13 eumelanic) from diverse herds from Argentina were collected by jugular puncture. Total genomic DNA was isolated following the LiCl protocol described in Daverio MS (2015).

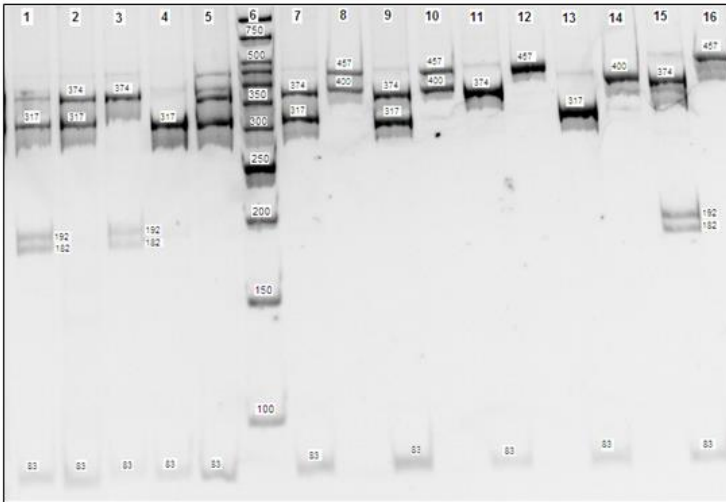
Using the PCR technique, exon 4 of the ASIP gene was amplified in the 33 samples and 18 controls of known sequence. Amplification reactions were carried out as described in Daverio et al., (2016), using as template 60-70 ng of genomic DNA. The PCR products were digested using the restriction enzyme Bvel (BspMI). The enzyme cuts the amplicon into two fragments, one of 83 bp and the other one of 374 bp or 317 bp depending on whether the allele carries or not the c.325\_381del. If the c.292C>T polymorphism is present, it creates a new cutting site for Bvel generating a different banding profile.

Digestion mix consisted of 6 µl of PCR product, 6.5 U of Bvel (Thermo Fisher Scientific Inc.), 0.3 µl of Oligos 50X, 1 µl of Buffer O 10X and 6 µl of bidistilled water that was incubated at 37 °C overnight. Restriction fragments were analyzed by electrophoresis on an 8 % polyacrylamide gel for 90 min at 200 V stained with GelRed™ and visualized using an UV trans illuminator. Fragment length determination was done by comparison with a 50 bp commercial ladder (Embiotec SRL).

### Results and Discussion

We adjusted amount of PCR product, digestion time and enzyme quantity to standardize PCR-RFLP conditions. Digestion of control samples yielded band patterns in all cases concordant with genotypes previously obtained by ASIP exon 4 sequencing. Homozygous genotypes for c.292T that did not have the c.325\_381del mutation (T/T, -/-) showed two fragments of 192 and 182 bp, in addition to the 83 bp band present in all digestions. Homozygous deletion genotypes (C/C, del/del), generated a 317 bp fragment while controls without the

deletion (C/C, -/-) showed a 374 bp band. Heterozygous individuals for one or both polymorphisms also had the expected combined patterns: 374 bp and 317 bp (C/C,-/del); 317 bp, 192 and 182 (C/T, -/del); 374 bp, 192 and 182 bp (C/T, -/-) (Fig. 1.)



**Figure 1:** Electrophoresis pattern of ASIP exon 4. Lines 1 to 5 and 7, 9, 11, 13, 15: digested PCR products. Lines 8, 10, 12, 14 and 16: PCR products before digestion. Line 6: molecular size marker of 50 bp. Line 5: incomplete digestion.

After confirming that digestion patterns were as expected, unknown samples were genotyped. PCR-RFLP analysis revealed 6 different genotypes (Table 1). No shared genotypes were found between the two color groups; three were exclusive of eumelanic samples and the remaining three of pheomelanic animals.

The two mutations here studied have been associated to the recessive black coat in alpacas (Feeley et al., 2011; Chandramohan et al., 2013) and llamas (Daverio et al., 2016). Substitution c.292C> T, causes a p.R98C change that introduces an additional cysteine into the region of ASIP which makes contact with MC1R (Kiefer et al., 1997). The other mutation, c.325\_381del, leads to the loss of 19 amino acids (p.C109\_R127del) within the same domain. Both mutations are predicted to be deleterious and to alter normal protein function. Therefore, the mutated ASIP protein is unable to bind MC1R to induce pheomelanin production and only eumelanin is synthesized, resulting in a black coat. In agreement with that, we found that black llamas were all homozygous for c.292T (T/T), homozygous c.325\_381del (del/del), or in a lower frequency (3/13), compound heterozygotes for both mutations, whereas these genotypes were not found in pheomelanic individuals. Thus, our results provide additional support to the previously reported

association between ASIP variants and coat color in domestic camelids (Feeley et al., 2011; Chandramohan et al., 2013, Daverio et al., 2016)

Table 1: ASIP exon 4 genotypes in eumelanic and pheomelanic llamas

c.292C>T	Genotype	Phenotype	
	c.325_381del	Pheomelanic	Eumelanic
C/C	del/del	0	6
T/T	-/-	0	4
C/T	-/del	0	3
C/C	-/-	9	0
C/T	-/-	2	0
C/C	-/del	9	0

del: deletion    -: non deleted variant

## Conclusion

The PCR-RFLP technique proved to be useful for genotyping c.325\_381del and c.292C> T polymorphisms. It offers the advantage of being based on a single PCR reaction and is suitable for reading on polyacrylamide gels.

The confirmation of the association between these variants and the black coat still requires the analysis in a larger number of animals and further family based segregation studies. The methodology presented here would be adequate for this purpose, since it allows to genotype the two variants simultaneously, without needing of DNA sequencing.

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# Heredabilidad estimada de fibras meduladas en alpaca huacaya

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**Abstract.** The aim was to estimate the heritability of medullated fibers in alpaca Huacaya. Samples from 36 fleeces of white males (0.4-10.4 years old), from Pacamarca farm (Puno, Perú) were taken. The diameter was recorded in each fiber (FD) following the IWTO-8-2011 regulation, and simultaneously the class of medullation (CM) was recorded as established in the literature (Villarroel, 1963) using the projection microscope (PM). For estimating genetic parameters of CM and FD was used an animal model with repeated measurement, the age was considered as a linear and quadratic covariate, the additive genetic effect and the permanent environmental as random effects. Continuous and threshold models were evaluated; considering 4 univariate models to estimate the heritability of CM and 4 bivariate models to estimate the heritability as well as the genetic correlation between CM and DF which were analyzed using TM software. The estimate heritability of CM was  $0.36 \pm 0.13$  under a bivariate continuous model where CM was non-medullated versus medullated. Using the same model, the estimate genetic correlation between CM and FD was  $0.93 \pm 0.12$ . In sum, if the amount of medullated fibers is reduced, the individual fiber diameter in alpaca fleece will decrease.

**Resumen.** El objetivo fue estimar la heredabilidad de fibras meduladas en alpaca Huacaya. Se tomaron muestras de 36 vellones blancos de alpacas machos (0.4-10.4 años) del fundo Pacamarca (Puno, Perú). Se registró el diámetro de cada fibra (DF) siguiendo la norma IWTO-8-2011, y simultáneamente se registró la clase de medulación (CM) según se describe en la bibliografía (Villarroel, 1963), mediante el microscopio de proyección (MP). Para la estimación de los parámetros genéticos de CM y DF, se usó un modelo animal con medidas repetidas. La edad se consideró como covariable lineal y cuadrática, el efecto gen-

ético aditivo del animal y su ambiente permanente como efectos aleatorios. Se evaluaron modelos continuos y umbrales, considerando 4 modelos univariados para estimar la heredabilidad de CM y 4 modelos bivariados para estimar las heredabilidades de CM y DF así como su correlación genética, mediante el software TM. La heredabilidad estimada de CM fue de  $0.36 \pm 0.13$  bajo un modelo continuo bivariado donde CM se consideró en dos categorías: no medulada versus medulada. Utilizando el mismo modelo se estimó una correlación genética de  $0.93 \pm 0.12$  entre CM y DF. En conclusión, si se reduce la cantidad de fibras meduladas por selección, también se disminuirá el diámetro individual de la fibra.

**Keywords:** alpaca, medullated fiber, heritability, genetic correlations

## Introducción

La calidad de la fibra de alpaca es considerada mundialmente como una de las mejores para la industria textil. Sin embargo, su precio aún está lejos de otras fibras finas como la cachemira. Quizás el principal factor que reduce su valor es la presencia de fibras meduladas por lo que se ha asociado con el factor de picazón en la prendas (Frank et al., 2014). En el vellón de alpacas, incluso las fibras más finas pueden tener medulación fragmentada o discontinua.

En el fundo Pacamarca se ha realizado grandes esfuerzos para eliminar esta característica en particular. Realizando el descordado manual (eliminación de fibras meduladas) se podría reducir inmediatamente la picazón por ende mejoraría el confort de las prendas (Laime et al., 2016). Sin embargo, el descordado manual no sería rentable económicamente (Quispe et al., 2015). Por lo que se busca animales con bajo contenido de medulación en el vellón y el propósito de este trabajo fue estimar la heredabilidad y la correlación genética entre la medulación y el diámetro de cada fibra.

## Materiales y métodos

Se registraron un total de 21600 fibras individuales de 36 muestras de vellones blancos de alpacas Huacaya machos entre 0.4 y 10.4 años del fundo experimental Pacamarca del Grupo Inca (Puno, Perú). El diámetro individual de fibra (FD) se registró aleatoriamente (600 fibras por animal) siguiendo la norma IWTO-8-2011 (IWTO, 2011); por microscopio de proyección (PM) se clasificaron en cinco categorías de medulación (Villaruel, 1963): no medulada, fragmentada, discontinua, continua y fuertemente medulada.

El pedigrí de los individuos registrados fue rastreado a los ancestros hasta los fundadores para completar un pedigrí de 121 individuos, con el 100 % de los padres conocidos, así como el 71 % de los abuelos, el 20 % de los bisabuelos y menos del 1 % de los bisabuelos identificados. La matriz de relaciones numéricas fue calculada utilizando el software Endog v4.8 (Gutiérrez y Goyache, 2005) donde se mostró una fuerte conexión entre los animales.

El grupo de machos fue tomado al azar, donde se observa una conexión entre ellos, justificando su representatividad en toda la población. Por otra parte, aun cuando el número de animales es escaso, el alto número de registros ayudó a obtener estimaciones confiables y la fiabilidad final de fue conocido mediante el enfoque Bayesiano que ayuda a cuantificar adecuadamente los niveles de incertidumbre, una vez que la convergencia fue probada.

La medulación aquí se ha codificado como una característica discreto. Por lo tanto, los modelos umbrales son los indicados para la estimación de los parámetros genéticos para este rasgo. Sin embargo, se ha demostrado que los modelos lineales tienen mejores resultados que los modelos umbrales cuando las bases de datos son pequeñas (Ibáñez et al., 2014).

Para la medulación las categorías evaluadas fueron: C2A, dos categorías: 1: agrupada no medulada, fragmentada y la discontinua, 2: agrupada la continua y la fuertemente medulada. C2B, dos categorías: 1: no medulada 2: agrupada todas las meduladas.

Las dos formas de clasificación se analizaron utilizando dos metodologías diferentes. El modelo lineal continuo (L) y el modelo umbral (I); la ecuación del modelo umbral fue idéntico al modelo lineal lineal, pero en este caso el vector  $y$  explica la categoría visible conocido como umbrales (Gianola, 1982). Finalmente, todas las combinaciones también fueron probadas usando 4 modelos univariados y 4 modelos bivariados que combina el diámetro de la fibra y la medulación. Así, se llevaron a cabo 8 estimaciones diferentes de componentes de varianza para la medulación.

La ecuación del modelo animal con medidas repetidas fue:

$$y = Xb + Zu + Wp + e$$

(1) donde  $y$  vector de observaciones (categoría de medulación o diámetro de fibra individual);  $b$  vector de efectos fijos: edad, edad al cuadrado;  $u$  vector de efectos genéticos aditivos;  $p$  vector de ambiente permanente;  $e$  vector de efectos residuales;  $X$ ,  $Z$  y  $W$  son matrices de incidencia de efectos fijos, efecto genético del animal y efectos del medio ambiente permanente, respectivamente.

La ecuación del modelo umbral fue idéntico, pero en este caso el vector  $y$  representa una variable subyacente llamada problema que explica la categoría visible correspondiente definida por valores específicos llamados umbrales (Gianola, 1982). El análisis se llevó a cabo con el software TM (Legarra et al., 2011). Un millón de iteraciones se realizaron para cada modelo con un período de ciclo de 100.000 y con un intervalo fino de cada 100 iteraciones.

## Resultados y discusión

Las estimaciones de los componentes de la varianza para diferentes agrupaciones de categorías de medulación, se muestran en la Tabla 1, junto con las respectivas desviaciones estándar de sus distribuciones marginales posteriores. Utilizando

diferentes modelos que combinan modelos univariados o bivariados con el diámetro de fibra, bajo modelos lineales o umbrales.

Las estimaciones de heredabilidad para el rasgo de la médula variaron de 0.30 a 0.36 en los modelos lineales y de 0.11 a 0.15 en los modelos de umbrales. Todas las estimaciones de heredabilidad para categorías de medulación pueden considerarse significativas de acuerdo con la magnitud de la desviación estándar de sus distribuciones marginales posteriores, mostrando que la cantidad de información utilizada fue suficiente para proporcionar estimaciones significativas.

Las estimaciones de la heredabilidad del diámetro de cada fibra fueron entre  $0.28 \pm 0.14$  y  $0.35 \pm 0.15$ , que son mucho menos precisos, de acuerdo con sus respectivas desviaciones estándar, que los de la medulación.

Por otro lado, la correlación genética estimada entre el diámetro individual y la medulación fue muy alta utilizando modelos continuos de 0.88 a 0.93, aunque el uso de modelos de umbral fue moderado e impreciso de 0.26 a 0.68. En ovejas Corriedale, Sánchez et al. (2016) reportaron una correlación genética de magnitud intermedia (0.50) entre el diámetro medio de fibra y la presencia versus ausencia de fibra medulada.

En ovejas Corriedale, utilizando un modelo animal multivariado, Sánchez et al. (2016) reportaron una heredabilidad de  $0.37 \pm 0.10$  para la presencia versus ausencia de medulación; similar a la heredabilidad estimada en el presente estudio ( $0.36 \pm 0.13$ ). Sin embargo, resulta mayor a  $0.23 \pm 0.02$  del contenido de medullation OFDA en cabras Angora, aunque similar a  $0.32 \pm 0.02$  del contenido de fibras kemp (Allain y Roguet, 2006). Se han reportado heredabilidades similares para el porcentaje de medulación total ( $0.29 \pm 0.04$ ) y de  $0.33 \pm 0.07$  para el porcentaje de fibras fuertemente meduladas (Frank et al., 2011).

Por último, una limitación de nuestra investigación fue un número limitado de animales (36) debido a que el tiempo requerido para procesar una muestra tomó alrededor de 1 día por PM y además requiere personal capacitado. También Sánchez et al. (2016) indicaron 1,5 días por animal. Sin embargo, por OFDA 100 analiza en menos de 8 minutos una muestra (Lupton y Pfeiffer, 1998), lo cual permite registrar más animales, basado en la opacidad ( $>80\%$ ) agrupando fibras meduladas continuas y fuertemente meduladas de forma similar a C2A que se registró por MP.

## Conclusión

Los modelos lineales continuos estimaron mejores heredabilidades de CM y altas correlaciones genéticas entre CM y DF. Así se reduciría la cantidad de fibras meduladas y el diámetro de cada fibra en el vellón de alpaca Huacaya.

**Tabla 1:** Parámetros genéticos estimados  $\pm$  desviación estándar de sus distribuciones marginales posteriores, para categorías de medulación y el diámetro individual de fibra bajo modelos lineales (L) y umbrales (T)

Modelo	Categoría de Medulación						Diámetro de Fibra					
	$\sigma_u^2$	$\sigma_{ep}^2$	$h^2$	$c^2$	R	$r_g$	$\sigma_u^2$	$\sigma_{ep}^2$	$h^2$	$c^2$	R	
Uni; L;	0.09 $\pm$	0.05 $\pm$	0.30 $\pm$	0.15 $\pm$	0.45 $\pm$	-	-	-	-	-	-	
C2A	0.04	0.04	0.12	0.11	0.06	-	-	-	-	-	-	
Uni; L;	0.14 $\pm$	0.07 $\pm$	0.35 $\pm$	0.17 $\pm$	0.52 $\pm$	-	-	-	-	-	-	
C2B	0.07	0.05	0.13	0.12	0.06	-	-	-	-	-	-	
Bi; L; C2A	0.10 $\pm$	0.07 $\pm$	0.30 $\pm$	0.20 $\pm$	0.50 $\pm$	0.91 $\pm$	25.5 $\pm$	17.0 $\pm$	0.34 $\pm$	0.23 $\pm$	0.58 $\pm$	
	0.05	0.04	0.12	0.11	0.07	0.13	14.14	11.63	0.15	0.15	0.07	
Bi; L; C2B	0.16 $\pm$	0.10 $\pm$	0.36 $\pm$	0.21 $\pm$	0.57 $\pm$	0.93 $\pm$	26.0 $\pm$	17.0 $\pm$	0.35 $\pm$	0.23 $\pm$	0.58 $\pm$	
	0.08	0.06	0.13	0.12	0.07	0.12	14.09	11.88	0.15	0.14	0.07	
Uni; T;	0.13 $\pm$	0.08 $\pm$	0.11 $\pm$	0.07 $\pm$	0.14 $\pm$	-	-	-	-	-	-	
C2A	0.07	0.06	0.06	0.05	0.04	-	-	-	-	-	-	
Uni; T;	0.19 $\pm$	0.09 $\pm$	0.15 $\pm$	0.07 $\pm$	0.22 $\pm$	-	-	-	-	-	-	
C2B	0.09	0.07	0.06	0.06	0.05	-	-	-	-	-	-	
Bi; T; C2A	0.14 $\pm$	0.11 $\pm$	0.11 $\pm$	0.08 $\pm$	0.19 $\pm$	0.26 $\pm$	24.1 $\pm$	14.6 $\pm$	0.34 $\pm$	0.21 $\pm$	0.55 $\pm$	
	0.08	0.06	0.05	0.05	0.05	0.51	12.76	10.60	0.15	0.14	0.07	
Bi; T; C2B	0.17 $\pm$	0.11 $\pm$	0.13 $\pm$	0.09 $\pm$	0.21 $\pm$	0.56 $\pm$	24.6 $\pm$	15.5 $\pm$	0.34 $\pm$	0.22 $\pm$	0.55 $\pm$	
	0.09	0.07	0.06	0.05	0.05	0.45	14.08	11.63	0.16	0.15	0.07	

$\sigma_u^2$ : varianza genética aditiva,  $\sigma_{ep}^2$ : varianza ambiental permanente,  $c^2$ : varianza de ambiente permanente/varianza fenotípica,  $h^2$ : heredabilidad, R: repetibilidad,  $r_g$ : correlación genética, Uni: univariado, Bi: bivariado, C2A: agrupada sin medulación, medulación fragmentada y discontinua vs medulación continua y fuertemente medulada; C2B: sin medulación vs medulación

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# Performance Evaluation of Llama, Alpaca and Sheep Herds of a Community in Pasco, Peru

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**Abstract.** In the Peruvian highlands livestock production is especially developed in the South and Center of the country between altitudes ranging from 2,200 to 4,500 meters above sea level. Community-owned pasture is the main source of animal feed in this region. Llama rearing is commonly combined with other species, such as alpacas and sheep. One way to technically evaluate livestock systems is by calculating technical parameters. These indicators allow to synthesize the information contained in the production records and to standardize the evaluation criteria of the production unit. The following technical parameters were used in this study: Annual average capital (AAC), gross birth rate (%GBR), real birth rate (%RBR), mortality (%M), harvest (%H), gross increase (%GI), real increase (%RI) and livestock efficiency (%LE). In the present study, the technical evaluation of livestock was carried out using the monthly records of llamas, alpacas and sheep of the Communal Cooperative San Pedro de Racco, located in Pasco Region, Peru. The evaluation period for llamas and alpacas was from 2012 to 2015, whereas for sheep the evaluation period was from January 2014 to December 2015. It was concluded that the Community Cooperative San Pedro de Racco presented values of technical parameters corresponding to a good technological level company, possibly due to the capacity development of human resources in terms of animal and grassland management.

**Resumen.** La ganadería altoandina en el Perú se desarrolla especialmente en el centro y sur del país, entre los 2.200 a 4.500 metros sobre el nivel del mar. Los pastos comunitarios son la principal fuente de alimentación animal en esta región. La crianza de llamas es comúnmente combinada con otras especies, como alpacas y ovejas. Una forma de evaluar

técnicamente a los sistemas ganaderos es mediante el cálculo de índices técnicos. Estos indicadores nos permiten sintetizar la información contenida en los registros de producción y normalizar los criterios de evaluación de la unidad de producción. En el presente estudio se utilizaron los siguientes parámetros técnicos: Capital promedio anual (CPA), natalidad bruta (%NB), natalidad real (%NR), mortalidad (%M), saca (%S), incremento bruto (%IB), incremento real (%IR) y eficiencia ganadera (%EG). En el presente estudio, se realizó la evaluación técnica del ganado mediante registros mensuales de llamas, alpacas y ovejas de la Cooperativa Comunal San Pedro de Racco, ubicada en la región de Pasco, Perú. El período de evaluación para llamas y alpacas fue del 2012 a 2015, mientras que para ovinos el período de evaluación fue de enero del 2014 a diciembre del 2015. Se concluyó que la Cooperativa Comunal San Pedro de Racco presentó valores de parámetros técnicos correspondientes a una empresa de buen nivel tecnológico, posiblemente debido al desarrollo de capacidades de recursos humanos en materia de manejo de animales y pastizales.

**Keywords:** llama, alpaca, sheep, performance evaluation

## Introduction

In the Peruvian highlands livestock production is especially developed in the South and Center of the country, between altitudes ranging from 2,200 to 4,500 meters above sea level. Livestock is usually raised by peasant communities who have pastures as the main resource for feeding their animals (Flores, 1992).

In Peru llama rearing takes place in large areas of highland grasslands where the possibility of developing agriculture is almost non-existent. Llamas convert very efficiently poor pastures at high altitudes into high quality products such as meat (Quispe et al., 2008). In the Central Andes, the llama management system is extensive, based on the use of communally-owned grasslands and its main objective is the meat production. Many farmers keep mixed herds composed of llamas, alpacas, sheep, and cattle. The predominant llama type is the K'ara variety, which is mainly considered for its good qualities for meat production (Mendoza, 2013).

The majority of alpacas and llamas are raised by smallholder farmers in a low-input system. Disease control measures are non-existent in most cases, as well as the grassland management. The other 20 % of the population belong to individual small and medium farmers and big enterprises. In communities, there is a tendency to possess many animals above the carrying capacity of pastures, which leads to overgrazing and consequent degradation of this resource (Coughenour, 1991). The absence of disease control and prevention measures results in high mortality rates as well as low growth and birth rates, all of which result in low levels of production and productivity. Additionally, farmers have many difficulties for the commercialization of products that depend on a chain of intermediaries. The result is a low level of income for families that has an impact on their standard of living. This is the most relegated and unprotected sector of camelid breeders but at the same time the one that has enormous potential for development due to the high number

of livestock it owns and a valuable local knowledge that farmers keep (FAO, 2005b).

Sheep rearing in Peru is mainly developed in the highlands, mostly under extensive grazing based on degraded natural pastures with low nutritive levels which is reflected in the production of wool, meat and hides (Aliaga, 2006). The sheep sector in the country supplies meat but most of it is informal, which accounts for 70 % of the national meat production, which is mostly for self-consumption (FAO, 2004). The effect of falling wool prices on the international market by adding the low-quality wool produced by small farmers and communities, as well as the presence of wool substitutes such as synthetic fibers and other natural fibers such as cotton has not allowed to increase the demand in the consumption of wool (Mesias, 2001). Sheep in Peru are certainly the savings box of the rural Andean farmer whose one of their customs is to sell them if there is an urgent need for money (Diaz, 2007).

One way to technically evaluate livestock systems, basically productivity, is by calculating technical parameters. These indicators allow us to synthesize the information contained in the production records and to standardize the evaluation criteria of the production unit (Pumayalla, 1980). The parameters show the efficiency with which the herd is being managed, as well as the success or failure of the management that is carried out (Cuadros, 1981). Gutiérrez (1993) points out that the following parameters should be used to perform a technical evaluation: Annual average capital (AAC), gross birth rate (%GBR), real birth rate (%RBR), mortality (%M), harvest (%H), gross increase (%GI), real increase (%RI) and livestock efficiency (%LE). The objective of the present study was to evaluate the performance of llama, alpaca and sheep herds of the Communal Cooperative San Pedro de Racco, located in Pasco Region, Peru.

## Materials and Methods

Performance evaluation of livestock was carried out using monthly records of llamas, alpacas and sheep of the Communal Cooperative San Pedro de Racco. This cooperative is located in the province of Cerro de Pasco in the Central Andes at an altitude of 4,000 m. The evaluation period for llamas and alpacas was from November 2012 to October 2015. In contrast, the sheep evaluation period was from January 2014 to December 2015. The technical parameters were calculated using the following formulas:

- Annual average capital (AAC):  $\left( \frac{\text{initial existence} + \sum \text{final existence}}{13} \right)$
- Gross birth rate (%GBR):  $\left( \frac{N^{\circ} \text{ births}}{N^{\circ} \text{ mated females}} \right) * 100$
- Real birth rate (%RBR):  $\left( \frac{N^{\circ} \text{ births}}{\text{AAC}} \right) * 100$
- Mortality (%M):  $\left( \frac{N^{\circ} \text{ dead}}{\text{AAC}} \right) * 100$

- Harvest (%S):  $\left(\frac{N^{\circ} \text{ sold} + \text{slaughter} + \text{donations}}{AAC}\right) * 100$
- Gross increase (%GI):  $\left(\frac{\text{final existence} - \text{initial existence}}{AAC}\right) * 100$
- Real increase (%RI):  $\left(\frac{\text{final existence} - \text{initial existence} - \text{sold}}{AAC}\right) * 100$
- Livestock efficiency (%LE): %RI + %H

The AAC is an indicator that expresses the monthly average number of animals that exists in the herd during the year. The % GBR is an indicator of reproductive efficiency in breeding, while the %RBR expresses the relation between the number of born and the AAC. The %M indicates the percentage ratio of the number of deaths among the AAC and the %H considers the number of animals sold, benefited and includes those destined for self-consumption and donations. The %GI expresses the increase of animals per year in the herd and the %RI expresses the increase of the population due exclusively to the technical management of the herd. Finally, %LE is an indicator of efficiency in the technical management of the herd.

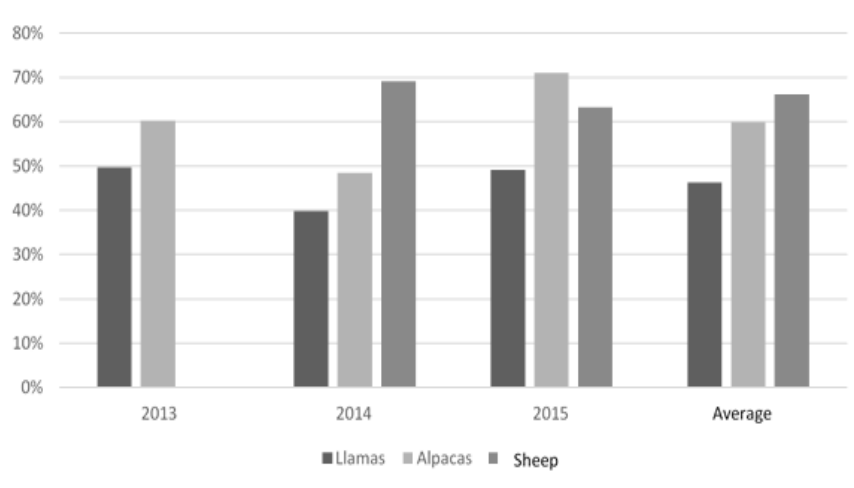
## Results and Discussion

### *Annual average capital*

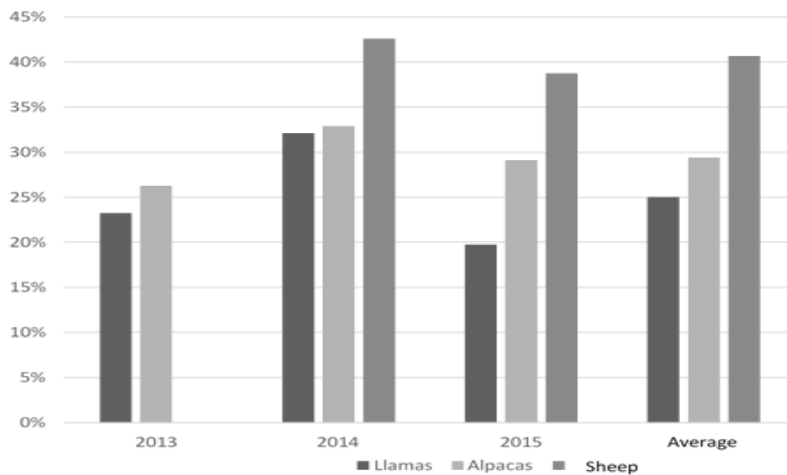
The results showed that llama and alpaca populations are reduced during the periods of 2012 to 2014, but increase in the years 2014 and 2015; this increase was also observed in the sheep population. This could be due to the grassland management strategies that the cooperative began to use at the end of the year 2013 (LEUP, 2013). It was found that the average AAC was 507.8, 3,086.2 and 4,683 animals, for llamas, alpacas and sheep, respectively.

### *Gross and real birth rate*

The average gross birth rates (%GBR) during the evaluated years were 46.21 %, 59.82 % and 66.21 % for llamas, alpacas and sheep, respectively (Figure 1); while the average real birth rates (%RBR) were 25.03 %, 29.43 % and 40.68 % (Figure 2). The birth rate obtained in the present study was lower than that reported by Pantoja et al. (2012) of 78.29 % for llamas in Pasco, however it is necessary to specify that in their study, they only evaluated the year 2011. A similar result was obtained by Novoa and Leyva (1996) who report a birth rate of 50 %. In the Turco region (Bolivia), Rodriguez and Quispe (2007) indicate birth rates of 61 % and 66 % for llamas and alpacas, respectively.



**Figure 1:** Gross birth rates (%GBR) of llamas, alpacas and sheep from the Community Cooperative San Pedro de Racco from 2013-2015



**Figure 2:** Real birth rates (%RBR) of llamas, alpacas and sheep from the Community Cooperative San Pedro de Racco from 2013-2015

On the other hand, the gross birth rate of alpacas reported in the present study was below the one calculated for alpacas Huacaya (89.34 %) and Suri (83.54 %) in the Research and Production Center “La Raya” in Cusco (Gallegos, 2013). One possible explanation for these differences might be the different weather conditions. “La Raya” is located in the wet puna, whereas the Community Cooperative San Pedro de Racco is located in the dry puna. Therefore, pasture productivity and

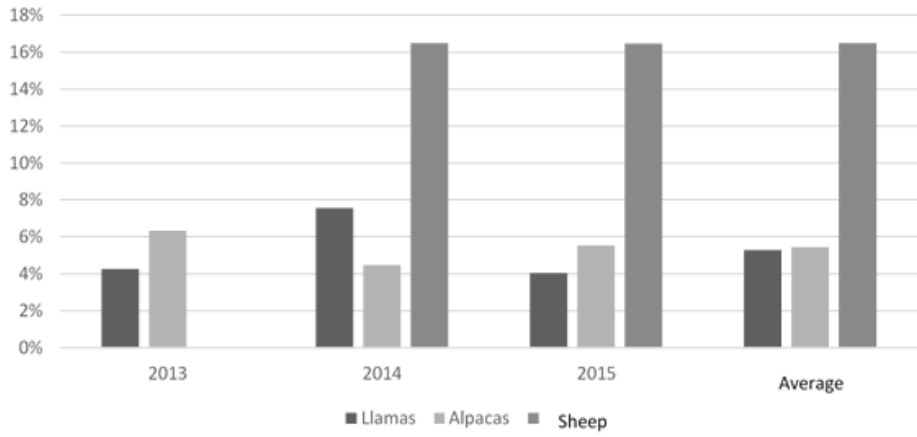
conditions are more favorable in “La Raya”. But also, different management strategies could be responsible for these observed differences.

### *Mortality*

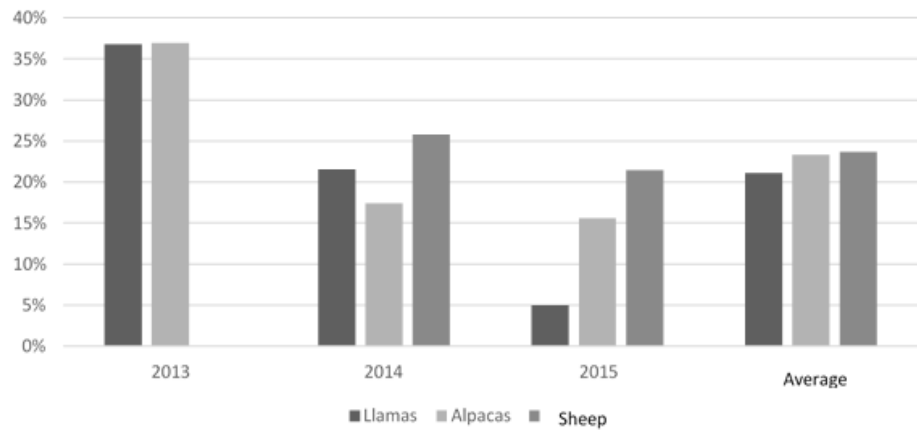
The average mortality rate during the evaluation period was 5.28 %, 5.45 % and 16.47 % for llamas, alpacas and sheep, respectively. Mortality was found to be higher in sheep than in camelids; however, most of the llama deaths occurred in the period 2013-2014, while in the period 2012-2013 there were more deaths of alpacas (Figure 3). Regarding mortality by age, a mortality of 2.47 %, 0.47 % and 2.34 % was found in offspring, ancutas (llamas between weaning and first mating) and adult llamas, respectively. In the case of alpacas, the mortalities found were 3.7 %, 0.67 % and 1.07 % in offspring, tuis (alpacas between weaning and first mating) and adults; while for sheep, mortalities of 11.28 %, 1.8 % and 3.39 % were calculated for lambs, young sheep and adults, respectively. The mortalities found for llamas in this study were lower than those used in the development of a simulation model of the llama population under environmental and market restrictions in the Bolivian highlands (Treydte et al., 2011) of 29 % and 6 % for juveniles (less than 2 years) and adults (greater than 2 years), respectively. The high rates of llama mortality in Bolivia are due to inadequate management, as well as lack of sanitary controls, such as feeding programs, which result in high incidences of diarrhea and malnutrition (Lopez, 2004, Whitehead and Anderson, 2006). Ruiz et al., (2015) report higher mortalities than those of the present study of 7.47 % and 6.94 % for two associations of alpaca farmers in the Pasco Region, shows that good pasture conditions and proper management of the animals result in lower mortality rates. On the other hand, it was observed that sheep had higher mortality rates than llamas and alpacas, demonstrating that camelids have some resistance to diseases such as pulmonary adenomatosis, pododermatitis and epididymitis, which are diseases with a high incidence in sheep, but are rarely seen in camelids (Sumar and Camino, 1992).

### *Harvest*

An average harvest of 21.11 %, 23.33 % and 23.64 % was determined for llamas, alpacas and sheep, respectively. Figure 4 shows that the largest amount of camelid harvest occurred in the period 2012-2013 and that the lowest harvest occurred in the period 2014-2015 with 4.99 %. In addition, it was observed that there is no defined month to perform the harvest. The percentage of llama harvest obtained in this study was higher than the 11.4 % found in Bolivia, which is attributed to low fertility and high mortality (FAO, 2005a).



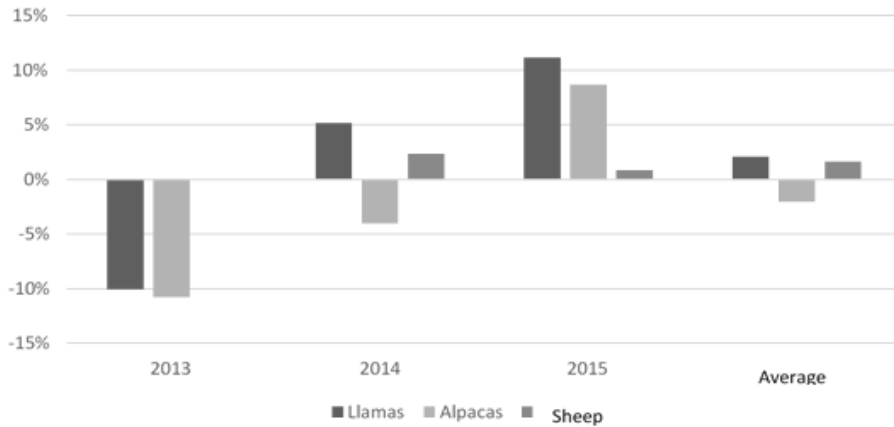
**Figure 3:** Mortality (%M) of llamas, alpacas and sheep from the Community Cooperative San Pedro de Racco from 2013-2015



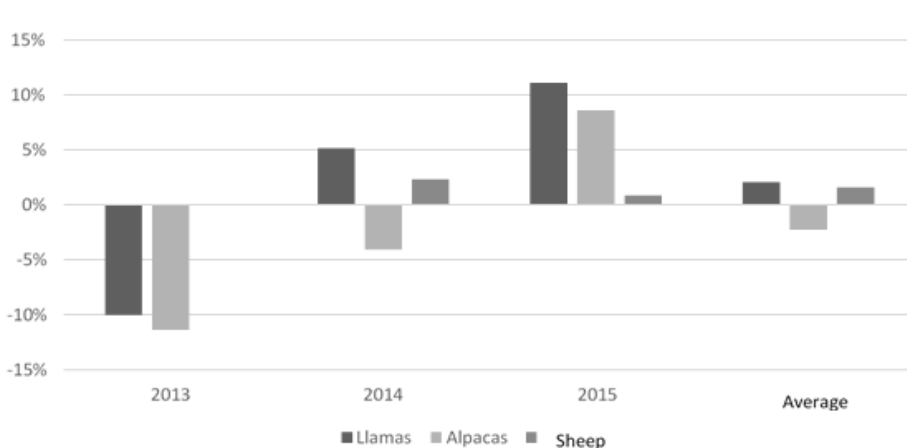
**Figure 4:** Harvest (%H) of llamas, alpacas and sheep from the Community Cooperative San Pedro de Racco from 2013-2015

*Gross and real increase*

It was found that llamas had an average GI greater (2.09 %), unlike alpacas with -2.05 % and 1.6 % for sheep (Figure 5). During the evaluation years the gross increase rates were the same as the real increase rates in each species. Except for the alpaca period 2012 - 2013, where the % GI was -10.75 and the % RI was -11.35 % since twenty born animals were not reported (Figure 6).



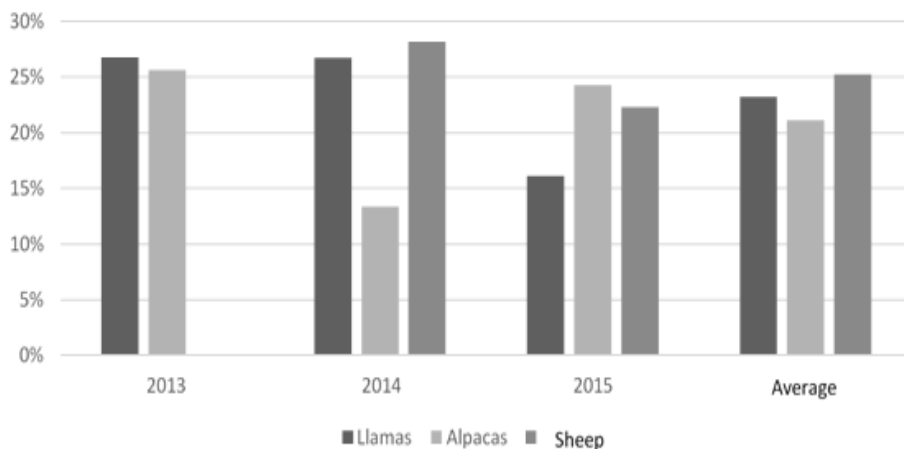
**Figure 5:** Gross increase (%GI) of llamas, alpacas and sheep from the Community Cooperative San Pedro de Racco from 2013-2015



**Figure 6:** Real increase (%RI) of llamas, alpacas and sheep from the Community Cooperative San Pedro de Racco from 2013-2015

*Livestock Efficiency*

In the 2012-2013 period, the highest LE was found for camelids, with 26.76 % for llamas and 25.61 % for alpacas, whereas for sheep a LE of 28.16 % was obtained in 2014. Livestock efficiency averages of 23.2 %, 21.08 % and 25.24 % were obtained for llamas, alpacas and sheep in the evaluated years (Figure 7).



**Figure 7:** Livestock efficiency (%LE) of llamas, alpacas and sheep from the Community Cooperative San Pedro de Racco from 2013-2015

Table 1 shows all the technical parameters of the Communal Cooperative San Pedro de Racco during the evaluated years, finding that its management system has a medium to high technological level, according to Esponda et al. (2004). This could be a result of keeping records containing the complete information of the animals, such as calving, mating, mortality and harvest records (Condorena, 1987; Cruz, 2012). In addition, studies have shown that communal cooperatives in the Pasco Region exhibit adequate rangeland condition and trend parameters, adequate carrying capacity and forage balance (Barrantes, 2012; Zarria et al., 2012), which would explain the efficient management done by the Communal Cooperative San Pedro de Racco.

Regarding llama management systems in Peru, there have been no recent studies of performance evaluation, however Lopez (2004), reports a birth rate of 34.01 % and a mortality of 2.58 % for llamas in Oruro province, Bolivia.

In the case of alpacas, Palacios (2009) reported technical parameters of 70.94 %, 29.68 %, 18.56 %, 6.33 %, 4.75 % and 23.31 % for %GBR, %RBR, %H, %M, %RI and %LE respectively for Huacaya alpacas from the Mallkini Ranch (Azángaro, Puno) during the period 2001 to 2008.

**Table 1:** Average technical parameters from the Community Cooperative San Pedro de Racco from 2013-2015

Technical parameter	Llamas (2012-2015)	Alpacas (2012-2015)	Sheep (2014-2015)
%AAC	507.8	3086.2	4683.0
%GBR	46.0	60.0	66.0
%RBR	25.0	29.0	41.0
%M	5.3	5.4	16.5
%H	21.1	23.3	23.6
%GR	2.0	-2.0	2.0
%RI	2.0	-2.0	2.0
%LE	23.0	21.0	25.0

Likewise, Ruiz et al., (2015) reported average technical parameters of two associations of alpaca farmers in the Pasco Region during the years 2012-2014 of 47.1 %, 26 %, 7.2 %, 18.8 %, 0.2 %, 0.0 % and 19 % for %GBR, %RBR, %M, %H, %GI, %RI and %LE, respectively. This revealed that small community organizations can be as efficient as a company with a medium to high technological level. This can be mainly attributed to the capacity development of human resources.

On the other hand, Cayo (2001) reported 76.76 %, 35.76 %, 10.74 %, 24.37 %, -0.3 %, -0.51 % and 23.87 % for %GBR, %RBR, %M, %H, %GI, %RI and %LE, respectively for sheep Corriedale of Pachacutec Agricultural Society, located in Junin province (Central Peru). This data supports the findings of the present study because both, the farmers' associations and agricultural society use records and perform pasture improvement practices, which is reflected in their technical parameters.

## Conclusion

The Community Cooperative San Pedro de Racco presented values of technical parameters corresponding to a company with good technological level. These results might be attributed to the capacity development of human resources in terms of animal and grassland management.

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# The Camelid Registry LAREU: What Are We Breeding In Europe?

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**Abstract.** The Llama and Alpaca Registry Europe (“LAREU”) is providing an online registration system for breeders and owners of South American camelids (SACs), free of charge. Founded in the year 2005, over 19,000 animals from more than 10 European countries are stored in LAREU’s database by spring 2017, with a yearly growth of about 10 percent. In this article we will present statistical material from the LAREU database with the aim to provide some insight into the breeding goals of the European camelid owners. The main subjects of the data analysis are concerned with the distribution of the different types of alpacas (huacayas and suris) and of llamas (light, medium, heavy wool, and suri), as well as the distribution in the various color classes (for alpacas), according to the color code scheme defined by ARI. Information is also presented about the frequency of parentage for sires and dams, giving some idea about the fraction of “proven” reproducers.

**Resumen.** El Registro de Llamas y Alpacas de Europa (“LAREU”) ofrece un sistema de introducción de información gratuito en línea para criadores y propietarios de camélidos sudamericanos (SAC). Tras ser fundado en 2005, la base de datos de LAREU en la primavera de 2017 contenía registros de más de 19.000 animales de más de 10 países europeos en la primavera de 2017, con un crecimiento anual aproximado del 10 por ciento. En este artículo se presentan estadísticas de la base de datos LAREU con el ánimo de sugerir objetivos de mejora a los propietarios de camélidos europeos. La información principal se refiere a la distribución de los diferentes tipos de alpacas (huacayas y suris) y de llamas (ligeras, medianas, pesadas de lana y suri), así como la distribución de los diversos colores de capa (para alpacas) de acuerdo con la clasificación de colores definida por ARI. También se presenta información sobre la el parentesco entre padres y madres, dando idea de la fracción de reproductores “probados”.

**Keywords:** Alpacas, llamas, statistics, LAREU registry

## Introduction

With the increasing numbers of alpacas and llamas kept by European owners over the last two decades, also an increase in the number of national camelid associations spread over Europe was encountered. The increase of the latter was fre-

quently motivated by the associations' diverging breeding goals, but also sometimes because of incompatibility in the views of the associations' leaders towards future directions and developments of the associations, let alone the difficulties related to the different languages spoken in the associations within Europe. Almost inevitably, every association established its own registration database, with no possibility of cross-linking to the databases of the other associations. Since nevertheless quite often the animals have been transferred between owners of different associations, also across national borders, most of the "history" of the animals, in particular the pedigrees, got lost. In addition, the different associations were usually working with different DNA laboratories using different marker sets for DNA typing, making a reliable parent verification across association borders impossible.

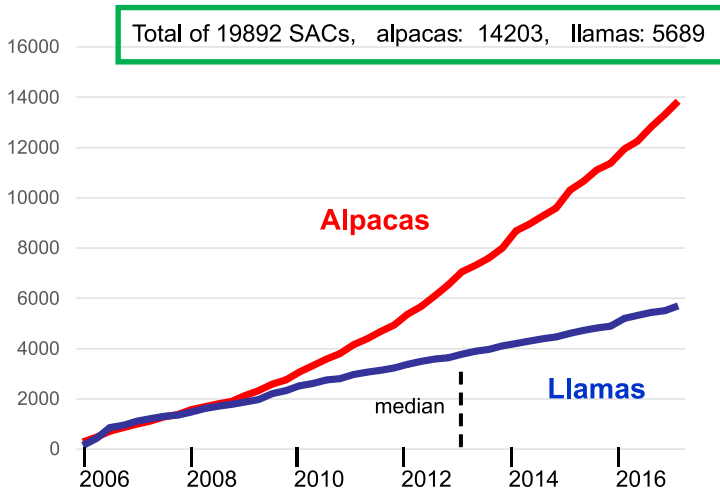
Given this unsatisfactory situation, LAREU was founded in 2005 as a truly European registry, open to individual breeders and owners of South American camelids. The main idea of LAREU was to provide an internet-based registry without human intervention from the side of the registry (except providing the registration page and its software and working on improvements of the informatics part). An additional requirement of LAREU was to keep the registration process free of cost. This was made possible by an offer of the animal welfare organization TASSO, based in Germany [TASSO, 2017], to host the LAREU database and to provide the computing infrastructure for servicing the LAREU registration webpages. TASSO itself is an organization for registering and localizing run-away pets, with a database of a few million animals, mostly dogs and cats. The data of the animals registered with LAREU are entirely separated from the TASSO database, also the LAREU registration software is being developed independently as a voluntary, unsalaried project by members of TASSO and LAREU. In contrast to most other registries, LAREU allows filling only the parents for each animal, which in turn are (or should be) registered in LAREU as well. With this system unique ancestry trees are possible, providing reliable statistics over many generations, in principle resulting in unlimited ("infinite") pedigrees.

The transparent borders within the European Union are offering breeders of SACs great opportunities for improving the genetics of their animals, both with respect to fiber quality as well as to conformation and health. LAREU has recognized from the beginning the importance of a Europe-wide registry and of a worldwide standard of the DNA markers used for SACs. Upon initiative of LAREU the "International Society of Animal Genetics (ISAG)" developed in the years from 2008 until 2012 a unique marker panel for alpacas and llamas. LAREU is working together with three laboratories in Germany, France and the Netherlands, who have been part of the early research to establish the unique marker set. In the meantime this set is used by most DNA laboratories worldwide, so that the transfer of DNA data from other laboratories into the common LAREU database and their evaluation for parent verification is now becoming a meaningful routine. Clearly, the DNA typing is a service provided by a qualified laboratory, which is reimbursed for its services directly through the animal owner.

It should be stressed here that all animal data in the LAREU database are entered by the animal owners. Also, there is no requirement for completeness of the animal data at the time of registration. However, there are a few automatic consistency checks, for example, the transponder number is cross-checked for uniqueness. All missing information can be entered later, once available. For this reason, the DNA information is, at this stage, far from being complete for all the animals registered in LAREU. Nevertheless, the large numbers of animals already in the database (more than 19,000 in spring 2017, increasing each year by more than 2,000) makes LAREU by now the largest database on SACs in Europe.

In the following, we will first show some statistics on the registration activity and the numbers of animal registrations for those European countries with the highest activities. We then turn to some interesting questions concerning the distribution of types of alpacas and llamas registered. For the case of alpacas, we present distributions of the various colors bred in huacayas and suris, and show these distributions for the four European countries with the largest numbers of registered animals. Then we turn to the data analysis with respect to possible trends visible in the breeding program for color. Finally, some data related to possible breeding strategies is shown, and a “demographic” statistics concludes the article.

### Registration Activity and Country Share



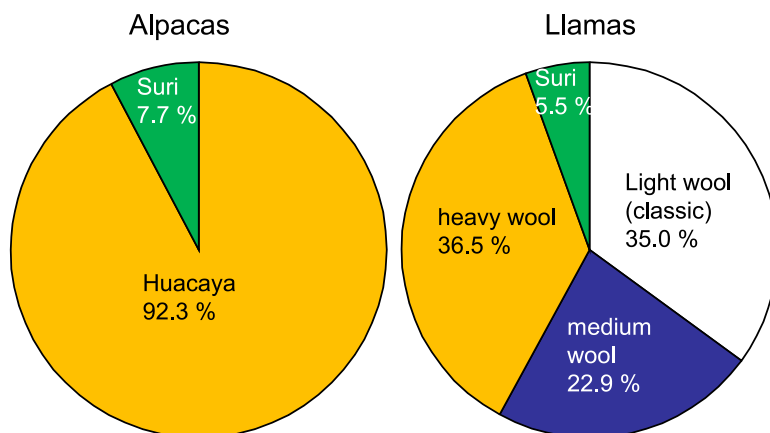
**Figure 1:** Time development of the animal registrations in the LAREU database. The registry was founded in 2005, registrations were started in 2006. The numbers shown on top of the figure reflect the status of the registration in April 2017.

During the year of foundation in 2005, the initial code of the LAREU registry was developed. First registrations were possible in the beginning of 2006. During the first years, the numbers of llamas and alpacas registered were quite similar, the

llamas were dominating until the year 2008. From then on the alpacas became more and more popular and their numbers increased steadily, overtaking the llamas. Today there are more than double the number of alpacas registered compared to the llamas. The registration activities over the years are shown in fig. 1, separated into llamas and alpacas, until April 2017. At this point almost 20,000 SACs were registered, out of them over 14,000 alpacas and more than 5,600 llamas. While the rise of the llama registrations appears almost linear since about 2010, the increase of the alpacas is following a rate of about 10 % per year. Since the beginning of 2013, slightly more than four years ago, the number of registered animals has doubled. If the trend holds, one can expect another doubling by the year 2022.

**Table 1:** Breakdown of animal registrations in the various European countries, ordered by the numbers of animals. Also given are the numbers of registered owners and the average numbers of animals registered per owner.

Country	Animals	Fraction (%)	Alpakas	Llamas	Owners	Animal/Owner
DE	5967	30.0	4872	1095	774	7.7
FR	5812	29.2	3362	2450	1096	5.3
CH	1330	6.7	1110	220	151	8.8
IT	1008	5.1	479	529	109	9.2
AT	668	3.4	470	198	138	4.8
NL	545	2.7	520	25	73	7.5
BE	358	1.8	334	24	73	4.9
FI	188	0.9	179	9	43	4.4
SE	140	0.7	66	74	23	6.1
CZ	90	0.5	35	55	18	5.0
others	417	2.1	350	67	23	18.1
no owners	3369	16.9	2426	943		
Sum	19892		14203	5689	2583	7.7

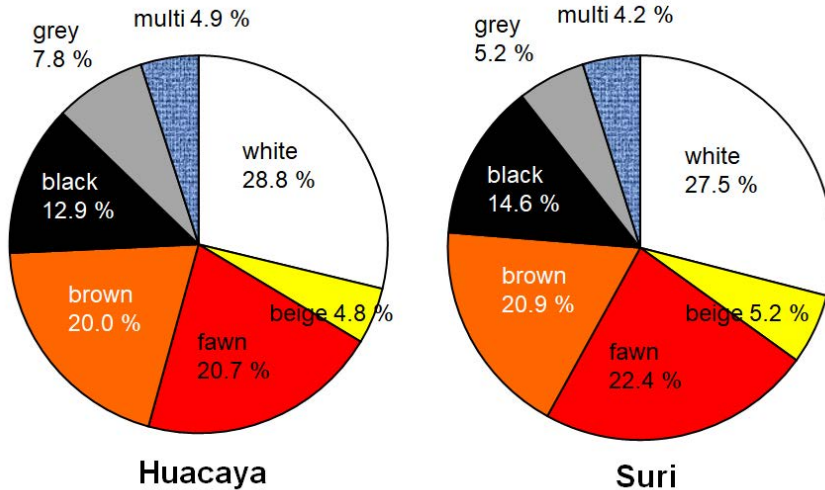


**Figure 2:** Breakdown of types for alpacas and llamas, taking into account all registered animals since the start of the LAREU registry. The type “suri” exists for both species, both alpacas and llamas show similar fractions of suris.

A breakdown of the registrations (status of April 2017) into the individual European countries is shown in Table 1, ordered in descending numbers of registered animals. The most active countries are Germany and France, who have registered about 30 % each of the total number of animals in the LAREU database, followed by Switzerland, Italy and Austria. The Netherlands and Belgium have also registered substantial numbers. There is also a relatively large fraction of animals not attributed to any country (“no owners”). These animals have been transferred from their original breeders to other owners who do not (yet) register their animals with LAREU. The transfer of an animal to a new owner is initiated by the previous owner: After the transfer all animal data, including the pedigree, are visible in the account of the new owner and have disappeared from the list of animals of the previous owner. If the new owner is not registered with LAREU, the animal data are transferred to a special “no owner” area. Once the new owner registers with LAREU, the animal data can be retrieved from the “no owner” area.

#### *Gender and Type Distributions*

The different types of wool for alpacas and llamas are shown in fig. 2. While for the alpacas only two types exist (huacaya and suri), the llamas are traditionally separated into four classes. In recent years, the “classic llama” has become more rare and is being overtaken by the woolly type, at least one can observe this in the registration activity, but also at shows. A similar situation can be found for the suris, which have seen a relative increase in numbers with respect to the total number of SACs registered with LAREU.



**Figure 3:** Breakdown of color classes for huacayas and suris. The similarity of the relative fractions within huacayas and suris is remarkable.

While the fraction of suri is pushing close to 10 % for the llamas in recent years, the fraction of suri alpacas remained pretty constant over time, as shown in Fig. 2.

Another interesting piece of data is the breakdown into gender, namely males, castrated males (“geldings”), and females. The fraction of geldings is small, but with a significant difference between llamas and alpaca. While only 3.9 % of geldings are registered for alpacas, a much larger fraction of 8.4 % is registered for llamas. Possible reasons for the larger fractions of geldings in llamas might lie in their use as companion animals in various recreational activities, such as trekking, or also in animal-assisted activities and therapy. Correspondingly, there is a difference in the registrations of males: 37.9 % for llamas and 40.3 % for alpacas, while the fraction of females is 55.8 % for the alpacas and 53.7 % for the llamas.

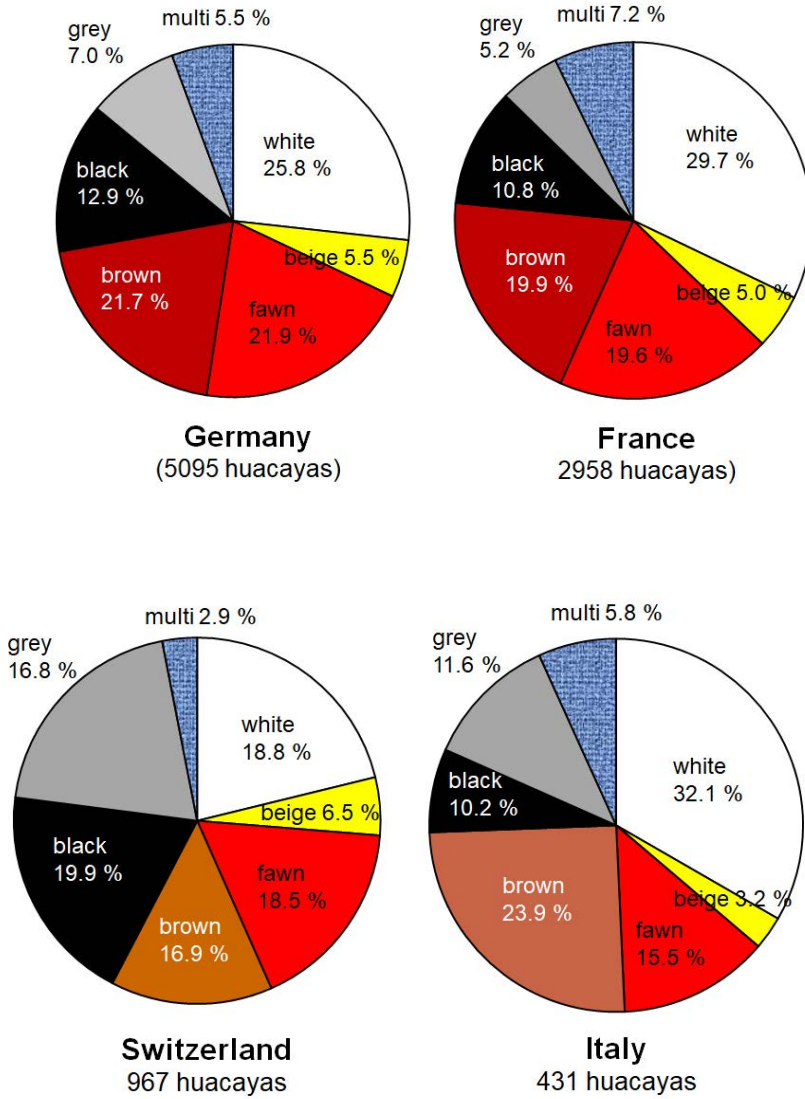
We now turn to the statistics concerned with the fiber which is an important marketing subject for the alpaca owners. Since wool characteristics related to the fiber diameter and comfort factor are usually dependent on the animal age and may vary substantially during the animal’s lifetime, these quantities are not stored in the LAREU database and cannot therefore be used here in a statistical analysis. Instead, we concentrate on the aspects of fleece color, which can be more reliably judged by the animal owner and is therefore a mandatory item in the LAREU database. For the various color shades we use the classification of ARI [AOA, 2014], which exhibits 16 natural colors and 3 additional multi-color classes. From these 19 classes for the alpacas we have summed up the main color traits, as shown in Fig. 3 (the fiber of llamas is not of so much importance as quality indicator, mainly due to the higher average fiber diameter, but also due to the large variation of color shades in the llamas). While the colors white and beige are unique, the colors

brown and fawn are divided into three further classes each: light, medium and dark. The grey color is divided into two main classes, rose grey and silver grey. These three are again subdivided into light, medium and dark. Finally, the color black is divided into true black and bay black. All subdivisions are summed up into the respective color classes shown in this and the following figures.

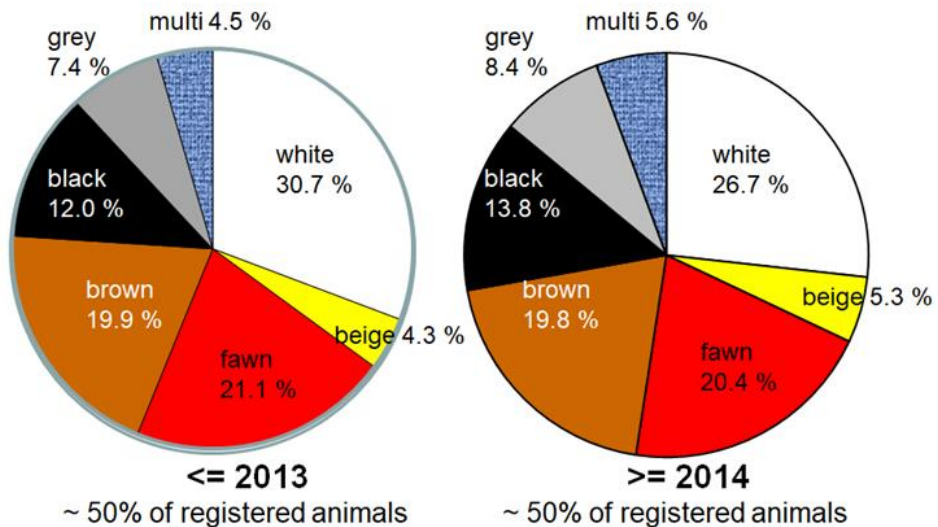
Comparing huacaya and suris shows a remarkable similarity in the relative fractions of colors. In both types the color white is dominating, for obvious reasons, since the early European alpaca population was imported from wool producing countries where emphasis is put on easy-to-stain fiber. Fawn and brown follow, black is still around 13-15 %, the grey shades, multi-color and beige are very similar, adding up to less than 20 % in total.

While the data in Fig. 3 are summed over all countries, it may be interesting to see whether there are any national differences in the color fractions. This is shown in Figure 4 for the huacaya types, displaying the four countries with the largest number of registrations, namely Germany, France, Switzerland and Italy (see also Table 1). From these data one finds that there are indeed differences among the four nations. The color white dominates in Italy, but is least prominent among the four in Switzerland. Switzerland, on the other hand, has the largest fraction of grey huacayas, whereas Germany and France have much less. Switzerland dominates in the black colors, and Italy has the largest fraction of brown animals. The largest fawn community one finds in Germany, Italy has the smallest fraction of fawn animals of the 4 countries. Since more detailed information is missing, it is not possible to reliably explain these regional differences. But it seems plausible that the differences have to do with the regional market situation and with the preferences of the animal owners and the intended use (wool, companionship etc.) of the animals.

The national differences observed in the color distributions of Fig. 3 may point to some possible trends in the selection of alpacas, or in other words, reflect the “taste” and the “preferences” of the animal owners. In order to address this interesting question, the various colors were counted starting on January 1, 2014, and were compared with the distribution before that date.



**Figure 4:** Breakdown of color classes for huacayas, comparing the four European countries with the largest numbers of registered alpacas. Significant differences are observed in the relative fractions between the countries.



**Figure 5:** Breakdown of color classes for huacayas, comparing the number of registrations before the year 2014 with those afterwards. January 1, 2014 marks the date when the half of the present animals in the database have been registered.

The significance of the date (January 1, 2014) comes from the fact that the same number of animals was registered before and after this date. This means that this date defines the median in the temporal frequency of registrations (see also Fig. 1, where the median is indicated). The result of the color count is shown in Fig. 5, where all European countries are summed up in order to see a general trend in the data. Here one observes that the color white is becoming less popular in the recent years, while the “colored” animals are increasing in fractions, most significantly black, grey, multi-colored and beige. This trend may indicate that the emphasis is slowly shifting from “finest fiber” (mostly found in white animals due to selection) towards “companion animals”, which are pleasing the eye and are more attractive.

### *Sires, Dams and Offspring*

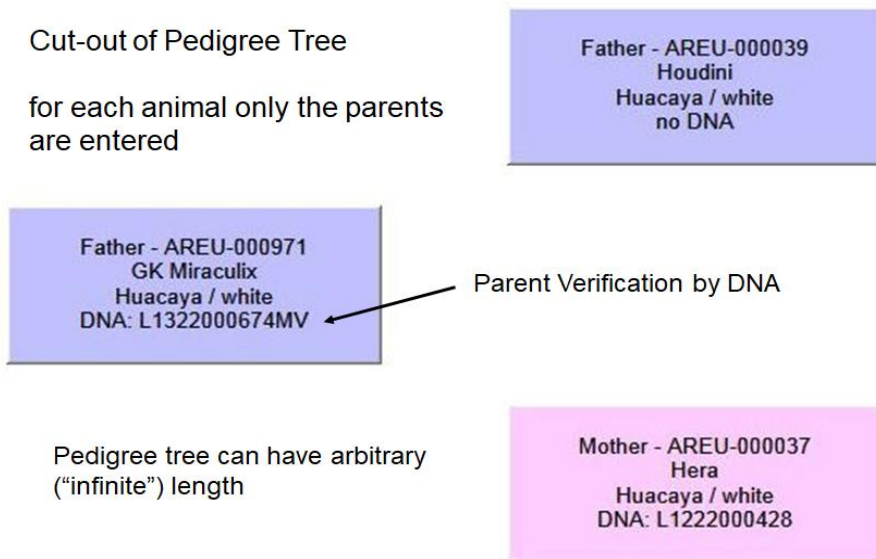
As mentioned in the beginning, the possibility to construct an “infinite pedigree” is one of the attractive features of the LAREU registry. The principle is shown again in Fig. 6: Each animal is linked to its parents (and only to its parents), and the animal information including the color type and the DNA typing (if existing) is displayed. Parent verification is indicated as well by adding the affixes “FV” for “father verified”, or “MV” for “mother verified (see arrow in Fig. 6). This information is filled into the database by the DNA lab and cannot be changed by the animal owner. The ancestry tree can be followed (on the web-browser) by

clicking on an animal within the tree, which then lists the three ancestry generation of the animal clicked.

The DNA information is not mandatory at the time of registration, but can be added afterwards by ordering DNA typing and parent verification from one of the DNA laboratories working with LAREU at any convenient time (import of previous DNA results into the database, launched by the DNA lab, is also possible). Despite all this, the number of DNA-verified animals is still small in the LAREU database. This is shown in Table 2: A total of 858 (2,643) alpaca sires (dams) and 474 (1,298) llama sires (dams) are entered in the database. The alpaca (llama) sires have 4,215 (2,939) offspring, the corresponding numbers for the dams can be found in the table. The table also shows clear indications for the frequent use of “proven” sires and dams: For example, in the alpaca sires 50 % of the offspring are fathered by only 127 sires, 33 % by only 62 sires (similar numbers for the llamas, see also the corresponding numbers for the dams of both types). The maximum number of offspring for the “most productive” sires is 54 for the alpaca and 84 for the llama. The most productive dams have 11 (13) offspring for the alpacas (llamas). One should note, however, that these numbers are entered by the owners, there is no unique proof about the parentage. The last row of each of the tables gives the truly verified parentage: Here one finds that only about 10 % of the sires, dams and their offspring are verified by DNA at present.

**Table 2:** Statistics of DNA-typed sires and dams and their verified offspring in the LAREU database in alpacas and llamas

	Offspring	Sires	Offspring	Dams
Alpacas	4215 (100%)	858	5384 (100%)	2643
	50%	127		669
	33%	62		370
Max. offsprings		54		11
Average		4.9		
DNA verified	389	129	456	321
Llamas	2939 (100%)	474	3227 (100%)	1298.0
	50%	54		318.0
	33%			
		27		174.0
Max. offsprings		84		13.0
Average		6.2		2.5
DNA verified	138	52	147	101.0

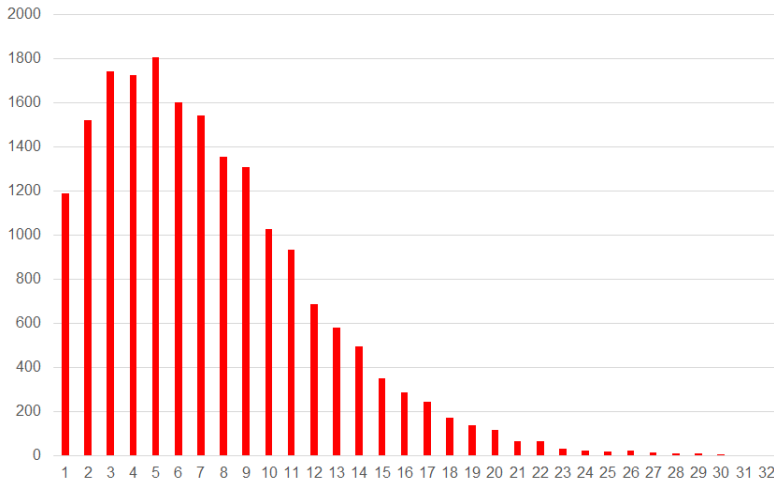


**Figure 6:** Example of part of the ancestry tree. Three ancestry generations are shown on the web browser

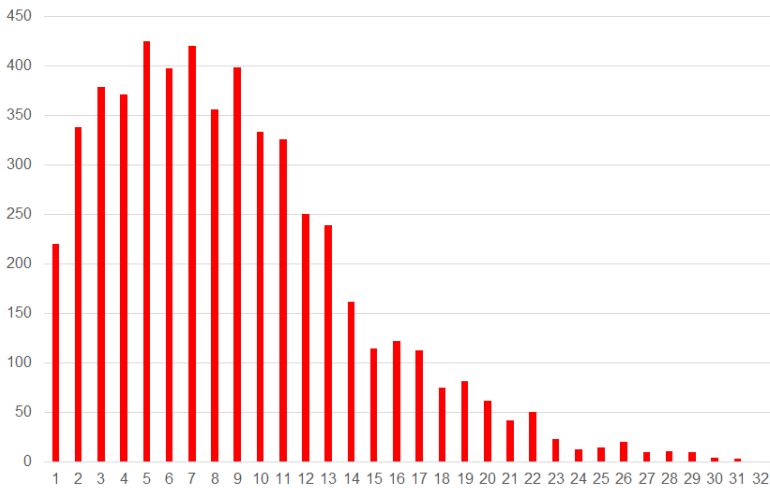
### *Age Distribution*

The registry logs the time of registration and demands the date of birth. Furthermore, the owner can mark an animal as deceased. With these informations one can generate an age distribution of the animals. This is shown, separately for alpacas and llamas, in Fig. 7. One observes that the alpaca population is peaking at about 4 years, while the llama population shows a broader peak, with a maximum of about 5-6 years. One also can see that there are apparently llamas and alpacas at a very advanced age (beyond 25 years). These extremely long lifetimes are most likely not real, but are the result of forgetting to mark the animal as deceased.

## a) Alpacas



## b) Llamas



**Figure 7:** Age distribution of the SACs in the LAREU database. Top: alpacas, bottom: llamas

## Summary and Conclusion

The European Camelid Registry LAREU is steadily growing with an increase of more than 2,000 animals per year. It is now the largest registry for camelids in Europe. With a total number of close to 20,000 animals (spring 2017), a number of significant statistics can be provided, giving some insight into the breeding program and the preferences for keeping these animals. In the recent years, alpacas

have clearly overtaken the llamas in numbers, which contribute now only one third of the total number of registered animals.

Using the large statistics of the LAREU registry it was shown that there are clear differences between the European countries concerning the distribution of colors in alpacas. Lacking further detailed information, no clear reasons for these differences can be given. In addition, one observes a significant trend in recent years towards “colorful” alpacas (beige, fawn, black, grey) which are becoming more popular at the cost of the white types.

The data also show that there is clear (and understandable) trend to use “proven” sires and dams in the breeding program. However, the data relies dominantly on the input of the animal owners, only a small fraction (about 10 % of the sires, dams and offspring) are DNA tested with verified parentage.

In the pursuit of a meaningful breeding program, breeders should be encouraged to clarify the parentage by taking DNA typing of their animals more serious.

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# Comparación de los Criterios de Selección de los Productores con el Reglamento Oficial para Llamas en el Perú

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**Abstract.** The objectives of this study are: 1) To know the selection criteria for K'ara llama breeders 2) To identify the relationship between the selection criteria of the breeders with the descriptors of the K'ara racial standard existing in the Regulation of Genealogical Records of Alpacas and Llamas of Peru – RGALLP and 3) Associate the selection criteria of the breeders and the descriptors of the racial standard with the corporal measurements of K'ara llamas. The study was carried out in four zones of the Pasco Region, collecting information from 19 breeders with the evaluation of 21 llamas. A ranking of animals (from first to sixth place) was based on visual evaluation of breeders and technicians who used the guidelines of the official national registration system (RGALLP). Body weight and different body measurements were taken of each animal. The three main selection criteria for the breeders were: large cannon bone, chest width and height at the withers. The correlation between the ranking of the breeders versus the ranking of the technicians ranged from 0.76 to 0.99, being significant ( $p < 0.05$ ). However, this association does not mean that the selection criteria are the same. The average measurements of the circumference of cannon bone, chest width, height at the withers and live weight, are consistent with the ranking of the breeders, but this does not occur in the ranking of the technicians. The ranking of llamas by farmers and as well by technicians shows a negative relationship with body measurements. This means that llamas with larger corporal dimensions are usually better ranked. In general, the selection criteria of the breeders lead to larger animals in their body measurements and these in turn are oriented to a greater production of meat and its use as pack animals.

**Resumen.** Los objetivos del estudio fueron: 1) Conocer los criterios de selección de los criadores en llamas K'ara del sexo macho, 2) Identificar la relación entre los criterios de selección de los criadores con los descriptores del estándar racial de llamas K'ara existente en el reglamento de Registros Genealógicos de Alpacas y Llamas del Perú (RGALLP) y 3) Asociar los criterios de selección de los criadores y los descriptores del estándar racial con

las medidas corporales de llamas K'ara. El estudio se realizó en cuatro zonas de la Región Pasco, recopilando información de 19 criadores con la evaluación de 21 llamas. La información de rankings (De primero a sexto lugar) de los animales fue por evaluación visual tanto de criadores como de técnicos (conocedores del RGALLP); Para determinar el peso corporal se utilizó una balanza tipo plataforma y para las medidas biométricas se utilizó un bastón zoométrico y una cinta inextensible graduada en centímetros. Los tres principales criterios de selección de los criadores fueron: cañas gruesas, ancho de pecho y altura a la cruz. La correlación entre el ranking de los criadores versus el ranking de los técnicos tuvo un rango de 0.76 a 0.99, siendo una asociación significativa ( $p < 0.05$ ), sin embargo, esta asociación no significa que los criterios de selección sean iguales. Las medidas promedio del perímetro de cañas, ancho de pecho, altura a la cruz y el peso vivo, son congruentes con el ranking de los criadores, más esto no ocurre en el ranking de los técnicos. La asociación entre el ranking de llamas, realizado por los criadores y los técnicos, con las medidas corporales son negativas, es decir las llamas con mayor dimensión corporal ocupan generalmente los primeros puestos en el ranking. En general, los criterios de selección de los criadores conducen a animales de mayores dimensiones en sus medidas corporales y estos a su vez están orientados a una mayor producción de carne y su adaptación a ser animales de carga.

**Keywords:** selection criteria, llamas, local knowledge

## Introducción

La mayor parte de la crianza de llamas a nivel alto andino en el Perú es realizada por pequeños productores bajo un sistema familiar. En general en el Perú hay escasa investigación realizada en llamas, ya que se da más importancia a la alpaca. La mayor parte de la investigación en llamas se ha realizado en la región sur del Perú. Las áreas de investigación son reproducción, salud animal, producción de fibra y carne, transformación y comercialización (Mendoza 2014). Lamentablemente se ha prestado poca atención al desarrollo de estrategias de selección, y al establecimiento de objetivos de mejoramiento genético en llamas.

Por otro lado, la llama viene perdiendo importancia como animal de fibra y como animal de carga de bienes, producto del desarrollo de la alpaca para producción de fibra y el incremento de vías de comunicación en las zonas rurales. Como resultado de esta orientación la población de llamas está en peligro de extinción, a menos que se hagan esfuerzos concertados urgentes para seleccionar, caracterizar y manejar adecuadamente los rebaños (Maquera 1991).

La llama K'ara en gran medida está dirigida a la producción de carne por lo que presenta un potencial genético para este tipo de producción. Sin embargo, para poder seleccionar a los mejores animales se requiere definir los criterios de selección más adecuados. En la región de Pasco existen llamas fenotípicamente diferentes (mayor altura a la cruz y mayor longitud corporal) respecto a la región sur. Para el diseño de un programa de mejoramiento genético se requiere conocer los criterios de selección e indicadores fenotípicos corporales que utilizan para la selección de sus animales dirigidos a la producción de carne y/o carga.

Los objetivos del estudio fueron: 1) Conocer los criterios de selección de los criadores en llamas K'ara del sexo macho, 2) Identificar la relación entre los criterios de selección de los criadores con los descriptores del estándar racial de llamas K'ara existente en el reglamento de Registros Genealógicos de Alpacas y Llamas del Perú (RGALLP) y 3) Asociar los criterios de selección de los criadores y los descriptores del estándar racial con las medidas corporales de llamas K'ara.

## Materiales y Methodos

### Localización

El presente estudio se realizó en las zonas de Iscaycocha, Tunacancha, Los Andes y Pucunan, ubicadas en la Región Pasco, Perú.

### De los animales

De una tropa de llamas, se preseleccionaron animales machos en tres categorías (Categorías: A= bueno, B=regular y C=malo) de 1 a 2 años de edad, que fueron identificados con aretes de plástico codificados con numeración correlativa, y para la evaluación se les colocó bretes numerados. La preselección se realizó semanas antes de realizar el trabajo de investigación para cada zona (Cuadro 1).

**Cuadro 1:** Distribución de llamas K'ara según zona geográfica

Zona	Sexo	Edad	n
Iscaycocha	Macho	1 – 2 años	3
Tunacancha	Macho	1 – 2 años	6
Los Andes	Macho	1 – 2 años	6
Pucunan	Macho	1 – 2 años	6
Total			21

### Evaluación de los animales

La evaluación visual de las llamas por los criadores se realizó de una manera participativa. Un grupo de cuatro a cinco criadores por zona, realizaron individualmente un ranking (del mejor al peor animal) de los animales de acuerdo al fenotipo del animal y a sus propios criterios de selección. Además, informaron de las principales razones para la ubicación de las llamas en el ranking que cada uno elaboró independientemente, estas fueron registradas en fichas individuales. Luego los criadores discutieron sus resultados y llegaron a un consenso para establecer las características fenotípicas de mayor y menor importancia.

La evaluación visual de llamas por los técnicos, se realizó en cada zona, participando dos a tres técnicos. La evaluación se realizó utilizando las llamas que fueron

previamente evaluados por los criadores, pero de manera independiente. Los técnicos realizaron la evaluación utilizando los descriptores del estándar racial de llamas K'ara del Reglamento de los Registros Genealógicos de Alpacas y Llamas del Perú (Minagri, 2011).

#### *Mediadas corporales*

Las medidas corporales ancho de pecho, altura a la cruz, y perímetro de cañas anteriores fueron medidas utilizando una cinta métrica de 1.5 metros y una regla de madera de una longitud de 2 metros con una escuadra incorporada móvil. Para medir el peso vivo de cada uno de los animales se utilizó una balanza ganadera digital electrónica tipo plataforma.

#### *Análisis de datos*

Los criterios de selección de los criadores, se obtuvo del consenso y discusión grupal que se realizó entre los criadores después de finalizada su evaluación, además se usó la información de las fichas de calificación donde cada criador registró sus rankings y las razones de la evaluación visual por cada animal en cada una de las zonas. Para realizar un ranking general de todos los criterios de selección de los criadores se determinó mediante un índice. Los índices se calcularon como: la suma de (8x para el ranking 1 + 7x para el ranking 2 + 6x para el ranking 3 + 5x para el ranking 4 + 4x para el ranking 5 + 3x para el ranking 6 + 2x para el ranking 7 + 1x para el ranking 8) multiplicado por 0.01 (Abegaz 2014).

Para determinar el grado de asociación entre los rankings de los criadores y los rankings de los técnicos se utilizó la correlación de Spearman y para la asociación de los criterios de selección de los criadores y los descriptores del estándar racial con las medidas corporales se utilizó la correlación de Pearson.

## **Resultados y Discusion**

#### *Criterios de selección*

Los criterios de selección utilizados por los criadores para la selección de llamas K'ara, se presentan en orden decreciente de acuerdo al índice obtenido (Cuadro 2). La característica "cañas gruesas" alcanza el índice más alto de 0.29 ocupando el primer lugar en orden de importancia; y la característica "presencia" alcanza un índice de 0.02 ocupando la última posición según el orden de importancia (Cuadro 2).

**Cuadro 2:** Ranking de criterios de selección de criadores según zonas

Características	Zona				Índice
	Iscaicocha (n = 5)	Tunacancha (n = 5)	Los Andes (n = 5)	Pucunan (n = 4)	
Cañas gruesas	1	1	1	1	0.29
Ancho de pecho	3	2	2	2	0.24
Alzada	2	3	3	3	0.22
Cuello largo	5	5	4	5	0.14
Longitud corporal	-	4	-	4	0.10
Conformación testicular	-	6	-	6	0.06
Tipo	6	8	7	8	0.04
Conformación de cabeza	-	7	-	7	0.04
Ancho de anca	4	-	-	-	0.03
Fortaleza	-	-	5	-	0.03
Presencia y otros	-	-	6	-	0.02

1 = Primer lugar, 2 = Segundo lugar, 3 = Tercer lugar, etc. (Ranking de las características); n = Numero de criadores.

Las características cañas gruesas, ancho de pecho y altura a la cruz, ocupan el 1er, 2do y 3er lugar, respectivamente; siendo este orden consistente en todas las zonas de estudio. Estas características fueron determinadas por los criadores de llamas K'ara como criterios de selección para la evaluación visual de reproductores orientados mayormente a la producción de carne. En este contexto, los criadores consideran que animales más gruesos (con pecho amplio, cañas gruesas, cuello ancho) y altos tendrán mayor producción cárnica, no tomando en cuenta ningún indicador zoométrico que tenga relación con una mayor masa muscular; ni la orientación de la selección en función de características de importancia económica como es el peso vivo para las llamas K'ara (Mamani et al., 2011).

Estos resultados concuerdan con los criterios de selección empleados por criadores de las zonas alto andinas de Arequipa (Torres, 2014), quienes mencionan criterios como llamas gruesas y altas, al igual que lo reportado para Pasco (Mendoza, 2014) como la alzada, conformación y tipo. En Bolivia además de la alzada se consideran otras características como largo de cuerpo, largo de cuello y ausencia de defectos congénitos (Rodríguez y Quispe, 2007, Saavedra et al., 2012, Sepúlveda, 2011), y también la conformación corporal, conformación testicular y salud (Markemann y Valle Zárate, 2009), además, Wurzinger et al. (2007), también reporto como único criterio el tamaño corporal.

*Correlación del ranking de los criadores vs el ranking de los técnicos*

Los valores de los coeficientes de correlación de Spearman entre los rankings de criadores versus el ranking de los técnicos vario de 0.76 a 0.99 (Cuadro 3). Estos valores relativamente altos, nos indicarían la existencia de una significativa asociación entre los rankings elaborados a partir de información de los criadores y de los técnicos. Si bien el ranking de criadores y técnicos tienen similar orden de los animales con fines de selección, ello no significa que los criterios de ambos sean similares. Los criadores utilizan criterios de selección diferentes a los que se describen en el estándar racial del RGALLP.

**Cuadro 3:** Coeficientes de correlación de Spearman entre los rankings de criadores con el de los técnicos, según zonas

	Zonas			
	Iscaycocha	Tunacancha	Los Andes	Pucunan
Número de criadores	5	5	5	4
Número de técnicos	3	3	3	2
Número de animales	3	6	6	6
Coef. de Spearman	0.99**	0.88*	0.89*	0.76

\* = Significativo ( $p < 0.05$ ), \*\* = Significativo ( $p < 0.01$ ).

*Medidas corporales y el peso vivo*

*Según ranking de criadores*

Las llamas que obtuvieron el primer lugar en el ranking de los criadores, son aquellas que registraron los mayores valores promedio en las tres principales características y en el peso vivo (Cuadro 4). Estos resultados nos indicarían que las evaluaciones visuales realizadas por los criadores son congruentes con mayores valores de las medidas corporales y peso vivo.

Varios autores reportan medidas corporales para llamas K'ara de uno y dos años, así en la región de Puno: Maquera (1991) reporta para altura a la cruz 91.65 y 98.60 cm y pesos vivos de 60.05 y 87.35 kg.; García y Franco (2006) también mencionan medidas en altura a la cruz 94.60 y 102.30 cm y en ancho de pecho 23.10 y 25.70 cm.; y en Oruro – Bolivia Cortez et al. (2006) para altura a la cruz 98.3 y 101.1 cm y peso vivo para llamas de 2 años de edad de 96.70 kg. Todos estos valores son inferiores a los reportados en el presente estudio, confirmando que las llamas de la región Pasco presentarían mayor altura, conformación y peso corporal, en comparación con llamas de otras regiones del sur del Perú y Bolivia

(Gutiérrez et al. 2012). Sin embargo, Cano et al. (2012) en Marcapomacocha – Junín, reporta valores para altura a la cruz de 110.70 y 115.10 cm., para ancho de pecho 33.80 y 36.10 cm y pesos vivos de 122.60 y 137.70 kg, en llamas K'ara, de uno y dos años de edad, respectivamente, los cuales son superiores a los valores hallados. Tales medidas, reportadas por Gutiérrez et al. (2012) y por Cano et al. (2012), nos indicarían que las llamas del Centro del País (Pasco y Junín) son mucho más desarrolladas, mostrándonos una mayor altura y un mayor peso vivo en relación a las llamas del sur, indicándonos que el estándar racial del RGALLP no concuerda con las características de las llamas K'ara de la Región Pasco. Nuestros resultados, y los reportes mencionados, podrían explicar la causa del porque los criadores eligen estas características como los principales criterios de selección los cuales están asociados directamente a una mayor producción de carne.

**Cuadro 4:** Medidas promedio de perímetro de cañas anteriores, ancho de pecho, altura a la cruz y peso vivo según el ranking de los criadores

Ranking general	n	Perímetro de cañas anteriores (cm)	Ancho de pecho (cm)	Altura de la cruz (cm)	Peso vivo (kg)
Primero	4	14.89	28.42	109.42	122.53
Segundo	4	14.05	26.16	107.68	112.56
Tercero	4	13.26	25.84	106.63	101.26
Cuarto	3	13.14	25.00	103.43	99.04
Quinto	3	13.57	24.79	101.64	94.09
Sexto	3	12.79	23.29	100.21	89.30

#### *Según ranking de técnicos*

Las llamas que obtuvieron el primer lugar en el ranking de los técnicos (Cuadro 5), no siempre son aquellas que registraron los mayores valores promedio, a excepción de la característica “ancho de pecho”. Consecuentemente, se aprecia valores desordenados que no están de acuerdo al ranking general, claramente se aprecia que no hay un patrón en forma descendente, diferente a los observado para el caso de los criadores. Probablemente estos resultados se deban a que los descriptores del estándar racial del RGALLP se basa en criterios de selección distintos y abstractos, cuyos puntajes no reflejan las características corporales y peso vivo de los animales evaluados (Cuadro 5).

**Cuadro 5:** Medidas promedio de perímetro de cañas anteriores, ancho de pecho, altura a la cruz y peso vivo según el ranking de los técnicos

Ranking general	n	Perímetro de cañas anteriores (cm)	Ancho de pecho (cm)	Altura de la cruz (cm)	Peso vivo (kg)
Primero	4	14.25	27.50	108.00	112.15
Segundo	4	14.75	26.50	110.25	121.20
Tercero	4	13.00	26.50	106.50	100.60
Cuarto	3	13.33	25.67	106.33	108.17
Quinto	3	12.33	21.67	101.33	87.63
Sexto	3	13.00	24.00	98.33	83.93

*Asociación de los criterios de selección de los criadores con las medidas corporales*

Los coeficientes de correlación entre el ranking general de los criadores con las medidas corporales (perímetro de caña anterior, ancho de pecho y altura a la cruz) y el peso vivo, resultaron ser negativos (Cuadro 6). Los coeficientes de correlación indican que si el animal esta mejor posicionado en el ranking (es decir más próximos al primer lugar) las medidas corporales y el peso vivo serán de mayor magnitud.

**Cuadro 6:** Coeficientes de correlación de Pearson del ranking de los criadores versus medidas corporales según zona

Ranking de la zona	n	Perímetro de caña anterior	Ancho de pecho	Altura a la cruz	Peso vivo
Iscaycocha	3	-0.87	-0.87	-0.72	-0.87
Tunacancha	6	-0.53	-0.63	-0.71	-0.23
Los Andes	6	-0.47	-0.40	-0.66	-0.60
Pucunan	6	-0.56	-0.54	-0.76	-0.75
Promedio		-0.61	-0.61	-0.71	-0.61

*Asociación de los descriptores del estándar racial con las medidas corporales*

Los promedios de coeficientes de correlación entre el ranking general de los técnicos basados en los descriptores del estándar racial de llamas K'ara con las me-

didadas corporales (perímetro de caña anterior, ancho de pecho y altura a la cruz) y el peso vivo, también son negativas (Cuadro 7).

**Cuadro 7:** Coeficientes de correlación de Pearson del ranking de los técnicos versus medidas corporales según zona

Ranking de la zona	n	Perímetro de caña anterior	Ancho de pecho	Altura a la cruz	Peso vivo
Iscaycocha	3	- 0.87	- 0.87	- 0.76	- 0.87
Tunacancha	6	- 0.68	- 0.62	- 0.60	- 0.49
Los Andes	6	- 0.82	-0.76	- 0.84	- 0.85
Pucunan	6	- 0.75	- 0.45	- 0.46	- 0.48
Promedio		- 0.78	- 0.68	- 0.67	- 0.67

Al igual que en el caso de los criadores estos coeficientes nos indican que si el animal esta mejor posicionado en el ranking (es decir más próximos al primer lugar) las medidas corporales tanto perímetro de caña anterior, ancho de pecho, altura a la cruz y peso vivo serán de mayor magnitud (es decir mayor perímetro de caña anterior, ancho de pecho y altura a la cruz en centímetros y mayor peso vivo en kg).

## Conclusiones

En la región Pasco, los criadores que participaron en el estudio identificaron los siguientes criterios de selección según su importancia: 1º) perímetro de cañas, 2º) amplitud de pecho y 3º) la altura a la cruz (talla). La correlación entre el ranking de los criadores versus el ranking de los técnicos varió de 0.76 a 0.99, indicando diferencias menores entre ellos. El valor promedio del perímetro de cañas, ancho de pecho, altura a la cruz y el peso vivo, son congruentes con el ranking de los criadores, pero no con el ranking de los técnicos. En general, los criterios de selección de los criadores conducen a animales de mayores dimensiones en sus medidas corporales y estos a su vez están orientados a una mayor producción de carne y su adaptación a ser animales de carga.

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# Selection and Evaluation of Fiber Characteristics of an Extreme Fine Alpaca Strain at Victory Farm in Missouri

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**Abstract.** Data recordings of animal breeding, fleece production, and fiber characteristics in an alpaca herd were analyzed and presented. A selective mating strategy was applied while establishing an extremely fine fiber alpaca strain at Victory Farm over 10 years. The herd size was expanded from one dozen breeding females and five males to 200 head in 2016. All animals were recorded for sires, dams, and registered pedigree, birth weight, weaning weight, shearing weight, and fleece weight. Mid-side flank fleece samples were measured for Optical-based Fibre Diameter Analyser (OFDA) fiber characteristics, including mean fiber diameter, length, and curvature. Live weight, fleece weights, and fiber characteristics were analyzed using SAS® GLM procedures. Mean birth weight, weaning weight, mixed age shearing weight, fleece weight, fiber diameter, coefficient of fiber diameter variation, fiber length, and fiber curvature were 6.82 kg, 25.27 kg, 48.84 kg, 0.85 kg, 16.67  $\mu\text{m}$ , 25.2 %, 64.6 mm, and 56.9 deg/mm, respectively. In addition, the finest 25 percent of the herd's fleeces were tested and found to have an average fiber diameter of 14.17  $\mu\text{m}$ , which was within vicuna fiber diameter ranges. These characteristics did not significantly increase with age. Heritability estimates for the fleece weight and fiber diameter, length, and curvature were 0.40, 0.65, 0.29, and 0.50, respectively. The average fiber diameter measurements of the herd at Victory Farm were significantly ( $p < 0.001$ ) finer than the comparison herds. This study found that strict selective breeding is effective for genetic gains in ultralow fiber diameter and fleece quality in an extremely fine fiber alpaca strain.

**Resumen.** Se analizó y se present información sobre cía de animales, producción de vellones y características de fibra en un rebaño de alpacas. Se ha creado una variante de alpacas extremadamente finas mediante estrategias de apareamiento selectivo durante 10 años en la Granja Victory. El rebaño partió de una docena de hembras y cinco machos reproductores, llegando en 2016 a 200 cabezas. Se registró el padre y la madre y el pedigrí completo de todos los animals, así como el peso al nacimiento, al destete y los pesos de esquila y del vellón. Se analizaron muestras de vellón del costillar medio para obtener el diámetro, la longitud y la curvatura medios de la fibra mediante un Analizador de Diámetro de Fibra Óptico (OFDA). El peso vivo, los pesos del vellón y las características de la fibra se analizaron con el procedimientos GLM del software SAS®. Las medias del peso al nacimiento, al destete, peso a edad mixta de esquila, peso del vellón, diámetro de fibra, coeficiente de variación, longitud de fibra y curvatura de la fibra, fueron 6,82 kg, 25,27 kg,

48,84 kg, 0,85 kg, 16,67  $\mu\text{m}$ , 25,2 %, 64,6 mm y 56,9  $^{\circ}/\text{mm}$  respectivamente. Además, se analizó únicamente el 25 % mejor de los vellones del rebaño y se obtuvo un diámetro de fibra medio de 14.17  $\mu\text{m}$ , valor dentro del rango de la fibra de la vicuña. Estas características no aumentaron significativamente con la edad. Las heredabilidades estimadas para peso del vellón, diámetro, longitud y curvatura de la fibra fueron 0,40, 0,65, 0,29 y 0,50 respectivamente. El diámetro medio de fibra de la Granja Victory fueron significativamente ( $p < 0,001$ ) más finas que los rebaños de comparación. Este estudio demostró que la mejora selectiva estricta es efectiva para obtener ganancias genéticas en ultrabajo diámetro de y de calidad del vellón en una variante de alpaca de fibra extremadamente fina.

**Keywords:** Vicugna pacos, alpaca strain, extremely fine, selective breeding, heritability

## Introduction

Although camelids are indigenous to the Andean highlands of South America, now they are distributed in many parts of the world. The alpaca (*Vicugna pacos*) is the most numerous fiber-producing member of the four South American camelid species: llama, guanaco, alpaca, and vicuna (Kadwell et al., 2001). Two types of alpacas have been introduced into the United States, namely, huacaya and suri; however, the majority of alpacas (80 %) are huacaya. Whereas Peru owns 75 percent of the alpaca population and produced about 90 percent of the world's camelid fiber in the past (Morante et al., 2009), the alpaca population introduced into nonnative regions has increased steadily in the last 30 years, especially in Australia, Canada, the United Kingdom, New Zealand, and the US. Alpacas are becoming an important specialty fiber production livestock species in these regions beyond the South American Andes. The alpacas registered to the Alpaca Registry, Inc. (ARI) in the US from 1986 to 2017 have increased to 255,128 head and the total number of fiber-growing camelids has been estimated at passing the 300,000 population mark in the US (Alpaca Consulting Services USA, 2017). Alpacas can be found in every state of the US and are farmed in various geographical environments, ranging from the desert to high mountain ranges (Wuliji, 2012). Coat color varies widely in camelids, ranging from white to black and various shades, including 22 natural color categories (Hoffman, 2003). Fleece production and fiber traits have been reported in some studies in the alpacas' native land (Pumayalla & Leyva, 1988; Frank et al., 2006; Montes et al., 2008; Morante et al., 2009; Cervantes et al., 2010). Animal husbandry and reproduction, especially fleece and fiber characteristics, were studied in these nonnative environments (Wuliji et al., 2000; McGregor, 2004; McColl et al., 2004). Profiles of the fiber characteristics of both huacaya and suri breeds farmed in the US have been reported (Lupton et al., 2006; Wuliji, 2012). Some of the literature and production records indicate that the introduced camelids had significantly improved body weight and fleece weight performance, whereas the fiber diameter had become coarser when grown on more nutritive grazing lands than the high Andes. The owners of Victory Farm

initiated an alpaca breeding venture in Braymer, Missouri, with just one dozen females and five males 10 years ago. By persistently acquiring breeding stock from fellow breeders and through restrictive selection within their animals, their herd size increased to 200 head in 2016. The goal was to establish an extremely fine alpaca herd by corrective breeding and intensive selection within the US camelid population. Therefore, this study was conducted to record, evaluate, and summarize the progress of breeding, production, and management practices established at Victory Farm. Animal production performance was presented for birth weight (BW); weaning weight (WW); shearing live weight (SW); fleece weight (FW); staple length (SL); and fiber characteristics, including fiber diameter (FD), fiber diameter variation (FDsd and FDcv), curvature (CUR), and medullation rate (MD).

## Materials and Methods

### *Animal husbandry, welfare, and health management*

Victory Farm, owned by the Smith family, is located in Missouri at 39.5870° N, 93.7960° W, at an elevation of 233 m above sea level, with a landscape of rolling hills, with an annual average precipitation of 914 mm as rain and 406 mm as snowfall. Monthly average temperature ranges were recorded at -8 °C to 3 °C (January), 6 °C to 18 °C (April), 20 °C to 32 °C (July), and 7 °C to 20 °C (October). As of April 2016, the herd size had increased to 200 animals, including males, females, and crias. All animals were identified by implanted microchips for pedigrees, parentage, and breeding society registrations. Animals were grazed as a mixed-age herd on pastures during warm seasons and fed on dry feeds under shelter during the winter months. Pasture plant species on the farm are a mixture of grasses and legumes, including fescue, orchard, brome, bluegrass, and white clover. Animals were grazed on pastures and rotated more frequently and efficiently at a stocking rate of 10 head per acre. Adult males and females were penned and fed separately in a drylot during winter. High-quality forages, clean water, and mineral supplements were provided ad lib. Alfalfa hay containing 12 to 16 % crude protein was fed to females, yearlings, and weanlings, whereas brome or orchard grass hay with 10 % crude protein was fed to males during drylot feeding. Animals were regularly graded for body condition scores as nutrition and health status indicators. Animal welfare and health care were contracted with the local veterinary practitioner. A routine vaccination for recommended preventable illnesses was practiced, such as an injection of *Clostridium perfringens* type C + D and tetanus (CD&T) at annual intervals and an injectable dewormer (Noromectin®) at six-week intervals during seasons prevalent with internal parasites. Over 10 years of operation, the animal mortality rate was kept below 2%. Other animal welfare and health care was performed, such as timely

inspection for eye infections, scours, and bloating as well as toenail trimming and tooth grinding at shearing operations.

### *Production, selection, and breeding*

Animal selection criteria were set for three objective measurements and two subjective traits, namely, fiber diameter (FD,  $\mu\text{m}$ ), staple length (SL, mm), curvature in degree (CUR, deg/mm), coat color, and fleece density. The first breeding age was normalized at three years or older for males and two years for females; however, a female can be bred at about 18 months old if her live weight reaches 45 kg. Most animals were fall bred and fall born; however, a small number of animals could be spring bred if they were a noncarrier or not a bearer from fall breeding. Breeding was practiced as a single-pair mating in pens. Studs were fed and managed in separate barns and halter-trained prior to breeding. The breeding pair match-up was decided with reference to the offspring from previous mating allocations. Regular animal performance records were made on-farm, including birth date, sex, sire and dam, pedigree, birth weight (BW), weaning weight (WW), shearing body weight (SW), and fleece weight (FW). Animal breeding, pedigree, and production performance data were submitted to Colorado State University (Fort Collins, CO) to generate breeding value estimates. Data was recorded in an Excel spreadsheet for retrieval, communication, storage, and analysis. Animal registry information included animal ID (microchip), pedigrees, health records, and fiber test records.

Animals were estimated for breeding values using the expected progeny difference (EPD), which was updated annually to serve as a reference for individual animal sire or dam selection. As alpacas are self-induced ovulators, the estrus cycle occurs after breeding. Therefore, the female was reintroduced to a male seven days later but would reject mating if she was pregnant; otherwise, she would submit to breeding. The pregnancy was confirmed by observation of her “spitting off” for three consecutive weeks, and predicted cria due dates could then be placed on the birthing calendar. The gestation length averaged 345 d on-farm but was observed as early as 317 d to as late as 388 d for normal birthing. From weaning age, animals were trained for weighing, catching, and releasing as well as halter-training so they could develop an early interaction with humans and become less stressed by handling.

### *Blood pack cell volume and cell type measurements*

In 2017, blood samples were taken from 24 animals (12 males and 12 females, with each sex including four crias) representing the herd to determine blood pack cell volumes and blood cell profile survey measurements. Blood samples were drawn from the jugular vein from the right side of the neck (the C6 vertebra) using venipuncture fitted with a needle and collected into heparinized tubes (purple top).

A number of blood volume and cell parameters, including white blood cell (WBC) count and types (i.e., neutrophil, lymphocyte, eosinophil, and monocyte), red blood cell (RBC) count, hematocrit (HCT%), and platelet concentration were examined using the HEMAVET® HV950 hematology instrument (Drew Scientific, Inc.).

#### *Shearing, fleece classing, and fiber trait measurements*

Animals were scheduled to shear annually in April. Animals were sorted into coat color groups and penned under shelter overnight to prevent overfeeding or wetting of the fleece. The shearing floor space was cleaned and covered with a tarp/canvas sheet that was used for animal restraining and for fleece collections. Shearing started with the lighter shade fleeces, such as white, cream, tan, and light brown to dark brown. The shearing procedure followed an established alpaca shearing technique, which is similar to the procedure described by Wuliji et al. (1992), namely, the animals were shorn lying on their side on a tarp/canvas sheet-covered floor, with their two hind legs tied to a wall. Electric clippers with a sheep shearing comb and cutters were used to conduct shearing, while an assistant held the alpaca by both front legs, stretching and rolling the alpaca to expose the unshorn side fleece for the shearer. The saddle (blanket) fleece part was shorn first, followed by the rest of the fleece coat. The blanket and saddle fleece parts were weighed separately, recorded, bagged individually, labeled, and stored for further fiber classing, processing, and/or marketing. A small batch of mid-side fiber sample was collected from each animal for fiber trait measurements at each shearing and tested by the Yocom-McColl Testing Laboratories Inc. (Denver, CO). Yocom-McColl uses the Optical-based Fibre Diameter Analyser (OFDA 100) to measure a set of traits, such as the fiber diameter in microns, which is the ASTM standard (D2252) for alpaca fiber. Fiber measurements included FD, FD standard deviation (FDsd), and FD coefficient of variation (FDcv), SL, F30 (percentage of fiber diameter greater than 30 micron), CF (comfort factor, calculated as  $100 - F30$ ), curvature (CUR), and percentage of medullation rate (MD).

#### *Specialty camelid fiber collection, measurement, and comparison*

As a reference analysis, 15 fiber samples (dehaired) of Bactrian camel from Inner Mongolia and 17 fiber specimens of vicunas from private collectors and zoos were donated for this in-house study. These samples were measured and compared for FD and staple length with the fiber from the extremely fine fiber animals (25 %) from the Victory Farm herd in 2016 and 2017. Staple length was measured in mm, with five replicates. Fiber diameter was measured according to a standard microscopic method that measures 250 individual fibers randomly per specimen prep slide, following the standard ISO 137:2015 Wool – Determination of Fibre Diameter – Projection Microscope Method (2015). Eight of those vicuna fiber specimens were also tested at Yocom-McColl and validated for the accuracy of the

test by microscope procedures, which showed a 99.9 % agreement between the two methods. The FD, FDsd, FDcv, and SL of the extremely fine groups (2016 and 2017) were selected from the Yocom-McColl lab test results. The fiber test results of the Victory Farm herd (shearing 2016) are included in a multi-ranch reference comparison (Table 6).

#### *Expected progeny difference and heritability estimate*

The herd-expected progeny difference (EPD) and heritability ( $h^2$ ) values were estimated using a data pool of the breeding group's herds, which Victory Farm had organized for the collaborative breeding program. The pedigree and performance data of 760 animals with 2,915 performance records were included in this analysis. The expression  $h^2$  was estimated as the proportion of the difference in performance that is explained by a transmittable genetic difference. Multiple trait evaluation procedures were applied to estimate the correlation coefficient ( $r$ ) among these traits, which may have a positive or negative effect on each other.

#### *Data analysis and presentation*

Victory Farm alpaca herd data files for birth weight, weaning weight, and shearing weight were retrieved and pooled for data analysis and presentation. Data were analyzed and compared among production year, sex, and age groups for body weight as well as fleece and fiber characteristics. Fleece weight and fiber characteristics were pooled and analyzed on 1,230 records of 11 annual shearings (2007 to 2017). However, 1,021 observations were used for most traits, as some fleece weight or fiber characteristic measurements were either incomplete or missing for the production years 2007 and 2008. This resulted from the small herd size, so data from those years were excluded from the comparisons. There were 173 birth weight (2010 to 2016) and 166 weaning weight records available for this analysis. Shearing live weight (SW) records of 478 animals were available from 2014 to 2016, which were compared by age groups with their repeated measurements. The FD, FDcv, SL, F30, CUR, (deg/mm), and MD rates were provided as part of the systematic standard measurements by the Yocom-McColl test lab, except for the farm reference study specimens. Production performance data by year, age, and sex were extracted from the Victory Farm database. SAS® statistical procedures (i.e., GLM, ANOVA, CORR, and t-test) were used to process those data sets. The main effects were primarily compared for production year, sex, and age; however, the EPD calculation and  $h^2$  estimates were adopted from the Victory Farm group breeding annual reports (Enns & Speidel, 2017).

## Results and Discussion

Fleece weight and fiber characteristics are summarized by production years in Table 1. There were several significant differences ( $p < 0.05$ ) in SL, FD, FDcv, and CUR. The FW and SL decreased gradually in the past few years, whereas the FDcv slightly increased over time in the herd. There was no significant difference in the FW, except for the 2009 shearing, which was highly skewed due to the shearing regimen and the small number of animals. The measure for the CUR and F30 remained steady, with only a small fluctuation. Except in 2010, when there was a significant surge, the F30 regularly tested low (i.e., from 0.96 to 1.62 %), which was interpreted as the average CF of the fleeces for the herd over all the years of the study, which ranged from 98.4 to 99.9 %. The FD of the herd gradually decreased, while the number of animals in the herd increased, and the FD decreased significantly ( $p < 0.05$ ) in production years 2016 and 2017.

**Table 1:** Fleece weight (FW), staple length (SL), mean fiber diameter (FD), coefficient of fiber diameter variation (FDcv), percentage of fiber coarser than 30 micron (F30), and fiber curvature (CUR) measurements by production years

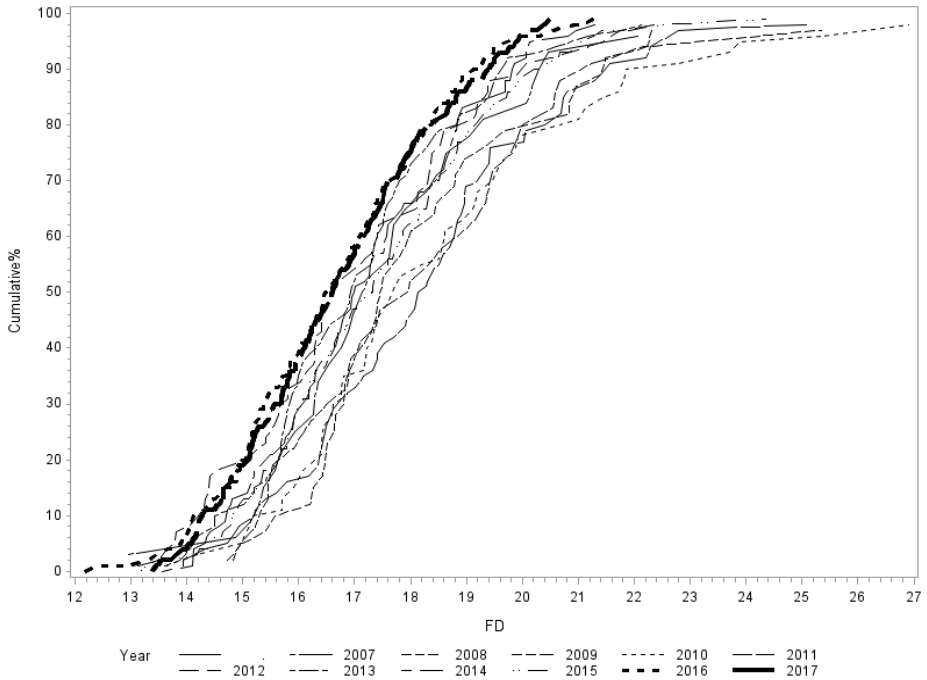
Year	Numbers	FW (kg)	SL (mm)	FD ( $\mu\text{m}$ )	FDcv (%)	F30 (%)	CUR (deg/mm)
2009	35	1.43 <sup>n*</sup>	87.4 <sup>c</sup>	18.02 <sup>bc</sup>	25.2 <sup>abc</sup>	1.43	57.61 <sup>ab</sup>
2010	59	1.11	75.2 <sup>bc</sup>	18.37 <sup>cd</sup>	25.5 <sup>ab</sup>	2.16 <sup>n*</sup>	53.17 <sup>a</sup>
2011	67	1.08	64.2 <sup>ab</sup>	18.19 <sup>bc</sup>	23.0 <sup>a</sup>	1.49	53.81 <sup>a</sup>
2012	68	1.04	65.5 <sup>ab</sup>	16.95 <sup>a</sup>	24.0 <sup>ab</sup>	1.13	57.81 <sup>ab</sup>
2013	77	0.85	57.2 <sup>a</sup>	17.03 <sup>ab</sup>	24.1 <sup>ab</sup>	1.03	61.49 <sup>bc</sup>
2014	168	0.83	56.7 <sup>a</sup>	17.13 <sup>ab</sup>	24.8 <sup>abc</sup>	1.05	58.15 <sup>bc</sup>
2015	143	0.78	57.5 <sup>a</sup>	17.36 <sup>bc</sup>	26.6 <sup>bc</sup>	1.32	53.87 <sup>a</sup>
2016	164	0.76	57.8 <sup>a</sup>	16.58 <sup>a</sup>	26.4 <sup>bc</sup>	0.96	57.66 <sup>ab</sup>
2017	171	0.73	59.9 <sup>a</sup>	16.67 <sup>a</sup>	27.2 <sup>bc</sup>	1.05	58.30 <sup>bc</sup>

<sup>abcd</sup>: means bearing a different superscript differ within the column at the  $p < 0.05$  level

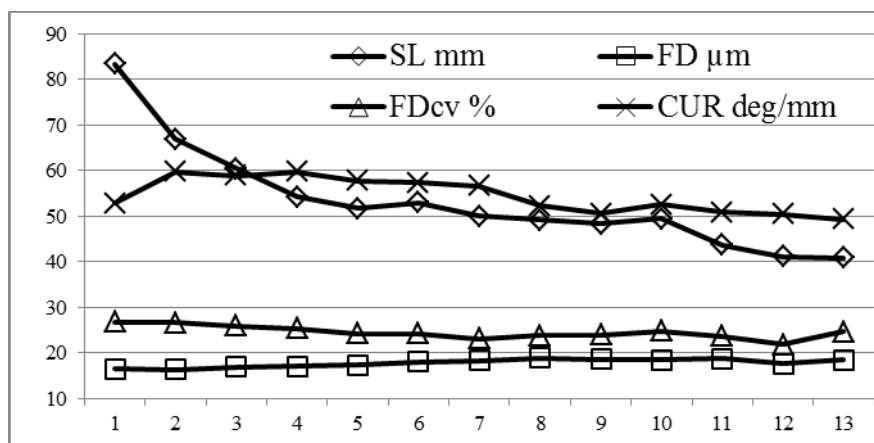
<sup>n\*</sup>: significantly differed ( $p < 0.05$ ) from the rest of the column

The effect of age was more pronounced on the SL, and the CUR decreased with increasing age. There was no difference in the SL by sex; however, for crias, the SL was reported to grow longer in the warm seasons than in the cold seasons in Peru (Quispe-Peña et al., 2014). Both the FD and FDcv in the herd showed minor fluctuations with increasing age, which was significantly less varied compared to other huacaya or suri alpacas (Wuliji, 2012). The Victory Farm alpaca herd's cumulative FD measurements were plotted by production year (Figure 1). The mean FD of the herd decreased gradually and consistently from 2007 to 2017, while the herd size increased. The apparent annual average reduction in the FD was 0.21 microns per year in the last eight fleece measurements, which reduced the herd average FD

to 16.58 and 16.68 in 2016 and 2017, respectively. The FW and F30 fluctuated and slightly decreased with age, whereas the MD rose with age. There were almost no changes in the FW and F30 measurements based on age but several significant ( $p < 0.05$ ) surges in the MD corresponded only to the eight-year-old age group (data were not shown). The herd average fiber diameter, staple length, fiber curvature, fiber diameter, and variation analysis by age group is shown in Figure 2.



**Figure 1:** Scatter plot of individual animal FD measurements (cumulative percentage of the herd) in the Victory Farm alpaca herd by production year (2007–2017).



**Figure 2:** Staple length, fiber diameter and diameter variation, and fiber curvature measurements in alpaca age groups.

**Table 2:** Fleece weight and fiber characteristic measurements compared by sex (pooled for years and ages)

Sex	Freq. No	FW (kg)	SL (mm)	FD (μm)	FDcv (%)	F30 (%)	FC (%)	CUR (deg/mm)	MD (%)
Female	576	0.84	60.0	17.59	24.7	1.39	98.61	56.3	2.1
Male	443	0.85	61.3	16.69	26.2	1.03	98.97	57.8	1.3
SD		0.29	18.8	2.05	4.6	1.16	1.16	10.8	5.9

Fleece weight and fiber characteristics are summarized by sex in Table 2. There was no difference for the FW, SL, and CUR, but the FD, F30, and MD were higher for females than males, and the FDcv was coarser for males than females. BW and WW were presented for birth years by sex, and the means were pooled (Table 3). There was no difference based on sex, however, there was a small but significant difference in the mean BW and WW by birth years, and both the BW and WW were higher for females than males. There were a number of significant positive or negative correlations among fleece weight and fiber characteristics (Table 4). There was a significant and positive or negative correlation ( $p < 0.01$ ) among all traits except MD with SL; there were no significant corrections with the FDcv. There were positive and significant correlations found among FW, SL, FD, F30, and MD, whereas there were some negative and significant associations between the FD and each of the following: FDcv, F30, FC, CUR, and CUR standard deviation (CURsd).

**Table 3:** Birth weight (kg), weaning weight, and the mean weights of alpacas recorded for six consecutive years after birth

Birth weight (BW)	2010	2011	2012	2013	2014	2015	2016
Female BW	6.8 <sup>ab</sup>	6.5 <sup>ab</sup>	6.5 <sup>ab</sup>	6.5 <sup>ab</sup>	7.0 <sup>ab</sup>	6.3 <sup>a</sup>	7.5 <sup>b</sup>
Male BW	6.8	6.7	6.8	7.3	6.9	6.6	7.4
Mean BW	6.8 <sup>ab</sup>	6.6 <sup>a</sup>	6.7 <sup>ab</sup>	6.9 <sup>ab</sup>	7.0 <sup>b</sup>	6.4 <sup>a</sup>	7.4 <sup>b</sup>
SD	1.1	1.1	1.0	0.9	0.9	0.9	1.0
Weaning weight (WW)							
Female WW	23.5 <sup>ab</sup>	26.6 <sup>b</sup>	25.2 <sup>bc</sup>	26.8 <sup>b</sup>	29.8 <sup>c</sup>	22.2 <sup>a</sup>	27.9 <sup>bc</sup>
Male WW	21.8 <sup>a</sup>	25.5 <sup>ab</sup>	23.9 <sup>ab</sup>	25.1 <sup>ab</sup>	27.3 <sup>bc</sup>	22.8 <sup>a</sup>	26.3 <sup>bc</sup>
Mean WW	22.9 <sup>ab</sup>	25.9 <sup>b</sup>	24.7 <sup>ab</sup>	25.9 <sup>b</sup>	28.3 <sup>bc</sup>	22.5 <sup>a</sup>	26.7 <sup>bc</sup>
SD	2.5	3.2	1.9	3.3	3.7	3.8	3.5

abcd: means bearing a different superscript differ within rows at the  $p < 0.05$  level

**Table 4:** Correlation coefficient estimates among fleece weight and fiber characteristics

Traits	SL	FD	FDcv	F30	FC	CUR	CURsd	MD
FW	0.42**	0.22**	-0.14**	0.20**	-0.24**	-0.21**	-0.12**	0.16*
SL		-0.12**	0.18**	0.25**	-0.18**	-0.28**	-0.17**	-0.14
FD			-0.34**	0.59**	-0.55**	-0.63**	-0.53**	0.68**
FDcv				0.28**	-0.12**	0.16**	0.30**	0.06
F30					-0.79**	-0.53**	-0.36**	0.55**
FC						0.45**	0.32**	-0.46**
CUR							0.83**	-0.35**
CURsd								-0.23**

\* significant association ( $p < 0.05$ ); \*\* significant association ( $p < 0.01$ )

Correlations among the FW, FD, FDcv, and CUR were of a similar magnitude to those found in previous analyses of US alpacas (Lupton et al., 2006; Wuliji, 2012). The mean fiber diameter (FD), minimum and maximum variant, FD variations (FDsd and FDcv), and staple length (SL) measurements in specialty reference fibers are compared in Table 5. The mean FD was significantly lower in vicuna fiber compared to camel and alpaca fibers. The extremely fine alpaca fiber diameter measured 3.2 microns less than camel hair and only one micron coarser than vicuna fiber ( $p < 0.01$ ). The FD measured for the reference vicuna fiber specimens was nearly the same as the saddle fleeces of vicunas examined in Peru (Quispe et al., 2014). However, the FD measured for extremely fine fiber groups of the Victory

Farm herd in the 2016 and 2017 shearings tested within the range (i.e., 14.15 and 14.29  $\mu\text{m}$ ) of the fiber diameter variation among the vicunas of South America.

**Table 5:** Comparison of the mean fiber diameter (FD), minimum and maximum variant, FD variations (FDsd and FDcv), and staple length (SL) measurements in specialty reference fibers (camel or vicuna fiber) with extremely fine selection groups (25 %) of the Victory Farm herd for 2016 and 2017

Camelid Species	Num- bers	FD ( $\mu\text{m}$ )	Minimum ( $\mu\text{m}$ )	Maximum ( $\mu\text{m}$ )	FDcv (%)	SL (mm)
Vicuna fiber	17	13.07 <sup>a</sup>	11.54	15.29	22.69 <sup>a</sup>	34.7 <sup>a</sup>
Extreme fine 2016	34	14.15 <sup>b</sup>	12.17	15.05	28.12 <sup>b</sup>	60.6 <sup>b</sup>
Extreme fine 2017	34	14.29 <sup>b</sup>	13.38	14.99	29.25 <sup>b</sup>	66.9 <sup>b</sup>
Camel fiber	15	17.43 <sup>c</sup>	15.35	21.09	31.83 <sup>b</sup>	80.0 <sup>c</sup>

abc: means bearing a different superscript differ within the column at the  $p < 0.01$  level

The Victory Farm FD test results from 2016 were compared with results from eight randomly tested alpaca farms in the US (Table 6). The Victory Farm herd's average FD in 2016 was significantly ( $p < 0.01$ ) finer than all of the eight alpaca farms (Wuliji, 2012). The FD of the Victory Farm herd ranked first in micron fineness, which was 4.74  $\mu\text{m}$ , 6.10  $\mu\text{m}$ , 7.82  $\mu\text{m}$ , 8.81  $\mu\text{m}$ , 8.96  $\mu\text{m}$ , 10.26  $\mu\text{m}$ , 12.35  $\mu\text{m}$ , and 12.42  $\mu\text{m}$  lower than the FD tested in the other farm herds, respectively. Blood cell counts and cell type measurements in a selected number of animals are shown in Table 7. There was no difference between the sexes for any of the blood parameters measured and mean blood parameters were within normal ranges.

**Table 6:** Victory Farm (1) FD test results (2016) compared with eight alpaca farms in the US

Farms	1	2	3	4	5	6	7	8	9
Animals	164	81	104	202	65	27	239	91	58
FD( $\mu\text{m}$ )	16.58 <sup>a</sup>	21.32 <sup>b</sup>	22.68 <sup>b</sup>	24.40 <sup>c</sup>	25.39 <sup>c</sup>	25.54 <sup>c</sup>	26.84 <sup>c</sup>	28.93 <sup>d</sup>	29.00 <sup>d</sup>
SE	0.32	0.45	0.39	0.30	0.50	0.78	0.26	0.43	0.53

abc: means bearing a different superscript differ within the row at the  $p < 0.01$  level

**Table 7:** White blood cell (WBC) count, hematocrit (HCT), and cell types measured for the Victory Farm herd (2017)

Alpacas	WBC (K/ $\mu\text{L}$ )	NE (%)	LY (%)	MO (%)	EO (%)	RBC (M/ $\mu\text{L}$ )	HCT (%)	PLT (K/ $\mu\text{L}$ )
24	10.26	76.59	6.46	15.93	0.90	13.03	28.95	174
SD	2.64	13.63	5.91	9.30	0.48	1.62	3.37	54

NE: neutrophils; LY: lymphocytes; MO: monocytes; EO: eosinophils; RBC: red blood cell count; PLT: platelet count; K = 1 thousand; M = 1 million.

The calculated EPD for six traits, including FW, SL, FD, FDsd, CUR, and FD was in the range from 19 to 76 % (Table 8). Heritability and correlations among fleece weight and fiber trait estimates for extremely fine alpaca breeding herds are listed in Table 8. The  $h^2$  estimate indicated that the FD and CUR were highly ( $\geq 0.5$ ) heritable traits, but the FW, FDsd, and SL exhibited only moderate levels of heritability ( $\leq 0.4$ ). These results were closely comparable to published estimates for camelids (Wuliji et al., 2000; Gutierrez et al., 2009). The phenotypic correlation estimates (Table 4) for the FW and SL, and also for the FD and CUR, were in agreement with the current genetic correlation estimation (Table 9).

**Table 8:** Expected progeny difference (EPD) in fleece weight and fiber characteristics in the Victory Farm alpaca herd (Enns & Speidel, 2017)

EPD traits	Average	SD	Minimum	Maximum	Accuracy %
Fleece weight (kg)	- 0.06	0.04	- 0.07	0.10	19 - 66
Staple length (mm)	- 0.83	4.65	- 9.71	12.45	21 - 71
Mean curvature (deg/mm)	0.79	3.33	- 10.21	7.51	24 - 74
Fiber diameter ( $\mu\text{m}$ )	- 0.21	0.99	- 1.84	3.94	25 - 75
FD standard deviation ( $\mu\text{m}$ )	- 0.10	0.21	- 0.51	0.77	22 - 71
FD rate of change ( $\mu\text{m}$ )	- 0.01	0.09	- 0.20	0.43	22 - 76

**Table 9:** Heritability (the values along the diagonal) and correlations among fleece weight and fiber traits estimated for group breeding herds (Enns & Speidel, 2017)

Fiber traits	FW	FD	FDsd	SL	CUR
FW	0.40	0.54	0.36		
FD		0.65	0.75	0.58	-0.82
SL			0.39		
FDsd				0.29	-0.82
CUR					0.50

The efficiency of selection and genetic gain in an extremely fine fiber diameter depends on the strength of heritability as well as correlations among the selection traits, such as the FD, FW, SL, CUR, and threshold-subjective traits. In addition to the strict selection and corrective mating in the herd, a tandem culling for some undesirable traits, such as unfitness, poor reproduction, and high medullation, should support the long-term selection goals.

## Conclusions

For the alpacas on the Victory Farm, the birth weight, weaning weight, and shearing weight were similar to the published camelid literature; however, the fiber weight and fiber characteristics of the Victory Farm herd were lower than typical huacaya and suri alpacas. Specifically, the fiber diameter, staple length, softness, and coat color traits appeared more similar to domesticated vicuna-like characteristics. The heritability, genetic, and phenotypic correlations among fleece and fiber traits were moderate to high for the major selection traits. This study indicated that strict selection and corrective breeding in the Victory Farm herd was effective for genetic gain in the fiber diameter to establish an extreme fine fiber alpaca strain.

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# Merino Breeding Program Improves Wool Quality in US Wool Sheep Flocks

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**Abstract.** A Merino breeding resource flock was established at Rafter 7 Ranch through cooperation with the University of Nevada and The Edwin L. Wiegand Trust. Initially, 500 Rambouillet ewes were purchased from two established breeders. These ewes were bred naturally or by artificial insemination (AI) to imported Australian Merino rams and to rams selected from within the flock. Over 16 years, 16 rams and semen from 41 Australian Merino rams were imported from Australia. Selection was based on objective wool measurements and phenotypic performance traits. The wool fiber diameter of the current flock decreased by 3 microns on average compared with the foundation flock (23 microns). The flock was expanded to 1,300 ewes and wasbred in 30 single-sire mating groups in 2006. The Merino flock is managed in two breeding lines, one as a registered Merino flock (n=650) and the other as a Rafter 7 line (n=650), which is selected for wool, lambing, and meat production traits. Sheep producers from 18 states, Canada, and Mexico have purchased breeding rams and ewes from Rafter 7 Ranch. The resource flock has made significant progress over the foundation ewe flock during the upgrading phase in major selection traits and is disseminating Merino genes in US wool sheep flocks.

**Resumen.** Se creó un rebaño de recursos para mejora de Merino en el Rancho Rafter 7 en cooperación con la Universidad de Nevada y el Edwin L. Wiegand Trust. Inicialmente, se compraron 500 ovejas Rambouillet de dos criadores reconocidos. Estas ovejas se reprodujeron por monta natural o inseminación artificial (IA) procedente de carneros merinos australianos importados y de carneros seleccionados del propio rebaño. Durante 16 años se importaron 16 carneros y semen de otros 41 carneros merinos australianos. La selección se basó en mediciones objetivas de lana y caracteres de rendimiento. El diámetro de la fibra de la lana actualmente fue 3 micras inferior en promedio al de los animals fundadores (23 micras). El rebaño se expandió hasta 1.300 ovejas y la reproducción se realizó en 30 grupos con un único carnero en 2006. La reproducción se realiza independientemente en dos líneas, una registrada como rebaño Merino (n=650) y la otra como Rafter 7 (n=650), y la selección se basa en caracteres de lana, partos y producción de carne. Otros productores de 18 estados, Canadá y México han comprado carneros y ovejas del Rancho Rafter 7. El rebaño de recursos para mejora de Merino ha logrado un progreso significativo en relación a los animals fundadores en los principales caracteres de selección y difunde genes de raza Merino en rebaños de ovejas de lana de Estados Unidos.

**Keywords:** merino sheep, breeding program, wool quality

## Introduction

In the United States, sheep and lambs are raised primarily in small farm flocks in the Midwest and East, and on large ranching operations in the West. Sheep grazing in the western rangelands can be profitable and environmentally sustainable (Glimp & Swanson, 1994). Most of the 7 million head of sheep in the country is in wool/meat dual-purpose flocks. However, over the last two or three decades, the wool clips in areas of major production have become progressively finer, while textile technology has improved for processing superfine wool types. Consequently, the raw wool premium prices set for fine and superfine wool categories increased significantly over other types of wool during those years. Two decades ago, the University of Nevada-Reno and Rafter 7 Ranch established a Merino breeding program at Rafter 7 Ranch near Yerington, Nevada. The purpose was to introduce superior fine wool Merino genetics from Australia and provide genetically improved and adapted breeding rams and ewes for the US western range regions. All animals born on the ranch are provided an individual metal ear tag at birth and an electronic ear tag number incorporated into their scrapie tag at weaning at approximately 90-120 days of age. Sheep numbers have steadily increased to meet the needs for breeding, replacements, and distribution of breeding stock to sheep producers. The flock is physically inventoried annually at breeding (approx. Nov. 20), shearing (approx. March 20), and lamb weaning/ewe culling (approx. Aug. 15). This paper describes a wool sheep selection program at Rafter 7 Ranch and the impacts of Merino genetics dissemination into western US range sheep flocks. Animal performance and wool characteristics were analyzed and presented for two selection flocks, namely, Rafter 7 Merino and Rafter 7 Line.

## Materials and Methods

### *Range and Pasture*

A flock of 1,300 breeding ewes (Rambouillet and Merino crossbred) and 35 stud rams are maintained at Rafter 7 Ranch, a University of Nevada-Reno (UNR) cooperative sheep station owned by The Edwin L. Wiegand Trust. The ranch includes 1,400 hectares of private land and grazing permits on 40,500 hectares of Bureau of Land Management Lands, and 1,800 hectares of USDA forestland. The flat pasture elevation is 1,200 to 1,500 m, with the high desert range elevation extending to 3,000 m. The annual precipitation within the area is less than 200 mm, mostly as winter snowfall, with unpredictable frosts and wind patterns. Desert shrubs include black greasewood, basin big sagebrush, black sagebrush, bud sage, white sage, and ephedra. Grass species include Indian ricegrass, bottlebrush

squirreltail, and cheatgrass. The established pastures were primarily tall fescue, overseeded with ladino clover. Improved irrigated pastures include a mix of tetraploid perennial ryegrass, improved fescue cultivars, and a variety of alfalfa and ladino clover. An additional 50 hectares of irrigated land is used for alfalfa hay production and aftermath grazing. Irrigated pastures, 35 pastures of 2-6 hectares, are set-stocked during breeding and lambing, with an intensive rotation during the rest of the grazing season.

#### *Animal Breeding and Ram Distribution*

Natural mating and AI were used alternately during the upgrading phases. A computerized record and database program that includes individual animal pedigree, sex, birth date, birth and rearing rank, weaning, and yearling performance is maintained on the ranch. Two seasonal lambing management options were adopted since 2006, although the majority of lambs are scheduled to be born during spring lambing. Animal selection was made each year prior to the breeding season on a multi-trait performance index in conjunction with independent culling for undesirable traits, such as poor conformation and structure, wool face cover, jaws, infertility, and colored fibers. Animal selection was based on objective wool measurements as well as subjective assessment, growth rate, and reproductive performance traits. The Merino flock was fully inspected, pedigreed, and registered with the Texas Delaine-Merino Record Association. Flock management is in two breeding lines, one as a registered Rafter 7 Merino flock (n=650) and the other (Merino x Rambouillet) as the Rafter 7 Line (n=650), both of which are selected for high fleece weight and quality, twinning, and growth traits. The spring lambing flock was wintered on desert rangelands and grazed on irrigated pasture from shearing through lambing and early weaning. Lambs were subjected to preselection culling at weaning and final selection based on yearling performance, including body and fleece weight as well as wool characteristics. A final selection performance index was derived by various adjustment weightings made to birth and rearing ranks, age, body weight, weight gain, fleece weight, fiber diameter, and length. Ram distribution catalogs for selected rams and ewes with a comparative performance index were posted to potential sheep producers/clients two to four weeks before the ranch held a ram sale field day. Selected rams and ewes were presented along with their IDs, pedigree and yearling performance data sheets, and health certificates in subdivided pens on the field day. Typically, about 70-100 producers participated in the annual ram sale, and several dozen ranchers purchased their choice of breeding rams and ewes by open bidding at the field day auction. The genetic distribution and impact on range wool sheep production was monitored on a number of ranches that consistently used Rafter 7 Ranch rams. Two ranches' fleece tests and breeding animal replacement data were monitored and shared as client feedback information. Four of these associated ranches (located in Nevada at Reno, Ely, Fernley, and at Rafter 7 Ranch) were surveyed for

their superfine category (19 microns or less) wool lot weight ratios in the clips, using the wool warehouse records and public auction information from 2004 to 2009 wool sale catalogs.

#### *Wool Production and Clip Preparation*

Individual fleece weight and wool characteristics were recorded for the lifetime of each breeding ewe and ram. Pre-shearing, mid-side wool staples were collected from each sheep, and a set of programmed wool tests for wool characteristics, including average fiber diameter (FD), fiber diameter variation coefficient (FDcv), average staple fiber length (SL), and estimated comfort factor (CF) were measured using an Optical-based Fibre Diameter Analyser OFDA 2000 (BSC Electronics, Western Australia) instrument. Shearing was scheduled at least four weeks prior to lambing. Fleeces were classed according to the pre-shear test classification with some subjective alternatives, such as length, discoloration, or tensile strength. Wool clip volumes and sale values were recorded and presented for each of the five years. At the 2009 spring shearing, approximately 3,000 fleeces at Rafter 7 Ranch were pretested, shorn, and classed into five category lots, namely, ultrafine, superfine, fine, medium, and coarse lines. These were baled and transported to the wool warehouse, which provided a sale lot test certification.

#### *Measurements and Statistics*

Data was recorded for each animal in the selection flock, including post-shearing body weight (BW), greasy fleece weight (FW), and wool characteristics. Wool characteristics, including FD, FDcv, CF, and SL were measured using an OFDA 2000 instrument on pre-shearing, mid-side wool staple samples at the UNR Wool Research Lab (Reno). Lamb fleeces were classed separately from the adult fleeces into three classes of ultrafine ( $< 17 \mu\text{m}$ ) superfine ( $< 19 \mu\text{m}$ ), and fine ( $< 21 \mu\text{m}$ ) wool lots, whereas mixed-age sheep fleeces were subjectively examined at wool skirting tables and pooled into the appropriate classes according to the pre-shearing fleece test results. The post-shearing body weight, fleece weight, and wool characteristics of two flocks (2009,  $n=2,218$ ) were analyzed. In addition, the annual fleece FD was measured for 600 ewes from two breeding flocks, which were born between 2001 and 2004. Their fleece FD was monitored for five years of shearing within the period from 2002 to 2009. The changes in FD by age were available for 563 animals every year from the first (lamb wool) to the fifth shearing (Age I, II, III, IV, and V), consecutively. These data were analyzed using a linear regression equation and a calculated fleece FD change prediction formula (index) for lifetime wool classing. The FD change prediction indexes were calculated by subgrouping animals from FD classes into fiber diameter ranges corresponding to US standards for grades of wool (USDA, 1968). The SAS® (SAS Institute Inc., Cary, NC) procedures GLM, CORR, REG, and GLIMIX were followed for the data analysis

of the body weight, fleece weight, fiber characteristics, and fiber diameter variations relative to birth year and age. Heritability ( $h^2$ ) and genetic correlation coefficients were cited from the previous analysis in the flocks by a multi-trait animal model statistical procedure (Rauw et al., 2010).

## Results and Discussion

Summary data of body weight, fleece weight, and wool characteristics are shown in Table 1. There are significant ( $p < 0.05$ ) differences between the two selection lines for BW and FW. The pure Merinos produced more wool than the Rafter 7 Line, even though their wool was slightly finer. The Rafter 7 Line sheep were significantly heavier ( $p < 0.05$ ) than the Merino sheep. The mean greasy fleece weight of both the Rafter 7 Merino and Rafter 7 Line flocks were higher than in the published data (Lee et al., 2000) of typical Rambouillet ewes. The earlier analysis of Merino crossbred ewes showed that wool fiber density, clean wool yield, staple length, and greasy fleece weight increased by 41 % per unit area of skin, 15 %, 2.5 cm, and 1.14 kg per head shorn, respectively (Glimp, 2006). Over the years of upgrading, the Rafter 7 Line has closed the gap with the Merinos in average fiber diameter, while still maintaining a significant advantage in BW and other subjective traits, such as having a larger body frame and structure. However, no differences were found between the FD, FDcv, SL, and CF of the flocks.

**Table 1:** Means of body weight (BW), fleece weight (FW), fiber diameter (FD), fiber diameter variation (FDcv), staple length (SL), and comfort factor (CF) in flocks (2009)

	No Obs.	BW (kg)	FW (kg)	FD ( $\mu\text{m}$ )	FDcv (%)	SL (mm)	CF (%)
R7 Merino	1291	66.5 <sup>b</sup>	5.32 <sup>a</sup>	19.4	17.2	86.0	99.0
R7 Line	1947	72.2 <sup>a</sup>	4.63 <sup>b</sup>	19.5	17.4	82.0	98.9
SE		0.9	0.9	0.1	0.05	0.5	0.05

ab: Column means with different superscript letters are different ( $p < 0.05$ ).

Changes in the FD were shown to exist among ages and birth year ( $p < 0.05$ ) (Table 2), which reflected environmental variations and the interaction of birth year and age groups.

**Table 2:** Least squares means of the average fiber diameter of Rafter 7 Ranch breeding ewes by flock and age group (n =556)

Flock	Means/ Flock	Means by Age Group					SE
		Age I	Age II	Age III	Age IV	Age V	
R7 Merino	20.5	18.4 <sup>c</sup>	20.9 <sup>c</sup>	20.7 <sup>c</sup>	21.4 <sup>a</sup>	21.4 <sup>a</sup>	0.1
R7 Line	20.6	18.7 <sup>d</sup>	21.1 <sup>b</sup>	20.8 <sup>c</sup>	21.6 <sup>a</sup>	21.5 <sup>a</sup>	0.1
Means	20.5	18.5	21.0	20.7	21.5	21.4	0.1

<sup>abcd</sup> Means with a different superscript letter differ significantly at the  $p < 0.05$  level within and between rows for age groups; there is no statistical difference between the pooled flock means.

Weaning weight, yearling weight, and fleece weight of the Rafter 7 Ranch sale rams have improved over the years (Table 3). The BW and FW for sale rams of birth years 2005-2007 did not differ between the two lines. The BW measures in sale rams showed a trend of the two breeding flocks converging in terms of BW and FD, although the Merinos produced more and finer wool. Weaning weight of the Rafter 7 Ranch flocks were analyzed previously (Rauw et al., 2007a), which showed that ram lambs weighed more than ewe lambs, single-reared lambs were heavier than multi-litter lambs, and lambs born from two-year-old ewes were lighter than lambs born from ewes of other age groups.

**Table 3:** Body weight (BW) at weaning (WW), yearling (YW), 16-months-old (SW), and 10-month-old fleece weight (FW) of the Rafter 7 Ranch sale rams (n = 120/year)

Year of Birth	Flock	WW (kg)	YW (kg)	16-mo SW (kg)	10-mo FW (kg)
2005	Merino	30.8	60.1	68.5	2.72
	R7 Line	31.3	60.0	71.7	2.63
2006	Merino	27.7	50.9	75.5	3.55
	R7 Line	28.6	52.7	78.6	3.64
2007	Merino	32.7	67.3	74.0	4.09
	R7 Line	31.4	72.7	79.1	4.09

Pearson correlation coefficients were calculated between body weight, fleece weight, and wool characteristics and are presented in Table 4. There is a significant ( $p < 0.01$ ) high correlation between FD and CF and also high correlations among FW, FD, and SL; a low-to-moderate correlation exists between BW and FW, whereas small or negative ( $p < 0.05$ ) correlations were observed between FDcv and FD, BW, FW, FD, SL, and CF.

**Table 4:** Pearson correlation coefficients (R-values) of body weight (BW), fleece weight (FW), fiber diameter (FD), staple length (SL), and comfort factor (CF) characteristics

Traits	FW	FD <sub>cv</sub>	FD	SL	CF
BW	0.20	- 0.06	0.21	- 0.13	- 0.10
FW		- 0.20	0.64	0.45	- 0.36
FD <sub>cv</sub>			- 0.16	- 0.09	- 0.16
FD				0.31	- 0.70
SL					- 0.13

Note: all R-values are significant at  $p < 0.01$ .

The heritability and genetic correlative values are listed in Table 5. The  $h^2$  value was low to moderate for BW, SL, and FW but high for the FD and FD<sub>cv</sub> traits. The  $h^2$  and genetic correlations among wool traits were generally lower but comparable to the previous findings in fine wool breeds by Wuliji et al. (2001) and Safari et al. (2005).

**Table 5:** Heritability and genetic correlations for body weight (BW) change, fiber diameter (FD), fiber variation (FD<sub>cv</sub>), staple length (SL), and greasy fleece weight (FW) estimated in Rafter 7 flocks (Rauw et al., 2010).

Traits	BW	FD	FD <sub>cv</sub>	SL	FW
BW	0.29	- 0.23	- 0.21	0.17	- 0.21
FD		0.51	0.49	0.37	0.21
FD <sub>cv</sub>			0.50	- 0.05	0.29
SL				0.39	0.34
FW					0.36

The sheep breeding, wool selection, and genetic resource distribution of the Rafter 7 Ranch flock impacts and the breeding stock distribution for western range sheep flocks (Wuliji et al., 2007) and the grazing efficiency of the ewes on the range were discussed previously (Rauw et al., 2007b). The genetic merit distribution and impacts on western range wool sheep breeding was presented for Borda's Ranch, Nevada, operation (Table 6). The number of two-year replacement ewes tested for fiber traits exponentially increased from 2003 to 2005 and remained high to 2008; however, the average FD of selected animals decreased significantly, from 23.9  $\mu\text{m}$  to 20.9  $\mu\text{m}$  in six years, whereas the CF and SL increased.

**Table 6:** Fleece measurements of two-year-old replacement ewes at the Borda's Ranch

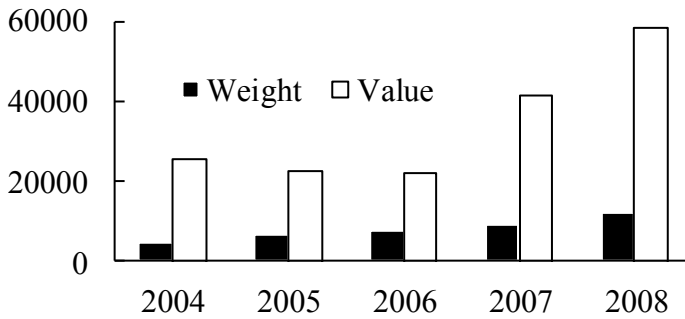
Year	No. of Sheep	FD ( $\mu\text{m}$ )	FDcv (%)	Comfort Factor (%)	SL (mm)	Mean Fiber Ends ( $\mu$ )
2003	292	23.9	16.2	92	70	23.5
2005	1457	22.4	16.0	96	77	22.1
2006	1252	21.6	16.0	97	80	21.3
2007	848	20.8	17.6	98	75	20.9
2008	626	20.9	16.7	98	87	20.7

A summary of two (Borda's Ranch and Volger's Ranch) of the client ranches' wool classing lines and clip production are presented in Table 7. Both ranches produced and classed fleeces into three sale lots, which showed a gradual reduction in FD and an increase in fine class wool lot volumes, even eliminating the coarse line lot in later years.

**Table 7:** Annual wool class FD lines and portions of clip changes in Rafter 7 Ranch client flocks in shearing and wool clips (2004 – 2008)

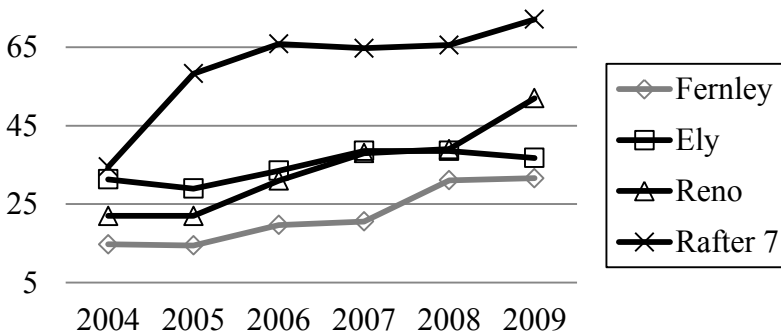
Wool Class	2004	2005	2006	2007	2008	2004	2005	2006	2007	2008
	Fiber diameter of wool lots ( $\mu\text{m}$ )					Portion of wool clips (%)				
Borda's Ranch (NV) wool flock records										
Fine	21.0	20.1	19.7	19.3	20.4	15	15	20	32	35
A	22.9	22.4	22.4	21.8	22.2	79	59	43	38	39
C	25.0	24.8	24.2	22.3	23.7	6	26	37	30	26
Volger's Ranch (NV) wool flock records										
Fine	20.3	20.6	20.6	20.5	20.3	31	29	58	35	30
A	22.0	22.4	22.0	21.6	22.1	39	31	35	65	70
C	23.0	23.1	22.4	-	-	30	19	7	0	0

Sheep flock performance and wool sale information show consistent improving trends within Rafter 7 Ranch flocks and associated clients' flocks. Wool sales from Rafter 7 Ranch increased significantly in clean wool clip weight and price values (Figure 1).



**Figure 1:** Rafter 7 Ranch clean wool clip weight (kg) and value (US dollar).

The gradual and continual increase in superfine and fine wool ratios of the clips at Rafter 7 Ranch was consistent with an earlier within-flock analysis (Wuliji et al., 2008). For eight-year production performance, the Rafter 7 Ranch wool clips received the highest price of any wool grown in the US. Sheep producers in 18 states, including Nevada, and in Mexico and Canada have regularly purchased breeding rams and ewes from Rafter 7 Ranch. Approximately 1,000 breeding rams and 500 replacement ewes were distributed to range flocks in the western states in the 2000s, which made a notable improvement in the fleece weight, fiber diameter, and yield in the clients’ flocks (Wuliji et al., 2009). Superfine lot portions of wool clip sales at four farms in Nevada are shown in Figure 2.



**Figure 2:** The superfine ratio (%) of wool clips on four farms (in Reno, Ely, Fernley, and at Rafter 7 Ranch) by production years (2004 – 2009).

The superfine wool ratio increases in the ranches wool sales is associated with the dissemination of the introduced Merino genetics of Rafter 7 Ranch into their sheep flocks. This has produced an improvement in wool quality by increasing the superfine ratio of the wool clip in the associated sheep producers’ ranches, with an especially rapid rise in superfine production at Rafter 7 Ranch. This trend is expected

to strengthen a long-term competitive advantage for the western US states' wool sheep enterprises.

The class of FD ranges, standard wool grades, means, and the prediction formula for mixed-age ewes for lifetime fleece classing and assigned wool class color tag codes are listed in Table 8. The FD changes by age groups were small to moderate, which is similar to the pattern of increase in the fleece weight, which showed a larger increase from the first shearing to the second but small changes until four years of age. Such features of FD and FD<sub>cv</sub>, and inter-trait correlations were also observed in ultrafine Merino flocks (Wuliji et al., 1999). These indexes appeared reasonable and capable of predicting future fleece FD ( $\hat{y} = a + bx$ ) using a single FD test ( $x$ ) of individual animals at two years old.

It is recommended that the formula derived for the particular wool grade or FD range be used; however, if there is a limited flock size or operation, flock mean formulas are more suitable to cover a wide range of FDs in small flocks. The X values (the second shearing FD value) were highly and significantly ( $p < 0.01$ ) correlated to the recorded and derived dependent variates (Y1 and Y2). With 95 % confidence, the fit plot analysis (Y1 and Y2) indicated a coefficient of determination expressed by the R-squared value of 0.53 and 0.71, which are equal to the x and y correlative associations at 74 % and 85 % ( $r = 0.73$  and  $0.84$ ), respectively. The FD prediction formula was used for Rafter 7 Ranch commercial flock wool classing operations. Several wool sheep ranches, including Borda Ranch, had adopted the one test for lifetime wool classification procedures using an OFDA analysis of the FD for their wool clip classing or breeding ewe replacements.

The selection efficiency in premium wool characteristics and rapid genetic gain were reported for various wool breeding demonstration flocks (Wuliji et al., 1999; Swan & Purvis, 2005; Brien et al., 2005). These characteristics showed moderate-to-high heritability (Atkins, 1997; Okut et al., 1999; Wuliji et al., 2001; Hansford et al., 2004). Therefore, we predict an increased likelihood of a higher rate of genetic dissemination into commercial sheep flocks followed by rapid genetic gains in wool quality traits.

**Table 8:** US wool grade, FD micron range ( $\mu\text{m}$ ), wool class code (ear tag color), and the derived FD prediction formula for lifetime fleece classes (mixed-age ewes)

Wool Grade	FD Range	Class Code	N	Mean X	Mean Y1	Mean Y2	$\hat{y}_1 = a + bx$	$\hat{y}_2 = a + bx$
80s	< 19.14	White	63	18.4	19.3	19.1	$6.14 + 0.72x$	$4.61 + 0.79x$
70s	19.15-20.59	Green	93	19.7	20.4	20.2	$11.72 + 0.44x$	$8.79 + 0.58x$
64s	20.60-22.04	Orange	152	20.6	20.9	20.8	$7.63 + 0.65x$	$5.72 + 0.74x$
62s	22.05-23.49	Yellow	112	21.6	21.6	21.5	$6.46 + 0.70x$	$4.84 + 0.78x$
60s	23.50-24.94	Red	94	22.6	22.6	22.5	$8.45 + 0.63x$	$6.34 + 0.72x$
58s	24.95-26.39	Black	49	23.9	23.0	23.2	$20.11 + 0.12x$	$15.1 + 0.34x$
Flock Mean Formula			563	21.0	21.3	21.2	$7.16 + 0.67x$	$5.37 + 0.75x$

Wool grade (80s): based on the spinning count of one pound clean wool that yields 80 hanks (or 44,800 yards); Mean X: the second shorn fleece FD values; Mean Y1: the average FD of three years of tests; Mean Y2: the average FD of four years of tests;  $\hat{y}_1$ : the predicted FD estimated by Y1;  $\hat{y}_2$ : the predicted FD estimated by Y2; a: intercept value; b: slope value; x: the second shearing fleece's FD.

## Conclusions

The Rafter 7 Ranch Merino flocks have made significant progress during the crossbreeding and upgrading phase as well as in the objective selection of major selection traits, including fleece weight and fiber diameter. The ranch is now disseminating elite genetics to many western range sheep flocks. The dissemination of introduced Merino genetics in the western range sheep flocks have improved wool quality and clip profits, which will strengthen a long-term competitive advantage for the US wool and sheep production sectors.

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# Selection Strategies for Fiber Quality in Alashan Cashmere Goat

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**Abstract.** Over the last ten years, many authors have attempted to study various genetic patterns and how they relate to improved cashmere fiber production. Unfortunately, the way the sector is currently structured hinders any “on the field” attempts to apply modern quantitative and molecular techniques to improve fiber characteristics through selection. The process is complicated by the fact that cashmere fiber quality is normally assessed when the animal is approximately a year old. The study investigates the prediction of cashmere quality in adult Alashan cashmere goats from measurements on young animals. Ten fleece samples were collected from 12 kids until 14 months of age (1,441 records). Fiber quality, determined by OFDA, was measured monthly. The statistical analysis indicates that the kid random effect is highly significant ( $P \leq 0.0001$ ) throughout the test period and thus confirms a genetic effect on cashmere fleece characteristics; the age effect was also found to be consistently significant. The measured traits showed an increasing trend of mean values up to the 5th -7th month. The fiber girth stabilises by the fifth month reaching a thickness of 14.46  $\mu\text{m}$ . All studied traits show a strong correlation with fiber quality beyond the fifth month. Furthermore, although adult fleece quality can be predicted beyond the fifth month of age, this usually coincides with the warm summer period and hence is an unsuitable season for the evaluation of fleece. Under such situations it may be recommended to postpone the evaluation beyond the age at eight months during the cooler months.

**Resumen.** En los últimos diez años, muchos autores han intentado estudiar diversos patrones genéticos y cómo estos se relacionan con la producción mejorada de la fibra de cashmere. Desafortunadamente, la forma en que está estructurado actualmente el sector dificulta cualquier intento de aplicar técnicas cuantitativas y moleculares modernas "en campo" para mejorar las características de las fibras mediante selección artificial. El proceso se complica porque la calidad de la fibra de cashmere se evalúa normalmente cuando el animal tiene aproximadamente un año de edad. Este estudio aborda la predicción de la

calidad de cashmere en cabras adultas Alashan a partir de mediciones en animales jóvenes. Se recogieron diez muestras de lana de 12 cabritos de hasta 14 meses de edad (1441 registros). La calidad de la fibra se valoró mensualmente mediante OFDA. En el análisis estadístico el efecto aleatorio cabrito resultó altamente significativo ( $P \leq 0.0001$ ) a lo largo de todo el período, confirmando la presencia de efecto genético sobre las características del vellón de cashmere. El efecto de la edad resultó también fuertemente significativo. Los caracteres medidos mostraron una tendencia creciente de valores medios hasta el 5°-7° mes de edad. La curvatura de la fibra se estabiliza en el quinto mes alcanzando un espesor de 14.46  $\mu\text{m}$ . Además los caracteres estudiados mostraron una fuerte correlación con la calidad de la fibra después del quinto mes. Aunque la calidad del vellón adulto se podía predecir después del quinto mes de edad, este período suele coincidir con el verano, temporada considerada inadecuada para la evaluación del vellón. Por ello, se recomienda aplazar la evaluación a partir de los ocho meses coincidiendo con un período más frío.

**Keywords:** animal breeding, early selection, rural production, cashmere, goat, fiber

## Introduction

The cashmere goat is to be found over most of Central Asia; including countries such as: Tibet, Mongolia, China (mainly in Inner Mongolia, Qinchai, Xinjiang, Yinghai, Ningxia, Hebei, Shanxi, Shang Dong, Liaoning provinces); and are also common in Afghanistan, Iran, Nepal, Pakistan, India, Turkey and in some regions of the ex-Soviet Union (Uzbekistan, Turkmenistan, Tajikistan, Kazakistan, Kirghigistan). A small population is also to be found in Australia and New Zealand.

Although official cashmere production data is not readily available, the production of raw/greasy fleece quantities can be estimated as follows: People's Democracy of China (8-9,000 tons); People's Republic of Mongolia (2,700-3,000 tons); Iran and Afghanistan (1,500-2,000 tons); Pakistan, Nepal and India (800 tons) and some 80 tons originating from other countries (Turkey, Australia, New Zealand) (<http://www.cashmeregoatassociation.org/>). The global production of cashmere is estimated at about 15,000 tons and amounts to about 0.2% of the total produced fibers (<http://www.naturalfantasy.it/>).

The Alashan region is located at an altitude of 1,000-1,400 m above sea level and extends over 670,000 km<sup>2</sup> of desert area characterised with continental type of climate having an average temperature of 7 °C, with a minimum of -13 °C in January and a maximum of 24 °C in July. The Alashan region produces one of the best cashmere fiber in the People's Democracy of China (Zizhi and Degang, 2006).

Over the last 10 years many authors have studied different genetic aspects of the cashmere goat in an attempt to enhance quality and quantity of fiber production. Some studies using microsattellites to evaluate the ethnological structure of the Chinese cashmere goat populations infer that different goat groups can actually be considered as being separate breeds (Di et al., 2011; Liu et al., 2013) that are highly

to moderately polymorphic (Xu et al., 2010). Others have focused on the association between productive traits and genetic markers. Shen et al. (2004) reports a connection linking body weight, cashmere yield and fineness at certain blood protein loci. Jin et al. (2006a) also found favourable genotypes at six blood protein loci for body weight and fiber yield in Liaoning cashmere goat; and obtained comparable results with the use of microsatellites on the same breed (Jin et al., 2006b). The SNPs association with cashmere production was studied by Lan et al. (2008); and found higher cashmere yields in TC genotype of a SNP in LALBA gene and established (Lan et al. 2009) that there exists a significant effect of 12 SNPs of POU1F1 gene on raw/greasy fleece weight and fiber length in Liaoning and Mongolian white cashmere goats. Moreover, Lan et al. (2012) found a significant effect of 28 bp insertion on the body mass of yearlings in the Inner Mongolia white cashmere breed. Pan et al. (2011) proposes the use of the polymorphism in Six6 gene as suitable marker for fiber length in Inner Mongolia white cashmere goats. Wang et al. (2012) found that the KAP9.2 gene could be a suitable marker in the selection of cashmere goat studs.

Other researchers have investigated a variety of correlations linked with productive traits. The quantitative approach was used to compare breeding and phenotypic value for body weight and fiber yield in Mongolian cashmere goats (Li et al., 2000; Junyan et al., 2006); and it was also used to estimate the genetic parameter of growth traits in Raini cashmere goat (Barazandeh et al., 2012; Mohammadi et al., 2012). Other researchers (Zhou et al., 2002) evaluated the genetic parameters of the production traits in Inner Mongolia Cashmere goats and found that heritability estimates were 0.28, 0.23, 0.32 and 0.10 for cashmere weight, fiber length, fiber diameter and live bodyweight respectively. Wang et al. (2012) studied the genetic parameters that contribute towards fleece traits; and later Wang et al. (2015) investigated the effects of a procedure of early selection for fleece traits in a monitored population consisting of 3,257 goats divided in 12 flocks.

Unfortunately, the current industrial structure and set up in the Alashan region does not allow for the “in the field” application of current quantitative and molecular techniques to enhance fiber characteristics through the identification and selection of superior sires. This poor industrial organisation is evident through the lack of any scientific approach or application in sire / dam evaluation criteria, and the normal practice of random matings. Furthermore, selection of potential reproducers is also done randomly, sometimes at too early an age when the fiber characteristics quality are still not fully expressed while others when the animals are over 1 year of age. Recently a record of performance and a genetic amelioration program on the cashmere goat population in Alashan has been put in place (Valbonesi et al., 2012). The objectives of this selection program are to evaluate cashmere diameter and its coefficient of variation.

The aim of this study is to study the probability of predicting cashmere quality in the adult Alashan cashmere goat from parameters measured on the young animal.

## Materials and Methods

Whole fleece samples including both the undercoat and out coat were collected from 12 kids on a monthly basis starting in April 2011.

The first sample was obtained at birth (MONTH0) and every month thereafter for the next 13 months (MONTH14). All animals were reared in the same flock and housed at the Animal Improvement Station of Alashan Left Banner in Alashan region.

A total of 1441 records were available for analysis (some records were missing) and the following variables were reported in the original record data file: age at sampling in months (0,...,13); LC: length of cashmere (under coat) (cm); LK: length of hair (outer coat) (cm); D: diameter of cashmere ( $\mu\text{m}$ ); R: LK/LC ratio; CV: coefficient of variation of D; CF: comfort factor (% of fiber  $\leq 30 \mu\text{m}$ ).

Due to practical constrains, only the data related to the first eleven sampling events were considered for evaluation having a total of 1210 records. All the fiber parameters were evaluated with an Optical Fiber Diameter Analyser (OFDA) (Peterson and Gheradi, 1996) at the labs of the Environmental Sciences School of Camerino University (Italy). It is relevant to note that fiber quality improves as LC, LK and CF values increase and D, R and CV values decrease.

All the statistics were computed by SAS (SAS, 2000). The main statistical inference parameters were analysed, and the effects of age and kid were computed according to the following linear model:

$$y_{ijk} = \mu + \text{month}_i + K_j + e_{ijk}$$

Where:

$y_{ijk}$  = experimental observation (LC, LK, D, R, VC, CF);

$\mu$  = overall mean;

$\text{month}_i$  = fixed effect of monthly age (1, ..., 11);

$K_j$  = random/fixed effect of kid (1, ..., 12);

$e_{ijk}$  = residual

The same model was run twice, once setting the K factor as casual and again setting K as fixed effect. In the first case, the K factor is assumed to be the only indicator of the genetic variability, which due to the lack of genealogical information is actually not possible to estimate. In the second case the K factor is considered as fixed so as to estimate the GL means, the option "repeated" on PROC GLM (SAS) was used correctly since each animal had eleven observations according to age. The correlations between the observations at the more mature age (MONTH10) and the younger ages (except for the diameter measures) were computed (PROC CORR, SAS), at all the ages to observe the relationship between the baby cashmere produced at young ages and the regular cashmere produced at the more mature age. The linear, quadratic and cubic regression models were

compared through  $R^2$  coefficient in order to evaluate the best one to predict fiber quality in the adult goat on the base of younger observations.

## Results and Discussion

The number of kids that were able to be sampled for fleece collection was not always constant throughout the experimental period. In fact Table 1 shows that at the several ages, fiber had a decreasing trend from MONTH0 to MONTH6, followed by an increasing one up to MONTH10. BY MONTH11 only six kids gave suitable samples of fiber. Thus in the analysis only samples from MONTH0 to MONTH10 were considered.

**Table 1:** Number of kids sampled at several ages

Month	Traits					
	LC	LK	R	D	CF	CV
0	12	12	12	12	12	12
1	12	12	12	12	12	12
2	10	11	10	12	12	12
3	2	10	2	4	4	4
4	7	10	7	11	11	11
5	7	5	5	8	8	8
6	6	6	6	6	6	6
7	12	10	10	12	12	12
8	12	12	12	12	12	12
9	12	12	12	12	12	12
10	11	11	11	12	12	12
11	6	6	6	6	6	6

LC = length of cashmere; LK = length of hair; R = LK/LC ratio; D = diameter of cashmere; CF = comfort factor; CV = coefficient of variation.

The trend described above is most likely the result of the climatic conditions of the region. The kidding season starts in April (MONTH0), subsequent sampling up to MONTH6 correspond chronologically from April to September when temperatures get progressively warmer along the test period. One has to keep in mind that cashmere kids have ample fleece at birth (cashmere baby wool) that is gradually lost on the onset of photoperiod inversion which triggers off the development of new follicular activity that becomes very low in June (2 samples), therefore the ages after MONTH10 were not considered.

Secondary follicular growth was studied by Henderson and Sabine (1991) who established that the greatest development of secondary hair follicles occurred at twenty weeks of age in three groups of Australian cashmere twin goats. This result should indicate that the selection for fiber characteristics could be defined on samples collected between the fifth and tenth week of age.

In the selection of other fiber producing species, such as sheep, fiberwool quality is normally assessed at approximately one year of age during the time of first shearing (Ozcan et al., 2005; Mandal et al., 2009). This strategy requires that all the young males be reared together until this age. Under this situation it is therefore crucial to be able to identify at the most earliest stage those individuals that can accurately predict the adult fiber quality, so as to reduce the holding costs of the flock under evaluation.

In Merino sheep, 5 months could be a suitable age to evaluate fiber for an early selection; as a matter of fact, in Australian Merinos the secondary follicles are fully developed by the 17-18 week (Burns 1949, 1953, 1954a, b; Fraser, 1952, 1954; Schinckel, 1955). Despite that, in Merinos, the 1st shearing at 1 year of age is considered as the suitable age for fiber evaluation in the Merino wool improvement selection program.

In the domestic Camelids of South American (SAC) Antonini et al. (2004) defined in all three types of SAC investigated the fleece structure is established and fiber analysis could be performed as tool for an early criteria of selection on animals that were two months old.

In Table 2 the significance level of the kid (random) and age effect on the studied traits are reported. The kid factor is always highly significant ( $P \leq 0.0001$ ), hence it imparts a confirmed genetic effect on the characteristics of cashmere fleece. Similarly to what was reported by others (Wang et al., 2012); the age effect was also always significant, but at a lower level P value in CF. The statistical model worked well on traits LC, LK and D; obtaining high coefficient of determination ( $R^2$ ) values of between 0.6 and 0.7; but had a lower relevance for R, CF and CV (0.34-0.49).

**Table 2:** Significance and  $R^2$  of the variability factors in the studied traits

	Traits					
	LC	LK	R	D	CF	CV
KID*	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
MONTH	0.0001	0.0001	0.0001	0.0001	0.0163	0.0001
$R^2$	0.67	0.60	0.43	0.68	0.34	0.49

\*: random

LC = length of cashmere; LK = length of hair; R = LK/LC ratio; D = diameter of cashmere; CF = comfort factor; CV = coefficient of variation.

The mean values of the traits (Table 3) increase progressively with age and tended to stabilise by the age of 4-6 months (MONTH5-MONTH7); after which, the results indicate that there are no further significant improvements. At MONTH0 (at birth) the length of cashmere is 2.28 cm and maintains a steady rate of growth until it tapers off by the age of 5 months (MONTH6) reaching a length of 4.35 cm. A similar trend is observed in LK, starting at 8 cm at MONTH0 and achieves the largest length by the 5th month of age (16.01 cm). This value is very close to the

reported length at 1 year of age as observed by Wang et al. (2015). R achieves a value close to 3 cm by the 4-5 month of age, and could represent the beginning of new cashmere growth corresponding to the loss of baby fiber coat. Fiber diameter (D) reaches 14.46  $\mu\text{m}$  by the third month and remains relatively constant thereafter; similar values were those observed by Wang et al. (2015) in the same breed. Beyond the second month, the CF registers values exceeding 99 % and CV reduces consistently implying that the fiber becomes progressively more uniform and homogeneous. Baby cashmere, which is obtained at an early age (2-4 months), has a diameter of about 13  $\mu\text{m}$ ; and is used in the spinning of high quality yarn.

**Table 3:** LSmeans ( $\pm$ SD) of the studied traits at the several ages

Month	Traits					
	LC	LK	R	D	CF	CV
0	2.28 $\pm$	8.00 $\pm$	3.67 $\pm$	14.27 $\pm$	98.17 $\pm$	35.80 $\pm$
	0.20	0.97	0.34	0.17	0.24	1.32
1	2.34 $\pm$	9.47 $\pm$	4.35 $\pm$	13.90 $\pm$	98.58 $\pm$	30.89 $\pm$
	0.20	0.97	0.34	0.17	0.24	1.32
2	2.06 $\pm$	8.94 $\pm$	5.59 $\pm$	13.61 $\pm$	99.33 $\pm$	26.87 $\pm$
	0.22	1.06	0.37	0.18	0.27	1.45
3	3.21 $\pm$	7.66 $\pm$	2.68 $\pm$	13.04 $\pm$	99.57 $\pm$	29.15 $\pm$
	0.49	2.38	0.83	0.41	0.60	3.26
4	3.63 $\pm$	12.35 $\pm$	3.59 $\pm$	14.46 $\pm$	99.08 $\pm$	30.84 $\pm$
	0.26	1.26	0.44	0.22	0.32	1.73
5	4.02 $\pm$	13.93 $\pm$	3.77 $\pm$	14.24 $\pm$	99.23 $\pm$	27.90 $\pm$
	0.30	1.50	0.52	0.26	0.38	2.06
6	4.35 $\pm$	16.01 $\pm$	3.82 $\pm$	14.52 $\pm$	99.26 $\pm$	27.21 $\pm$
	0.28	1.37	0.48	0.24	0.35	1.45
7	4.17 $\pm$	13.65 $\pm$	3.26 $\pm$	14.41 $\pm$	98.50 $\pm$	27.81 $\pm$
	0.22	1.06	0.37	0.18	0.27	1.45
8	5.19 $\pm$	13.93 $\pm$	2.84 $\pm$	14.34 $\pm$	99.18 $\pm$	28.40 $\pm$
	0.20	0.97	0.34	0.17	0.24	1.32
9	4.55 $\pm$	12.69 $\pm$	2.94 $\pm$	14.39 $\pm$	99.28 $\pm$	26.97 $\pm$
	0.20	0.97	0.34	0.17	0.24	1.32
10	5.00 $\pm$	12.59 $\pm$	2.57 $\pm$	14.73 $\pm$	98.68 $\pm$	31.10 $\pm$
	0.21	1.01	0.35	0.17	0.25	1.38

LC = length of cashmere; LK = length of hair; R = LK/LC ratio; D = diameter of cashmere; CF = comfort factor; CV = coefficient of variation.

The correlations reported in Table 4 were an attempt to identify any potential correlation between final fiber quality and parameters measured on the coat of young kids.

**Table 4:** Correlations between the traits at MONTH10 and at other ages

Month	MONTH10					
	LC	LK	R	D	CF	CV
0	- 0.35	0.33	0.24	0.24	0.17	0.07
1	- 0.24	0.31	0.35	0.31	0.26	0.11
2	- 0.15	0.40	0.35	0.35	0.36	0.40
4	0.54	0.72	0.49	0.51	0.53	0.55
5	0.78	0.76	0.60	0.61	0.61	0.65
6	0.82	0.77	0.57	0.72	0.62	0.62
7	0.77	0.81	0.59	0.75	0.73	0.68
8	0.05	0.83	0.80	0.81	0.67	0.59
9	0.70	1.00	0.88	0.72	0.83	0.82

LC = length of cashmere; LK = length of hair; R = LK/LC ratio; D = diameter of cashmere; CF = comfort factor; CV = coefficient of variation.

Due to lack of sufficient data in the MONTH3 group, the correlations related of this age were not computed. This lack of data was expected as the MONTH3 coincides with the month of July when the baby cashmere coat is lost and the growth of the new fiber is about to start. All the coefficients become relevant after MONTH4; this situation leads to a practical problem that can have two possible solutions. The kids to be select for breeding can be identified at 5 months of age, hence the rest of the group can be culled. The main constrain in this solution is that MONTH4 is achieved at the late summer, when potentially some animal could still be carrying the undercoat. Alternatively, selection can be done when the animals are 7 months old, in this option the holding time is extended by a further three months, but this will provide sufficient time for all the animals to express their fiber qualities. To solve this uncertainty, the provisional suitability of linear, quadratic and cubic regression models of MONTH4 and MONTH7 on MONTH10 were estimated for all traits and results presented in Table 5.

**Table 5:** R<sup>2</sup> (%) of the regression models to predict traits at MONTH10 by traits at MONTH4 and MONTH7

MODEL	Traits											
	LC		LK		R		D		CF		CV	
	M4	M7	M4	M7	M4	M7	M4	M7	M4	M7	M4	M7
Linear	94	93	81	91	78	84	99	99	96	99	99	99
Quadratic	84	84	72	83	57	68	99	99	92	95	99	99
Cubic	73	71	64	76	37	52	98	99	86	88	99	99

LC = length of cashmere; LK = length of hair; R = LK/LC ratio; D = diameter of cashmere; CF = comfort factor; CV = coefficient of variation.

The linear regression proved to be the better predictor; in fact, the  $R^2$  values were almost always greater than 90 %. In the case of trait R except for obvious differences, no real tangible differences are observed between MONTH4 and MONTH7 models; hence the results do not allow for the identification of the best time to measure as an indicator of mature fleece quality. Another indication on the suitability of early age selection is presented in Table 6 that shows correlations between fiber diameters at different ages.

Results indicate that this single trait may be the most important indicator in defining the fleece quality. Table 6 presents the correlations between measurements records at the baby cashmere production stage (from birth till the age of 2 months) in *italic* while the coefficients between the diameters in the young cashmere stage (at ages from 4-10 months) are indicated in normal fonts. The coefficients between the data at baby and young ages are represented by bold fonts. If the correlations between baby and young are significant and high, it should be possible to assume a very early selection of the best animals. From the general point of interest, it has to be noted that the estimated correlation coefficients are similar to those reported by Wang et al. (2015) in adult Inner Mongolia Cashmere goats.

In the baby phase, the correlations are often significant and range from 0.14 (not significant) to 0.40. In the young phase these coefficients are also often significant with high values (0.97: 4-6 months, 0.84: 8-10 months; 0.81: 6-8 months). The correlations between the baby and young cashmere production ages are not significant and in many cases get values close or over 0.50. These results are encouraging and need to be verified on a larger population to assess the possibility if selection for cashmere fleeces quality at early age.

**Table 6:** Correlation coefficients between the fiber diameter at several ages

MONTH	1	2	4	5	6	7	8	9	10
0	<i>0.14</i>	<i>0.39***</i>	<b>0.05</b>	<b>0.50***</b>	<b>0.17</b>	<b>0.44***</b>	<b>0.53***</b>	<b>0.49***</b>	<b>0.64***</b>
1	<i>1</i>	<i>0.40***</i>	<b>0.37***</b>	<b>-0.36***</b>	<b>0.53***</b>	<b>0.35***</b>	<b>0.41***</b>	<b>0.40***</b>	<b>0.61***</b>
2	<i>1</i>	<i>1</i>	<b>0.30***</b>	<b>0.14</b>	<b>0.46***</b>	<b>0.41***</b>	<b>0.62***</b>	<b>0.21**</b>	<b>0.66***</b>
4			<i>1</i>	<b>-0.008</b>	<b>0.97***</b>	<b>0.20*</b>	<b>0.17</b>	<b>0.39***</b>	<b>0.31***</b>
5				<i>1</i>	<b>-0.38**</b>	<b>0.17</b>	<b>0.22*</b>	<b>0.19</b>	<b>-0.01</b>
6					<i>1</i>	<b>0.68***</b>	<b>0.81***</b>	<b>0.55***</b>	<b>0.72***</b>
7						<i>1</i>	<b>0.60***</b>	<b>0.59***</b>	<b>0.75***</b>
8							<i>1</i>	<b>0.53***</b>	<b>0.84***</b>
9								<i>1</i>	<b>0.72***</b>

*Italic:* between baby cashmere ages; **Bold:** between baby and young cashmere ages; Normal: between young cashmere ages. \*:  $P \leq 0.05$ ; \*\*:  $P \leq 0.01$ ; \*\*\*:  $P \leq 0.001$

## Conclusion

This study represents the first scientific approach to the practice of selection for fiber quality in the Alashan cashmere goat in its local managerial environment. More data is required to generate and validate information that can be applied in practice. Some possible considerations as a result of this investigation are: 1) fiber diameter in the kid (0-2 months) is quite correlated to the fiber diameter in older animals (4-10 months), 2) adult fleece quality can be predicted after the fourth month of age, but this age usually coincides with the warm summer period, an unsuitable season to evaluate the fleece. Therefore, it may be prudent to postpone the evaluation age to seven months, a time that usually occurs past the summer season. Probably, in the current state of reality, the breeders will select the animals for reproduction according to their own management condition and constraints.

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# Interaction between ASIP and MC1R in Black and Brown Alpaca

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**Abstract.** Agouti (ASIP) and Extension (MC1R) are genes known to be involved in coat colour through pigmentation pathways by regulating type, amount and distribution of eumelanin and pheomelanin pigments in melanocytes. In alpaca genotype of ASIP and MC1R genes have already been analysed distinctly in previous studies, but so far their epistatic interaction have not been evaluated. In this study have been assessed their segregation more insights on black and brown phenotypes. In several mammals MC1R is epistatic over ASIP, id est recessive allele in Agouti (a) and dominant allele in Extension locus (E) produces black phenotype. That is confirmed in alpaca where black coat has aH/a $\Delta$ 57 and aH/ahT genotype on ASIP and E/E or E/e genotype on MC1R locus. Otherwise ASIP and MC1R in brown, have a dominant profile at least in one allele as A/A, A/ahT on Agouti and E/e on Extension. Genotype and phenotype comparison clears that receptor and ligand are in concordance to produce pheomelanin and eumelanin in alpaca. Segregation analysis of 12 alpaca families genotyped by coat color, confirm the dominance of brown over black and could be helpful for coat colour classification and genotyping.

**Resumen.** Se sabe que los genes Agouti (ASIP) y Extension (MC1R) están implicados en el color de la capa al intervenir en las vías de pigmentación regulando el tipo, la cantidad y la distribución de los pigmentos de eumelanina y feomelanina de los melanocitos. Los genotipos disponibles para estos genes ASIP y MC1R ya han sido analizados en profundidad previamente, pero no se ha estudiado su interacción epistática hasta la fecha. En este estudio se estudia la segregación de estos genes en la aparición de fenotipos negro y marrón. MC1R es epistático sobre ASIP en varios mamíferos, produciendo el fenotipo negro la combinación del alelo recesivo del Agouti (a) con el alelo dominante del gen Extensión (E). Esto se confirma en la alpaca ya que la capa negra tiene un genotipo aH/a $\Delta$ 57 o aH/ahT en el locus ASIP y E/E o E/e en el locus MC1R. Por el contrario, ASIP y

MC1R producirán capa de color marrón cuando el individuo porte al menos un alelo dominante siendo A/A o A/ahT para el locus Agouti y E/e en el locus Extension. La comparación entre genotipos y fenotipos permite concluir que el receptor y el ligando están en concordancia para producir feomelanina y eumelanina en alpacas. El análisis de segregación de 12 familias de alpacas genotipadas para el color de la capa, confirma el predominio del marrón sobre el negro y podría ser útil para la clasificación del color de la capa y el genotipado.

**Keywords:** Alpaca, Genetic interaction, ASIP, MC1R, Black, Brown.

## Introduction

Fiber harvested from alpaca (*Vicugna pacos*) is sold as luxury yarn. Peru hosts the largest biological reserve of alpaca in the world. Alpaca are raised in a variety of coat phenotypes and, recent studies have investigated various candidate genes possible involved in coat color variation in alpaca (Feelay et al., 2011). Recently a scheme for coat colour classification was defined by the International Committee for Animal Recording (ICAR) (Antonini, 2009). As regards potential colour, it could be affected by the pigmentation process. The synthesis of both eumelanins and pheomelanins are under control of a group of genes acting at the melanosomes level. The principal genes are Extension (MC1R protein) and Agouti (ASIP protein). Melanocortin 1 receptor (MC1R) and its peptide antagonist agouti-signaling-protein (ASIP) are well known in the regulation of the eumelanin/pheomelanin switch involved in the base color expression. As in other animals, the epistatic interaction of MC1R and ASIP, could be visible as hues palette of phenotypes, although genotype segregation patterns is not revealed. In alpaca, three significant mutations in the Agouti locus causing black and brown phenotypes were identified. In particular the SNPs (g.3836C>T and g.3881G>A) and an in-frame 57 bp deletion (g.3866\_3923del57) in exon-4 are predicted to independently cause functional changes to ASIP protein. Missense mutation g.3836 C>T involve in an amino acid substitution in R98C, would predict a change of arginine (R) to Cysteine, which seems to have minimal/ partial effect on the functional property. This is evident in one of the allele ahT associate with g.3836C>T mutations seems to have partial/minimal functionality. Missense mutation at g.3881G>A suggests that it may produces a substitution at the amino acid position R118H changes the R to histidine (H) in the cystine-rich domain, which disrupt the highly conserved Arg-Phe-Phe (R-F-F) motif in the protein. Other analysis with cloning experiment showed that one of the allele marked with g.3896G>A (aH) seems to be associated to non-functional ASIP due to the R-F-F motif disruption. Deletion of 57 bp. would result in a short 114 amino acid containing agouti protein, which lacks 19 amino acids from the cysteine (C) rich domain, which is critical in agouti function and ASIP is supposed to be not functional (Table 1). For the locus Extension (MC1R) in alpaca, four missense

mutation are relevant with the black and brown phenotypes. In particular the g.901C>T nucleotide mutation resulting in the R301C amino acid change shows that all the brown animals were heterozygous to C901T (Powell et al., 2008). The interesting fact observed with our molecular segregation analysis is that the animals homozygous to the mutations A82/ A259/ A376/ C901combination expressed black skin phenotypes with an EE genotype. The brown phenotypes were observed to have the heterozygous condition for the observed mutations (A82G/ A259G/ A376G/ C901T) with Ee genotypes (Table 2). In alpaca mutations of this two genes were analysed separately in two previous studies performed by our research group (Bathrachalam et al., 2011; Bathrachalam et al., 2013), but so far their epistatic interaction have not been evaluated. In this regard, the present study aims to fill the knowledge gap on the genetic interaction between ASIP and MC1R in alpaca and, to provide novel information useful for both applicative and basic science issues. Our outcomes provide further insights into the mechanisms of pigmentation in alpaca as well as provide "molecular tools" for the development of an efficient marker assisted breeding program for coat colors in this animal.

**Table 1:** The ASIP geotypes and phenotype of Peruvian alpacas.

Phenotype	g.3836C>T p.R98C	g.3896G>A p.R118H	g.3866-3923del57 p.C109_R127del	Allele
Black	C	A	-	<sup>H</sup> <i>a</i>
	C	-	Yes	<sup>Δ57</sup> <i>a</i>
Black	C	A	-	<sup>H</sup> <i>a</i>
	T	G	-	<i>a</i> <sup>HT</sup>
Brown	C	G	-	A
	T	G	-	<i>a</i> <sup>HT</sup>
Brown	C	G	-	A
	C	G	-	A

## Materials and Methods

### *Sampling and processing of the samples*

Alpacas were sampled from the Quimsachata Experimental Station, Peru. The Station is located on the Andean Plateau at 4,300 m under the management of the Instituto Nacional de Innovacion Agraria (INIA). The trials have been organized in a hierarchical scheme. Five Black rams have been mated to 10 black dams, and for them and their crias (baby alpaca) the colors have been assigned. Brown animals have not been crossed in this mating plan. Skin biopsies were obtained after antisepsis, and local anesthesia was injected at the border of the sampling site. Samples have been collected from parents and crias by disposable biopsy punch

(8 mm diameter) and have been stored in All Protect (Qiagen). Then, the skin fragments have been removed from preservative reagents and stored in liquid nitrogen. For molecular genetic analysis all samples have been transferred to the laboratories at the School of Environmental Sciences, University of Camerino, Italy. The genomic DNA was isolated from the skin biopsies by using DNAeasy tissue kit (Qiagen S.A., Courtaboeuf, France) according to the instruction of the industry. The DNA samples with good quantity and quality have been stored at -80 °C for further analysis. The genotyping assays have been performed for ASIP and MC1R in all the informative phenotypes according to method reported in the previous Bathrachalam works (Bathrachalam et al., 2011; Bathrachalam et al., 2013). Then the genetic interaction between ASIP and MC1R have been analysed (Table 3).

**Table 2:** The MC1R genotypes and phenotype of Peruvian alpacas.

g.A82G (p.T28A)	g.A259G (p.V87M)	g.A376G (p.G126S)	g.C901T (p.R301C)	Phenotype	MC1R Genotype
A/A	A/A	A/A	C/C	Black	<i>EE</i>
A/G	A/G	A/G	C/T	Black	<i>Ee</i>
A/G	A/G	A/G	C/C	Black	<i>Ee</i>
A/G	A/G	A/G	C/T	Brown	<i>Ee</i>

#### *Ethics statement*

In agreement with the new European Directive on the protection of animals used for scientific purposes (Directive 2010/63/EU, Article 15, Annex VIII), all animal procedures used in the study are classified as ‘mild’ (i.e., procedures with no significant impairment of the well-being or general condition of the animals) and were preemptively approved by the Animal Ethics Committee of the University of Camerino.

**Table 3:** ASIP and MC1R genotype interaction and the following phenotype of Peruvian alpacas.

Genotype ASIP (Ligand)	MC1R(Receptor)	Phenotype
$a^{A57}/a^H$ ,	E/E, E/e	Black
$a^H/a^{hT}$	E/E, E/e	Black
$A/a^{hT}$	E/e	Brown
A/A	E/e	Brown

## Results

### *ASIP – MC1R combined Genotype*

In alpaca, the black animals observed to have two agouti genetic backgrounds. One seems to have completely recessive alleles i.e. aH/a $\Delta$ 57 and another with a recessive and a partial dominant allele i.e. aH/ahT. The MC1R genotypes of black with two agouti genetic background were completely in association, thus black phenotypes with aH/a $\Delta$ 57 and aH/ahT had either E/E or E/e (MC1R) genotypes (Table 3), thereby the receptor and ligand genetic background is perfectly balanced to produce eumelanin in black animals. Furthermore, black animals were heterozygous to a $\Delta$ 57 allele with ahT or aH, heterozygous to both ahT and aH, homozygous to the mutation C901 (EE) and heterozygous to C901T (Ee). Brown animals were heterozygous to ahT, homozygous for aH, homozygous to g.3836C, wild allele (A). The a $\Delta$ 57 and the A were not observed. All the brown animals analysed in the present study were heterozygous to C901T (Ee).

### *Segregation Analysis*

Segregation analysis was performed on 10 alpaca families constituted from 5 male (rams), 10 female (dam) and 10 crias (baby alpaca). All of them presented a black phenotype, and the genotype at the Extension and Agouti locus have been evaluated (Table 4). In all the families the allelic variants at the Agouti locus was aH/ahT or aH/a $\Delta$ 57 and at the Extension locus was Ee or EE.

## Discussion

Molecular identification of the Agouti and MC1R genes provided much of the molecular groundwork for understanding the role of melanocortin signaling in pigmentation. In alpaca, the extension locus encoding for MC1R is epistatic over locus Agouti which means that a fully functioning MC1R receptor is necessary for the Agouti to be expressed. If MC1R is not functional then it cannot be activated or inactivated by either of two alternate ligands. In most of the mammals the recessive allele in agouti (a) and dominant allele in MC1R locus (E) produces black phenotypes. In black alpacas MC1R is functional at the least in one allele, and ASIP is in recessive form due to possible explained missense mutations. In these cases, ASP lost the ability to block  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$  – MSH). Improvement of the  $\alpha$ -MSH receptor induced cAMP production and up-regulated the transcription of MITF that activates the expression of the melanogenesis-related enzymes and regulating the gene expression of tyrosinase, TRP-1, and TRP-2. These pathways enhance the pigmentary function of melanocytes to produce more eu- and pheo-melanin and hair darkening.

**Table 4:** The Agouti and Extension genotype segregation in 10 black alpaca families.

Family n	Code	Color	ASIP	MC1R	Family n	Code	Color	ASIP	MC1R		
1	RAM	366203 <sup>4</sup>	Black	$a^H / a^{hT}$	E/e	2	RAM	95101 <sup>2</sup>	Black	$a^H / a^{\Delta 57}$	E/E
	DAM	301204	Black	$a^H / a^{\Delta 57}$	E/E		DAM	386301	Black	$a^H / a^{\Delta 57}$	E/E
	CRIA	138108	Black	$a^H / a^{\Delta 57}$	E/E		CRIA	81108	Black	$a^H / a^{\Delta 57}$	E/E
3	RAM	35104 <sup>1</sup>	Black	$a^H / a^{hT}$	E/E	4	RAM	95101 <sup>2</sup>	Black	$a^H / a^{\Delta 57}$	E/E
	DAM	83104	Black	$a^H / a^{hT}$	E/e		DAM	275204	Black	$a^H / a^{\Delta 57}$	E/e
	CRIA	78108	Brown	$a^H / a^{hT}$	E/E		CRIA	287208	Black	$a^H / a^{\Delta 57}$	E/E
5	RAM	35104 <sup>1</sup>	Black	$a^H / a^{hT}$	E/E	6	RAM	237204 <sup>3</sup>	Black	$a^H / a^{hT}$	E/E
	DAM	348204	Black	$a^H / a^{hT}$	E/E		DAM	283205	Black	$a^H / a^{\Delta 57}$	E/e
	CRIA	264208	Black	$a^H / a^{hT}$	E/E		CRIA	160108	Black	$a^H / a^{\Delta 57}$	E/E
7	RAM	244203 <sup>5</sup>	Black	$a^H / a^{\Delta 57}$	E/E	8	RAM	237204 <sup>3</sup>	Black	$a^H / a^{hT}$	E/E
	DAM	75103	Black	$a^H / a^{\Delta 57}$	E/e		DAM	480100	Black	$a^H / a^{\Delta 57}$	E/E
	CRIA	241208	Black	$a^H / a^{\Delta 57}$	E/E		CRIA	437308	Black	$a^H / a^{\Delta 57}$	E/E
9	RAM	244203 <sup>5</sup>	Black	$a^H / a^{\Delta 57}$	E/E	10	RAM	244203 <sup>5</sup>	Black	$a^H / a^{\Delta 57}$	E/E
	DAM	2160200	Black	$a^H / a^{\Delta 57}$	E/e		DAM	130105	Black	$a^H / a^{\Delta 57}$	E/e
	CRIA	262208	Black	$a^H / a^{\Delta 57}$	E/E		CRIA	89108	Black	$a^H / a^{\Delta 57}$	E/E

<sup>1</sup>Ram1. <sup>2</sup>Ram2. <sup>3</sup>Ram3. <sup>4</sup>Ram4. <sup>5</sup>Ram5.

In alpaca, the black animals observed to have two agouti backgrounds that seems to have completely recessive alleles i.e.  $a^{\Delta 57} / a^H$ , and  $a^H / a^{hT}$ . The MC1R genotypes of black with two agouti backgrounds were completely in association. All the brown animals analysed in the present study were heterozygous at the extension locus E/e and present SNP g.901C>T in one allele. Furthermore, Agouti gene has the A/ahT, A/A genotype. In this case, at the least one allele of both genes have to be functional to express this phenotype that is the typical wild-type pigmentation pattern for the alpaca. This phenotype also occurs in domestic sheep (Våge et al., 2003), in which it is understood to be a product of positive artificial selection for novel colour variants during domestication. In fact when MC1R is bound by ASIP, eumelanin synthesis is inhibited because of the down-

regulation of several melanogenic factors, and pheomelanin production relies on the chemical status of the cell environment, i.e. the content of available tyrosine and tyrosinase activity. The depigmentation induced by ASIP results from the inhibited synthesis of both types of melanins concomitant with the dramatic decrease of tyrosinase activity. That leads preferentially to the suppression of eumelanin production during the synthesis of mixed-melanins than pheomelanin. Different shades of brown phenotypes observed in alpaca could be due to the interactive effect between agouti and MC1R followed by a mixed amount and type of melanins production. The dominance of brown phenotype over black coat was confirmed by segregation analysis. In fact occurrence of both black and brown baby alpaca (crias) in crosses of black parents could be explained by the absence of black offspring in crosses involving brown parents (Valbonesi et al., 2011). Furthermore black phenotype is under control of an allelic heterogeneity at ASIP locus. An effort of segregation about black phenotype was confirmed by genotype analysis in Agouti and Extension (MC1R) locus in 10 families. (Table 4)

## Conclusions

Finally, clearly interaction of agouti and MC1R is in concordance with the black and brown phenotypes not the only genetic interaction through which black and brown pigments are synthesized in alpaca. As verified by the segregation analysis the black colour in alpaca is a recessive pattern. However, this research could have several implications: in fact standardize fibre production based on natural coat colour, could surely help herders farm. Breeders that perform mating program based on colour genotyping of their breeding alpacas could have a large amount of fleece with similar tone and shades. Select coat colour through biomarker assisted selection could increase breeders' income. That is considerable in Peru, where rear alpaca is popular and poverty prevails. Furthermore natural coloured fibre does not need to be dyed and that improve the performance of eco-friendly, low economic impact and compete with synthetically dyed products, that fade with time and sometimes causes harmfulness to human body. Further studies exploring other candidate genes, especially those with regulatory functions are likely to provide great insights into our understanding of the black and brown phenotypes in alpaca.

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# Alpaca FGF5: Hypothetical Post-Transcriptional Readthrough Regulation in Skin Biopsies

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**Abstract.** Two different phenotypes are described in alpaca, identified as Suri and Huacaya which differ for the type of the fleece. The Huacaya fleece is characterised by compact, soft and highly crimped fibres while the suri fleece is longer, straight, less-crimped and lustrous. In our study, the Fibroblast growth factor 5 (FGF5) was investigated as a possible candidate gene for hair length in alpaca (*Vicugna pacos*). Total RNA purified from alpaca Suri and Huacaya skin biopsies, was reverse transcribed to cDNA using oligo dT priming and subsequently amplified by using FGF5 specific primers. Further, the resulting amplicons were cloned and sequenced. As previously identified in others mammals, our results also show that the alpaca FGF5 gene, give rise to a short (FGF5S) and a long (FGF5) isoform. Interestingly, in the long isoform, we observed a point mutation (i.e. a transition C>T at position 499 downstream of the ATG codon) able to generate a Premature Stop Codon (PSC). The highly conserved nucleotide and aminoacid sequence after PSC suggested, and western blot analysis confirmed, a Readthrough event (RT). The analysis of mRNA sequence revealed motifs and characteristics that correlate with mRNA that undergoing RT (i.e. the higher “leakyness” of UGA Stop Codon, leakyness” due to the position -1, -2 in respect the UGA PSC with purines and +4 with pyrimidines, presence of suzesky sequences, pseudoknots in 3'UTR and tandem repeat Stop Codon after the canonical TAA, presence of long intron in the gene and long 3'UTR). To the best of our knowledge this is the first case of readthrough event on PSC reported for FGF5 gene.

**Resumen.** Existen dos fenotipos diferentes de alpaca llamados Suri y Huacaya que únicamente difieren en el tipo de vellón. El vellón de Huacaya se caracteriza por sus fibras compactas, suaves y altamente rizadas, mientras que el vellón de Suri es más largo, recto, menos rizado y más brillante. En nuestro estudio, el factor de crecimiento de fibroblastos 5 (FGF5) se investigó como un posible gen candidato para la longitud de la fibra de alpaca (*Vicugna pacos*). El ARN total purificado obtenido de biopsias de piel de alpaca Suri y

Huacaya, se transcribió de manera inversa a ADNc utilizando primers oligo(dT), y posteriormente se amplificó utilizando cebadores específicos de FGF5. Los amplicones resultantes fueron posteriormente clonados y secuenciados. Al igual que en otros mamíferos los resultados también mostraron que el gen FGF5 de alpaca da lugar a una isoforma corta (FGF5S) y otra larga (FGF5). Curiosamente se observó una mutación puntual (es decir, una transición C> T en la posición 499 por debajo del codón ATG) en la isoforma larga, capaz de generar un codón STOP prematuro (PSC). Las secuencias de nucleótidos y de aminoácidos altamente conservados después del PSC sugirió, y el análisis de transferencia Western confirmó, un evento de lectura (RT). El análisis de la secuencia del ARNm reveló motivos y características que se correlacionan con el ARNm relativo al RT (es decir, la mayor "fuga" de UGA del codón STOP, "fuga" en la posición -1, -2 con respecto al UGA PSC en bases púricas y +4 en pirimidínicas, presencia de secuencias suzesky, pseudonodos en el extremo 3'UTR y repeticiones en tándem del codón STOP después del TAA canónico, presencia de un intrón largo del gen y un largo extremo 3'UTR). Hasta donde conocemos, éste es el primer caso de lectura del evento PSC citado para el gen FGF5.

**Keywords:** alpaca, suri, huacaya, FGF5

## Introduction

This paper contains the results of the research on FGF5 gene of the alpaca carried out at the Italian University of Camerino. The results are published in Pallotti et al. (2018).

Regulation of gene expression is found at different stages, one of which operates at the level of termination in protein synthesis. Sometimes translation continues reading through stop codons due to different kinds of events such as misreading by some natural non-cognate tRNAs or natural suppressor tRNA. This phenomenon, called readthrough (RT), allows the ribosome to pass through the termination codon in mRNA to continue translation until the next stop codon. Translational readthrough is widespread in viruses, fungi and *Drosophila*. However, its prevalence in mammals is not clear. (Schueren, F., & Thoms, S. 2016).

Fibroblast growth factor 5 gene (FGF5) belongs to the FGF family composed by at least 23 members that binds four receptors and perform different biological functions (Zhang et al., 2006; Beenken and Mohammadi, 2009; Sasaki et al., 2011). FGF5, first identified as human oncogene (Zhan et al., 1988), is expressed in different tissues such as the brain, heart, liver, spleen, muscle, rumen and skin (He et al., 2014; Zhang et al., 2015).

In mouse, FGF5 mRNA is highly expressed in the hair follicle as two isoforms, identified as FGF5 and FGF5S (Suzuki et al., 2000), and the latter is due to the alternative splicing of exon 2 (Hattori et al., 1996). Both isoforms, through binding to FGF receptor 1 and 2, regulate the hair follicle growth cycle during the anagen stage: FGF5 actively inhibits cell proliferation and the synthesis of hair fibers, while FGF5S antagonizes the inhibitory effects of FGF5 through competi-

tively binding the FGF receptors (Suzuki et al., 2000; Ota et al., 2002; He et al., 2016).

Previous studies showed how FGF5 gene is a crucial regulator of hair length in a wide variety of mammals such as human (Higgins et al., 2014), rabbit (Mulsant, 2010), cats (Kehler et al., 2007), dogs (Cadieu et al., 2009), cetaceans (Chen et al., 2013) and sheep (Hu et al., 2017; Li et al., 2017).

Alpaca (*Vicugna pacos*) is a South American camelid specialized in fiber production (Bonavia, 1996). Based on the fleece type, two different phenotypes are identified for alpaca, known as huacaya and suri. While the huacaya coat consists of compact, soft and highly crimped fibers, the suri coat consists of straight, less-crimped, lustrous and silky fibers (Antonini, 2010). An important phenotypic feature is the longer staple length of the alpaca suri compared to huacaya (Lupton & McColl 2011; Ferguson et al., 2012). Several studies on segregation analysis were carried out in order to understand the genetic control of fleece characteristics in alpaca and, in particular, the mode of inheritance of the suri phenotype (Ponzoni et al., 1997; Renieri et al., 2009; Sponenberg, 2010; La Manna et al., 2011). In a recent study by Presciuttini et al (2010), the data supported a genetic model in which two linked loci must simultaneously be homozygous for recessive alleles in order to produce the huacaya phenotype, while the suri phenotype is determined by the presence of a dominant allele at either locus.

The primary aim of the study was to investigate FGF5 transcript variability in suri and huacaya alpaca and to assess possible association between the identified transcript variants and differences in hair length showed by the two phenotypes. Studying these transcripts, we found evidence of post-transcriptional readthrough regulation.

## Materials and Methods

A total of 20 animals, consisting of 10 suri and 10 huacaya, were sampled from the Quimsachata Experimental Station, Instituto Nacional de Innovacion Agraria (INIA), Peru. The same animals were used in two studies by Chandramohan et al., (2013; 2015).

The sample was structured in order to obtain two sets of records. The first set was represented by 3 families (9 individuals) and the second set composed of 11 genetically unrelated animals (five huacaya and six suri).

For collection of skin biopsies, trichotomy was performed using disposable stainless-steel blades. Skin biopsies were obtained after antiseptis, and local anesthesia with 2 % lidocaine was injected at the border of the sampling site. Two samples were collected from each animal by disposable biopsy punch (8 mm diameter) and were stored in All Protect (Qiagen). Then, the skin fragments were removed from preservative reagents and stored in liquid nitrogen.

RNA and proteins were extracted using a AllPrep DNA/RNA/Protein Mini Kit (Qiagen, Germany), and the first strand cDNA was synthesized using Prim

Script™ Reverse Transcriptase (Takara Biotech, Japan), according to the manufacturer's protocol. To identify both FGF5 isoforms, 9 primers were designed according to orthologous sequences published for other mammals retrieved from the NCBI GenBank. Primers were designed to amplify the complete FGF5 isoforms. The first strand cDNA was synthesized with 1 µg of total RNA using 10 pmol oligo dt primer, 0.5 mM dNTPs, 1X RT buffer, 20 U RNase inhibitor and 200 U PrimScript™ Reverse Transcriptase (Takara Biotech, Japan) in 20 µl total reaction volume according to the manufacturer's instructions. The reaction mixture was incubated for 45 min at 50 °C and then at 70 °C for 15 min.

The PCR reactions were performed using Bio-Rad thermal cycler (Bio-Rad) in a 25-µl reaction final volume consisting of 0.5 U TaqDNA polymerase (Thermo Scientific, Milano, Italy), 50 ng DNA, 1X GC Buffer (Takara, Saint-Germain-en-Laye, France), 2 µM dNTPs, 10 µM each of forward and reverse primer, and RNase free water. The cycle conditions were set as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 10 s, annealing for 20 s (at different T° depending on primers used) and extension at 72 °C for a time depending on the length of expected amplicon. Amplicons were then separated by agarose gel electrophoresis and purified using Nucleospin columns (Macherey Nagel, Germany). The purified amplicons were cloned using CloneJET PCR Cloning Kit (Takara, Saint-Germain-en-Laye, France) and sequenced by StarSEQ GmbH (Mainz, Germany).

ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit (Epicentre Bio) was used for the identification of the 5'UTR while the amplification of 3' UTR was performed using modified oligo (dt). Sequences were visualized with sequencing chromatogram trace viewer FinchTV v. 1.4.0 (Biosoft, Cambridge). The FGF5 nucleotide sequences were determined using NCBI BLAST ([www.http://blast.ncbi.nlm.nih.gov/Blast](http://blast.ncbi.nlm.nih.gov/Blast)). The amino acid sequence was deducted by the Translate tool of ExpASy (<http://www.web.expasy.org/translate>). Alignment of nucleotide and amino acid sequences was conducted using MAFFT version 7 (<http://mafft.cbrc.jp/alignment/server>).

The protein concentration was determined by Bradford assay (Bradford, 1976). Equal amounts of protein lysates (30 µg) were electrophoretically separated on SDS-PAGE 12 % PA gel and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). The membrane was blocked with 5 % non-fat dried milk for 1 h at room temperature and then incubated at room temperature for 1 h with a monoclonal anti-FGF5 antibody No. H00002250-M01 produced in mouse (Abnova), binding the epitope AA 159-268 of FGF5 protein. After three washes, the blots were incubated with an anti-Mouse IgG anti-body produced in rabbit (1 µg/ml, Sigma) for 1 h at room temperature and subsequently visualized using the Odyssey Infrared Imaging System (Li-Cor, Lincoln, NE, USA), according to the manufacturer's instructions.

To predict eukaryotic selenoproteins and SECIS elements along FGF5 nucleotide sequences, we used Seblastian tool on Selenoprotein Prediction Server

(<http://sebastian.crg.es/>) (Mariotti et al., 2013). To predict and visualize the FGF5 RNA secondary structures and pseudoknot, the online versions of DotKnot (Sperschneider and Datta 2010; Sperschneider et al., 2011 (<http://dotknot.csse.uwa.edu.au/>)) and PseudoViewer (Byun and Han 2006) ([http://pseudoviewer.inha.ac.kr/PVWebService/WSPV\\_input.asp](http://pseudoviewer.inha.ac.kr/PVWebService/WSPV_input.asp)) were used.

## Results and Discussion

### *cDNA analysis of the FGF5 gene*

We amplified and sequenced the complete coding region by PCR using skin RNA from ten suri and ten huacaya.

The alpaca FGF5 gene gave rise to two transcripts similar to those of other mammals represented by the short (FGF5S) and the long isoform (FGF5), which were present in both suri and huacaya phenotypes.

In both phenotypes, the FGF5S transcript presented an open reading frame of 375 bp, encoding a protein of 125 amino acids (Fig. 1), while the FGF5 transcript presented an open reading frame of 498 bp due to a premature termination codon (PTC), which was predicted to be translated into a protein of 166 amino acids (Fig. 2). The FGF5s showed two 3'UTRs of 713 and 542 bp, respectively. In contrast, a single 3'UTR of 713 bp has been identified for the FGF5 isoform. FGF5s showed a 166 bases 5'UTR while FGF5 showed two different 5'UTRs of 166 and 205 bp (see supplementary material published in Pallotti et al., 2018).

Concerning the FGF5 transcript in both phenotypes, downstream to the premature termination codon in position 499, the sequence was highly conserved until the next stop signal in position 811, as also seen in other mammals (Fig.3). The highly conserved and functional sequence of amino acids downstream to the premature termination codon (PTC) suggested RT phenomenon and the synthesis of an entire FGF5 protein. To evaluate the role of the FGF5 gene for the hair length in alpaca, we compared the cDNA sequences from suri and huacaya. This analysis could not detect polymorphisms in either the coding regions or in the UTRs, which could explain the different hair length in the two phenotypes.

```

gttcgggtggcctctctctctccccctctcccagctctcttccaagctatgtccac -106
ccggtgcgcgaggggggagcgcagaggtacgcagccgcgcggggctgaggagccca -46
gaaccagccctacaagatgcgcttaggacccccgcggctggaagaATGagcttgtccttc 15
                                     M S L S F 5
ctcctcctccttctgctcagccacctgatcctcagcgcctgggctcgcggggagaagcgc 75
L L L L L L S H L I L S A W A R G E K R 25
ctcgtccccaagggaaccccgaccggctgccactgctaggaacccgggagggcgcagc 135
L V P K G Q P G P A A T A R N P G G A S 45
agcagctggagcagcagaagcagcagctctcctctcttctcctctgctcctcctcccc 195
S S W S S R S T T S S S S S S A S S P 65
gcggtctctctgggaagccaaggaagcgcttggagcagaccagtttccagtgagcccc 255
A A S L G S Q G S G L E Q T S F Q W S P 85
tcggggcgccgaccggcagcctctactgcagagtgggcatcggttccatctgcagatc 315
S G R R T G S L Y C R V G I G F H L Q I 105
taccggatggcaagtcaatggctcccacgaagccaatatgtaagccagatttacaga 375
Y P D G K V N G S H E A N M L S Q I Y R 125
TGActgcaagtgcaggagtgatttcaagaaacagctataaacctatgctcagtgat 435
stop
acacagaactgagaacacggggcgagggtggtacgtggccctgaacaagagggggaaagc 495
taaaggggctgcagccccgggttaaccccagcacgtctccactcactttctgccaag 555
atttaagcaatcgagcagccggaactttctttcacagttactgttctgaaaagaaaa 615
accacctaaccatcaagccaaggtcccccttccacatctcggagaagccccagcac 675
ggtgaagtacagactcaagtttcgcttggataattatcatcttggcgtgaggaaccgc 735
gccgtccctcaagagtttccctaggtgtttttagtgccctgaagacagagtttcggaca 795
cagcttcatctctactacactgcacgaagccaggtcatttgttcagctctgactgaaac 855
aaaaacgttttctgataggaactgaactggaatcttttgtagcaaatgaagagcgcact 915
gctcagttcagtgccacataaagccttttgctttatgcttgacgcacctcgtgactctg 975
gttccagaaagtgaaatgatgggaactatgtacttttctgacttttacagatcaacctga 1035
aaaaacatacatggtacatttttattttgggttccaaagaatattttgatgcagataa 1095
aaatattttatctaacttttgttttttgtaaaaaaacataacctctgacttgagca 1155
tattttctacttttatttttaattaataagacataaaacaacctttgaatacagtt 1215
tatgaattataaatatggttatagccatttgtaaacagtggtttccctttcatttttc 1275
ttattcattgcacttttaattttattattattatttttaccataacctcagatcatg 1335
taagtttagtttacactcttaaatgtttaaattctctctcatagcaaaaaaaaaaaaa 1395

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**Figure 1:** FGF5 Short isoform nucleotides and amino acids sequence. The sequence was taken from accession MF497582. The start codon and the stop codon are highlighted in gray.

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actggcgtataaaatcccagtgccagcgccgagatccgttogggtggcctctctctctc -145
tccccctctcccagctcttcccaggctatgtccaccgggtgcggcgagggggcgag -85
cgccagaggtacgcagcgcgcgggggtgcgagcccagaaccagccctacaagatgcg -25
cttaggaccccccgggctggaagaATGagcttgccttctcctcctcctctctcttctgctcagc 36
      M S L S F L L L L L L S 12
cacctgatcctcagcgcctgggctcgcggggagaagcgcctcgtccccaagggaacc 96
H L I L S A W A R G E K R L V P K G Q P 32
ggaccggtgccactgctaggaaccgggagggccagcagcagctggagcagcagaagc 156
G P A A T A R N P G G A S S S W S S R S 52
acgagcttctcctctctcctcctcctcctcctcctcctcctcctcctcctcctc 216
T T S S S S S S A S S S P A A S L G S Q 72
ggaagcggctggagcagaccagtttccagtgagccctcggggcggcggaccggcagc 276
G S G L E Q T S F Q W S P S G R R T G S 92
ctctactgcagagtgggcatcggtttccatctgcagatctaccggatggcaaatcaat 336
L Y C R V G I G F H L Q I Y P D G K V N 112
ggctcccacgaagccaatgttaagtattttggaatatttgcgtgtctcaggggatt 396
G S H E A N M L S I L E I F A V S Q G I 132
gtaggaatacggagagttttcagcaacaaattttagcagatgtcaaaaaaggaatact 456
V G I R G V F S N K F L A M S K K G K L 152
catgcaagtgcagatttacagatgactgcaagttcagggagTGAtttcaagaacagc 516
H A S A R F T D D C K F R E - F Q E N S 172
tataaacctatgcctcagtgatacacagaactgagaacacggggcgggagtgatcgtg 576
Y N T Y A S V I H R T E N T G R E W Y V 192
gccctgaacaaggggaaagctaaacggggctgcagccccgggttaaaccacagcac 636
A L N K R G K A K R G C S P R V K P Q H 212
gtctccactcactttctgccaagatttaagcaatcgagcagcgggaaactttcttcaca 696
V S T H F L P R F K Q S E Q P E L S F T 232
gttactgttctctgaaaaaaccacctaaccctcaagccaaggtccccctttcc 756
V T V P E K K K P P N P I K P K V P L S 252
acatctcggagaagcccagcaggtgaagtacagactcaagtttctgcttggatTAAatta 816
T S R R S P S T V K Y R L K F R F G stop 270
tcatcttggcgtgaggaacgcgctccctcaagagtttccctagtggttttagtg 876

ccctgaagacagagtttcggacacagcttcatctctactacactgcatcgaagccaggtc 936

at ttgtttcagctgactgaacaaaaacgttttctgataggaactgaactggaattcct 996

tgtaccaacatgaagagcgcactgcttcagttcagtgccacataaagccttttgctttat 1056

gcttgacgcactcctggtactctggttccagaaagtgaatgatgggaactatgtaactttt 1116

ctgacttttacagatcaacctgaaaaacatacatggtacatttttttttttggtttcc 1176

aaagaatattttgatgcagataaaaaatattttataacttttgttttttggttttttaaa 1236

aaaacatacctctgcattgagcataattttctacttttattatttttaataataagacat 1296

aaaacaccatttgaatacagtttatgaattataaatatgtttatagccatttgaaca 1356

agtgatttccctttcatttttcttattcattgcacttttaattttttattattattatta 1416

ttttaccatacctcagatcatgtaagtttagttttacaactttaaagtgttaattct 1476

cttcatagcaaaaaaaaaaaaaaa 1501

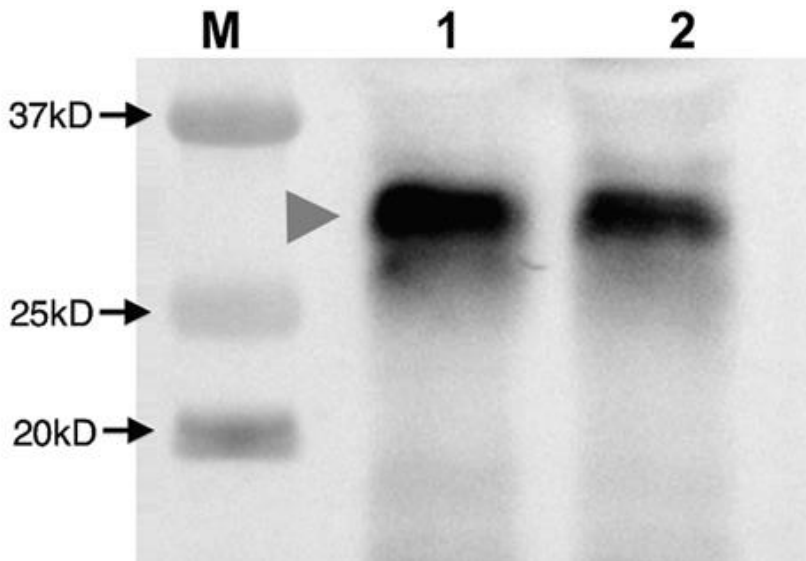
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**Figure 2:** FGF5 Long isoform nucleotides and amino acids sequence. The sequence was taken from accession MF497584. The start codon, the TGA PTC, the TAA canonical stop codon and the Skuzeski sequences of the form CARYYA and the “CAGCAGCA” sequence by Beier and Grimm (2001) are highlighted in gray (for the colored version see Pallotti et al., 2018). The (-) symbol at position 167 of the protein sequence indicates the unknown amino acid.

**A)**

Ovis aries	MSLSFLLLLLFLSHLILSAWAQGEKRLAPKGQPGPAATERNPGGASSRRSSSTVSSSSSP	60
Capra hircus	MSLSFLLLLLFLSHLILSAWAQGEKRLAPKGQPGPAATERNPGGASSRRSSSTASSSSSP	60
Bos taurus	MSLSFLLLLLFLSHLILSAWAQGEKRLAPKGQPGPAATERNPGGASSRRSSSTATSSSSP	60
Vicugna pacos	MSLSFLLLLLFLSHLILSAWARGEKRLVPGQPGPAATARNPGGASSWSRSSTSSSSS *****:*****:****.***** ** *.:****.	60
Ovis aries	ASSSSAASRGGPGSGLEQSSFQWSPSGRRTGSLYCRVIGFHLQIYPDGKVNGSHEANML	120
Capra hircus	ASSSSAASRGGPGSGLEQSSFQWSPSGRRTGSLYCRVIGFHLQIYPDGKVNGSHEANML	120
Bos taurus	ASSSSAASRGGPGSSLEQSSFQWSPSGRRTGSLYCRVIGFHLQIYPDGKVNGSHEANML	120
Vicugna pacos	ASSSPAASLGSQSGLEQTSFQWSPSGRRTGSLYCRVIGFHLQIYPDGKVNGSHEANML ***.*** *. **.:*****:*****	120
Ovis aries	SILEIFAVSQGIVGIRGVFSNKFAMSKKGLHASAKFTDDCKFRERFQENSINTYASAI	180
Capra hircus	SILEIFAVSQGIVGIRGVFSNKFAMSKKGLHASAKFTDDCKFRERFQENSINTYASAI	180
Bos taurus	SILEIFAVSQGIVGIRGVFSNKFAMSKKGLHASAKFTDDCKFRERFQENSINTYASAI	180
Vicugna pacos	SILEIFAVSQGIVGIRGVFSNKFAMSKKGLHASARFTDDCKFRE(-)FQENSINTYASVI *****:*****:*****.*****.*****.	180
Ovis aries	HRTEKTGREWYVALNKRKAKRGCSPRVKQHVSTHFLPRFKQSEQPELSFTVTVPEKKK	240
Capra hircus	HRTEKTGREWYVALNKRKAKRGCSPRVKQHVSTHFLPRFKQSEQPELSFTVTVPEKKK	240
Bos taurus	HRTEKTGREWYVALNKRKAKRGCSPRVKQHVSTHFLPRFKQLEQPELSFTVTVPEKKK	240
Vicugna pacos	HRTEKTGREWYVALNKRKAKRGCSPRVKQHVSTHFLPRFKQSEQPELSFTVTVPEKKK *****:*****:*****.*****.*****.	240
Ovis aries	PPNPLKPKVPLSAPRRSPNTVKYRLKFRFG	270
Capra hircus	PPNPVKPKVPLSAPRRSPNTVKYRLKFRFG	270
Bos taurus	PPNPVKPKVPLSAPRRSPNTVKYRLKFRFG	270
Vicugna pacos	PPNPIKPKVPLSTRRSPTVKYRLKFRFG ****:*****:****.*****	270
Ovis aries	MSLSFLLLLLFLSHLILSAWAQGEKRLAPKGQPGPXATERNPGGASSRRSSSTVSSSSSP	60
Capra hircus	MSLSFLLLLLFLSHLILSAWAQGEKRLAPKGQPGPAATERNPGGASSRRSSSTASSSSSP	60
Bos taurus	MSLSFLLLLLFLSHLILSAWAQGEKRLAPKGQPGPAATERNPGGASSRRSSSTATSSSSP	60
Vicugna pacos	MSLSFLLLLLFLSHLILSAWARGEKRLVPGQPGPAATARNPGGASSWSRSSTSSSSS *****:*****:*****.***** ** *.:****.	60
Ovis aries	ASSSSAASRGGPGSGLEQSSFQWSPSGRRTGSLYCRVIGFHLQIYPDGKVNGSHEANML	120
Capra hircus	ASSSSAASRGGPGSGLEQSSFQWSPSGRRTGSLYCRVIGFHLQIYPDGKVNGSHEANML	120
Bos taurus	ASSSSAASRGGPGSSLEQSSFQWSPSGRRTGSLYCRVIGFHLQIYPDGKVNGSHEANML	120
Vicugna pacos	ASSSPAASLGSQSGLEQTSFQWSPSGRRTGSLYCRVIGFHLQIYPDGKVNGSHEANML ****.*** *. **.:*****:*****	120
Ovis aries	SQIYR	125
Capra hircus	SQIYR	125
Bos taurus	S	121
Vicugna pacos	SQIYR *	125

**Figure 3:** Alignment of FGF5 proteins sequences in different species. Identical amino acids among the two sequences are indicated by (\*), whereas those with high or low similarity are indicated by (:) and (.), respectively. (A) FGF5 Long Sequences. The (-) symbol highlighted in gray at position 167 of the Vicugna pacos protein sequence indicates the unknown amino acid. (B) FGF5 Short Sequences.



**Figure 4:** Western blot analysis of FGF5. The gray arrowhead indicates the FGF5 protein. Lane M, molecular weight marker; Lane 1, huacaya; Lane 2, suri.

#### *Analysis of the FGF5 protein*

To test the possibility of the translation of the entire FGF5 protein in alpaca, we performed the western blotting analysis starting from the proteins extracted from the skin biopsies.

The western blotting result showed FGF5 proteins of approximately 30.4 kDa in both suri and huacaya, which was consistent with the predicted sizes of 267 aa based on the cDNA sequence data (Fig.4). The presence of the predicted 30.4 kDa protein was the main evidence of a stop codon readthrough in alpaca.

#### *Readthrough signals analysis*

As shown in Figure 2, the alpaca FGF5 mRNA presents characteristics that make the transcript a good RT candidate. The efficiency of RT depends on a variety of factors, including the type of the termination codon and its surrounding mRNA sequence context (Dabrowski et al., 2015), the presence of sequence elements (Skuzesky et al., 1991; Harrell et al., 2002; Beier and Grimm 2001), a long 3'UTR (Jungreis et al., 2011), which reduces termination efficiency, and finally the secondary structure of the transcript (Bertram et al., 2001, Firth et al., 2011).

First, the identity of the stop codon is crucial (Dabrowski et al., 2015) for RT. In genes undergoing RT, the UGA PTC is present 10 times more than others stop codons, as their order of “leakiness” in eukaryotes is UGA>UAG>UAA (Firoozan et al., 1991; Jungreis et al., 2011; Dabrowski et al., 2015).

The efficiency of suppression of PTC varies with the nucleotides and sequence motifs surrounding the stop codon (McCaughan et al., 1995; Pedersen et al., 1991). The nucleotide immediately downstream the UGA PTC influences the translational termination in the expression of mammalian genes. This base compromises the efficiency of suppression and positively influences the RT with different forces: C > U > G > A (Jungreis et al., 2011). In alpaca, the FGF5 transcript U was the nucleotide at position +4 after UGA. Additionally, the nucleotides upstream to the PTC play an essential role in the termination of protein synthesis. According to Dabrowski et al. (2015), Bonetti et al. (1995), Tork et al. (2004) and Jungreis et al. (2011), the presence of adenine at -2 and purine at -1 positions immediately upstream to the PTC is a determinant for RT.

Special sequence elements are known to affect translation termination while positively influencing RT. The FGF5 mRNA presented four Skuzeski sequences (Skuzeski et al., 1991), a hexanucleotide sequences of the form CARYYA (R= purine; Y=pyrimidine), of which one is adjacent to the stop codon. According to Skuzeski et al. (1991) and to Harrel et al. (2002), these sequences could influence the binding of release factor or directly interact with the ribosome stimulating RT in different RNA viruses and eukaryotic organisms. Likewise, the “CAGCAGCA” sequence at 128 bp downstream to ATG is known to facilitate readthrough of a stop codon as proposed by the paper by Beier and Grimm (2001).

The physical separation of the PTC from the poly (A) tail due to a long 3' UTR can directly lead to RT mechanism (Jungreis et al., 2011). An increase in the distance between a PTC and the poly(A) leads to a reduction in the fidelity of termination due to reduced interaction between eRF3, a component of the termination complex, and the poly(A)-binding protein (Amrani et al., 2004; Kobayashi et al., 2004). In FGF5 transcript, the PTC was 1,050 bp upstream to the poly (A), likely leading to inefficient termination and consequent RT.

Finally, RNA secondary structure can play a major role in the regulation of translational termination. In eukaryotes, the UGA stop codons along nucleotide sequences can be translated as selenocysteines if mRNA harbors the secis sequence (selenocystein elements), which is a secondary structure involved in non sense codon suppression with cognate tRNA<sup>sec</sup> (Walczak et al., 1996). From the computational scanning of FGF5 mRNA, no secis elements were detected. RT can also be stimulated by the ability of the transcript to form pseudoknot structures that pause the ribosome to induce frameshift (Somogyi et al., 1993), or by distorting the mRNA structure to favor tRNA interaction over eRF1 binding at the A-site in the termination step (Bertram et al., 2001, Firth et al., 2011).

In FGF5 mRNA, seven hypothetical pseudoknots were found starting from position 28 downstream to UGA PTC (these results are available in Pallotti et al., 2018). The more stable pseudoknot is found from position 59 to 148 due to its lowest estimated free energy (-43.46 kcal/mol).

## Conclusions

Taken together, our results demonstrate a post-transcriptional readthrough regulation in FGF5 gene of alpaca.

In a pool of FGF5 mRNA, RT allows synthesis of different proportions of functional extended polypeptide and truncated protein (Harrel et al., 2002), this phenomenon it may act as a crucial regulatory mechanism of FGF5 gene expression in the development of the different alpaca phenotypes. As suggested by Beier and Grimm (2001) and Roy et al. (2015), during the polypeptide synthesis, the UGA PTC may mediate translational termination or trigger incorporation of arginine, cysteine or tryptophan.

Differences in steric hindrance, charge and polarity of these three amino acids may affect the functionality of FGF5 in its role of binding and activating the FGFRs; in fact the Arg is the wild type amino acid at position 167 aa immediately upstream to the Glu residue which is the amino acid fundamental in the receptor binding (Plotnikov et al., 2000) of FGF family proteins. This regulation of FGF5 can in turn arrest the follicle development during the anagen phase or retard its progression, thus explaining in part the different hair length between suri and huacaya. Recently, the same mutation was described for the long-fiber llama (Daverio et al., 2017). These data suggest that a common domestic fiber-producing ancestor could be shared by the llama and alpaca. Further studies will be required to determine the real role of this mechanism in the translation of FGF5, in addition to the possible differences in the incorporation of the aa 167 between suri and huaca.

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# Alpines Steinschaf (Alpine Stonesheep)

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**Abstract.** A breeding programme was established in 2010 to conserve the Alpine Stonesheep, a rare breed originating in the Neolithicum. Recently, an Alp index was developed as new selection criteria, involving different breeding lines in a cross-border project. The number of Alpine Stonesheep increased from 120 sheep in 2000 to 800 sheep in 2016. The conservation program also includes wool examinations, development of high-quality products made from the wool of this sheep breed, and the Alpine Stonesheep information centre in the Berchtesgardener Land /Bavaria, the natural habitat of the Alpine Stonesheep.

**Resumen.** En 2010 se estableció un programa de conservación genética para la raza autóctona Alpine Stonesheep, una raza que procede en origen de una raza neolítica. Recientemente, se ha desarrollado un nuevo índice de selección llamado índice Alp, para ser utilizado como criterio de selección para un proyecto transfronterizo con varias líneas de mejora. El número de ovejas Alpine Stonesheep aumentó de 120 en 2000 a 800 animales en 2016. El programa de conservación también incluye exámenes de lana, desarrollo de productos de alta calidad a base de lana de esta raza y el centro de información de la raza Alpine Stonesheep en Tierra de Berchtesgardener/Baviera, el hábitat natural de esta raza.

**Keywords:** conservation, sheep, traditional breeds

## Introduction

### *History of the breed*

The Alpine Stonesheep originated from the Neolithic Torfschaf, only a few breeds can be traced back directly to this sheep. The original stature of the Stonesheep is small, almost delicate with very thin, but very strong legs and firm claws. The breed is mainly found in Berchtesgarden, the surroundings of Traunstein and south-east of Rosenheim in the state of Bavaria in Germany.

*Breed description*

Alpine Stonesheep are very smart animals, small to medium sized with a compact body. Within the breed, two types are defined: a larger and a smaller type. The face, the inner side of the thighs and legs are free from wool in these animals. The Alpine Stonesheep has short and pointed ears that are slightly hanging and stuck out sideways. The ears are longer when compared to the Carniolian Sheep. The Alpine Stonesheep has very hard claws with sharp edges. This makes it a confident climber despite the extremely thin legs. Another trademark of the Alpine Stonesheep is his long and woolly tail which is bended and mostly white at the end. Among both sexes, horned as well as unhorned animals exist. However, the male horn is considered stronger and mostly shaped in a spiral. The horns of ewes are smaller and slightly curved. Still, most ewes don't have horns at all. Typical for this kind of breed is the dual coated fleece with pithy, long coarse hair and fine wavy short bottom hair. In addition, the wool grease prevents the sheep from getting wet in snow and rainfall. The wool fineness is between 38-52 microns and depends on sex, age and the selection process of the breeder. Generally, the Alpine Stonesheep needs its wool clipped twice a year. The colour ranges from white to black and everything in between. The Alpine Stonesheep is well adapted to rough weather conditions. It is a great climber and is well suited for grazing on rocky alps. The sheep have an intensively developed herd instinct and will protect their offspring at any costs. Towards familiar persons they can show affection. Ewes weight around 45-60 kg while rams weight around 60-75 kg. In 2006, the preservation project to save the Alpine Stonesheep was started with 120 sheep. Today, in 2017, we can proudly count 680 sheep in the flockbooks.

**Projects and research topics***Breeding project*

A strong aim of ours was also to not only save the Alpine Stonesheep but also to establish a breeding program. The breeding program was started in 2010. The core part of the breeding program is the "Alp Index" together with wool examinations and the Alpine Stonesheep information centre.

Within the breeding program, we found eight different ram lines, based on data of old flockbooks: German ram lines A, M, B, H and N, and Austrian ram lines J, K and S. These ram lines help us to stop inbreeding and are an easy guideline for breeders to know which ram to buy for his ewes or at e.g. sheep shows.

*The alp project – Activating alpine pastures*

The Bavarian State Institute of Agriculture (LfL) at the Institute of Animal Breeding in Grub used the “Activating alpine pasture – new ways to diversity” of the Bavarian Academy for Nature Conservation and Landscape Management (ANL) to establish a special breeding project with the Alpine Stonesheep. The focus of the project is the preservation of the “Kleinrechenbergalm” by the grazing of young rams. The alp has not been used for over 20 years until the project started in 2012. The alp is located in the district of Traunstein in Upper Bavaria. The alp is located at approximately 1400 m above sea level and includes 5 hectares of land. The alp season starts in June and ends in the middle of September. The pasture of the alp provides enough food for 14-16 rams. In the end of May, all the selected rams are driven to Grub. At Grub all rams are weighted, dewormed and their claws are cut. After a short time for the rams to get used to each other, the breeders drive them to the alp and walk with them to the pasture for about an hour. After arriving at the alp pasture, the breeders need to install and repair the fence and then leave the rams on the alp till September. Every weekend another breeder goes up to the pasture to check on the rams. Beginning to Mid-September, the breeders hike up to the alp in the early morning, put down the fences for winter and then walk back into the valley with the rams. In the valley, other breeders that didn't put a ram up the alp prepare the event called “Almabtrieb” – the rams come down after their time on the alp to get examined and graded. Also the “Almabtrieb” is a festivity at which people can buy products of the Alpine Stonesheep like woollen sweaters or get information about the breed and the preservation work. The rams get graded on their weight gain, claws, wool, muscularity and appearance. The alp index is based on this grading.

To know if the rams can still „survive“ in their natural habitat, we developed the Alp Index, a tool to track their development and changes while on the Alp. The alp index is calculated as follows:

- Weight gain during the summer on the alp (1-18 points; the more gain the better)
- Claws (grades 1-9, times 2)
- Wool (grades 1-9, times 2)
- Muscling (grades 1-9, times 2)
- Over all appearance (grades 1-9, times 2)
- Ranking of the rams (First place = 10 points ... 10th place = 1 point, further places 0 points)

Nine points is the highest grade a ram can score. The average alp index of all alp rams evaluated between 2013 and 2016 was 71.2. Further examples of alp index results (2014-2016) are given in Table 1.

**Table 1:** Examples of alp index results (2014-2016)

Name	Line <sup>1</sup> (Country)	Weight 1 (kg)	Weight 2 (kg)	Weight gain (kg)	Wool fineness ( $\mu$ )	Claw grade	Wool grade	Muscling grade	Appearance grade	Alp index	Year
A-Bulgur	A (D)	59.4	63	3.6	41.8	11	16	16	14	72	2014
Helmüt	H (D)	62.4	59	-3.4	43.2	10	14	12	12	49	2014
Michl	M (D)	59.9	65	5.1	40.7	8	10	14	10	51	2014
Josef	J (A)	63.2	66	2.8	47.0	14	14	16	12	65	2014
Sugar	S (A)	52.5	58	5.5	39.8	16	14	14	16	77	2015
Bartl	B (D)	68.5	74	4.7	43.2	15	14	16	16	80	2015
Hector	H (D)	45.0	51	6.0	48.5	12	16	16	16	80	2015
Nathan	N (D)	46.4	50	3.6	34.4	14	14	12	14	62	2015
Augustus	A (D)	47.0	57	10.0	36.0	7	8	7	8	83	2016
Bono	B (D)	60.0	66	6.0	51.1	8	8	7	8	78	2016
Nagid	N (D)	48.3	60	11.7	38.5	7	8	8	7	81	2016
Gustl	G (A)	69.2	72	2.8	44.0	5	7	7	6	51	2016

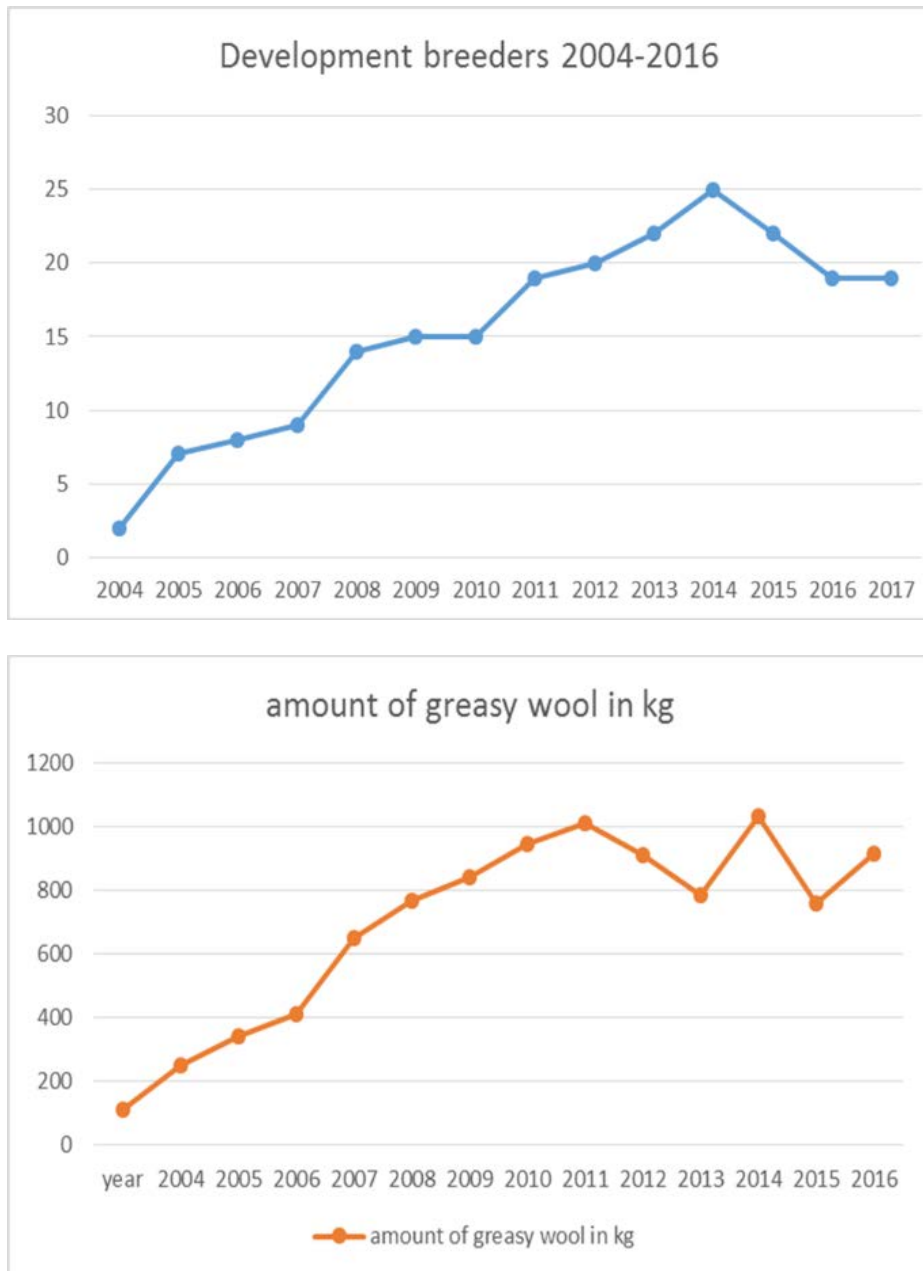
<sup>1</sup>D = Germany A = Austria

**Table 2:** The relation between the wool grade and the wool fineness (in microns)

Wool grade	Number	Fineness ( $\mu$ )	Comments
9	2	40.1	40-44 $\mu$
8	12	41.8	is ideal
7	22	43.5	
6	8	39.9	wool is not
5	1	37.7	typical

*The wool project and its impact on the preservation of this breed*

Another huge impact on preserving a breed has the ability to find ways to use the breed and its products. With the Alpine Stonesheep the breeders association decided to remember old traditions and ways of using the Alpine Stonesheep. For example “Loden” is a product people used to make out of the Alpine Stonesheep wool. In 2006 we started with the wool project with 200 kg of greasy wool and today we have around 1 ton of greasy wool. At the start there were only 3 breeders involved today over 25 breeders bring their greasy wool to the annual breeders meeting in April (Fig.1). The main focus of the wool project is that not only the breeders bring their wool but also sell their products at fairs and in their farm shop. The Alpine Stonesheep wool project is part of “Kollektion der Vielfalt” (Fig.2).



**Figure 1:** Development of number of breeders (above) and amount of greasy wool (below, kg) from the start of the project in 2004.



**Figure 2:** Some of our wool products. (Photos: Isabelle Ketterle)

### **Conclusion and outlook**

We plan on continuing the alp project in order to monitor changes and development of the breed of the Alpine Stonesheep. Another future goal of ours is to focus even more on the different genetic lines and do more research into that area. Additionally, we are planning to organise wool workshops for breeders to disseminate more information and education on the topic of wool.

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## Reproduction and Pathology





# The Alpaca Cria, Clinical and Immunological Aspects

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**Abstract.** Alpaca crias are born after a long period of gestation and they are subjected to many noxas according to the place where they are born. In this paper, the author gives a brief summary of some key events of neonatology. Body temperature regulation, hypothermia, colostrum intake and its dynamics and failure to passive transfer are covered.

**Resumen.** Las crías de alpaca nacen después de un largo período de gestación y están sometidas a muchas noxas de acuerdo al lugar donde nacen. En este artículo, el autor hace un breve resumen de algunos eventos clave en neonatología. El resumen abarca la regulación de la temperatura corporal, la hipotermia, la ingesta del calostro y su dinámica, y el fallo de la transferencia pasiva.

**Keywords:** alpaca cria, neonatology, hypothermia, colostrum

Alpaca offspring, cria, as called in South America, is born in an advanced state of maturity, weighing from 4 to 10 kg. Heavy crias weigh 9 kg and come from 8-9 years old dams. At birth, neonates are covered by an extra membrane, the epidermal membrane, which covers the whole body with the exception of the natural openings. Rectal temperature of the neonate is similar to their dams, 37.5 °C; this temperature drops transiently to 35 °C during the first 30 to 45 minutes, then rises to 37.5 °C and then remains as such. Sixteen percent of crias remain at 35 °C, becoming hypothermic, and would need immediate assistance. Normal vital signs at birth include a heart rate of 60 to 90 beats per minute, and a respiratory rate of 10 to 30 per minute. Colostrum intake occurs an hour after birth, then occurs every 30 minutes for the first 4 hours of life, end then every hour. Colostrum is the primary source of antibodies, being IgG the main and represents 80 % of all immunoglobulins. The highest IgG concentration is registered 24 to 48 hours after birth and is 2,500 to 3,000 mg/dL. Life for the neonate is better if the neonate sucks colostrum during the first 8 hours. However, if suckling occurs after 24 hours of birth, concentration of IgG is very poor,

175 mg/dL. Failure of passive transfer of IgG requires the administration of plasma from an adult animal. This transfer could be by intravenous and/or intraperitoneal transfusion. Crias are subject to different insults according to the country where they are born. Nonetheless, its care is vital and would have a positive impact on the number of weaned crias.

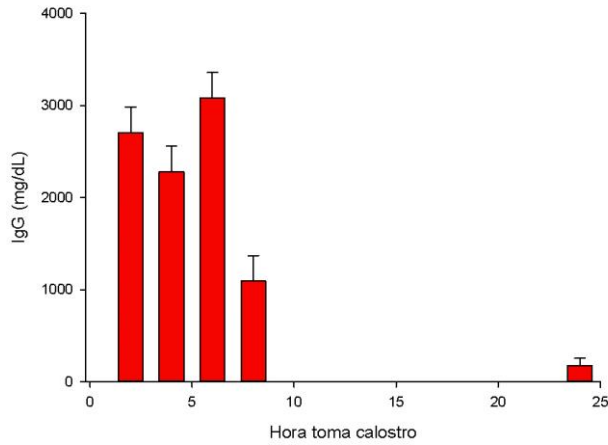
Alpaca crias are born in the morning hours wherein temperature conditions are suitable for survival. Eighty percent of crias are born between 8 and 12pm. Isolated cases of birthing are recorded between 4 and 6 pm, or even the next morning at 6 a.m. These last three cases may represent dystocia cases that went by unsuspected by the owner or caretaker. Weight of the cria is influenced by age of the dam. Heavier crias, 8.3 kg, are born from 8-9 year old dams, whereas crias weighing 6.9 kg are born from 2-3 year old dams (Bravo et al., 2009).

At birth neonates are covered by an extra membrane, the epidermal membrane. This membrane covers the entire body with the exception of natural openings like mouth, nostrils, eyes, ears, prepuce, penis and vulva. This membrane is also thick at the distal end of both toes, and is thin, transparent and facilitates fetal expulsion from the dam by uterus and abdominal contractions. It disintegrates as the cria begins to move and during the process of standing up.

Neonate rectal temperature is similar to its dam, 37.5 °C, and then drops to 35 °C by 30 minutes and then by three hours goes back to 38 °C. This rapid and transient drop in rectal temperature is normal, however, 16 % of crias develop a hypothermic state and in those crias, temperature does not reach 38 °C even by 24 hours of life. This is a reminder that when crias are born under adverse environmental conditions, they should be dried immediately. In this sense, crias dried with a hair dryer may survive; however, crias which are not dried may succumb to the process of hypothermia (Garnica et al., 1992).

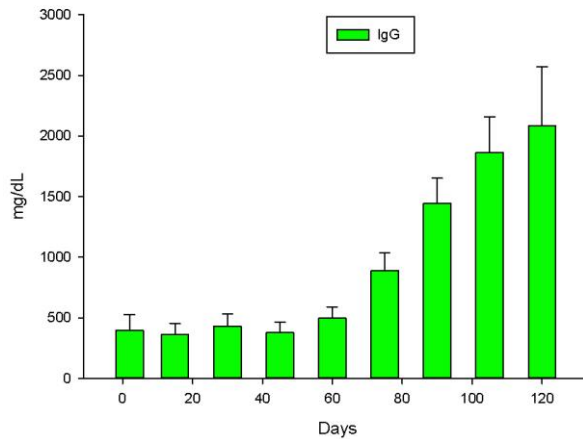
Colostrum intake is vital for the newborn. Colostrum is the primary source of antibodies necessary to fight many diseases and because the epitheliochorial placentation does not allow the passage of antibodies from the dam to the fetus. Studies done previously, indicate that 80 % of antibodies fall into the immunoglobulin type G, commonly known as IgG, and this IgG stays in the blood of the cria for longer period of time than other Igs, like IgA, IgM. As soon as crias stand up, they try to suckle the vital colostrum. It is not achieved on the first intent, but by the third attempt, the goal of suckling is reached. Crias are born without any IgG, i.e., agammaglobulinemic. IgG is absorbed and is present in the blood stream as soon as 1 hour after suckling, and it reaches a peak, 2,500 mg/dL, by 24-48 hours of life. It stays high for the next 10 days, and then starts to decline steadily to day-60 of life (Bravo et al., 1997). The time of first suckling is also important to consider. First successful suckling by 2-4 hours is far better than initiation of suckling by 6 hours. Recent data indicates that when neonates suckle by 2-4 hours after birth, IgG concentrations are greater than 1,200 mg/dL, by contrast when suckling occurs by 6 to 8 hours, IgG concentrations are 1,000 mg/dL. These values again indicate that suckling as soon as possible is more beneficial than delaying suckling

(Figure 1). A relationship between IgG concentrations and occurrence of suckling is observed naturally. The cria alpaca suckles every 30 minutes for the first 4 hours, and then suckling occurs every hour.



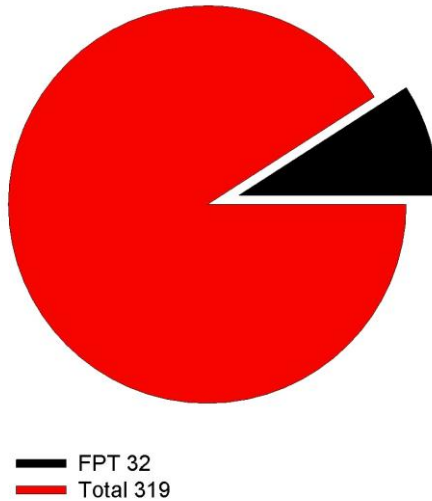
**Figure 1:** Concentrations of IgG in alpaca crias at 48 hours after colostrum suckling at 2, 4, 6, 8 and 24 hours of life.

A second aspect is the time when a cria alpaca is able to produce its own IgG, since there is passive transfer from colostrum dam to the cria via colostrum. Results indicate that by day 90 the cria produces its own IgG, see Figure 2 (Quispe, 2009).



**Figure 2:** Time of alpaca IgG production in crias following cow colostrum administration at birth.

The clinical consequence of a deficient IgG absorption or availability is called failure of passive transfer (FTP) and is depicted in Figure 3 wherein there is a clear difference of IgG concentrations between normal and crias with failure to transfer. In addition, FTP could be responsible for hypothermia, lack of gaining weight and cria mortality at least for the first 30 days.



**Figure 3:** Percentage of FTP in alpaca crias under clinical conditions. FTP was considered as concentrations of IgG were less than 900 mg/dL.

Concentrations of IgG in milk of alpacas for the period of lactation have been also reported (Ampuero et al., 2008). Low IgG concentrations exist from the first through the fourth month of lactation, averaging 180 mg/dL, in contrast to a slight increase from the fifth through the eighth month, 300 mg/dL. The presence of IgG in milk and during lactation is unknown, but indicates that the mammary gland is still synthesizing this antibody.

In summary, alpaca crias are born in an advanced state of maturity. There is a transient decrease in temperature which is common especially when climate conditions are adverse for cria survival. Those conditions could trigger hypothermia in 16 % of crias if colostrum is not suckled. Concentrations of IgG are vital for cria survival. As soon as the cria starts suckling, IgG increases, reaching a peak 24 to 48 hours after birth. It is better if first suckling begins 2-4 hours rather than 6-8 hours. A cria alpaca begins producing its own IgG at 2.5 months. FTP is a clinical entity present and it may affect up to 10 % of alpaca crias.

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# Addition of Seminal Plasma to Frozen-Thawed Llama Spermatozoa does not Preserve Sperm Motility

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**Abstract.** In South American Camelids (SAC) sperm survival is low after thawing. The aim of this study was to determine the effect of the addition of seminal plasma (SP) on the survival of frozen-thawed llama spermatozoa. Sixteen ejaculates from four llama males were obtained by electroejaculation, under general anesthesia. Ejaculates were diluted 4:1 in 0.1 % collagenase in HEPES-TALP and incubated 4 minutes at 37 °C, then centrifuged and re-suspended in lactose, egg yolk and 7 % dimethylformamide, equilibrated at room temperature, and frozen in 0.50-ml straws. After thawing, samples were divided into three aliquots for addition of SP: 0 % (control), 10 % and 50 % (final concentrations) and then incubated 3 h at 37 °C. Sperm motility, viability, membrane function, acrosome status and DNA quality were evaluated in raw and post-thaw samples at 0, 1.5 and 3 h. A split-plot design was applied, blocking the males and using the treatment as one factor (levels: 0, 10 and 50 % SP) and time as the other factor, (levels: 0, 1.5 and 3 h). After thawing, all samples maintained sperm viability, membrane function, acrosome status, and DNA quality ( $p > 0.05$ ) over the incubation time. Sperm motility significantly declined ( $p \leq 0.05$ ) at 3 h of incubation in all treatments. The rapid loss of motility of post-thaw llama spermatozoa while maintaining other seminal characteristics when in the presence of SP seems important to highlight and requires further studies. To conclude, post-thaw addition of 10 % and 50 % seminal plasma was unable to preserve sperm motility or improve the survival of llama frozen-thawed spermatozoa.

**Resumen.** La supervivencia de espermatozoides descongelados es baja en Camélidos Sudamericanos (CSA). Nuestro objetivo fue determinar el efecto de la adición de plasma seminal (PS) sobre la supervivencia de espermatozoides congelados-descongelados de llama. Dieciséis eyaculados de 4 llamas fueron obtenidos mediante electroeyaculación bajo anestesia general. Los eyaculados fueron diluidos 4:1 en colagenasa al 0,1 % en HEPES-TALP, incubados 4 minutos a 37 °C, centrifugados y resuspendidos en lactosa, yema de huevo y dimetilformamida al 7 %. Se equilibraron a temperatura ambiente y se criopres-

ervaron en pajuelas de 0,5 ml. Las muestras descongeladas fueron divididas en 3 para agregar PS: 0 % (control), 10 % y 50 % (concentraciones finales) y posteriormente incubados 3 h a 37 °C. En semen fresco y descongelado se evaluó movilidad, viabilidad, funcionalidad de membrana, estado acrosomal y calidad del ADN espermático a las 0, 1,5 y 3 h de incubación. Se aplicó un diseño de parcelas divididas en el tiempo bloqueando los machos, tomando el factor tratamiento (niveles: 0, 10 y 50 %) y el factor tiempo (niveles: 0, 1,5 y 3 h). Después del descongelamiento, todas las muestras mantuvieron la viabilidad, funcionalidad de membrana, estado acrosomal y calidad del ADN ( $p > 0,05$ ) a lo largo de la incubación. La movilidad espermática disminuyó significativamente a las 3 h de incubación ( $p \leq 0,05$ ) en todas las muestras. Es de destacar la rápida pérdida de movilidad espermática después del descongelamiento, conservando las demás características en presencia de PS, requiriendo más estudios. En conclusión, la adición posdescongelado de 10 % y 50 % de PS no preservó la movilidad ni mejoró la supervivencia en espermatozoides de llama descongelados.

**Keywords:** llama, frozen-thawed semen, seminal plasma

## Introduction

To date, no protocol for cryopreservation of SAC spermatozoa produces pregnancy rates that make application of this biotechnology commercially viable. Different diluents have been used, several cryoprotectants have been tried in different concentrations and diverse freezing curves have been tested (Morton et al., 2007; 2010; Santiani et al., 2005, 2013; Giuliano et al., 2010; Carretero et al., 2015). However, results are still discouraging when cryopreserved semen is artificially inseminated, obtaining between 0 and 26 % pregnancy rates (Bravo et al., 2000; Aller et al., 2003; Vaughan et al., 2003; Giuliano et al., 2012). Giuliano et al. (2012) demonstrated that to obtain pregnancy with refrigerated semen, it is necessary to inseminate female llamas after detecting ovulation. In 2015, Carretero et al. compared two cryoprotectants: glycerol (that is widely used in this species) and dimethylformamide, both at 7 %. The study revealed that glycerol causes severe damage to sperm DNA. These could be some of the possible reasons for the poor results obtained with this biotechnology and current research is trying to further elucidate this matter. It is acknowledged that SP is more than just a medium to transport spermatozoa through the reproductive tract. It has been proved that it has a role on osmotic stability of spermatozoa and works as a buffer, is implicated in sperm capacitation through its protein components, it is involved in female physiology through the ovulation factor it provides and has an important role in forming the sperm reservoir (Ratto et al., 2005; Adams et al., 2013; Apichela et al., 2014; Tribulo et al., 2015; Silva et al., 2015; Berland et al., 2016). Several studies had been carried out in productive species in order to elucidate the interaction between SP and spermatozoa, with differing results (Graham, 1994; Aurich et al., 1996; Maxwell et al., 1999; Moore et al., 2005; Domínguez et al., 2008; Leahy et al., 2010; Morrel et al., 2010; Kershaw-Young and Maxwell, 2011;

Carretero et al., 2015; 2016). In llama and alpaca there are studies that evaluated the effect of SP on raw sperm over time (Kershaw Young and Maxwell, 2011; Carretero et al., 2015). In the case of cryopreserved semen, one study has evaluated the effect of cooling llama spermatozoa to 5 °C for 24 h in the presence or absence of SP (Carretero et al., 2016). They observed that the samples refrigerated with SP showed a significantly lower total and progressive motility while preserving sperm viability. Most refrigeration and freezing techniques used so far in SAC have been adopted from those used in bulls and rams and most cryopreservation protocols dilute semen without removing SP before performing the freezing curves (Bravo et al., 2000; Vaughan et al., 2003; Aller et al., 2003; Santiani et al., 2005; Giuliano et al., 2010). Thus, while the presence of SP in the media could be interfering with the cryoprotectants reaching and protecting spermatozoa during temperature descent, on the other hand it may be needed at the time of AI to carry out its role in the female reproductive tract. Hence, the objective of our study was to evaluate over time the effect of adding, post-thaw, different dilutions of seminal plasma to llama spermatozoa that were cryopreserved without SP.

## Materials and Methods

### *Animals and location*

The study was carried out at the Faculty of Veterinary Sciences of the University of Buenos Aires, in Buenos Aires, Argentina. The city is situated at sea level, latitude 34°36' and longitude 58°26'. For the study, 4 male *Lama glama* ranging between 8 and 12 years of age were used. Animals were kept out at pasture in pens and supplemented with alfalfa; they also had free access to fresh water throughout the study.

### *Semen collection*

A total of 16 ejaculates (n=4; r=4) were collected, processed and evaluated. Semen collections were carried out using electroejaculation under general anesthesia, according to the technique described by Director et al. (2007). All procedures were approved by the Committee for the Use and Care of Laboratory Animals (CICUAL) of the Faculty of Veterinary Sciences of the University of Buenos Aires (protocol 2011/18).

### *Semen evaluation*

The sperm characteristics studied were: motility, membrane function, viability and DNA (condensation and fragmentation). Sperm motility was evaluated on a warm stage (37 °C) using a phase contrast microscope (100x); motility was classified as either oscillatory (OM) or progressive (PM) and total motility (TM) was also

estimated (Giuliano et al., 2010). Membrane function was evaluated using the HOS test carried out according to Giuliano et al. (2008). Sperm showing the characteristic swelling of the tail (HOS positive) were classified as having a functional plasma membrane. Fluorochromes 6-carboxyfluorescein diacetate (CFDA) and propidium iodide (PI) were used for evaluating membrane integrity (viability) and this technique was carried out according to Giuliano et al. (2008). Spermatozoa that fluoresced green were classified as being viable (intact membrane) while sperm nuclei that fluoresced red were classified as nonviable (damaged membrane). For the DNA evaluation, two techniques were used: the Toluidine blue (TB) stain, to determine chromatin condensation, and the Sperm Chromatin Dispersion (SCD) assay, to analyze DNA fragmentation. The TB stain was carried out according Carretero et al. (2009). Briefly, each sample was smeared on clean, non-greasy slides and once dried, fixed with ethanol 96° and stained with a working solution of 0.02 % TB. Preparations were observed directly under immersion oil (1000x) evaluating a minimum of 200 spermatozoa per smear. Spermatozoa were classified into three groups according to the degree of chromatin condensation: light blue (negative, no chromatin decondensation), light violet (intermediate, some degree of decondensation) and dark blue-violet (positive, high degree of decondensation). Sperm in both positive and intermediate groups were considered to have altered chromatin condensation. Dithiothreitol (DTT) 1 % in distilled water was used as a positive control for the TB stain. The SCD assay to evaluate the degree of DNA fragmentation was carried out according to Carretero et al. (2012). Briefly, each sperm suspension was mixed with low melting point aqueous agarose and pipetted onto a glass slide. Each slide was incubated in different lysing solutions, dehydrated in sequential ethanol baths and stained with Giemsa. Preparations were observed directly under immersion oil (1000x) evaluating a minimum of 200 spermatozoa per smear. Spermatozoa were classified into four patterns according to the size of the halo: 1- nuclei with large DNA dispersion halos (LH); 2- nuclei with medium halos (MH); 3- nuclei with small halos (SH) and 4- nuclei with no halo (NH). The first two patterns (1 and 2) were considered spermatozoa without DNA fragmentation and the other two (3 and 4), spermatozoa with DNA fragmentation. Semen incubation with NaOH was used as a positive control of sperm DNA fragmentation.

#### *Freeze-thawing of llama semen*

With the objective of decreasing thread formation and facilitating manipulation of the samples, each ejaculate was diluted 4:1 in 0.1 % collagenase in HEPES-TALP (HP) medium and incubated 4 minutes at 37 °C according to Giuliano et al. (2010). Afterwards, the ejaculates were centrifugated at 800 g during 10 minutes to separate the spermatozoa from the seminal plasma and the enzymatic medium. Then the cryopreservation extender (lactose-EDTA-egg yolk with 7 % of dimethylformamide) was added to the pellet and the samples were equilibrated for

20 minutes at room temperature. Freezing was carried out according to the manual method described by Miragaya et al. (2001). Briefly, temperature descent was carried out in three phases by placing the straws, submerged in a mixture of ethanol: acetone (1:1), in a bronze canister with a graduated handle and holding them over liquid nitrogen vapors. Temperature phases were as follows: a) from room temperature to  $-15^{\circ}\text{C}$ , temperature descent was at a rate of  $10\text{--}12^{\circ}\text{C}$  per minute; b) from  $-15^{\circ}\text{C}$  to  $-120^{\circ}\text{C}$ , temperature descent was at a rate of  $25\text{--}40^{\circ}\text{C}$  per minute; and finally c) the straws were plunged into the liquid nitrogen at  $-196^{\circ}\text{C}$  and stored until evaluation. The samples were thawed at  $37^{\circ}\text{C}$  in water bath for 60 seconds. After thawing, the samples were divided into three aliquots for the addition of the different concentrations of SP: 1) 0 % SP (control), 2) 10 % SP and 3) 50 % SP. Samples were then incubated 3 h at  $37^{\circ}\text{C}$  and were evaluated at different time periods: 0, 1.5 and 3 h, using the same evaluations that were carried out in the raw semen.

### *Statistical analysis*

A Kruskal-Wallis test was carried out to evaluate motility (oscillatory, progressive and total) between treatments (0, 10 and 50 % SP) and times of incubation (0, 1.5 and 3 h). To evaluate the others seminal characteristics, a split-plot design was applied, blocking the males and using the treatment as one factor with three levels (0, 10 and 50 % SP) and time as the other factor, also with 3 levels (0, 1.5 and 3 h).

## **Results**

The seminal characteristics of raw semen were (media  $\pm$  SD): volume:  $1.9 \pm 1.5$  ml; oscillatory motility:  $57.9 \pm 17.4$  %; no progressive motility was observed in raw semen samples; membrane function:  $31.8 \pm 10.6$  % (HOS positive spermatozoa); membrane integrity:  $56.9 \pm 11.8$  % (live spermatozoa); normal chromatin condensation:  $92.3 \pm 7.8$  % and DNA integrity:  $92.3 \pm 7.8$  %. After treatment of raw semen with collagenase, total motility was  $62.0 \pm 9.7$  % ( $25.8 \pm 14.8$  % was progressive motility and  $36.2 \pm 11.0$  % was oscillatory). A significant decrease of total motility, membrane function, viability and DNA integrity was observed in all post-thaw spermatozoa when compared to the raw semen samples ( $p < 0.05$ ).

Oscillatory motility was not observed or was almost null in all post-thaw samples (0, 10 and 50 % SP) at all incubation times (0, 1.5 and 3 h). A significant decrease in PM and TM was observed in all samples over time ( $p = 0.001$ ), but no significant differences ( $p > 0.05$ ) were observed in either PM or TM between post-thaw samples (0, 10 and 50 % SP) at any of the times of evaluation (Table 1; Figure 1). No significant differences ( $p > 0.05$ ) were observed in the percentages of live spermatozoa nor in the percentages of sperm with membrane function between post-thaw samples and both seminal parameters were conserved over time in all samples (Table 2). Nevertheless, a tendency to decrease the percentage

of spermatozoa with functional membranes was observed along the incubation period in all treatments (0, 10 and 50 % SP).

**Table 1:** Percentages of sperm with OM, PM and TM in raw llama semen, post-collagenase and post-thaw samples incubated at 37 °C with different percentages of SP (0, 10 and 50 %) and evaluated at different times (0, 1.5 and 3 h).

Time of Incubation (h)	PS (%)	OM (%)	PM (%)	TM (%)
0	0	0.0 ± 0.0 <sup>a</sup>	29.5 ± 5.7 <sup>a</sup>	29.5 ± 5.7 <sup>a</sup>
	10	0.0 ± 0.0 <sup>a</sup>	28.0 ± 12.3 <sup>a</sup>	28.0 ± 12.3 <sup>a</sup>
	50	0.0 ± 0.0 <sup>a</sup>	18.9 ± 12.6 <sup>a</sup>	19.1 ± 12.7 <sup>a</sup>
1.5	0	0.0 ± 0.0 <sup>a</sup>	16.1 ± 5.5 <sup>b</sup>	16.1 ± 5.5 <sup>b</sup>
	10	0.0 ± 0.0 <sup>a</sup>	17.2 ± 11.4 <sup>b</sup>	17.2 ± 11.4 <sup>b</sup>
	50	0.2 ± 0.5 <sup>a</sup>	9.9 ± 10.5 <sup>b</sup>	9.9 ± 10.5 <sup>b</sup>
3	0	0.0 ± 0.0 <sup>a</sup>	8.7 ± 4.6 <sup>c</sup>	8.7 ± 4.6 <sup>c</sup>
	10	0.0 ± 0.0 <sup>a</sup>	8.4 ± 4.4 <sup>c</sup>	8.4 ± 4.4 <sup>c</sup>
	50	0.6 ± 1.2 <sup>a</sup>	4.6 ± 1.7 <sup>c</sup>	4.9 ± 2.2 <sup>c</sup>

<sup>a,b,c</sup> Different letters within columns indicate statistical differences between samples and evaluation periods for each motility pattern ( $p < 0.05$ ).

No significant differences were observed ( $p > 0.05$ ) in sperm chromatin condensation between post-thaw samples at any of the evaluations over time (Table 2).

When DNA fragmentation was evaluated, no statistical differences ( $p > 0.05$ ) were observed between post-thaw samples (0, 10 and 50 % of SP). A significant increase ( $p < 0.05$ ) in the percentage of spermatozoa with DNA fragmentation was observed in all post-thaw samples (0, 10 and 50 % SP) when compared with raw semen. This increase was observed specifically in the category of nuclei with small halos in all post-thaw samples (Table 3).

**Table 2:** Percentages of sperm viability, membrane function and chromatin condensation in raw llama sperm and postthawed spermatozoa incubated with different amount of SP (0, 10 and 50 %) evaluated in different times of incubation at 37 °C (0, 1.5 and 3 h).

Time of Incubation (h)	SP (%)	Sperm viability (%)	Membrane function (%)	Normal chromatin condensation (%)
0	0	19.9 ± 9.5 <sup>a</sup>	21.5 ± 9.6 <sup>ab</sup>	92.8 ± 6.7 <sup>a</sup>
	10	19.6 ± 11.1 <sup>a</sup>	21.0 ± 9.4 <sup>ab</sup>	92.1 ± 6.9 <sup>a</sup>
	50	16.8 ± 9.2 <sup>a</sup>	20.0 ± 9.5 <sup>bc</sup>	93.1 ± 6.6 <sup>a</sup>
1.5	0	17.7 ± 11.8 <sup>a</sup>	12.6 ± 6.4 <sup>bc</sup>	92.2 ± 1.1 <sup>a</sup>
	10	16.8 ± 11.9 <sup>a</sup>	14.1 ± 6.6 <sup>bc</sup>	96.8 ± 1.2 <sup>a</sup>
	50	20.0 ± 8.9 <sup>a</sup>	13.7 ± 10.4 <sup>bc</sup>	96.0 ± 2.6 <sup>a</sup>
3	0	15.0 ± 7.7 <sup>a</sup>	13.1 ± 4.8 <sup>bc</sup>	91.5 ± 9.4 <sup>a</sup>
	10	15.5 ± 7.5 <sup>a</sup>	12.9 ± 4.5 <sup>bc</sup>	92.1 ± 7.3 <sup>a</sup>
	50	15.7 ± 10.2 <sup>a</sup>	12.6 ± 5.3 <sup>c</sup>	91.8 ± 9.0 <sup>a</sup>

<sup>a,b,c</sup> Different letters within columns indicate statistical differences between post-thaw samples and evaluation periods.

**Table 3:** DNA fragmentation of fresh llama semen and postthawed llama spermatozoa incubated with different percentages of seminal plasma (0, 10 and 50% of SP)

	LH	MH	LH+MH	SH	NH	SH+NH
0% SP	32.7 ± 24.7 <sup>a</sup>	40.8 ± 11.5 <sup>a</sup>	73.6 ± 16.8 <sup>a</sup>	23.4 ± 14.8	2.9 ± 2.4	23.4 ± 16.8
10% SP	30.5 ± 22.8 <sup>a</sup>	45.6 ± 14.7 <sup>a</sup>	76.1 ± 21.0 <sup>a</sup>	19.8 ± 18.4	4.1 ± 3.7	23.9 ± 21.0
50% SP	27.2 ± 22.6 <sup>a</sup>	49.7 ± 18.7 <sup>a</sup>	76.9 ± 23.4 <sup>a</sup>	21.2 ± 21.4	1.9 ± 2.4	23.1 ± 23.4

<sup>a,b,c</sup> Different letters mean statistical differences between treatments

## Discussion

Poor reproductive results after AI with cryopreserved semen have been reported for several species (boar, donkey, camelids, etc.) and consequently this biotechnology can't be commercially applied. Active research is therefore currently being carried out to determine where the problem could possibly be. In the case of SACs, multiple roles have been attributed to SP in their reproductive physiology (Ratto et al., 2005; Adams et al., 2013; Apichela et al., 2014; Tribulo et al., 2015; Silva et al., 2015; Berland et al., 2016) but there are still various unanswered questions. Most cryopreservation protocols in camelids add the extenders to the whole ejaculate without removing SP (Bravo et al., 2000; Aller et al., 2003; Vaughan et al., 2003; Santiani et al., 2005; Giuliano et al., 2012; Carretero et al., 2015). In the present study, SP was removed before cryopreservation of llama semen and then different percentages of SP were added after freeze-thawing the samples to determine its effect on sperm parameters (motility, viability, membrane

function and DNA quality). We used different final dilutions of SP (0, 10 and 50 %) and further incubated the samples 3 h at 37 °C and observed that none of them were able to preserve total sperm motility over time (0 % SP:  $29.5 \pm 5.7$  % vs  $8.7 \pm 4.6$  %; 10 % SP:  $28.0 \pm 12.3$  % vs  $8.4 \pm 4.4$  %; 50 % SP:  $19.1 \pm 12.7$  % vs  $4.9 \pm 2.2$  % (values are mean  $\pm$  SD)). Sabatini et al. (2014) evaluated the effect of the addition of SP to post-thaw donkey spermatozoa and they observed that total and progressive sperm motility significantly decreased ( $p < 0.05$ ) after 4 hours of incubation when 5 and 20 % SP was added compared to samples without SP (0 %). They also observed this effect at time zero when they added higher percentages of SP (70 %). When we added 50 % SP, at time zero we also observed lower percentages of sperm total motility compared to the other post-thaw samples (0 and 10 %), but this was not statistically significant ( $p > 0.05$ ). The study of Sabatini et al. (2014) established that motility of post-thaw donkey spermatozoa decreased when SP is added and that the effect is seen more rapidly when a higher final proportion of SP is used. These results are similar to ours with the difference that we observed this decrease progressively over the incubation time (0, 1.5 and 3 h) in all post-thaw samples, which could be a difference due to the differences between species.

Conversely, studies in rams obtained better sperm motility results since they reported that progressive and total motility were higher when they added SP after thawing (Ledesma et al., 2016). These authors also observed that intracellular Reactive Oxygen Species (ROS) and membrane stability (viability) were higher with the SP treatment compared to the controls (SP absent) and compared to the addition of seminal plasma vesicles. This contrast in results could be due either to the different physiology of ram spermatozoa or to differences in SP components, as it has been demonstrated that camelid SP differs from that of other species (Kershaw-Young and Maxwell, 2012). In addition, Ledesma et al. (2016) used a different final SP concentration in their study (25 %) from the ones that we used (10 and 50 %), a fact which also could have influenced the results.

Regarding llama sperm viability, membrane function and DNA quality, these parameters did not significantly change ( $p > 0.05$ ) between post-thaw samples (0, 10 and 50 % of SP) over the incubation. Zea et al. (2015) studied cryopreserved alpaca spermatozoa collected from a deviation of the deferent duct. They performed three treatments: without SP, with SP added before cryopreservation and with SP added after freezing-thawing. They observed a significant decrease in motility, membrane function and sperm viability in the samples where SP was added post-thaw. However, the authors did not mention the final proportions of diluent, spermatozoa and SP or the amount of time the samples were incubated. Similar to our study, the addition of SP from the sperm rich fraction of boar ejaculates to post-thaw sperm samples did not have an influence on sperm membrane integrity or lipid peroxidation after 2 h of incubation (Torres et al., 2016). With regard to DNA quality, the cryopreservation protocol did not affected chromatin condensation ( $p > 0.05$ ) but produced a significant increase in DNA

damage ( $p \leq 0.05$ ) compared to the raw semen. Other than this result, we did not observe a significant difference between post-thaw samples (0, 10 and 50 % of SP) regarding chromatin condensation and fragmentation. In donkey thawed semen, the degree of DNA fragmentation was not affected by the addition of SP immediately after dilution (Sabatini et al., 2014).

To conclude, post-thaw addition of 10 % and 50 % seminal plasma was unable to preserve sperm motility or improve the survival of llama frozen-thawed spermatozoa. The rapid loss of motility of post-thaw llama spermatozoa, while maintaining other seminal characteristics in the presence of SP, seems important to highlight and requires further studies.

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# Alpaca Semen Quality throughout the Breeding Period

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**Abstract.** Semen characteristics were determined in twelve male alpacas (4 young, 4 adult and 4 old) during an entire breeding period, two months, at La Raya research station. Characteristics included volume (ml), motility (%), and concentration (million spz/ml). This center is located at 4,200 m sea level and on the Southern hemisphere. Males were separated at random from a group of 150 breeding males and were maintained under natural pastures, with no nutritional supplementation. Three-age males were considered: young (3 years old), adult (6-7 years old), and old males (10-12 years old). Semen was collected by vaginal aspiration following natural breeding to a receptive female. Semen volume was determined reading from the 15 ml collecting tube. Semen motility, percentage of spermatozoa with tail movement was determined on a microscopic field. Semen concentration, million spz/ml, was determined using the hemocytometer method. Data were analyzed using the NCSS software and with general linear method. There were some males, independent from age and number of breeding that did not yield a semen sample and were not considered in the analysis. Ejaculated volume increased from 0.5 ml at the beginning of breeding to 5 ml at the end of the breeding period; however, sperm motility showed an opposite trend, decreased from 50 to 17 % ( $P \leq 0.05$ ). Sperm concentration started at 5 million spz/ml, increased to 70 million a month later, and by the end of breeding decreased to 16 million spz/ml ( $P \leq 0.05$ ). The length of breeding, 2 months, affected differently to ejaculated volume, motility and concentration under field conditions which definitively could affect male fertility.

**Resumen.** Se determinaron las características del semen de 12 alpacas macho (4 juveniles, 4 adultas y 4 ancianas) durante un período completo de cría de dos meses de duración en la estación de investigación La Raya. Los caracteres estudiados fueron el volumen (ml), la motilidad (%) y la concentración (millones de esp/ml). Este centro se encuentra a 4.200 metros sobre el nivel del mar en el hemisferio sur. Los machos se escogieron aleatoriamente de un grupo de 150 machos reproductores y se mantuvieron en pastos naturales sin suplemento nutricional. Se hicieron tres grupos de edades: jóvenes (3 años), adultos (6-7 años) y ancianos (10-12 años). El semen se recolectó de hembras receptoras mediante as-

piración vaginal después de la monta natural. El volumen de semen se determinó mediante lectura de un tubo de 15 ml. Se utilizó un microscopio para la determinación de la motilidad del semen y del porcentaje de espermatozoides con movimiento en la cola. La concentración de semen en millones de esp/ml se determinó usando el método del hemocitómetro. Se utilizaron modelos lineales generalizados para analizar los datos mediante el software NCSS. Se eliminaron los dados de machos que no produjeron muestra de semen independientemente del grupo de edad. El volumen eyaculado se incrementó desde 0.5 ml hasta 5 ml del comienzo al final del período reproductivo, pero la motilidad de los espermatozoides presentó la tendencia contraria, disminuyendo del 50 % a 17% ( $P \leq 0,05$ ). La concentración de esperma inicial fue de 5 millones de esp/ml, 70 millones un mes más tarde, y 16 millones de esp/ml al final del período ( $p \leq 0,05$ ). La duración de 2 meses del período reproductivo afectó el volumen eyaculado, la motilidad y la concentración en condiciones pudiendo afectar definitivamente a la fertilidad.

**Keywords:** semen, breeding period, motility, concentration

## Introduction

The breeding period, under South American conditions, lasts for about two months and takes place during the rainy summer days. When large females are kept, males are divided into two breeding teams, 2.5 % of females on each team. One team breeds for a week and the next team on the following week while the first team rests. This sequence of alternating males is maintained during a period of 2 to 2.5 months. Under this system, fertility is around 75 to 80 % (Novoa et al., 1973). An improved fertility rate (86 %) was also reported when males were exposed to females for 2 days and rest for 4 days (Condorena and Velasco, 1979).

Semen characteristics have been determined on field conditions and using the vaginal aspiration method immediately following a natural breeding to a receptive female (Neely and Bravo, 1998). This method avoids the use of an artificial vagina, stricture on the latex to simulate the female's cervix, and a heating pad to maintain the artificial vagina for the length of copulation. In addition, with this method suitable and comparable semen characteristics have been reported lately (Alarcon, Garcia and Bravo, 2012). The aims of this study were to follow semen characteristics throughout a breeding period of 2 months, as used in South America. Semen characteristics were volume, motility and concentration.

## Materials and Methods

This study was carried out at La Raya research center, located in the Peruvian highlands, at 4,200 m sea level, 70 °W longitude and 14 °S latitude.

Twelve males, four young three-years old, first time breeders; four adult 6-7 years old, and four old, 11-12 years old were separated at random from a group of 150 breeding males, and considered in this study. Males were maintained grazing natural pastures and with no nutritional supplementation. Females bred by the

males according to the management of the research station were located into four different groups, open from previous breeding periods, two lactating groups and a group of maiden females. The breeding period lasted two months, February and March.

Males were transported to the breeding females and allowed to a receptive female. A single breeding was allowed, and opportunities to breed were present all the time. Males accompanied the rest of 138 males to the different female groups. Generally, males bred females twice a week. Females bred (222) were marked and separated from males until the next scheduled breeding time.

Semen characteristics were determined following the protocol of Garnica, Achata and Bravo et al. (1993). Briefly, semen volume was read from the scale present of the 15-ml collecting tube. Motility was determined placing 10  $\mu$ l of sample on a pre-warmed glass slide and under a 10X magnification using a microscope. It was read as percentage of motile spermatozoa on a microscopic field. Concentration was determined by the hemocytometer method. 50  $\mu$ l of semen sample was mixed 1:100 with 3 % saline solution. 10  $\mu$ l of diluted sample was placed on a hemocytometer, allowed to rest for three minutes and then spermatozoa was counted using the grid of a Neubauer chamber.

Data was analyzed using the general linear model with the age of males as fixed factors and using the Number Crunching Statistical System, NCSS, software. Differences, if any, were considered at  $P < 0.05$ .

## Results and Discussion

### *Semen characteristics by age*

General volume, motility and concentration were 2.3 ml, 44.6 %, and 29.9 million spz/ml respectively. The effect of age on the same semen characteristics are presented in fig. 1, 2, and 3, respectively. Semen volume recovered was significantly greater ( $P < 0.05$ ) in young males than in adult and old males. Spermatozoa motility was significantly higher ( $P < 0.05$ ) in adult and old males than in young males. Spermatic concentration was different ( $P < 0.05$ ) in old males than in adult and young males.

Semen characteristics by age did follow different trends. Volume decreased by age, adult and old males were similar, by contrast volume was higher in young males. Although previous reports were on limited days, most of them only for 12 to 24 days, our results on volume were similar values were reported earlier in alpacas (Bravo, Flores and Ordoñez, 1997; Urquieta et al., 2005; and Alarcon, Garcia and Bravo, 2012).

Spermatozoa motility was less in young males than in adult and old males. This difference could be a reflection of sexual maturity. Young males were introduced to receptive females for the first time and their motility was not equal to older

males. Similar trend was also observed in alpacas (Bravo, 2002; Urquieta et al., 2005).

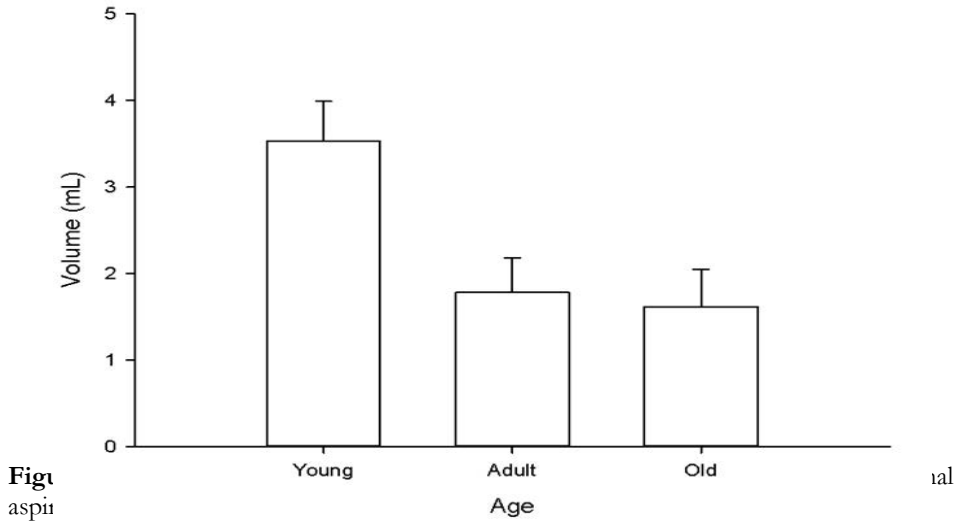
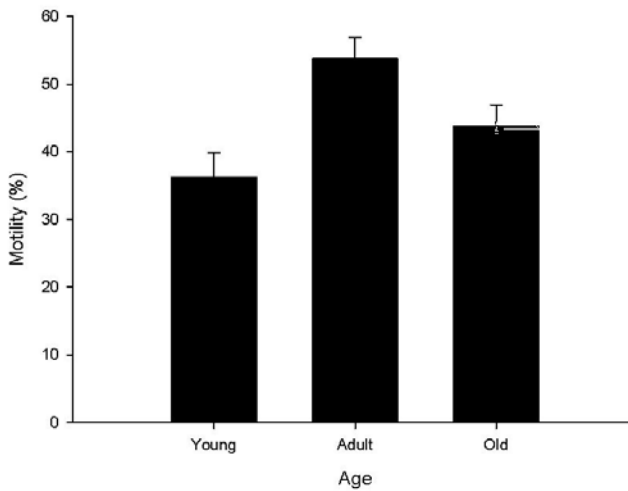
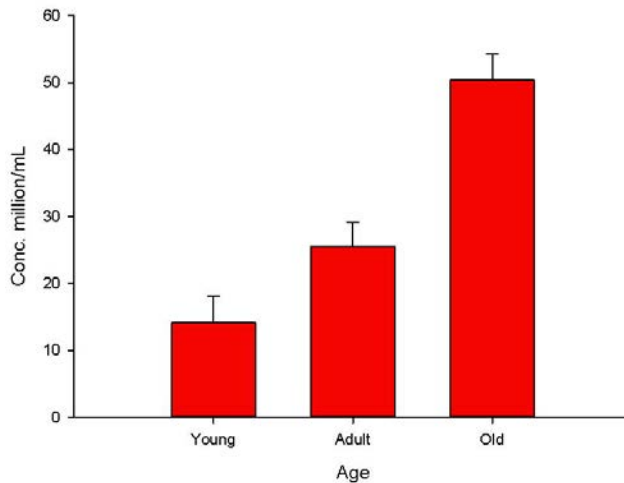


Fig  
aspi



**Figure 2:** Mean and standard error of spermatozoa motility on semen samples recovered by vaginal aspiration following copulation to a receptive female on three different ages of male alpacas.



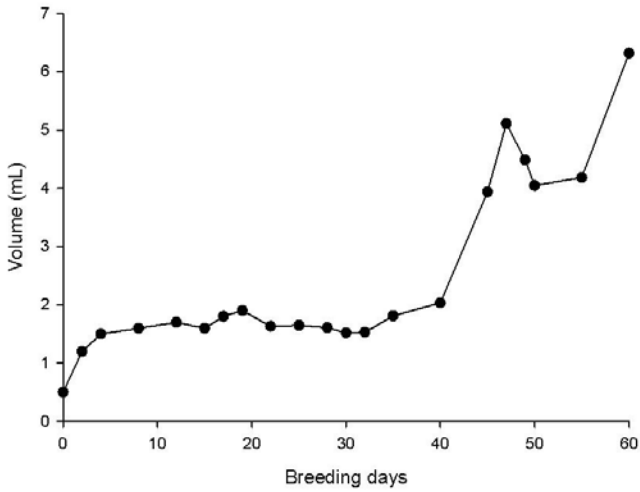
**Figure 3:** Mean and standard error of the mean of spermatic concentration in male alpacas of three different ages that bred for a period of two months.

Spermatic concentration varied like spermatozoa motility, being higher in old males, than in adult and young males. Young males, as stated earlier, might have been reaching sexual maturity and then their sperm concentration was less than in older males. Comparable results were reported earlier in alpacas (Bravo, 2002).

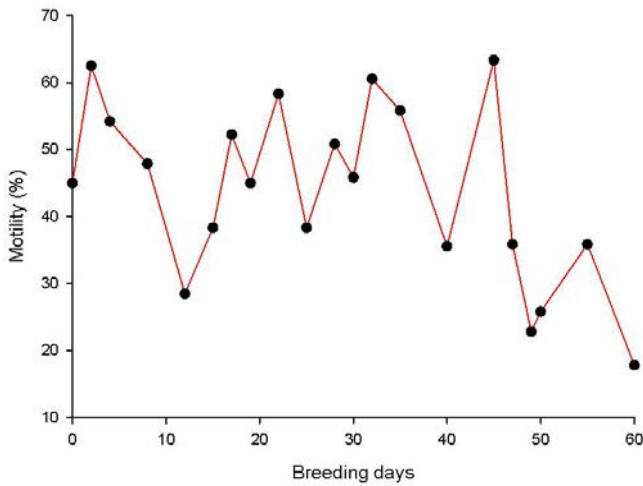
#### *Semen characteristics by day of breeding*

Means of semen volume, motility and concentration are presented in figures 4, 5, and 6, respectively. Semen volume was different ( $P < 0.05$ ) at the beginning of the breeding period (0.5 ml) in contrast to 6 ml by the end of the same period. Spermatozoa motility started by 45 %, remained at the same value, but was significantly lower (17 %) by the end of breeding period ( $P < 0.05$ ). Spermatic concentration started at 6 million spz/ml, reached a peak to 65 million, and then dropped to 17 million by the end of the breeding period ( $P < 0.05$ ).

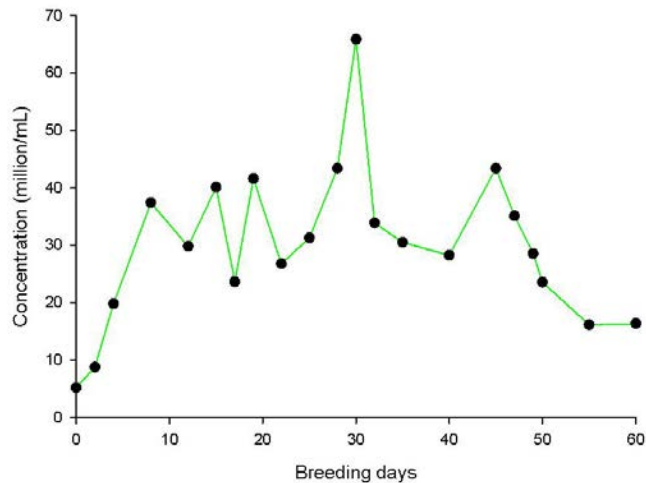
The effect of day on a breeding period of 60 days was variable and revealing. Semen volume collected started low, but to our surprise reached up to 6 ml by the end of the period of evaluation. Spermatozoa motility was maintained elevated for the first 45 days of breeding and then decreased dramatically by day 60 of breeding period. Sperm concentration was also different. It was low at the beginning of the breeding period, increased reaching a peak by day 30th, and then decreased slowly by the end of the evaluation period.



**Figure 4:** Mean semen volume recovered following breeding a receptive female for a period of 60 days.



**Figure 5:** Mean spermatozoa motility of male alpacas for a period of 60 days.



**Figure 6:** Mean spermatic concentration of male alpacas that bred for a period of 60 days.

It is the first time that alpaca semen was evaluated for 60 days; however, our results are comparable to previous work on alpacas (Bravo, Flores, and Ordoñez, 1997; Bravo, 2002) and llamas (vonBaer, 1998). Altogether, these results reflect that males may have a relative small pool of response to the length of breeding period. By the end, samples were less concentrated than at the beginning and volume increased. It should be remembered that alpacas have small testicles than other livestock species, and also may indicate a limiting factor on the testicular response to the number of receptive females.

Semen sample was not collected on 17 times, which represents 7 % of total of collecting times. There was no difference,  $P > 0.05$ , on lack of semen collection between aged-group males, 4 times on young males, 8 times on adult males and 5 times on old males. The fact that sometimes semen samples were not collected was real and is not significant, neither this lack of semen collection is different in the three group of males used in this study. It is unknown why sometimes semen is not collected, but also was reported whenever an artificial vagina was used in alpacas (Bravo, Flores and Ordoñez, 1997).

## Conclusion

In summary, semen characteristics were different in the three group of males used in this study. In addition, the length of the breeding period has definitively an effect on semen volume, spermatozoa motility and sperm concentration. The results of this study may have to be taken into consideration to evaluate the fertility of the male alpaca.

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# The Sperm Chromatin Dispersion Assay (HALO Test) Correlates with the Tunel Technique in Llama Sperm

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**Abstract.** Evaluation of routine seminal characteristics is used to predict fertility but cannot always reveal defects in genome integrity. Evaluation of sperm DNA becomes important for assessing ejaculates prior to applying assisted reproduction techniques. To evaluate llama sperm DNA fragmentation, we set up the Sperm Chromatin Dispersion assay (SCD). This technique does not require costly equipment and can be prepared using lab supplies without the need to buy special kits. Due to its objectivity and reliability, the TUNEL (terminal deoxynucleotidil transferase mediated dUTP nick end labelling) technique has become the assay of choice to measure sperm DNA fragmentation. The objective of this study was to use SCD to evaluate llama sperm DNA fragmentation in raw semen and correlate this technique with TUNEL. Sixteen ejaculates were obtained from 5 males using electroejaculation under general anesthesia. The DNA fragmentation was evaluated using SCD and TUNEL. Semen incubation with 0.3 M NaOH during 5 min was used in both techniques as a control of DNA fragmentation. Pearson's correlation test was used for statistical analysis. Sperm DNA fragmentation values were  $13.7 \pm 6.3$  % and  $8.6 \pm 5.6$  % for SCD and TUNEL respectively and in both assays, NaOH produced DNA fragmentation in 100 % sperm. Despite the greater fragmentation values in SCD compared to TUNEL, a highly positive correlation was observed between the techniques ( $r=0.80$ ,  $p=0.0002$ ). These results indicate that llama sperm DNA evaluation with SCD gives similar results to TUNEL and so could be used in andrology laboratories that don't have expensive equipment and even in high altitude labs, such as those in the Puna region.

**Resumen.** La evaluación de las características seminales de rutina es utilizada para predecir fertilidad. Sin embargo, puede no revelar defectos en la integridad del genoma. La evaluación del daño del ADN espermático constituye una herramienta potencial para eval-

uar los eyaculados antes del uso de técnicas de reproducción asistida. Para evaluar la fragmentación del ADN espermático de llama, se puso a punto la técnica de dispersión de la cromatina espermática (Sperm Chromatin Dispersion assay, SCD). Esta técnica no requiere de equipamiento costoso y puede realizarse mediante insumos de laboratorio, sin la necesidad de la compra de kits costosos. Debido a su objetividad y confiabilidad, la técnica de TUNEL (terminal deoxynucleotidil transferase mediated dUTP nick end labelling) se ha convertido en el ensayo de elección para medir la fragmentación del ADN espermático. El objetivo de este estudio fue utilizar el SCD para evaluar la fragmentación del ADN de espermatozoides de llama y correlacionar esta técnica con el TUNEL. Un total de dieciséis eyaculados fueron obtenidos de 5 machos utilizando electroeyaculación bajo anestesia general. La fragmentación del ADN fue evaluada a través de SCD y TUNEL. La incubación del semen con NaOH 0,3 M durante 5 min fue utilizada como control de fragmentación del ADN en ambas técnicas. Para el análisis estadístico se utilizó la correlación de Pearson. Los valores de fragmentación del ADN fueron  $13,7 \pm 6,3$  % y  $8,6 \pm 5,6$  % para SCD y TUNEL, respectivamente. En ambas técnicas el NaOH produjo un 100 % de espermatozoides con fragmentación del ADN. A pesar de observar mayores valores de fragmentación en el SCD en relación al TUNEL, se observó una correlación altamente positiva entre ambas técnicas ( $r=0,80$ ;  $p=0,0002$ ). Estos resultados indican que se observan porcentajes de espermatozoides con ADN fragmentado similares con el SCD y el TUNEL, y por lo tanto, podría utilizarse en laboratorios de andrología que no posean equipamiento costoso e incluso en laboratorios situados a altas latitudes como la región de la Puna.

**Keywords:** llama, sperm, SCD, TUNEL

## Introduction

Evaluation of routine seminal characteristics (concentration, motility, morphology and membranes) is used to predict fertility but they do not assess all aspects of sperm function. Poor sperm DNA integrity can negatively affect sperm fertilizing capacity *in vitro* (Henkel et al., 2004). Thus, evaluation of DNA becomes important for assessing ejaculates prior to applying assisted reproduction techniques. Cortés-Gutierrez et al., (2007) classified the techniques for evaluating sperm DNA fragmentation into two groups. The first group includes the methods used to mark single and double strand DNA breaks, for example, when enzymes are used to incorporate nucleotides *in situ*, such as Terminal dUTP Nick-End Labelling (TUNEL) or *in situ* Nick Translation (ISNT). Taking into account that DNA fragmentation increases chromatin susceptibility to denaturation, the second group of methods include those that measure this susceptibility after treatment. This group includes the Sperm Chromatin Structure Assay (SCSA), DNA Breakage Detection-Fluorescence *in situ* Hybridization (DBD-FISH), Single-Cell-Gel-Electrophoresis (SCGE) or Comet assay and the Sperm Chromatin Dispersion test (SCD) o Halo test. Many of these techniques are laborious, some depend on the irregular activity and accessibility of enzymes to DNA ruptures (Fernández et al., 2005) while others are costly and require complex equipment. To evaluate llama

sperm DNA fragmentation, we previously set up the SCD by optical microscopy (Carretero et al., 2012) and the TUNEL assay by flow cytometry and fluorescence microscopy (Carretero et al., 2015). The SCD technique does not require costly equipment and can be prepared using lab supplies without the need to buy special kits. On the other hand, due to its objectivity and reliability, the TUNEL technique has become the assay of choice to measure sperm DNA fragmentation. The objective of this study was to use SCD to evaluate llama sperm DNA fragmentation in raw semen and correlate this technique with the TUNEL assay.

## Materials and Methods

### *Animals and location*

The study was carried out at the Faculty of Veterinary Sciences of the University of Buenos Aires, in Buenos Aires, Argentina. The city is situated at sea level, latitude 34° 36' and longitude 58° 26'. For the study, 5 male *Lama glama* ranging between 8 and 10 years of age and weighing  $147.5 \pm 14.10$  kg (mean  $\pm$  SD) were used. Animals were kept out at pasture in pens and supplemented with bales of alfalfa; they also had free access to fresh water throughout the study. All males were shorn during the month of November.

### *Semen collection*

Semen collections were carried out between the months of April and October. Sixteen ejaculates were obtained from 5 males using electroejaculation under general anesthesia according to the technique described by Director et al. (2007). All procedures were approved by the Committee for the Use and Care of Laboratory Animals (CICUAL) of the Faculty of Veterinary Sciences, University of Buenos Aires (Protocol 2010/24).

### *Seminal characteristics evaluation*

The following routine seminal characteristics were evaluated in raw semen: volume, thread formation, concentration, sperm motility, membrane function and membrane integrity (viability). The ejaculate volume and thread formation were evaluated with a micropipette. Sperm numbers were calculated using a Neubauer hemocytometer. Sperm motility was evaluated using a phase contrast microscope (100 $\times$ ) and a warm stage (37 °C). The hypoosmotic swelling (HOS) test was used for assessing membrane function, and the fluorochromes 6-Carboxyfluorescein Diacetate (CFDA) and Propidium Iodide (PI) were used for assessing membrane integrity (viability). These techniques were carried out according to Giuliano et al. (2008).

### *Sperm DNA evaluation*

To reduce thread formation and improve handling, each ejaculate was diluted 4:1 in HEPES-TALP medium with 0.1 % collagenase and incubated at 37 °C for 4 min. Then, samples were centrifuged for 8 min at 800 × g and the pellets were resuspended in PBS (Giuliano et al., 2010).

The SCD assay was carried out according to Carretero et al. (2012) to evaluate the degree of DNA fragmentation. Briefly, each sperm suspension was mixed with low-melting-point aqueous agarose and pipetted onto a glass slide. Each slide was incubated in different lysing solutions, dehydrated in sequential ethanol baths and stained with Giemsa. A minimum of 200 sperm heads were observed under bright-field microscopy (1000x). Sperm were classified into four patterns according to the size of the halo: i) nuclei with large DNA dispersion halos; ii) nuclei with medium halos; iii) nuclei with small halos and iv) nuclei with no halo. The first two patterns (i and ii) were considered sperm without DNA fragmentation and the other two (iii and iv) sperm with DNA fragmentation.

The TUNEL assay was carried out using the Roche In Situ Death Detection Fluorescein® kit according to Carretero et al. (2015). Briefly, samples were fixed in 4% paraformaldehyde in PBS during 15 min at 4 °C, then were centrifuged and permeabilized in 0.1 % Triton X-100 in 1 % sodium citrate for 30 min at room temperature. After that, samples were centrifuged and incubated with the TUNEL reaction during 1 h at 37 °C in darkness. A minimum of 200 spermatozoa were evaluated per sample using an epifluorescence microscope with a rhodamine and standard fluorescein filter set. Sperm showing bright green fluorescence represented damaged cells (TUNEL positive), in which dUTP was incorporated to DNA breaks, in contrast to non-stained cells representing non-damaged sperm (TUNEL negative). Semen incubation with 0.3 M NaOH during 5 min at room temperature was used in both techniques as a control of DNA fragmentation.

### *Statistical analysis*

Pearson's test was used to correlate the SCD and TUNEL assays. All statistical analyses were performed using the R 2.2.1 Program (2005).

## **Results**

The percentages of routine seminal characteristics were (median ± SD): volume: 3.4 ± 2.7 ml; oscillatory motility: 23.7 ± 18.2 %; concentration: 56.1 ± 52.1 × 10<sup>6</sup> sperm/ml; membrane function: 29.8 ± 7.4 % and membrane integrity (viability): 47.5 ± 13.2 %. All samples showed thread formation, which disappeared after incubation with collagenase.

Sperm DNA fragmentation values were 13.7 ± 6.3 % and 8.6 ± 5.6 % for SCD and TUNEL, respectively. In both assays, 0.3 M NaOH produced DNA

fragmentation in 100 % sperm. A highly positive correlation was observed between the SCD and TUNEL techniques ( $r= 0.80$ ;  $p= 0.0002$ ).

## Discussion

Many of the techniques that evaluate sperm DNA are laborious and costly, requiring complex equipment such as flow cytometers and/or fluorescence microscopes. However, more simple techniques, that don't require complex equipment, exist. Two such are the Toluidine blue stain for evaluating the degree of chromatin condensation and the SCD assay for evaluating DNA fragmentation. In our laboratory, both these techniques have been adapted for use in the South American Camelids as well as in horses, dogs and cats (Sardoy et al., 2008; Carretero et al., 2009; 2010a; 2010b; 2010c; 2012; Monachesi et al., 2014; Allera et al., 2016). However, due to its accuracy and dependability, the TUNEL technique has become one of the assays of choice to measure sperm DNA fragmentation. For this reason, our laboratory adapted the TUNEL assay by flow cytometer and fluorescence microscopy to evaluate llama sperm DNA (Carretero et al., 2015). Various studies have been carried out to correlate different sperm DNA evaluation techniques in human andrology laboratories (Chohan et al., 2006; Zhang et al., 2010; Feijó et al., in press) and our aim was to do likewise for llama sperm.

The results for all raw semen parameters were within the normal range reported for raw ejaculates in this species (Bravo et al., 2000; Aller et al., 2003; Giuliano et al., 2008; Casaretto et al., 2012; Apichela et al., 2014; Carretero et al., 2015).

The percentages of DNA fragmentation observed with both techniques and in the controls, were comparable to those obtained previously (Carretero et al., 2012; 2015). Despite the greater fragmentation values obtained using the SCD assay as compared to TUNEL, a statistically significant positive correlation was observed between the two techniques for llama sperm DNA fragmentation. Similarly, in human sperm a significant correlation between TUNEL and SCD had also been observed (Zhang et al., 2010; Feijó et al., in press). Chohan et al. (2006) not only observed correlation between TUNEL and SCD, but also between these two techniques and the Sperm Chromatin Structure Assay (SCSA) in human spermatozoa. In addition, Fernández et al (2003) confirmed SCD test results for sperm DNA fragmentation in porcine, using breakage DBD-FISH. Sperm with a very small or absent halo showed extensive DNA fragmentation using DBD-FISH. Unlike the SCSA, TUNEL and DBD-FISH, the Sperm Chromatin Dispersion assay can be performed without complex or expensive instrumentation such as flow cytometry or fluorescence microscopy. We have shown that SCD can be carried out using either fluorescent or bright-field microscopy. In our laboratory we adapted the technique for evaluating equine and llama sperm (Carretero et al., 2010; 2012) and found that bright-field microscopy was more reliable than fluorescence microscopy. The reason for this is because bright-field microscopy allows one to distinguish more clearly the limits of the chromatin dispersion halos, permitting a more precise

classification of the different patterns (large, medium, small or no halos) (personal observations). In addition, evaluation with bright-field microscopy (as opposed to use of fluorescence) allows observation of the flagellum, thus confirming that in effect the halo belongs to a sperm cell and not any other DNA (Fernández et al., 2005).

The present study also demonstrated that the SCD test identified more spermatozoa with DNA damage than the TUNEL assay ( $13.7 \pm 6.3\%$  and  $8.6 \pm 5.6\%$ , respectively) suggesting that the SCD test may be more sensitive. Similar results were observed by Zhang et al. (2010) in sperm from infertile men ( $25.2 \pm 10.2\%$  for SCD and  $22.8 \pm 6.9\%$  for TUNEL using fluorescence microscopy), however these authors observed similar percentages between both techniques in fertile men ( $13.6 \pm 3.4\%$  and  $12.9 \pm 3.0\%$  for SCD and TUNEL, respectively). Chohan et al. (2006) observed similar values of fragmentation using TUNEL by fluorescence microscopy and SCD, both for infertile and fertile men ( $19.5 \pm 1.3\%$  and  $20.4 \pm 1.3\%$  for SCD and  $11.1 \pm 0.9\%$  and  $10.8 \pm 1.1\%$  for TUNEL, respectively).

The TUNEL assay quantifies the percentage of sperm with fragmented DNA by labeling the strand breaks with TdT, whereas the SCD test involves acid denaturation, which generates single-stranded DNA motifs from DNA breaks and further deproteinization of nuclear proteins suppresses the formation of a halo. Though these assays use different principles and protocols, and despite the greater fragmentation values in SCD compared to TUNEL, nevertheless the highly positive correlation observed between the techniques in raw llama semen ( $r= 0.80$ ;  $p= 0.0002$ ) was encouraging and further validates the use of the simpler SCD for DNA evaluation in llama sperm.

## Conclusion

These results indicate that DNA evaluation with SCD correlates with the TUNEL assay and so could be used in andrology laboratories that don't possess expensive equipment and could also be used in high altitude labs, such as those in the Puna region, making DNA evaluation more readily available.

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# Teeth in Camelids: Myths, Facts and Problems

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**Abstract.** Most owners of alpacas and llamas in Europe have small groups of these animals and most of them have no education in keeping and breeding agricultural animals. In addition, it is usually difficult for them to find correct information about alpacas and llamas in their native language. There are different tooth problems seen in camelids like prognatism, sharp fighting teeth, root infections and real or assumed problems with cheek teeth. In this paper “myths” about the reasons of these problems are presented and, in contrast, the real reasons for these problems are explained and some therapeutic proposals are added.

**Resumen.** La mayoría de los propietarios de alpacas y llamas de Europa poseen pocos animales y carecen de formación para mantenerlos y reproducirlos. Además, es difícil encontrar información correcta sobre alpacas y llamas en su lengua materna. Se observan diversos problemas dentales en camélidos, como prognatismo, dientes filosos, infecciones de las raíces y problemas reales o no en los carrillos. En este trabajo se presentan "mitos" sobre las razones de estos problemas mostrando de manera contrasada la verdadera naturaleza de estos problemas, sugiriendo propuestas terapéuticas.

**Keywords:** camelid teeth, anatomy, function, myths

## Introduction

Alpacas and llamas are kept in Europe since more than 20 years, but up to now breeding these species is a niche. Most owners have only small groups of these animals, and most of them have no education in keeping and breeding agricultural animals. In addition, it is usually difficult for the owners to find correct information about alpacas and llamas in their native language. The nutrition of camelids consists of grass and hay and they can ruminate. This eating technique is very efficient but it requires correct and healthy teeth. There are different tooth problems seen in camelids and one can find various “explanations”, better “myths”, for these tooth problems.

### *Eating Facts*

Camelids eat mostly grass, hay and minerals. They cut the grass with their incisors and use their lips to transport the grass (or hay) into the mouth. Then they chew the grass (hay) with the molar teeth as long as the “balls” reach a size which is the correct size to be swallowed. Later they ruminate the content of C1, chew it and swallow it again.

### *Anatomy of Camelid Teeth*

Alpacas and Llamas have incisor (J), canine (C) and cheek (P/M) teeth. The third incisor and the canine tooth in the maxilla and the canine tooth of the mandibular of each side are considered as fighting teeth.

The dental formula of each side in deciduous teeth is:

1-2 Jd, 1 Cd, 3 Pd

3 Jd, 1 Cd, 2 Pd

The dental formula of each side in permanent teeth is:

1 J, 1 C, 2 P, 3 M

3 J, 1 C, 1 P, 3 M

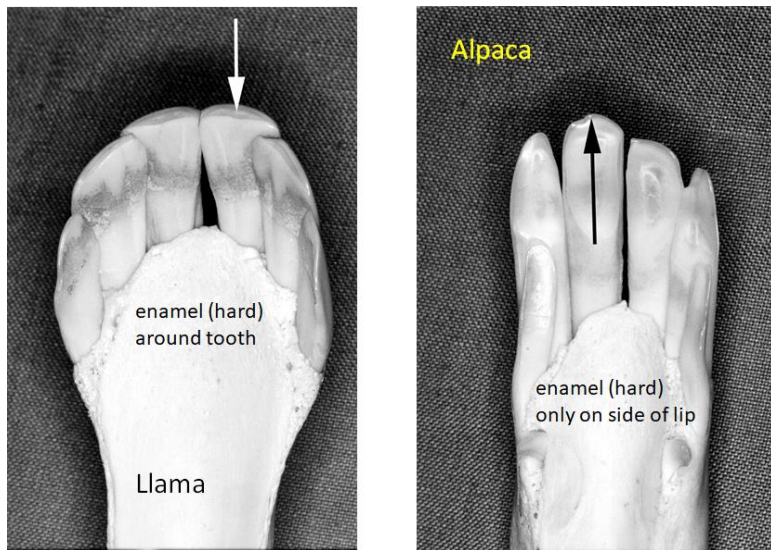


**Figure 1:** Skull of an adult male llama. Photo: Gunsser

Figure 1 shows the skull of a male adult llama with correct incisors and cheek teeth. In intact male alpacas and llamas the fighting teeth are more prominent than in females or geldings castrated before the influence of testosterone (1 – 1.5 years of age).

The incisors of alpacas are relatively long compared to the incisors of llamas. In llama incisors, the whole tooth is covered by enamel. In alpacas only the labial part of the incisors is covered by hard enamel. The lingual part of the incisors consists of softer dentin.

Figure 2 explains the differences seen in llama and alpaca incisors. Because of the softer dentin on the lingual part of the alpaca incisor the teeth are more easily worn down. For that reason incisors of alpacas are longer than llama incisors because they move up more to restore the contact with the dental pad.



**Figure 2:** Left incisor llama, right incisor alpaca. Photo: Gunsser

*Myth: "Incisors grow a life long"*

One such myth concerns the prognatism of incisors in some alpacas, stating that the incisors are growing during the whole life of the animal and that they protrude from the mouth because the grass and hay in Europe is not hard enough to keep them in the correct length. Fact is that, if used, the very long incisors move up during the lifetime of the animal to restore the contact with the dental pad (like in other species), but they do not grow permanently. The incorrect position of the incisors prevents contact with the dental pad and the dentin is not sufficiently worn down. Prognatism is an inherited problem. Too long prognate incisors impede the movement of the lips (Fig. 3) and make the cutting of grass and the transport of hay into the mouth difficult.



**Figure 3:** Long incisors impede movement of lips. Photo: Gunsser



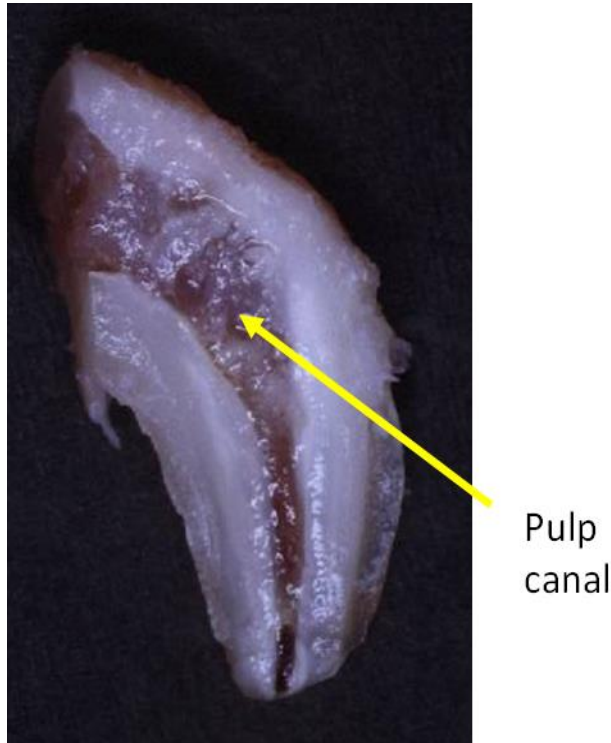
**Figure 4:** Incisor before (left) and after (right) cutting to restore a correct bite. Photo: Gunsser

Therefore cutting the incisors and restoring the correct bite (Fig.4) may in some cases help for a limited time. But one has to consider that there is a limited length of the incisors. Controlled breeding selection for anatomical correct teeth would be the better solution.

*Myth: "Cutting fighting teeth without anaesthesia does no harm to the animal"*

Another myth is that cutting fighting teeth without anesthesia is admissible. Fact is that fighting teeth have a pulpa with nerves, but flight animals do not express pain by screaming when the tooth is cut. Cutting teeth without anesthesia is not compatible with animal welfare. In Fig. 5 an example of a fighting tooth is cut

open to make visible the pulp canal. The pulp canal is usually filled with blood vessels and nerves.



**Figure 5:** Opened fighting tooth to show pulp canal. Photo: Gunsser

*Myth: "Sharp points of cheek teeth are the reason for not eating"*

Alpacas and llamas cannot open their mouths very wide and therefore the control of premolar and molar teeth by the animal owner or by the veterinarian is not easy. But the control is necessary, for example, when the animals do not want to eat. Young and middle age animals have cheek teeth with an oblique, very sharply pointed surface, necessary to chew the hay and grass. Fig. 6 shows a prepared skull with cheek teeth of the upper and lower jaw. The teeth in the mandibular are smaller than the teeth in the maxilla. While chewing, the mandibular teeth move oblique along the maxilla teeth and form the grass/hey to balls. With increasing age of the animal the surface of the cheek teeth will flatten out in the middle of the surface, the lingual edge (mandibular) and the labial edge (maxilla) will stay pointed for longer time. Some veterinarians (mostly horse practitioners) believe that these points may be a problem for the animal and will want to grind them off. But grinded teeth (like old used teeth) inhibit correct chewing. Insufficiently chewed

balls cannot be swallowed. Grinding of a cheek tooth is only necessary if the position of the tooth has changed and is going to block the movement of chewing.



**Figure 6:** Cheek teeth with sharp points. Photo: Gunsser

*No Myth but problems: Root infections*

The most important cheek teeth are the three molar teeth in the maxilla and mandibula of each side. Infections of the roots are a big problem for the camelids. Fig. 7 shows as example an x-ray of a young alpaca with infection in the region of the roots of pd1 and pd2 of the mandibula. Since root infections are seen mostly in more than one tooth, extractions may lead to chewing problems, because the teeth in the counterparts are missing. So regular tooth and mandibular/maxilla control and, if necessary, early conservative therapy should be preferred to conserve the functioning of the teeth as long as possible.

In old animals worn and lost teeth are the main reason for nutrition problems. Alpacas and llamas use the same eating techniques as ruminants, they chew everything twice. For this reason functioning teeth are very important. Most old animals die of starvation because they have lost cheek teeth or their teeth do not have sharp points to chop the food. Fig. 8 shows a mandibular of an old llama with missing cheek teeth and missing incisors.



**Figure 7:** X-ray of alpaca cheek teeth with infection of the roots. Photo: Gunsser



**Figure 8:** Mandibula old llama, 2 molars and all incisors lost. Photo: Gunsser

### **Conclusion**

Since alpacas and llamas are plant eaters and ruminates, correct and healthy teeth are very important for these species. The lifetime of alpacas and llamas depends on the quality of their teeth. In view of animal welfare correct conformation with correct teeth should therefore be a breeding goal. For that reason owners should have correct information and no “myths” about the anatomy and functioning of the teeth, and should be aware of potential tooth problems.



## Nutrition





# Advances in Nutrition on Chinese Cashmere Goat: A Review

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**Abstract.** China has the world's largest number of cashmere goat populations, and produces over 50 % the world's total cashmere. The quality of the cashmere is best, and output quantity is highest in the world. The Inner Mongolian, Liaoning and Hanshan cashmere goats are the most popular of the Chinese cashmere goats. Inner Mongolian cashmere goats are one of the best Cashmere goat breeds in China and the world. This paper reviews the progress in Chinese cashmere goat nutrition research.

**Resumen.** China tiene la población más numerosa de cabra cashmere del mundo, y produce más del 50 % de la lana cashmere total del mundo. La calidad del cashmere es la mejor, y el volumen de producción es el más alto del mundo. Las cabras cashmere de Mongolia Interior, Liaoning y Hanshan son las más populares en China. Las cabras cashmere de Mongolia Interior están entre las mejores razas de China y del mundo. En este trabajo se revisa el progreso en investigación nutricional de la cabra cashmere de China.

**Keywords:** cashmere goat, nutrition, hair follicle, diets evaluation

## Introduction

Inner Mongolian cashmere goats are one of the best Cashmere goat breeds in the world. Cashmere goat industry has made a great contribution in promoting local economic development and improving farmers' living standard through providing excellent cashmere raw material. For years, the cashmere goats' grazing and protecting the ecological environment of grassland have been in intense conflict due to overgrazing. The best choice to solve this problem is to carry out a confinement and semi confinement feeding instead of year-round grazing system only. But after the extension of the confinement feeding system, a considerable problem has been found: Feeding costs are increased and the economic returns are decreased compared with year-around grazing systems. Nevertheless, a profitable confinement system for cashmere goats can be established through manipulations

of nutrient partitioning. One is to develop techniques for using light duration or melatonin treatment, the other is to develop a basic technique of manipulating dietary nutrient balance. The purpose of this paper is to identify the nutritional factors responsible for increasing fiber production and economic returns.

### **Dietary Nutrient Balance Technique**

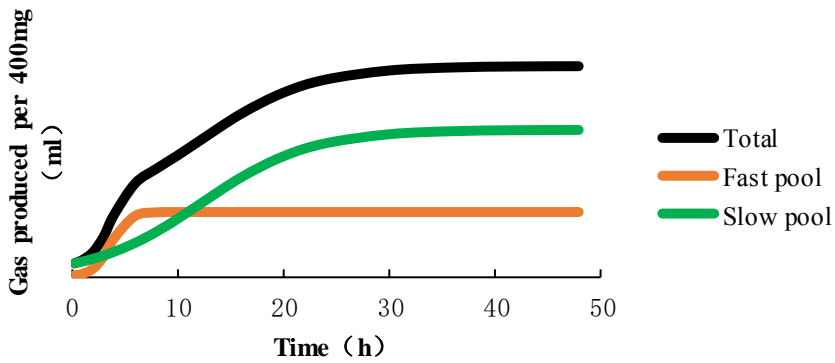
Feeding total mixed ration (TMR) is a widely-applied feeding strategy particularly on medium and large goat farms in China. The classic methods for evaluating TMR are *in vivo* techniques, but there are a lot of limitations because they are time-consuming, laborious and of poor accuracy. Now, it is popular using *in vitro* gas methods (Makkar et al., 2005) which allow to maintain experimental conditions more precisely than *in vivo* trials. It is a novel laboratory method utilizing a batch-culture, rumen-fluid, gas-fermentation system combined with mathematical curve-peeling techniques, allowing for the differentiation of rapid and slowly fermenting carbohydrate pools in TMR samples. Moreover, when animals are fed with TMR, digestive interactions can occur between single ingredients. These interactions, called associative effects, can modify the fermentation processes in the rumen in such a way that the response of cashmere goats to a combination of feed ingredients may differ from the components when considered individually.

A series of studies was undertaken to evaluate the associative effect on *in vitro* gas production from total mixed ration (TMR), based on alfalfa and corn-soybean concentrate, and separate TMR components (Table 1). These experiments showed that associative effects on gas production were much higher in early hours of incubation, and declined as time of incubation progressed, which is similar to results reported by Liu et al. (2002) in an *in vitro* system with sufficient N to support microbial growth. Associative effects commonly occur, when the digestion of one feedstuff is not independent of another (Niderkorn et al. 2009). Because microbial fermentation of individual and combined feedstuffs may diverge, the velocity of fermentation may be different, which may lead to a misjudgment of the risk for rumen disorders in cashmere goats.

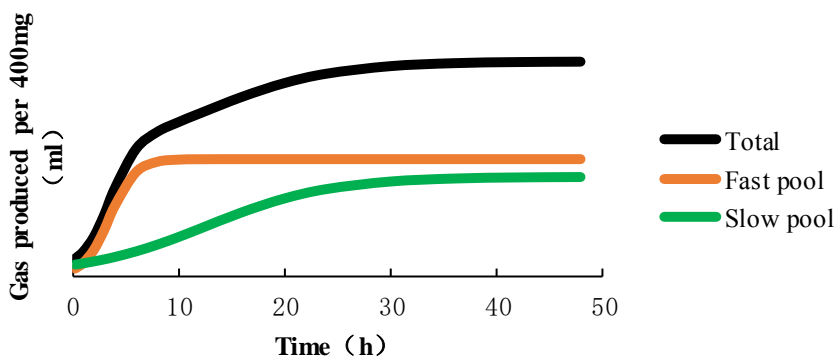
In addition, the amount (ml) and relative proportions of gas produced by each pool (fast and slow pool, gases derived from the degradation of the rapid or slow digestion soluble fraction, respectively) can help characterize the fermentation (Johnston, 2009). Field experience with hundreds of herds experiencing production challenges indicates, that the vast majority of problem herds are fed a TMR with an excessively fast "fast pool" and a relatively slow "slow pool" (Figure 1). The results also showed the extremely high gas production in the TMR, indicating that the cause of the excessively fast "fast pool" was primarily due to the B2 (soluble fiber) pool producing lots of methane and carbon dioxide gas (along with acetate) from rapidly digested soluble fiber rather than from excessively fermentable starch (B1).

**Table 1:** Associative effects of gas production of three TMR diets

NFC/NDF	Time (h)	Ratio TMR	Ratio Alfalfa	Ratio Concentrate	Calculated value	Associative effect (%)
2.00	2	27.33	22.89	30.95	29.33	- 6.80
	4	54.51	34.15	59.60	54.47	0.07
	8	96.46	49.59	113.95	100.98	- 4.48
	12	124.92	58.13	146.10	128.38	- 2.69
	24	178.14	72.98	211.11	183.28	- 2.81
	30	185.58	77.12	215.93	187.96	- 1.26
	48	191.18	89.05	221.99	195.20	- 2.06
2.35	2	43.40	22.89	39.68	36.97	17.39
	4	79.60	34.15	74.23	67.76	17.47
	8	132.31	49.59	136.18	122.22	8.26
	12	153.14	58.13	164.41	147.27	3.98
	24	184.45	72.98	210.41	188.24	- 2.02
	30	191.86	77.12	222.63	199.16	- 3.66
	48	199.74	89.05	230.21	207.44	- 3.72
3.00	2	33.36	22.89	31.06	29.83	11.85
	4	64.31	34.15	59.60	55.75	15.35
	8	116.21	49.59	117.41	107.16	8.44
	12	153.56	58.13	163.32	147.42	4.17
	24	219.17	72.98	232.67	208.52	5.11
	30	222.14	77.12	241.32	216.49	2.61
	48	228.25	89.05	246.80	222.95	2.38

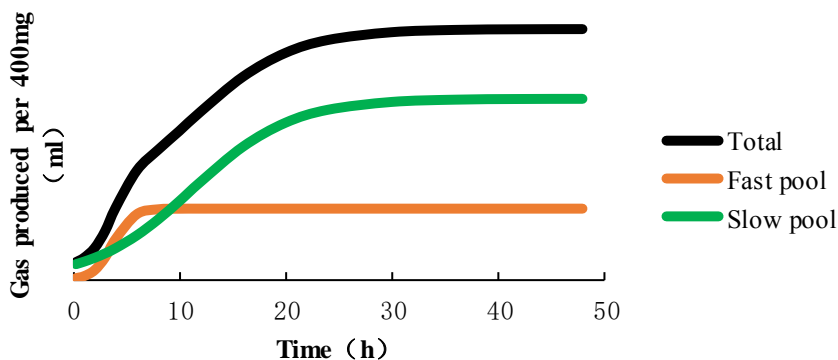


Relative pool contributions (NFC/NDF=2.00)		
	Gas(ml)	% of total
Fast pool	23.03	30.50
Slow pool	52.48	69.50
2-pool total	75.51	



Relative pool contributions (NFC/NDF=2.35)		
	Gas(ml)	% of total
Fast pool	43.03	54.43
Slow pool	36.02	45.57
2-pool total	79.04	

**Figure 1:** Application of a logistic-dual pool model to divide the cumulative gas production of three TMR diets during the incubation period



Relative pool contributions (NFC/NDF=3.00)		
	Gas(ml)	% of total
Fast pool	25.32	28.02
Slow pool	65.03	71.98
2-pool total	90.35	

**Figure 1 (continued):** Application of a logistic-dual pool model to divide the cumulative gas production of three TMR diets during the incubation period

### Regulation Technology of Cashmere Growth

The growth progress of cashmere in goat which is affected by photoperiod, nutrition, management, environment and genetic factors (Sun et al., 1998; Wang, 2004; Zhang et al., 2016), exhibits a seasonal pattern arising from circannual changes in the natural photoperiod, it also has been confirmed that the number, function and activity of the hair follicle cells in the skin influence cashmere growth (Sun et al., 1998; Duan et al., 2015; Zhang et al., 2017). However, the hair follicles in the skin undergo recurrent cycling of controlled anagen, catagen and telogen with a defined periodicity. The short duration of sunshine accelerates the development of hair follicles while the long duration of sunshine retards the development of hair follicles until it stops. For many years, a lot of techniques and methods have been explored to improve cashmere production. Until now, photoperiod alteration and melatonin (MT) implantation have been the best methods used. It was found that altering the photoperiod and implanting melatonin had potential use in cashmere goat feeding (Wang, 2004). More melatonin which was secreted by the pineal gland in short daily photoperiod might be the key factor for increased activity of secondary hair follicles (Wang, 2004; Wang et al., 2007; Hardeland et al., 2012; Liu et al., 2017). The study inferred that the secondary hair follicle activity of cashmere goats was increased under short daily photoperiod conditions, which might contribute to the pineal gland secreting

more melatonin (Zhang et al., 2017). In recent years, the skin has emerged as a model for studying the circadian clock regulation of the hair follicle cycle process (Tanioka et al., 2009; Lin et al., 2009; Plikus et al., 2015; Hardman et al., 2015). Previous studies of Liaoning cashmere goat have emphasized that not only *Bmal1*, *Clock*, *Cry1*, *Dbp*, *Nr1d1* were expressed in the skin, but also *Clock* genes expressions modified the secondary follicle cycle process (Shu, 2014). As these mitotic rhythms partly persist even in constant darkness, they might be under control of an endogenous clock. Recently, in liver (Eckel-Mahan et al., 2013; Jacobi et al., 2015; Dang et al., 2016), the circadian clock genes were shown to control the nutrients digestibility in goats (articles in press). Consequently, from my own perspective, providing a novel strategy for differential “peripheral clock” to research cashmere growth is a new development direction. The progression in photoperiod alteration, melatonin (MT) implantation and nutrition level used in cashmere goat feeding in 1998-2007 is collected in Table 2.

### **Regulation Technology of Functional Amino Acids**

Amino acids have enormous physiological importance, serving as building blocks for proteins and substrates for synthesis of low-molecular-weight substances (Wu et al., 2014). Especially, amino acids content in the diet during pregnancy are one important modifiable factor that can have a substantial influence on the viability and body composition of the newborn (Symonds et al., 2010). Furthermore, amino acids in histotroph of goat can activate mTOR cells signaling that stimulates migration, hypertrophy and hyperplasia of cells of the conceptus (Bazer et al., 2015).

The study indicated that the diet supplemental rumen-protected leucine (RPLeu) and HMB-Ca increased nitrogen intake, retained nitrogen and daily gain, and tended to decrease urine urea nitrogen excretion. RPLeu and HMB-Ca not only induced protein synthesis in skeletal muscle (Guo et al., 2009), but also played an important role in enhancing the body non-specific immune function (Guo et al., 2010). Gu et al. (2012) demonstrated that dietary N-Carbamylglutamate (NCG) supplementation increased meat rate of carcass and intramuscular fat, while dietary HMB-Ca supplementation increased the muscle fatty acids and decreased intramuscular fat in goats (Gu et al., 2012; Gu, 2012). Moreover, Wu et al. (2017) demonstrated that dietary arginine supplementation could help fetal growth and development, increase amino acid concentration in the blood, and induce the genes expression (OPN, VEGF, SLC7A and so on) of related conceptus development in the goat (Wu et al., 2017).

**Table 2:** The progress in photoperiod alteration, melatonin (MT) implantation and nutrition level using in cashmere goat feeding.

Target tissue	Test result	Author
Skin	The cashmere production and fiber length in Inner Mongolia Arbas white cashmere goat were significantly affected by protein and energy levels in the diet.	Sun et al., 1998
Skin	Study on the growth and activity changes of hair follicle in skin of Inner Mongolia Arbas white cashmere goat.	Sun et al., 1998
Tissue and organs	Experimental design to study the effects of different dietary MG levels on nutrient partitioning among tissue and organs in Cashmere goats.	Su, 2003
Gastrointestinal tract	The nitrogenous compounds partitioned more to cashmere growth when goats were treated by short daily photoperiod or melatonin implanted goats.	Wang, 2004
Gastrointestinal tract	The nitrogenous compounds partitioned more to body deposition when goats were treated by long daily photoperiod.	Wang, 2004
Skin and gastrointestinal tract	Not only the yield, diameter and length of cashmere fibres and nutrients digestibility of goat were changed significantly with short daily photoperiod, but also the cashmere growth performance and nutrients digestibility were improved significantly in high energy concentration diet.	Wang et al., 2007; Li et al., 2014; Kong et al., 2014; Zhang et al., 2017
Skin	Liaoning Cashmere goats have emphasized that not only Bmal1, Clock, Cry1, Dbp, Nr1d1 were expressed in skin, but also Clock genes expressions modified secondary follicle cycle process.	Shu, 2014
Blood	Analysis of metabolic pathways revealed that the energy levels affected valine, leucine, isoleucine biosynthesis, citrate cycle, glycolysis or gluconeogenesis, lactose metabolism, tyrosine metabolism, arginine and praline metabolism, cysteine and methionine metabolism, and the photoperiod influences oxidation of fatty acids, tyrosine metabolism, $\beta$ -alanine metabolism, pyruvate metabolism.	Zhang et al., 2016
Skin	The intrinsic oscillating molecular clock (Bmal1, Clock, Cry1, Per1 and Rev-erb $\alpha$ /five core clock genes) exists in skin hair follicles, which contributes to the regulation mechanisms of hair follicles cell proliferation.	Zhang et al., 2017

### Nutrition of the Grazing Goat

Dry matter intake and botanical pasture composition are the keys to assess the nutritional status of the Inner Mongolian Cashmere goats, so a series of studies were undertaken in different phases of pastures (sprout, growing and senescing period) (Sun, 1995; Bian, 1996; Guo, 2008). The results showed the seasonal variation of food intake and food composition of cashmere goats under natural grazing conditions using Alkane technique (Guo, 2008). The dry matter intakes (DMI) at different stages were 0.779, 0.845 and 1.632 kg/d respectively. Kenney et al. (1984) showed that the rate of intake of fresh forage increased as its water content increased, but the rate of dry matter intake declined once the dry matter content of the forage fell below 40 %. The feed intake of goats in the senescing period was higher than that of the sprout and growing period, which was also found in Liaoning Cashmere goats (Bian, 1996).

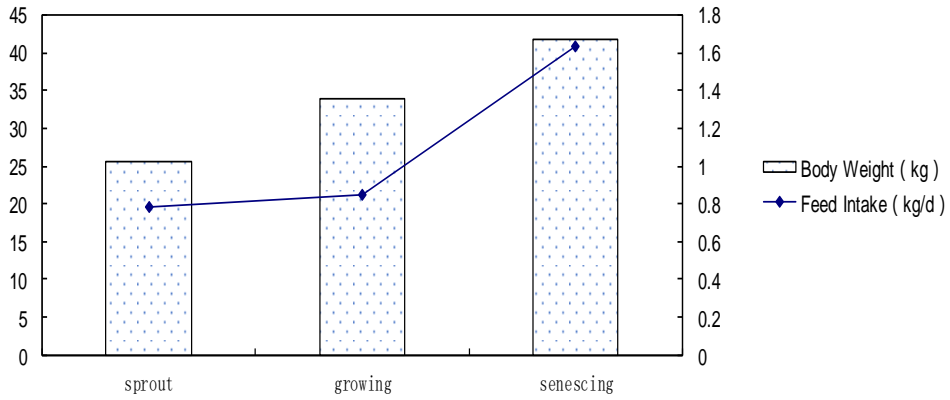
Pasture botanical composition were *Carex duriusula* 13.42 %, *Artemisia frigida* Willd 6.43 %, *Stipa breviflora* 71.85 %, *Iris bungei maxim* 3.93 % and *Peganum harmala* L 4.35 % in sprout period pasture and *Puncturevine* *Caltrop* Fruit 7.9 %, *Peganum harmala* L 42.28 % *Erodium stephanianum* Willd 8.78 %, *Allium victorialis* L 12.1 %, *Green Bristlegrass* Herb 8.56 % and *Poa alpigena* 20.4 % in growing period pasture, and *Sweet Wormwood* Herb 68.9 %, *Artemisia annua* L 13.3 % and *Carex duriusula* 17.82% in senescing period pasture, respectively (Table 3).

The nutritional values and dry matter intake of cashmere goats were affected greatly by the change of season (Figure 2), also for the nutrient digestibility of the herbage (Sun, 1995). When the nutrient digestibility of the herbage reached its peak, the grazing cashmere goats were fast growing. With the growth of the grass, the digestibility of various nutrients decreased and reached the lowest point in December or February respectively (Figure 3).

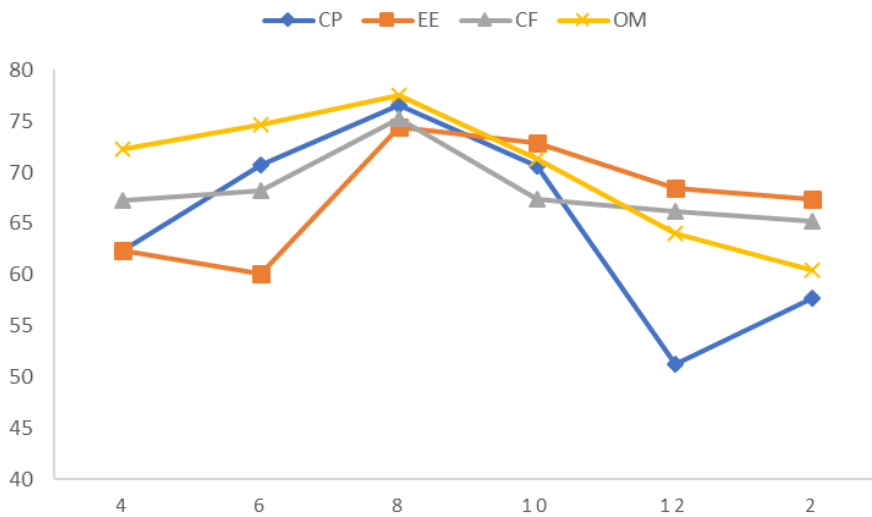
**Table 3:** Comparison of body weight, feed intake and pasture botanic composition in different pastures growing periods (means±SD).

Item	Sprout	Growing	Senescing	
Body Weight (kg)	25.7±1.3 <sup>a</sup>	34.0±2.53 <sup>b</sup>	41.7±3.8 <sup>c</sup>	
Feed Intake (kg DM/d)	0.779±0.23 <sup>a</sup>	0.845±0.31 <sup>a</sup>	1.632±0.21 <sup>c</sup>	
Feed composition	<i>Carex duriusula</i>	13.42	—	17.82
	<i>Artemisia frigida Willd</i>	6.43	—	—
	<i>Stipa breviflora</i>	71.85	—	—
	<i>Iris bungei maxim</i>	3.93	—	—
	<i>Peganum harmala</i>	4.35	42.28	—
	<i>Puncturevine Cal-trop Fruit</i>	—	7.90	—
	<i>Erodium stephanianum Willd</i>	—	8.78	—
	<i>Allium victorialis L</i>	—	12.10	—
	<i>Green Bristlegrass Herb</i>	—	8.56	—
	<i>Poa alpigena</i>	—	20.40	—
	<i>Sweet Wormwood Herb</i>	—	—	68.9
	<i>Artemisia annua L</i>	—	—	13.3

The crude protein and energy intake of the grazing cashmere goat in each period can be calculated from the feed intake and nutrient content. The nutrient requirements of protein and energy of angora goats are shown in Figure 4 and Figure 5 (Sun, 1995). It can be seen, that in winter and spring season, the protein and energy intakes of grazing cashmere goats from the pasture were less than their protein and energy requirements, even though supplementary feeding (250 g/d) was provided.



**Figure 2:** Food intake of goats in different pastures growing periods



**Figure 3:** The apparent digestibility of the herbage in different pastures growing periods

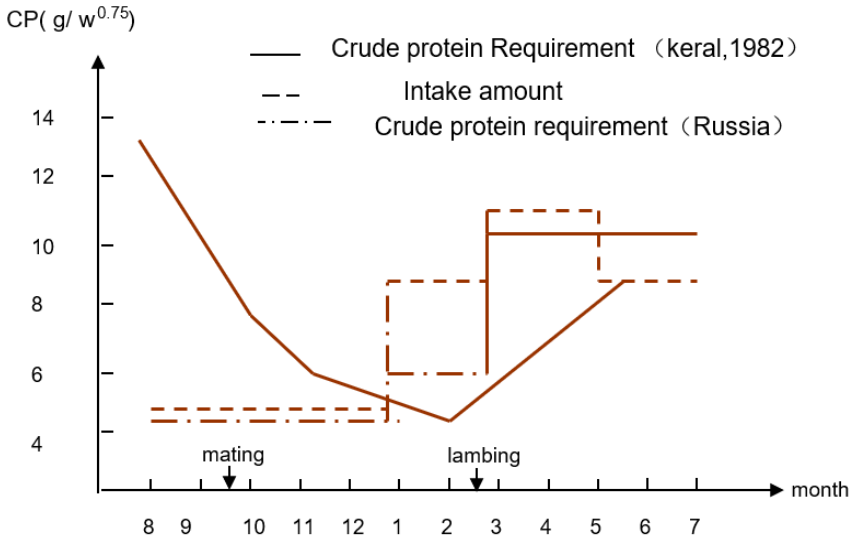


Figure 4: Daily crude protein intake of goats in different season

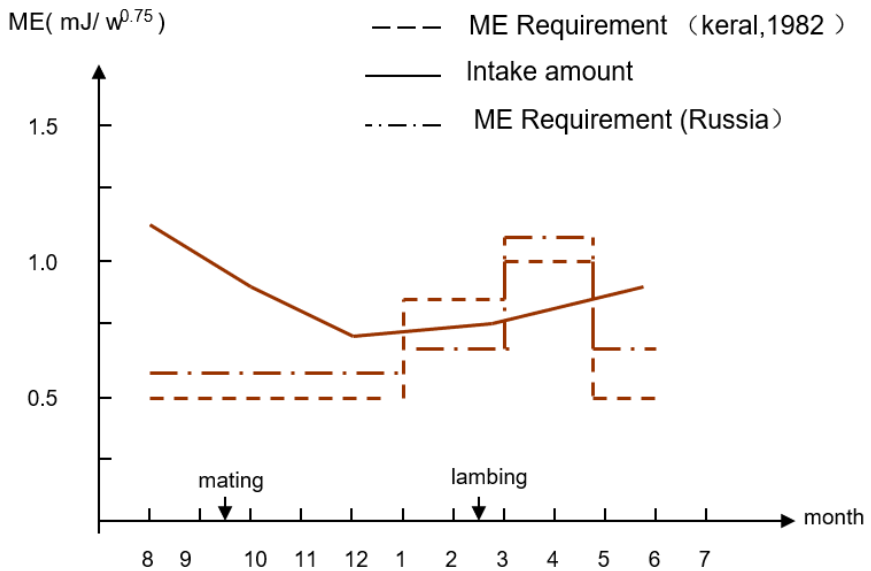


Figure 5: Daily energy intake of goats in different season

### Rumen Fermentation Regulation

As the forage ratio increased, the pH value of rumen improved (Shi, 2008; Ma, 2008), and the nutrient passage rates showed that rumen K<sub>p</sub> of feeding a 3:7 (concentrate to roughage ratio) diet was lower than that of feeding a 2:8 diet. After adding malate, K<sub>p</sub> in the rumen decreased (Shi, 2008). With the increasing of the forage in the diets, the *Entodinium* decreased; however, the *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* increased (Ma, 2008). Adding malate in the diets decreased rumen NH<sub>3</sub>-N concentration, lactate concentration and A/P ratio. The number of ruminal total bacteria, cellulolytic bacteria, and *S. Ruminantium* was higher than in the control group. Meanwhile, adding malate also could improve total flowing of DM, OM, NDF and ADF in the rumen, while the flowing of those in the duodenum was decreased. In other words, the amount of disappearance of NDF and ADF was increased in the gastric area. At the same time, daily gain and cashmere production of Cashmere goats was increased by adding malate in the diet (Shi, 2008). The gas production and methane emission of rumen were influenced strongly by addition of different doses of essential oil and antibiotics, and methane emission was decreased while gas production was increased (Xue, 2010).

### Nitrogen Digestion and Metabolism

The dietary nitrogen levels and partial ruminal defaunation have important effects on nitrogen metabolism, microbial protein synthesis and urea nitrogen recycling in the Inner Mongolia White Cashmere goats. Studies by Du (2010) have shown that the nitrogen intake, degradable nitrogen, retained nitrogen and retained nitrogen/metabolic body weight were increasing with the dietary crude protein increasing.

The ratios of retained nitrogen/metabolic body weight were significantly raised, but the plasma ammonia nitrogen was markedly reduced by feeding swelling corn compared to cracked corn. For the urea transporter-B (UT-B) expression in the gastrointestinal tracts, the high crude protein level of 13.5 % in the diet could enhance the expression of UT-B protein in fore-stomach, ileum, cecum, caecum, and kidney, while inhibiting the expression of the protein in duodenum. However, the parotid gland, the abomasum and the liver were less affected. Fan (2014) reported that the appropriate reduction of diet N was a benefit to improve the utilization of N when the microbial protein met the animals' demand. Partial defaunation improves the utilization of N in goats fed a low nitrogen diet while feeding oscillating dietary CP benefits the utilization of N and microbial protein synthesis.

### Conclusion

There is presently an export demand for both goat cashmere and meat, and Chinese scientists and producers face the exciting prospect being able to produce more and better cashmere at less cost. There is an increase in knowledge on the various factors that limit the performances of cashmere goats. Each of the

improvements mentioned in the paper, including dietary nutrient balance technique, cashmere growth regulation technology, functional amino acids regulation technology, nutrition of the grazing goat, rumen fermentation regulation and nitrogen digestion and metabolism will improve the animal performance greatly by integrating the fundamental knowledge about cashmere goat nutrition and physiology.

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# Alfalfa Hay Supplementation to Improve Llama Meat Production for Smallholders in Pasco Region, Peru

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**Abstract.** This study was carried out in Ninacaca, Pasco, Peru at an altitude of 4,350 meters above sea level. The scope of the study was to evaluate the effect of two feeding systems: (i) grazing on native pasture and (ii) grazing on native pasture plus alfalfa hay supplementation, on live weight, weight gain, biometric measurements and carcass dressing. Live body weight and biometric measurements were recorded every 15 days from 32 male llamas (8-12 months old). At 100 days of the experiment llamas were slaughtered, thus cold and hot carcass weight were recorded. Models used for analyzing final weight, total weight gain and weight gain by period included the feeding system as fixed factor and initial weight as covariate. Significant differences ( $p < 0.01$ ) were found due to the effect of alfalfa hay supplementation on final weight and total weight gain. Also, significant statistical differences were found between live weights per period of different feeding strategies. Supplementation with alfalfa hay showed better weight gain response at 13, 27 and 41 days after the beginning of the experiment. Supplementation with alfalfa hay improves the growing performance of young llamas kept on natural pasture.

**Resumen.** Este estudio se realizó en Ninacaca, Pasco, Perú a una altitud de 4.350 metros sobre el nivel del mar. El objetivo del estudio fue evaluar el efecto de dos sistemas de alimentación sobre el peso corporal, la ganancia de peso, medidas biométricas y rendimiento a la canal: (i) pastoreo en pasto nativo y (ii) pastoreo en pasto nativo más suplementación con heno de alfalfa. El peso corporal y las medidas biométricas de 32 llamas machos (8-12 meses de edad), fueron medidos cada 15 días. Las llamas se sacrificaron a los 100 días del experimento y se midió el peso de carcasa fría y caliente. Los modelos utilizados para analizar el peso vivo final, ganancia de peso total y la ganancia de peso por periodos incluyeron al sistema de alimentación como efecto fijo y el peso vivo inicial como covariable. Se encontraron diferencias significativas ( $p < 0,01$ ) debidas al efecto de la suplementación con heno de alfalfa sobre el peso vivo final y ganancia de peso total. También se

hallaron diferencias significativas entre los sistemas de alimentación evaluados para el peso vivo entre los períodos del experimento. La suplementación con heno de alfalfa tuvo mejores ganancias de peso a los 13, 27 y 41 días después del inicio del experimento. La suplementación con heno de alfalfa mejoró el crecimiento de llamas jóvenes criadas en pastos nativos.

**Keywords:** llama, nutrition, meat production, morphometric measurements, supplementation

## Introduction

In Peru, llama production is mainly located in the Center and South Andes at an altitude ranging from 3,800 to over 5,000 meters above sea level (Leyva, 1991; Westreicher, 2007). The production system faces different problems such as high mortality of offspring and poor meat quality due to the presence of sarcocysts. In addition, the impoverishment of grasslands of natural pastures due to overgrazing is another factor leading to low production (Buttolph and Coppock, 2004; Flores, 2012). All these limitations lead to low meat production and as a consequence the profitability for smallholders is also low (FAO, 2005).

Previous studies showed that smallholders in the Pasco Region in the Central Andes of Peru keep llamas mainly for meat production (Alvarez, 2001; Cano, 2009; Mendoza, 2013; Mendoza, 2015). Farmers usually sell older animals, which often demonstrate a high incidence of sarcocystis (Castro et al., 2004; Martin et al., 2016) and show low body condition. These factors reduce the value of sales and consequently the profits of the producer (Alva et al., 1980). Therefore, one main constraint for improving meat production in llamas is the availability of appropriate amount and quality of feed, mainly at the beginning of the dry season when most llamas are sold to the meat market. The aim of this study was to evaluate the effect of supplementation with alfalfa hay on body weight gain, biometric measurements and carcass weight in young male llamas selected for meat market.

## Materials and Methods

### *Study site and treatments*

The study was carried out on a smallholder community farm, located at 4,350 meters above sea level (latitude 10°45.524"S and longitude 76°3.338"W), in the district of Ninacaca, Province of Pasco, Peru. The annual average temperature is 4°C and the annual precipitation fluctuates between 650 and 900mm with a rainy season from November to March.

A total of 32 male llamas were randomly sampled from individual breeders in Pasco Province. Age of llamas ranged from 8 to 12 months. Llamas were identified with numbered ear tags and they were assigned randomly to the two treatments

(16 llamas per treatment). In treatment 1 only native pasture (NP) was provided and in treatment 2 native pasture was supplemented with alfalfa hay. The daily amount of alfalfa hay that each llama received was 30 % of dry matter requirements. The amount was calculated from the ME requirements for gaining about 200 g/day (NRC, 2007).

The llamas of the two treatments grazed together 9 hours per day (08:00 to 17:00) on the same native pasture. Llamas from treatment 2 were gathered in pens and alfalfa hay was offered daily from 17:00 onwards individually for each llama. Alfalfa hay intake was recorded daily. The experiment was carried out over a period of 100 days.

#### *Pasture characteristics*

The native pasture area covered 5 ha and it was divided into three plots and fenced with eucalyptus poles of 2.20 m height and a barbed wire fence. Each plot was grazed for 15 days with a resting period of 30 days. The stocking rate was 5.2 llamas/ha based on the calculation according to Flórez and Malpartida (1987). Native pasture was mainly composed of *Festucadolichophylla*, *Stipaichu*, *Muhlenbergia* spp., *Bro-musunioloides*, *Calamagrostis* spp., *Festucaorthophylla*. Llamas had permanent access to fresh water.

#### *Body weight and biometric measurements*

The body weight and body measurements were recorded at the beginning of the study, and thereafter every 15 days. All animals were weighed in the mornings (07:00) by an electronic weight scale (2,000 kg capacity, 1 kg precision). The daily weight gain was estimated from the difference of the initial weight and the final weight, divided by the number of days each test phase lasted. Different body measurements were recorded (Table 1).

#### *Carcass traits*

At the end of the trial, all llamas were weighed and transported together to a slaughterhouse, where they were fasted. Immediately after slaughter, the carcasses were weighed (hot carcass weight, HCW), and the dressing percentage was calculated as the ratio of hot carcass weight to live weight on farm (dressing percentage =  $\text{HCW} \times 100 / \text{slaughter weight}$ ). Cold carcass was weighed (CCW) and chilling loss calculated as the ratio of cold carcass weight to hot carcass weight ( $[\text{HCW} - \text{CCW}] / \text{HCW} \times 100$ ).

**Table 1:** Description of biometric measurements (adapted from Mendoza, 2013)

Biometric measurement	Anatomical reference	Measuring instrument
Height at head (cm)	Distance between the sagittal suture and the floor (perpendicular)	Ruler with square
Height at withers (cm)	Distance between the meeting of shoulder blades (thorny thoracic process) to the ground (perpendicular)	Ruler with square
Height at rump (cm)	Distance between the highest point of the sacral bone to the ground level (perpendicular)	Ruler with square
Thoracic perimeter (cm)	Circumference at the level of the anterior part of the thorax (immediately behind the axillary area) and the cross (encounter of the shoulder blades)	Measuring tape
Body length (cm)	Distance between the tip of the medial ischium to the tuberosity humeral scapula.	Measuring tape
Cane perimeter (cm)	Circumference at the height of the metacarpus (cane) anterior left	Measuring tape
Width of breast (cm)	Distance between the two scapula humeral joints	
Ear length (cm)	Measure taken from the base to the apex of the ear	Measuring tape

### *Data analysis*

The experiment was a completely randomized design with 2 treatments. Data were analyzed using SAS version 9.2 for Windows (SAS, 1996). Two models were used; both models included the feeding systems as fixed effects and initial body weight as covariate. Model 1: a fixed model fitted for final weight and total weight gain. Model II: a linear random coefficient regression model fitted for live weight over period. Differences between fattening periods were determined using the least significant difference (LSD) test.

## Results and Discussion

### *Pasture characteristics*

During the trial, dry matter availability on native pasture varied from 6,064.86 kg/ha initially and 4,807.73 kg/ha at the end of the experiment. When analyzing the chemical composition (Table 2), the native pasture showed lower crude protein content (7.48 %) and the higher crude fiber content (28.68 %) than alfalfa hay.

**Table 2:** Mean chemical composition of native pasture and alfalfa hay at initial and final stages of the trial

	Native pasture (initial)	Native pasture (final)	Alfalfa hay (initial)	Alfalfa hay (final)
Dry matter (%)	91.38	94.70	89.23	89.55
Crude protein (%)	7.48	3.58	15.97	15.80
Lipid (Fat) (%)	1.66	1.54	1.74	1.70
Crude fiber (%)	28.68	40.20	24.77	25.85
Ash content (%)	3.19	4.29	7.25	7.80
Carbohydrate (NFE) (%)	50.37	45.09	39.50	38.40

NFE=Nitrogen Free Extract

The chemical analysis of the natural pasture and the alfalfa hay showed differences in nutritional value. Natural pasture and alfalfa hay showed a protein composition of 7.48 % and 15.97 % at the initial stage of the trial, respectively. The higher crude protein content of the native pasture at the initial stage is due to the fact that plants are in a vegetative state of growth and flowering. While at the final stage of the trial the native plants are in a vegetative period of ripening and dormancy (Florez and Malpartida, 1987; San Martín, 1989; Farfán and Durán, 1998). The nutrients provided by natural pasture are insufficient to meet the requirements of llamas the dry season period.

### *Body weight, total gain and biometric measurements*

The average body weight of each treatment is shown in Table 3. The final body weight in alfalfa hay treatment showed a significant difference compared to the pasture-only feeding system ( $p < 0.05$ ). Thus, the total gain and daily weight gain of animals fed with supplementation was higher. Therefore, the percentage of weight gain for animals fed by native pasture was 15.75 % and 21.87 % for those fed native pasture plus alfalfa hay, respectively. Llamas that received alfalfa as a supplement had higher weight gains than llamas only fed on pasture ( $p < 0.01$ ).

Llamas fed with alfalfa hay had better performance, a higher final body weight, total weight and daily weight gain. This response is explained by the higher supply of nutrients in alfalfa hay which allowed covering the maintenance requirements in llamas and the additional requirements for growth (San Martin, 1989; Davies et al., 2007; Liu et al., 2009).

These results are in agreement with similar trials with llamas (Mamani-Linares, 2013; Pérez et al., 2000) and with alpacas (Smith et al., 2017). Assuming that there were no differences in pasture intake, the differences in final body weights found in this study (Tables 3) may be related to the greater input of nutrients from the alfalfa hay supplement, which resulted in a better performance.

**Table 3:** Body weights, total and daily weight gain (means  $\pm$  SD)

Treatment	Native Pasture (NP)		NP + Alfalfa Hay		Significance of differences
	Mean	SD	Mean	SD	
Initial live weight (kg)	56.39	12.88	55.79	12.12	***
Final live weight (kg)	65.27	12.94	67.99	15.13	***
Total weight gain (kg)	8.88	3.15	12.20	3.79	***
Daily weight gain (g)	89.65	31.85	123.23	38.29	***

\*\*\*  $p < 0.01$

The data from biometric measurements at initial and final stages are shown in Table 4. The thoracic perimeter was the only biometric measurement that was significantly different between the two treatments ( $p < 0.01$ ). This would indicate that the size of the thoracic perimeter is effectively affected by the feeding system and would be associated with the gain of weight in llamas. The thoracic perimeter increased with the weight of the animal as also reported by Mendoza (2013), Condori et al. (2003), Llacsá et al. (2007), Zea et al. (2007). However, body length and height at rump did not show significant differences with the feeding system, contrasting with the results of significant increases presented by Llacsá et al. (2007) and Zea et al. (2007). But these measures were reported for young animals of 1 to 8 months of age (Llacsá et al., 2007) and of 2 to 15 months (Zea et al., 2007). Probably, the time range (3 months) considered in the development of the animals in the present study was not sufficient to show the variability needed to find significant differences.

**Table 4:** Average size of biometric measurements at initial and final stages (means  $\pm$  SD)

Biometric measurements	Initial		Final	
	Mean	SD	Mean	SD
Height at head (cm)	147.69	10.77	156.31	9.98
Height at withers (cm)	92.09	4.18	96.06	4.95
Height at rump (cm)	92.09	4.18	96.06	4.95
Thoracic perimeter (cm)	90.78	6.82	96.38	6.22
Body length (cm)	83.19	5.95	88.22	6.40
Cane perimeter (cm)	10.56	0.88	11.00	0.88
Width of breast (cm)	18.53	1.83	19.78	1.75
Ear length (cm)	16.06	1.22	17.13	1.13

*Carcass traits*

Table 5 showed the values of hot and cold carcass weight, dressing percentage and chilling loss. Significant differences were found due to the effect of feeding systems ( $p < 0.01$ ). Alfalfa supplementation led to higher carcass weights and to higher dressing percentage ( $p < 0.05$ ). The carcass weights obtained in both feeding systems are similar to those reported in alpacas and llamas (Pérez et al., 2000; Cristofanelli et al., 2004).

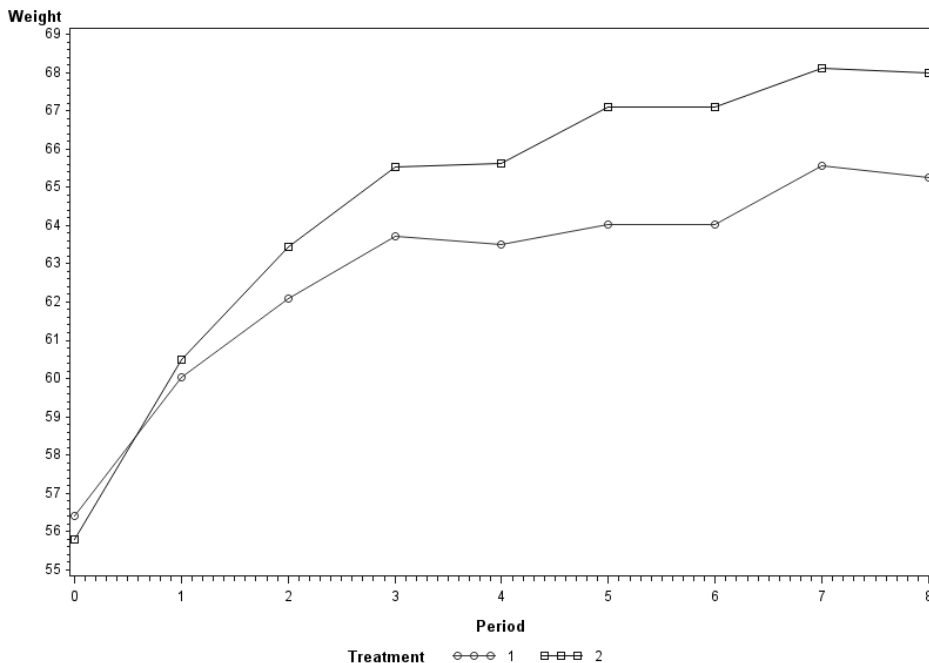
**Table 5:** Hot and cold carcass weight, dressing percentage and chilling loss (means  $\pm$  SD)

Treatment	Native Pasture (NP)		NP + Alfalfa Hay		Significance of differences
	Mean	SD	Mean	SD	
Hot carcass weight (kg)	34.65	7.71	36.26	8.15	**
Cold carcass weight (kg)	33.37	7.94	35.19	8.18	**
Dressing percentage (%)	52.92	3.00	53.38	2.69	*
Chilling loss (%)	50.81	3.31	51.71	2.68	**

\*\*  $p < 0.01$ , \*  $p < 0.05$

*Effect of supplementation in different periods of the experiment*

Also, significant statistical differences were found between live weights per period of different feeding strategies (Figure 1). Supplementation with alfalfa hay showed better weight gain response. The first three fattening periods (at 13, 27 and 41 days) show significant differences ( $p < 0.01$ ) respect to other periods. Supplementation with alfalfa hay improves the performance of young llamas kept on natural pasture.



**Figure 1:** Average growth (kg) curves per treatment (1 = Native pasture, 2 = Supplementation with alfalfa hay)

These results are in agreement with other studies conducted in llamas and alpacas (San Martin, 1996; Turin et al., 1999; Garcia et al., 2002). The differences among the first three fattening periods respect to the other periods could be due to the availability of natural pastures with a good nutritional value at the beginning of the experiment from March to April, which is the end of the rainy season (Florez and Malpartida, 1987; San Martin, 1996). In addition, llamas used in the present study came from places where the natural pastures were in poor condition. Therefore, the increase in body weight could be due to compensatory growth effect.

## Conclusion

This study showed that supplementation with alfalfa hay of young llamas grazing native pastures led to a higher live weight, total and daily weight gain and carcass weight. Feeding with natural pastures of good condition and supplementation with alfalfa hay results in a final live weight of 23 % more compared to the initial body weight in a period of 100 days. Highest gain of body weight occurred until 41 days of the beginning of the experiment for all the treatments.

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# Water Metabolism in South American Camelids

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**Abstract.** The present paper reviews the current knowledge on water metabolism, drinking behavior and water intake in South American camelids (SAC). Water requirements can be covered by intake of drinking water, water content of feed and water produced by metabolic processes. Body water turnover is closely related to body weight. But due to the complex role of water in thermoregulation, energy metabolism and other processes, many factors influence body water turnover. Water turnover rates in adult llamas were similar to those of goats. SAC have evolved efficient mechanisms using water, indicating an evolutionary adaptation to limited water supply in the high Andes. SAC and Old World camels share some physiological features with regard to their water efficiency, but they are less developed in SAC.

**Resumen.** El presente trabajo revisa los conocimientos actuales sobre el metabolismo del agua y el consumo de agua en camélidos sudamericanos (SAC). Los requerimientos de agua pueden ser cubiertos por el consumo de agua potable, el contenido de agua de los alimentos y el agua producida por procesos metabólicos. La tasa de rotación del agua corporal está estrechamente relacionada con el peso corporal. Sin embargo, debido al complejo papel del agua en la termoregulación, existen muchos factores que afectan al metabolismo energético y a otros procesos que modulan la tasa de rotación del agua corporal. Estas tasas de rotación de agua de las llamas adultas fueron similares a las de las cabras. Los SAC han desarrollado mecanismos para el uso eficiente del agua mostrando una adaptación evolutiva al limitado suministro de agua en los Andes. Los SAC y los camellos del Viejo mundo comparten características fisiológicas con respecto a la utilización eficiente del agua, pero están menos desarrolladas en los SAC.

**Keywords:** South American camelids, water metabolism, drinking behavior, water intake, salt tolerance

## Introduction

Water is an essential nutrient for all living beings. It is part of many body fluids such as blood and lymph. Water provides shape to the body cells. Biochemical and physiological reactions depend on a liquid medium and water functions as a solvent for transportation of nutrients and the excretion of waste products such as uric acid. The regulation of body temperature is an additional important role of water (Schmidt-Nielsen, 1997). Despite of the vital importance of water, only limited studies are available on water intake, water metabolism and water requirements of South American Camelids. The present paper reviews the current knowledge.

## Drinking behavior

South American camelids (SAC) suck in water with the mouth slightly opened. Interestingly, SAC are apparently unable to lick (San Martin & Bryant, 1989). The upper lip is divided by a labial cleft and each side of the lip can be moved independently, while the lower lip is less mobile. The upper lips are used as a tactile sense organ in food discrimination (Fowler, 2010). Thus, drinking from open water surfaces, such as ponds or running streams is most natural to SAC. Camelids have shown to use different types of automatic waterers, but are reluctant to use push valves water bowls where it is necessary to press a lever to have water dispensed. Float valve water bowls with an automatic float valve to maintain the water at a constant level are most suitable. SAC appear not to break through ice to obtain water and in cold climates heated waterers must be offered (Fowler, 2010).

In an ongoing experiment (unpublished data), we recorded drinking behavior of adult female llamas under temperate stable conditions. Water was offered in buckets with 10 l capacity *ad libitum*. The analyses of the 24 h video recordings revealed a large individual variation of drinking sessions/ day. Frequently, animals had only one drinking session/ day. Water intake occurred almost exclusively during the light hours of the morning and late afternoon while it was very rarely observed during the dark hours. Interestingly, llamas frequently raised the head and interrupted water intake for 10 to 30 sec, swallowed and masticated while looking around and then continued drinking. This behavioural pattern might indicate a vigilance strategy to prevent predation (Cappa et al., 2014).

## Total body water

Total body water in crias was found to be in the range of 70-72 % (Marcilese et al., 1994; Riek et al. 2007). Similar values were obtained by isotope labeling for kids (Makinde, 1993) and lambs (Dove, 1988). Dilution studies on adult llamas revealed lower values ranging between 57 to 70 % of the body mass (Rübsamen & Engelhardt, 1975; Riek et al., 2017). However, it is known that body water content decreases with age primarily due to fat accumulation (Ofteidal and Iverson, 1989).

### Water intake and water turnover

Water requirements can be covered by intake of drinking water, water content of feed and water produced by metabolic processes. Metabolic water is formed during the metabolic oxidation of hydrogen-containing organic nutrients. According to Van Es (1969), fat and protein produce 1.07 and 0.42 g of water/g material oxidized, respectively. Water is lost by four main avenues: evaporation (via skin and lungs), urine, feces and lactation.

Under temperate conditions, water intake of llama crias was found to be restricted to dams' milk during the first 10 weeks, whereafter they started to consume additional drinking water (Riek et al., 2007). In the study of Rüksamen and Engelhardt (1975), adult non pregnant llamas were kept in pens under temperate climate and fed a hay diet. The average amount of drinking water consumed was 42.5 ml /BW<sup>0.82</sup> per 24 h, being equivalent for example of 2.2 l for a 120 kg llama. At peak lactation, female llama dams drank approximately 9.8 l of water per day, whereas in late lactation they only consumed about 5.5 l (Riek et al., 2007) under temperate stable conditions and a hay based diet.

Body water turnover is an important parameter for characterization of water metabolism and is defined as the replacement of body water that is lost in a defined period of time. Species differ in their rates of water turnover, and in general animals adapted to dry environments display lower rates of turnover than those from temperate areas (King, 1979). Body water turnover is closely related to body weight (Rüksamen & Engelhardt, 1975). But due to the complex role of water in thermoregulation, energy metabolism and other processes, many factors influence body water turnover.

For estimation of water turnover in camelids, isotope labeling techniques have been applied (e.g., Rüksamen & Engelhardt, 1975; Riek et al., 2007, 2017). In an indoor experiment with crias aged between 17 and 128 days, the fractional water turnover decreased with increasing age. The highest fractional turnover at 17 days of age corresponded to a biological half-life of 4.3 days. With lower fractional turnover the biological half-life increased and accordingly the biological half-life corresponded to 7.9 and 12.6 days at later ages of 66 and 128 days, respectively. In contrast to growing llamas, Marcilese et al. (1994) showed that a lactating llama takes approximately 3.3 days to turn over half of the body water. This difference between growing and lactating llamas may be explained by the higher water demand due to milk production. In the study of Rüksamen and Engelhardt (1975), water turnover rates were 62.1 ± 8.8 ml/kg BW<sup>0.82</sup> per 24 h in adult non lactating llamas and were similar to those of goats (59.0 ± 10.9 ml/kg BW<sup>0.82</sup>).

For female llamas kept in year round outdoor housing in Germany, total water intake (from drinking water, preformed water ingested in food and metabolic water) was found to fluctuate considerably. Animals ingested more than double the amount of water (9.17 l/d) in summer compared to the winter season (4.22 l/d) (Riek et al., 2017). These differences can be attributed to an increased ingestion of drinking water during summer when animals had higher physical activities and the

environmental temperature increased above 25 °C. In addition, animals consumed more pasture in summer with a higher percentage of water compared to hay, which was the main food source in winter. Similarly, the water turnover rates in grazing llamas were found to be twice that recorded indoors (Rübsamen & Engelhardt, 1975).

As outlined before, water plays an important role in thermoregulation. SAC possess epitrachial sweet glands, which are especially numerous on the sparsely covered areas of the ventrum (Fowler, 2010). There was no detectable evaporative water loss in llamas kept at 20-25 °C. However, vapor loss increased to 100-240 g/m<sup>2</sup>/body surface/h when the ambient temperature was gradually raised to 40 °C (Allen & Bligh, 1969). Thus, substantial amounts of water will be dissipated with increasing ambient temperature, resulting in increased water ingestion and water turnover. In addition, the increased daily energy expenditure of grazing animals during summer months may also result in a greater production of metabolic water (Riek et al., 2017).

### **Water deprivation**

In the light of the vital importance of water for body functions, water deprivation poses a serious risk and animals have developed different mechanisms to prevent dehydration. Physiological strategies comprise decrease of renal urine flow, concentration of urine, and extraction of moisture from the feces. Among the channels of water loss from the body, less water tends to be lost through urine than through the feces, however, the scope for modulation of urine flow and concentration appears to be greater. In their normal habitat of the high Andes, SACs are confronted with conditions of poor vegetation, extreme temperatures and often limited water availability. For survival under these conditions, highly efficient adaptation constitutes a crucial prerequisite. The closely related Old World camels are legendary in their extremely efficient water metabolism and the question arises whether SAC possess similar physiological mechanisms to withstand dehydration.

In a comparative study, both llamas and goats reduced renal water losses when water intake was restricted (Rübsamen & Engelhardt, 1975). However, llamas were able to reduce their renal water loss to a greater degree than goats (17 vs. 29 % of total water loss). There were no significant differences between llamas and goats in their ability to concentrate the urine. Interestingly, under conditions of water restriction llamas were able to ingest more food than goats, thereby increasing their metabolic water gain. This may be an advantage when confronted with water deprivation. Guanacos demonstrated that they can withstand dehydration, but to a lesser degree and based on different mechanisms than the Old World camel (Mario & Morrison, 1963). When deprived of water for four days, while feed was offered ad libitum, a male guanaco lost 23.4 % of its body weight. However, body temperature was not elevated. The packed cell volume (PCV) increased from 30.6 to

43.6 %, indicating a reduction in blood plasma of about 40 %. In contrast, a camel only showed a 2.3 % reduction in blood plasma during similar dehydration underlining its better adaptation to dehydration (Mario & Morrison, 1963).

The high dehydration resistance in Old World camels might be explained by features of their erythrocytes which are able to avoid osmotic hazards by swelling to 240 % of their initial volume without rupturing. In other species, erythrocytes only swell to about 150 % (Fowler, 2010). It is assumed that South American camelids share some of these characteristics with Old World camels (Fowler, 2010). After severe dehydration, camels are able to consume high amounts of water at one drinking session to replace all the water lost (Engelhardt et al., 2006). Similarly, but to a lesser degree, a dehydrated guanaco (23.4 %) drank 9 l of water within 8 minutes, thus restoring 66 % of its deficit (Mario & Morrison, 1963). Such an amount of water would result in severe osmotic problems in other animals. The anatomy of the forestomach of camelids differs considerably from that of ruminants (Fowler, 2010). This fact may play an important role in slow absorption of the drinking water, allowing osmotic equilibrium to be established.

### **Saline water**

Due to global climatic changes, salinization of ground water and soil is an increasing worldwide phenomenon (IPCC, 2014; WWAP, 2015). In particular, Andean countries, such as Peru, are confronted with the challenge of soil degradation and salinization (Oldemann et al., 1991). Although sodium chloride salt (NaCl) is essential for regulating body water content, muscle and nerve functions and nutrient absorption (Suttle, 2010), excessive salt intake may affect feed and water consumption of animals or even cause severe health problems. Sheep have been shown to tolerate high salt concentrations between 5 % and 20 % in their diet (Digby et al., 2011). However, there is a different sensitivity to ingestion of salt either from food or drinking water (Masters et al., 2005). Drinking water with salt concentrations between 1.0 % and 1.3 % was tolerated by sheep (Peirce, 1968). However, ingestion of saline water with a salt concentration of 2 % or more led to severe reduction in food intake, and possibly death in sheep, goats and cattle (Peirce, 1957; Weeth and Haverland, 1961; Wilson and Dudzinski, 1973; Hamilton and Webster, 1987; McGregor, 2004).

It is obvious that Na<sup>+</sup> intake needs to be regulated by the animal because it is the major extracellular element influencing osmolarity of body fluids. A key physiological response to high salt ingestion is to increase water intake as shown e.g., for sheep (Peirce, 1957; Wilson and Dudzinski, 1973) to regain an isotonic state. However, this adaptive reaction is limited when only saline drinking water is available, as it increases salt load in the body fluids. In such cases, the animals have limited other adaptive responses to draw upon, beyond the excretion of increased amounts of salt via urine and a reduction of feed and water intake.

Camels demonstrated a remarkable high salt tolerance and tolerated more than 1.5 % salt in water (Abou El-Nasr et al., 1988), compared to sheep and goats (Abou Hussien et al., 1994; Assad and El-Sherif, 2002). Interestingly, the salt load controlling mechanisms differ between species (Abou Hussien et al., 1994). While sheep and goats reduce high salt loads by increased water intake, increased glomerular filtration rate and renal salt excretion through urine (Potter, 1968; Dunson, 1974), camels protect themselves from salt stress by reducing water consumption per unit of body size (Abou Hussien et al., 1994; Assad and El-Sherif, 2002). In a comparative study, camels, goats and sheep increased their total water intake by 130 ml/kg<sup>0.82</sup>, 376 ml/kg<sup>0.82</sup> and 500 ml/kg<sup>0.82</sup>, respectively, when exposed to water salinity of up to 1.7 %.

In this context, the Old World camel has evolved specific adaptive capacities. The kidneys are able to concentrate urine so that it becomes very thick. Salt content of camel urine may increase to twice the salt concentration of sea water (Fowler, 2010). This mechanism may partly explain the high salt tolerance in drinking water found in camels. However, SAC seem not to possess the same capacity, as llamas were not superior to goats in their ability to concentrate the urine (Rübsamen & Engelhardt, 1975).

There are anecdotal reports on guanacos, the wild ancestor of the llama, drinking saline water (Darwin, 1844). However, no studies are available on taste responses to sodium chloride in drinking water in llamas. For the study of taste discrimination in animals, different methods have been applied including electrophysiological methods (Bernard, 1964), recording of gustatory nerve impulses (Bell & Kitchell, 1966) or preference tests (Goatcher and Church 1970a,b). The principle approach in preference or choice tests is to provide a simultaneous choice of selections and then measure the animals' responses to each (Raffa et al., 2002). Goats have demonstrated their capacity to select a balanced diet according to their nutrient requirements (Fedele et al., 2002) and to avoid excessive intake of saline water (Runa et al., 2018) in a free choice experiment. The choice may be associated with sensory components (e.g., smell, taste) and post-ingestive effects. Interestingly, herbivores can recognize sodium directly by taste and show a specific appetite when it is in short supply (Denton, 1982).

In our ongoing study (unpublished), adult llamas were exposed to a two choice tests to evaluate their taste responses towards saline water. Drinking water with ascending salt concentrations (from 0.25 to 1.5 % NaCl) was offered in one container and unsalted tap water in the other. The position of the saline water was changed at random to counterbalance position effects. The reactions of the llamas suggest a rejection of saline water between 1.25 and 1.5 % concentration. Similar preferences for low salt concentrations of 0.85 % and 1.25 % were found for goats and sheep by Goatcher and Church (1970b). However, two choice preference tests only evaluate the short term taste discrimination and sensitivity thresholds. To determine the long-term salt tolerance in drinking water of llamas, further studies are required.

## Conclusion

SAC have evolved efficient mechanisms using water indicating an evolutionary adaptation to limited water supply of the high Andes. These strategies to withstand water scarcity appear to be partly superior to those of goats. Interestingly, SAC and Old World camels share some physiological features with regard to their water efficiency, but they are less developed in SAC. As water is an essential nutrient, SACs should have ad libitum access to clean drinking water. Animals adjust their intake according to their requirements. In particular during lactation, unrestricted water supply is essential. In the case of water scarcity, dehydration and hyperthermia may result (Fowler, 2010).

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## Meat and Fibre Production, Fibre Metrology





# Carne y charqui de llama

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**Abstract.** This paper summarizes several studies carried out over many years on the meat of South American camelids and llama meat in particular. The overview includes aspects of birth, growth, weaning, optimum age of slaughter, classification of the carcass, major and minor cuts, quality of the carcass and meat processing of charqui (traditional conservation of the meat), and technical aspects that have contributed to the legal commercialization of South American camelid meat in Bolivia.

**Resumen.** El presente trabajo es una recopilación de investigaciones realizadas durante muchos años sobre la carne de camélidos sudamericanos y específicamente de carne de llama. Se incluyen estudios desde el nacimiento, crecimiento, destete, edad óptima de faenamiento, clasificación de la canal, cortes mayores y menores, calidad de la canal, procesamiento de la carne en forma de charqui (conservación tradicional de la carne) y aspectos técnicos que han contribuido en la comercialización legal de carne de camélidos sudamericanos en Bolivia.

**Keywords:** meat, carcass quality, llamas, charqui

## Introducción

La problemática de la alimentación humana en el mundo presenta dos diferentes visiones: la de los países desarrollados en los que puede darse mal nutrición puntualmente, y la de los países pobres en los que se dan problemas de desnutrición. En la región de Altos Andes de Bolivia viven al alrededor de 70,000 familias. Están involucradas en la producción de alrededor de tres millones de cabezas de camélidos sudamericanos (CS), con una producción media de carne fresca de 13.884,19 TM/año y de 682.26 TM/año de charqui (carne salada y secada al sol), siendo importante también la producción de vísceras para el consumo humano. La cría de los CS garantiza el sustento alimenticio de estas poblaciones y desde hace menos de una década, la carne de CS en Bolivia se comercializa en forma legal. Antes de ello, la desinformación de sus características nutritivas y la

atribución de innumerables enfermedades zoonóticas, fueron causantes de grandes prejuicios para su comercio por décadas, a pesar de que históricamente, el consumo de carne fresca y procesada (Charqui), por cientos de años ha garantizado la salud y el bienestar de habitantes con culturas ancestrales en los Andes.

## Metodología

El presente artículo, resume resultados de varios trabajos de investigación desarrollados en Bolivia, que se enmarcan dentro de metodologías estandarizadas para la obtención de carne de CS y en específico de carne de llama, desde el nacimiento del animal, el proceso de crecimiento, la edad óptima de faenado, crecimiento alométrico, proceso de maduración de la carne, parámetros bromatológicos, calidad de la carne, clasificación de la canal, cortes mayores y menores, análisis microbiológicos, procesamiento del charqui (método de conservación de la carne mediante el secado) y análisis químicos y bromatológicos de este subproducto.

## Resultados

### *Curva de crecimiento*

El crecimiento y desarrollo en los animales es una característica propia de las especies. Así Hammond (1997), define al crecimiento como el aumento de peso de los animales desde el nacimiento hasta su estabilización en la edad adulta. Se determinó el ritmo de crecimiento en llamas, que nacen en un rango de 8 a 12 kg de peso vivo (PV). Al destete alcanzan un promedio de 30 a 35 kg de PV y a los 21 meses logran un peso de 63 a 72 kg para machos y 58-64,5 kg para hembras, no existiendo diferencias significativas entre machos castrados y enteros. La máxima ganancia de peso diario ajustado en pradera nativa fue de 129.11 g/d y de 114.65 g/d para machos enteros y castrados respectivamente. El crecimiento se ajusta a una curva sigmoidea (Figura 1), en la que se pueden distinguir dos partes: a) fase acelerada al principio de la vida, de crecimiento rápido y elevado por unidad de tiempo, y b) la fase auto-inhibición. En la pubertad el crecimiento se desacelera y las ganancias de peso son cada vez más pequeñas hasta que finalmente el animal alcanza la madurez.

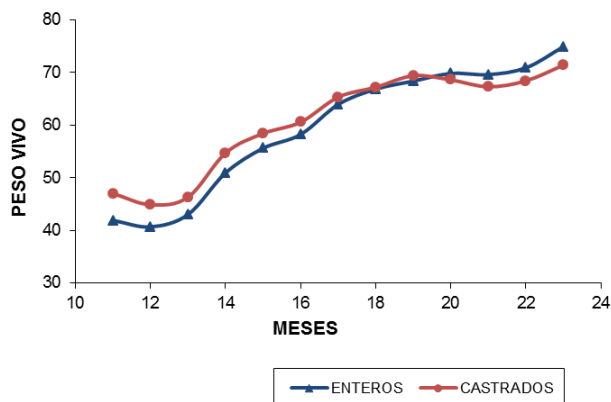
Se efectuaron dos métodos para evaluar el crecimiento: uno basado en el peso vivo y datos seriados del faenamiento. Para el ajuste del peso vivo (Y) en función de la edad (x) se utilizó una regresión polinómica de grado tres.

$$\text{Ecuación 1: } Y = -0.0077 x^3 + 0.2711 x^2 + 0.7488 x + 8.1006 \quad R^2 = 0.88$$

$$\text{Ecuación 2: } Y = -0.0041 x^3 + 0.1045 x^2 + 2.6005 x + 7.8848 \quad R^2 = 0.90$$

$$\text{Ecuación 3: } Y = -0.30x^2 + 10.06x + 14.28$$

Los animales presentaron diferencias altamente significativas ( $P < 0.01$ ) entre enteros y castrados a los 11 y 12 meses. A partir de los 13 meses y hasta los 15 meses de edad presentan diferencias menos significativas ( $P < 0.05$ ).



**Figura 1:** Curva de crecimiento en llamas de sexo macho, enteros y castrados

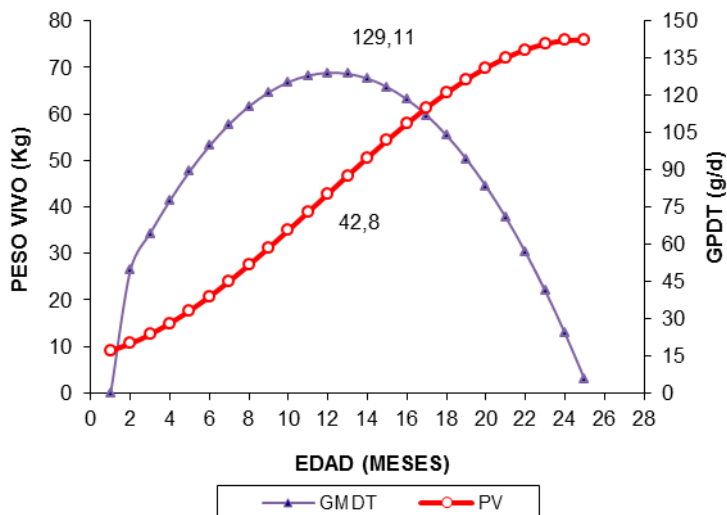
El cuadro 1 muestra las medidas biométricas tomadas desde los 12 hasta los 23 meses de edad de los animales.

**Cuadro 1:** Medidas biométricas (cm) en llamas de sexo macho

Carácter	12 Meses		23 Meses		Incremento	
	Enteros	Castrados	Enteros	Castrados	Enteros	Castrados
Perímetro torácico	85.55	86.76	101.33	100.78	15.78	14.02
Longitud de tronco	79.32	81.56	92.36	91.11	13.04	9.55
Ancho de anca	20.05	20.31	25.03	24.97	4.98	4.66
Altura a la cruz	84.41	85.76	97.11	96.88	12.7	11.12

#### *Ganancia de peso diario*

La máxima ganancia de peso diario en machos enteros de llama fue de 129.11 g/d, que se dio a la edad de 12 meses, con un peso vivo de 42.82 kg, lo que supone el 36 % del peso adulto (Figura 2). A partir de esta edad comienza el denominado crecimiento lento. En machos castrados la máxima ganancia de peso fue de 114 g/d a la edad de 9 meses, con un peso vivo de 36.72 kg, lo que corresponde con el 31 % del peso adulto. En otras especies la máxima ganancia de peso diario, coincide con el inicio de la pubertad Lloyd (1982). Sumar (1991) define el inicio de la pubertad en alpacas a partir de los 11 meses.



**Figura 2:** Peso vivo (Kg, PV) y ganancia de peso diario (g/d, GMDT) en llamas machos enteros

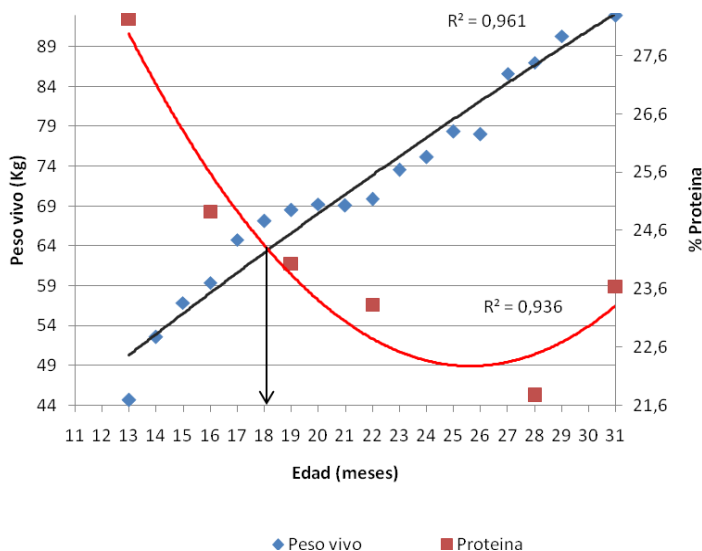
#### *Determinación de la edad óptima de faenamiento*

Se determinó la edad óptima de faenamiento entre los 18 y los 21 meses, en el punto de equilibrio en el porcentaje de proteína entre la canal y el peso vivo, y con un periodo de acabado de entre 45 y 60 días.

La Figura 3 muestra la relación de parámetros de productividad y calidad de la carne de llama para determinar la óptima edad de faenamiento.

#### *Medidas zométricas y sus correlaciones*

Las correlaciones entre las medidas biométricas (perímetro torácico, longitud de tronco, ancho de ancas y altura a la cruz) resultaron entre altas y muy altas: entre perímetro torácico y peso vivo fue de  $r = 0.94$ , entre peso vivo y carcasa en caliente  $r = 0.97$ , entre peso vivo y canal en frío  $r = 0.97$ , siendo inferior la existente entre la altura a la cruz y la longitud de cuello, que presentó un valor de  $r = 0.59$ . La correlación entre el peso vivo y la altura a cruz y el ancho de ancas fueron  $r = 0.74$  y  $r = 0.87$  respectivamente. El crecimiento alométrico en la fase I demuestra que existe pleno desarrollo en las extremidades anteriores y posteriores, propio de animales jóvenes. En la fase II el lomo y costilla presentan un crecimiento mayor longitudinalmente. Hacia los 25 meses el incremento en peso tiende a distribuirse en mayor medida en los lomos y cuello.

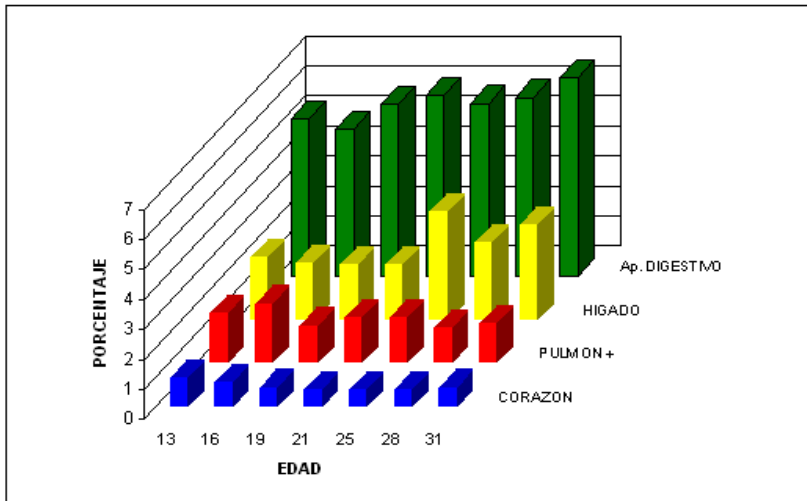


**Figura 3:** Determinación de la edad óptima de faenamiento

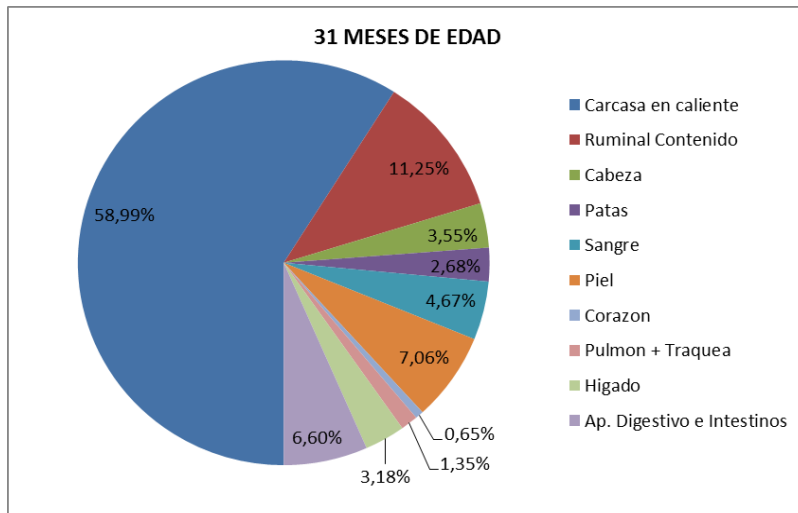
#### *Crecimiento alométrico de órganos y vísceras*

Se obtuvo una correlación muy alta ( $r = 0.86$ ) entre el crecimiento en peso del conjunto del sistema digestivo y el peso vivo del animal. La media del peso del tracto gastrointestinal a los 13 meses fue de 2.790 g y alcanzó un valor medio de 5.600 g a los 31 meses de edad siendo el incremento de 2810 g durante este periodo de crecimiento. La correlación entre el peso vivo y la piel fue muy alta ( $r = 0.88$ ). Sin embargo la correlación del crecimiento con el peso de la cabeza fue muy baja ( $r = 0.27$ ), al igual que con el peso de la sangre ( $r = 0.17$ ).

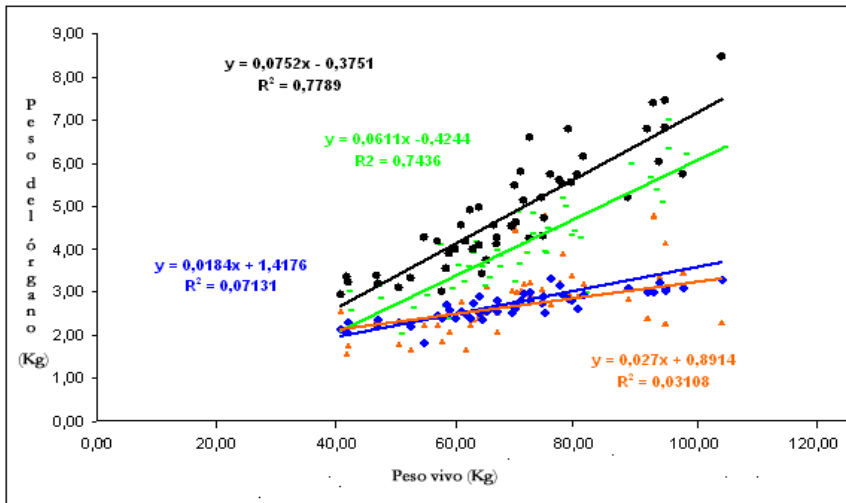
La Figura 4 muestra el desarrollo alométrico relativo de los principales órganos de la cavidad torácica y abdominal, comprendida entre los 13 y 31 meses de edad. Hammond (1960) estableció que los diferentes órganos, tejidos y piezas anatómicas no tienen la misma velocidad de crecimiento. Los nutrientes se distribuyen siguiendo prioridades. La Figura 5 muestra el porcentaje que ocupan los diferentes órganos en el organismo adulto. El orden en que los distintos tejidos culminan su crecimiento es: nervioso, óseo, muscular y graso. La Figura 6 muestra las correlaciones entre el peso vivo y el desarrollo de los órganos. La correlación entre el peso vivo y el de la piel fue  $r = 0.88$ , entre el peso vivo y el del aparato digestivo  $r = 0.86$ , entre el peso vivo y el de la cabeza  $r = 0.26$ , y entre el peso vivo y el de la sangre  $r = 0.18$ . La correlación entre el peso vivo y el de la canal en caliente fue  $r = 0.97$  y entre el peso vivo y el de la canal fría  $r = 0.97$ .



**Figura 4:** Desarrollo alométrico (%) de los órganos de la cavidad abdominal y torácica en función de la edad (meses)



**Figura 5:** Distribución porcentual de órganos y tejidos en un animal adulto de 31 meses de edad



**Figura 6:** Correlación entre el peso vivo y el de otros órganos

#### *Rendimiento a la canal*

Los valores promedios de rendimiento a la canal caliente y fría fue 57.48 % y 53.54 % respectivamente, similares a los reportados por Bravo (1981) de 58.1 % y 59.6 % en animales enteros y castrados, como muestra el cuadro 2 desde los 15 hasta los 25 meses de edad.

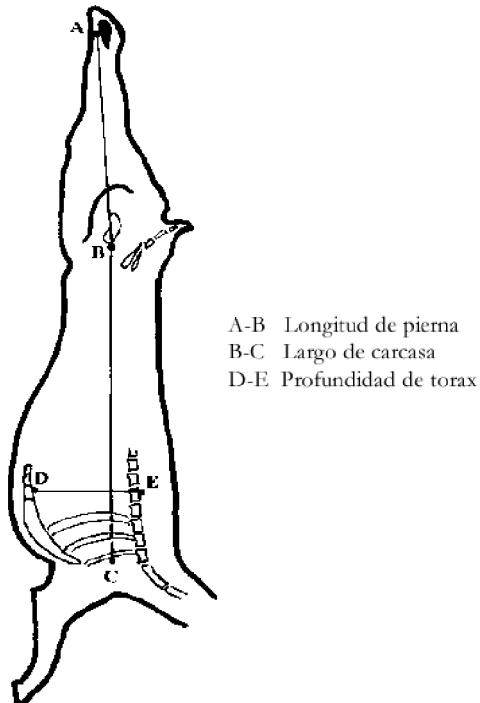
**Cuadro 2:** Rendimiento (%) de la canal caliente y frío según edad

Edad (meses)	Rendimiento (%)	
	Canal caliente	Canal frío
13	57.68	54.33
16	60.03	54.76
19	55.29	51.63
22	59.21	54.21
25	55.59	52.38
Promedio general	57.56	53.46

El Cuadro 3 y la Figura 7 muestran las diferentes medidas realizadas en la media canal de llamas machos faenadas en diferentes períodos de crecimiento. Las medidas en la canal (longitud de la canal, perímetro torácico, longitud del cuello y longitud de pierna), muestran una correlación alta a muy alta con las variables pesos de lomo grueso, pierna, brazuelo y cuello. Las variables longitud del cuello y peso de lomo fino presentaron un coeficiente de correlación inferior al esperado de  $r = 0.5$ .

**Cuadro 3:** Medidas de la canal (cm) en machos enteros (E) y castrados (C) según edad

Característica (cm)	Edad (meses)							
	13		16		19		22	
	E	C	E	C	E	C	E	C
Longitud de la canal	72	74	77	76	78	78	81	78
Profundidad de tórax	24	24	25	26	28	28	28	28
Longitud de la pierna	51	51	52	55	60	56	58	58



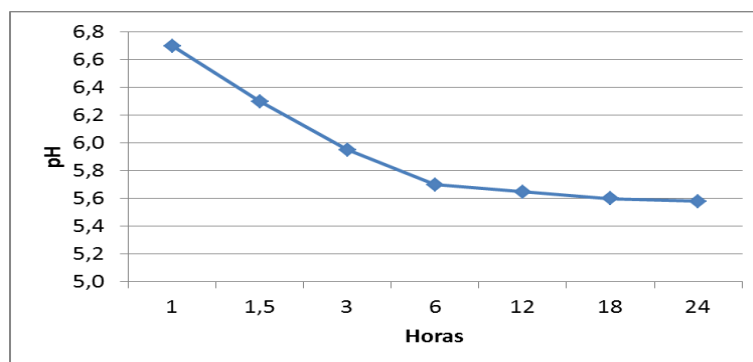
**Figura 7:** Medidas de la canal

### *Cortes comerciales*

Los pesos de cortes comerciales (pesos de lomo grueso, lomo fino, pierna, brazuelo y cuello) presentaron un coeficiente de correlación alto a muy alto con las medidas biométricas, a excepción del el peso del costillar que presentó un coeficiente de correlación muy bajo con las medidas biométricas.

### *Características físicas de la carne; pH*

El valor de pH tomó un valor de 5.5 24 horas después del sacrificio, siendo el pH final bajo (5.55) 48 horas después del sacrificio. La Figura 8 muestra los valores de pH cercanos a la neutralidad (pH = 7) o alcalinos (pH > 7), que favorecen la reproducción de microorganismos, entre los cuales están los que originan putrefacción (Aldana 1984).



**Figura 8:** Variación del pH de la carne en 24 horas

### *Capacidad de retención hídrica*

La canal caliente presenta proteínas con una alta capacidad de cohesión de agua, ya que aún no se ha formado el complejo actomiosina (Lieven 1986). La carne caliente en general presenta un pH = 6.2 lo que también favorece la cohesión de agua y grasas existiendo además una concentración limitada de ATP. Esto en vacunos sucede a las 4 horas y en cerdos en la primera hora. El análisis de varianza para la retención de agua a en la primera hora después del sacrificio mostró diferencias altamente significativas ( $P < 0.01$ ) para el factor edad, mientras que en las 6, 12, 24 y 48 horas siguientes no mostraron diferencias significativas ( $P < 0.01$ ) para los efectos edad, condición e interacción edad por condición (Cuadro 4 y Figura 9).

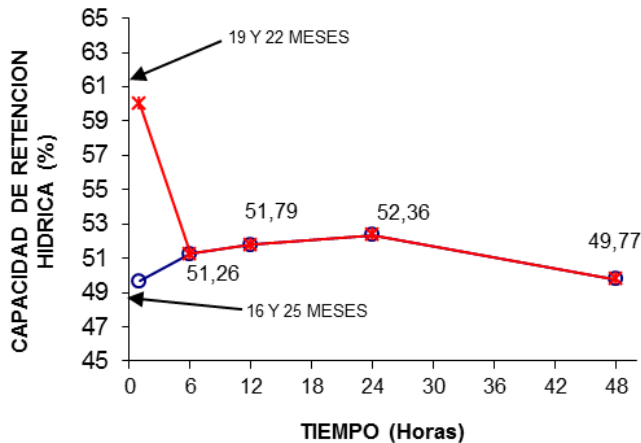
**Cuadro 4:** Capacidad de retención hídrica en la carne de llama

Edad (meses)	Tiempo en horas				
	1 <sup>ns</sup>	6 <sup>ns</sup>	12 <sup>ns</sup>	24 <sup>ns</sup>	48 <sup>**</sup>
16	6.60	6.52	5.91	5.64	5.59 ab
19	6.67	6.49	5.96	5.55	5.52 b
22	6.75	6.47	5.89	5.57	5.54 b
25	6.83	6.67	6.15	5.62	5.64 a
Promedio	6.71	6.53	5.97	5.59	a= 5.61 b=5.55

\*\* Diferencias altamente significativas para el factor edad ( $P < 0.01$ )

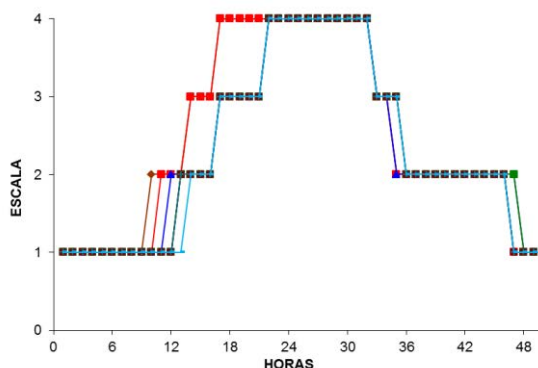
<sup>ns</sup> Diferencias entre edades no significativas ( $P < 0.01$ )

<sup>a,b</sup> Valores con letras iguales no tienen diferencias significativas ( $P < 0.01$ )

**Figura 9:** Capacidad de retención hídrica en carne de llama

#### *Rigidez cadavérica y maduración de la carne*

Al examen físico la rigidez cadavérica es instaurada por completo entre 16 a 20 horas, con un pH aproximado de 5.78. La rigidez cadavérica culmina cuando las articulaciones vuelven a ser flexibles a la presión (Aldana 1984). En la llama ocurre transcurridas de 32 a 42 horas (dura de 20 a 24 h) a una temperatura promedio de 15.29 °C. La ruptura del complejo actomiosina de acuerdo a Varnam (1995), es el período donde la maduración de la carne ha concluido, y la carne está lista para el consumo, como muestra la Figura 10.



**Figura 10:** Proceso de rigidez cadavérica después del sacrificio

La maduración de la carne concluye al mismo tiempo que la rigidez cadavérica al cabo de las 32 a 42 horas. La carne madurada y cocinada presenta una consistencia suave a la masticación por el hecho de que existe la degradación de proteínas miofibrilares que acompañan a una reducción de la dureza (Penny 1984). Esta degradación proteolítica según Varnam y Sutherland (1995) es el principal mecanismo de ablandamiento de la carne.

#### *Evaluación de componentes químicos de la carne*

El Cuadro 5 muestra la variación del contenido bromatológico de la carne de llama entre los 13 y los 22 meses de edad.

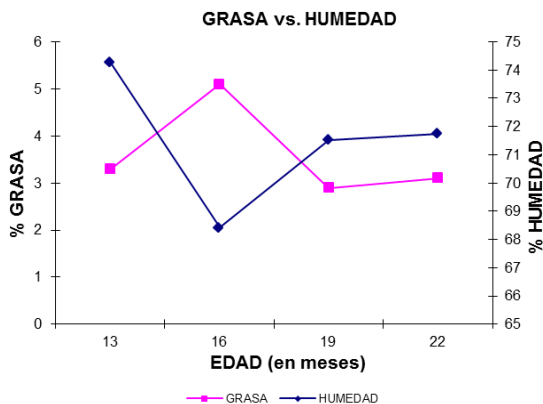
**Cuadro 5:** Componentes químicos de la carne de machos de llama enteros y castrados

Edad (meses)	Humedad (%)	Ceniza (%)	Grasa (%)	Proteína (%)	Colesterol (mg/100 g)
Enteros					
13	74.77	1.97	3.25	26.93	54.75
16	69.19	1.20	5.56	24.39	57.50
19	71.44	1.12	3.03	24.19	43.00
22	71.98	1.25	3.19	23.04	68.78
Castrados					
13	73.79	2.01	3.32	29.53	58.33
16	68.45	1.23	4.65	25.46	56.33
19	71.62	1.16	2.75	24.13	41.33
22	71.50	1.29	3.01	23.63	81.47

El mayor contenido de humedad se presenta en los machos enteros de 13 meses y el menor en los machos castrados de 16 meses (prueba de comparación de medias, Duncan).

### Contenido de lípidos

El bajo porcentaje de grasa en la carne de llama puede ser efecto de las condiciones del medio ambiente. La curva de la Figura 11 indica, que la proporción de agua en los músculos es menor cuanto mayor es el nivel de grasa en carne de llamas. El porcentaje de grasa varía en otras especies siendo 4.84 % en vacunos, 6.53 % en ovinos, 5.13 % en alpacas y 20.06 % en porcinos.



**Figura 11:** Niveles inversamente proporcionales de agua y grasa en carne de llama en función de la edad en meses

### Contenido de colesterol

La media del contenido de colesterol en carne de llama fue 42.16 mg/100 g en animales en edad de sacrificio. Es importante destacar que la carne de los CS tiene un menor contenido de colesterol que la de otras especies domésticas.

**Cuadro 6:** Niveles de colesterol a diferentes edades

Edad (meses)	Colesterol (mg/100 g)
13	56.54 <sup>b</sup>
16	57.08 <sup>a</sup>
19	42.16 <sup>b</sup>
22	74.79
Promedio	<sup>a</sup> = 56.81 <sup>b</sup> =49.35

<sup>a,b</sup> Valores con letras iguales no tienen diferencias significativas ( $P < 0.01$ )

El mayor contenido de colesterol en carne se encontró en animales de 22 meses y el menor contenido en animales de 19 meses (diferencias significativas mostradas por comparación de medias Duncan). El valor promedio fue de 56.81 mg/100 g, similar a lo reportado por Garnica (1993b) de 56.49 mg/100 g para alpacas. En llamas de 19 meses de edad el contenido de colesterol fue 42.16 mg/100 g, valor inferior al reporte de Garnica (1993b).

*Ácidos grasos*

Los ésteres del glicerol y los ácidos grasos conforman el 99 % de la grasa intramuscular. Sólo se encontraron tres o cuatro ácidos grasos en la carne de llama (oleico, palmítico, esteárico) y cuatro tipos de glicéridos (Cuadro 7).

**Cuadro 7:** Niveles de ácidos grasos en la carne de llama

Condición	Ácido Graso				
	Mir C:14	Pand C:15	Pal C:16	Est C:18	Ara C:20
Entero	0.061	0.036	0.334	0.721	0.353
Castrado	0.062	0.0	0.309	0.663	0.358
Promedio	0.061	0.018	0.321	0.692	0.355

En carnes de otras especies existen rangos como el caso del ácido mirístico de 0.9 % en vacunos a 3.26 % en cerdo, el palmítico de 22.34 % en cerdos a 24.14 % en pollos, el esteárico de 4.28 % en cerdo a 13.18 % en pollos y el araquidónico de 0.67 % en pollos a 2.30 % en cordero.

*Contenido de proteína*

El Cuadro 8 muestra que el valor promedio en la carne de llama fue 24.54 % de proteína, similar al 24.82 indicado por Pinto (1975). En otras especies el porcentaje de proteína es (Rivera *et al.*, 1996): vacuno (21.01 %), ovino (18.91 %), porcino (19.37 %) y alpaca (21.88 %). El valor de contenido proteico de la canal varía entre el 24.3 % y 25.2 % en machos enteros y castrados respectivamente.

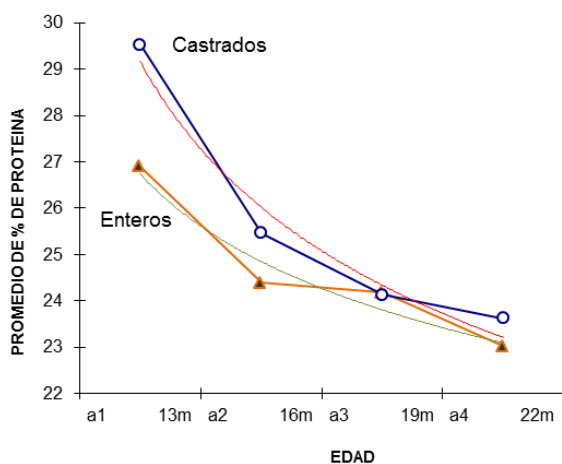
**Cuadro 8:** Porcentaje de proteína en la carne de llama

Edad (meses)**	Condición **	Proteína (%)	
13	Castrado	29.53	
13	Entero	26.93	
16	Castrado	25.46	a
16	Entero	24.39	a b
19	Castrado	24.19	a b
19	Entero	24.13	a b
22	Castrado	23.63	b
22	Entero	23.04	b
Promedio		24.54	a

\*\* Diferencias altamente significativas ( $P < 0.01$ )

<sup>a,b</sup> Valores con letras iguales no tienen diferencias significativas ( $P < 0.01$ )

La Figura 12 muestra la curva donde se observa una bajada en contenido de proteína hasta los 22 meses. Eso significa que a medida que incrementa la edad del animal, la proteína en la carne tiende a disminuir. Los resultados coinciden con Garnica (1993a), quien señala que a mayor edad existe menor contenido de proteína en carne de alpaca. Se observó una mayor ganancia de peso diario a 14 meses, así como un alto contenido proteico a los 13 meses de edad en llamas. Esto fue corroborado por Di Marco (1993) quien indica que la ganancia de peso en animales jóvenes se debe a la mayor retención de proteína. En animales adultos la ganancia de peso se debe al incremento de grasa de depósito en decremento de la retención proteica.



**Figura 12:** Niveles de proteína en la canal (%) según la edad de los animales

#### *Composición química de las vísceras*

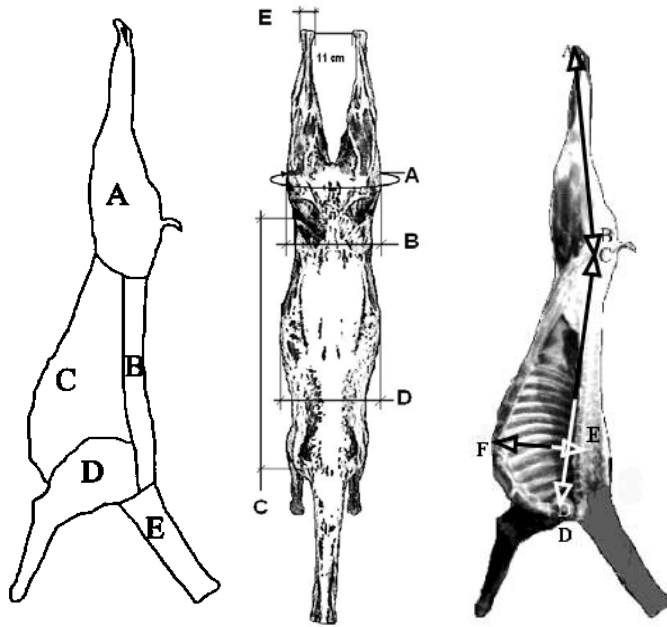
En los Andes, las vísceras son una alternativa nutricional importante para el consumo humano. Se ha publicado un rango del contenido de grasa de 3.44 a 8.34 %, proteínas de 19.0 a 21.84 %, colesterol de 147.3 a 328.40 mg/g, y un aporte energético de 95.29 a 365 cal/100 g (Cuadro 9).

**Cuadro 9:** Análisis bromatológico de órganos y vísceras de llama en fresco

Componentes		Hígado	Estómago	Intestino	Pulmón
Humedad	%	72.83	66.12	78.30	80.13
Grasas	%	3.65	8.34	5.27	3.44
Proteínas (*6.25)	%	21.84	21.20	20.72	19.50
Colesterol	mg/100 g	328.40	147.30	286.60	274.90
Valor Energético	cal/100 g	105.02	138.69	365.86	95.29

*Sistema de clasificación de canales de llama*

La Figura 13 muestra las regiones de evaluación de cobertura de grasa. La Figura 14 muestra la clasificación por conformación muscular y las medidas externas e internas en canales de llama.



**Figura 13:** Canales de llama clasificadas por cobertura de grasa

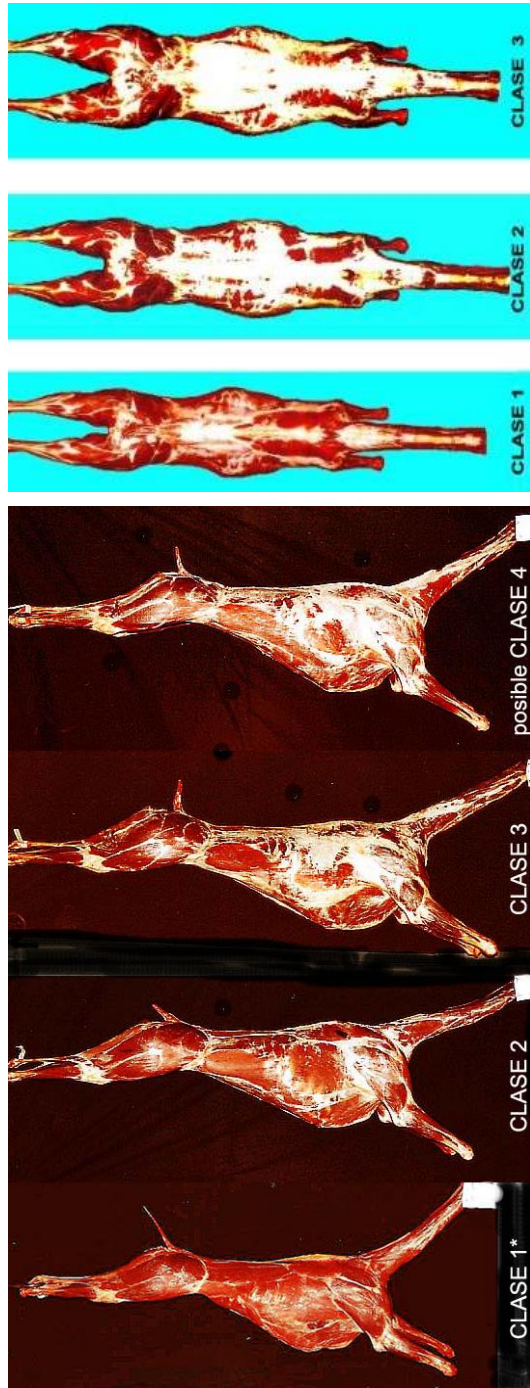


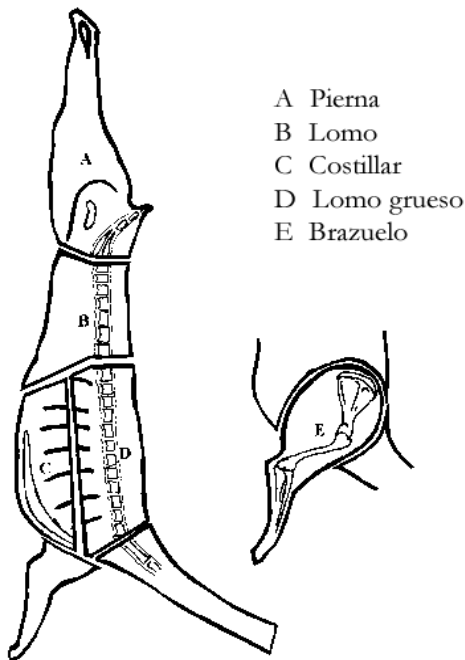
Figura No. 14: Clasificación de canales por conformación de músculos

*Cortes comerciales en canales de llamas*

En el Cuadro 10 y en la Figura 15 se presentan algunos resultados sobre cortes comerciales de canales de llamas.

**Cuadro 10:** Cortes comerciales en machos enteros y castrados

Corte comercial	Fase II (19, 22 y 25 meses)			
	Enteros		Castrados	
	Media (Kg)	Canal frío (%)	Media (Kg)	Canal frío (%)
Lomo grueso	1.97	6.76	2.79	7.83
Lomo fino	1.83	6.28	2.22	6.23
Pierna	5.13	17.57	6.16	17.26
Brazuelo	2.66	9.11	3.55	9.95
Costillar	1.72	5.90	1.36	3.82
Cuello	2.56	8.77	3.50	9.81

**Figura 15:** Cortes comerciales en canales frías de machos de llamas

**Cuadro 11:** Peso (Kg) y rendimiento (%) de los cortes mayores de llama (media y desviación estándar, DS)

Cortes mayores	Peso (Kg)		Rendimiento/ carcasa (%)	
	Media	DS	Media	DS
Cuello	2.96	0.17	9.69	0.85
Paleta	3.14	0.33	10.50	0.55
Paleta	3.11	0.22	10.43	0.71
Paletas	6.25	0.50	20.92	0.95
Medio lomo	2.94	0.38	9.83	0.86
Medio lomo	2.84	0.41	9.49	0.88
Lomo	5.78	0.61	19.32	0.53
Medio costillar derecho	1.97	0.41	6.53	0.81
Medio costillar izquierdo	1.97	0.33	6.54	0.61
Costillar	3.94	0.72	13.07	1.25
Pierna	4.98	0.49	16.66	0.60
Pierna	5.02	0.56	16.76	0.65
Piernas	10.00	1.03	33.42	1.05
Total cortes mayores	28.93		96.42	

#### *Proceso tradicional de conservación de la carne de llama (charqui)*

Etimológicamente la palabra “Charqui” proviene de la lengua Aimara, que significa “seco”. La técnica milenaria de los Altos Andes consiste en deshidratar la carne magra y saturada en sal, expuesta al sol y a heladas (- 2 °C) en áreas ventiladas, por un periodo de 7-15 días, permitiendo la deshidratación gradual y controlada de la superficie de los tejidos. Su durabilidad no está determinada, pero algunas experiencias indican una durabilidad de hasta 20 años en ambientes fríos y baja humedad. Comparada con las técnicas modernas de conservación de la carne, se trata de un proceso muy parecido a la liofilización, pero a costos bajos en comparación a éste y a otros métodos de conservación.

#### *Rendimiento de charqui*

El proceso comienza con el descarte de animales, se adecuan las prácticas de faenamiento, proceso de oreo y maduración de la carne. El proceso requiere el desgrasado de la carne con el fin de evitar el ranciamiento y olor desagradable del producto. Se realizan filetes o cortes finos de las partes más musculosas, de aproximadamente 1 cm. Luego se procede al salado donde se utilizan dos métodos: el primero usa aproximadamente 40 a 70 g de sal por Kg de carne y el segundo es por inmersión de la carne en salmuera durante 2 o 3 días. El secado de la carne dura 5 a 15 días por acción directa del sol, pérdida de agua, que junto con los

cambios de temperatura hasta heladas de  $-2\text{ }^{\circ}\text{C}$ , ayudan a inhibir el desarrollo de la actividad microbiana. El rendimiento de canal a charqui es del 34 % con un contenido de grasa de 5.96 % y de proteínas de 60.27 %, haciendo que el producto sea de alta calidad nutritiva (Cuadro 12 y 13).

**Cuadro 12:** Rendimiento de charqui según edad

Categoría	Edad (años)	Peso vivo promedio (Kg)	Peso canal (Kg)	Rendimiento canal (%)	Rendimiento charqui (%)
Adultos ♂	> 7	75	38.0	52.0	24.5
Adultos ♀	> 7	62	31.8	51.3	23.0
Jóvenes ♂	2-3	58	30.9	53.4	22.7
Jóvenes ♀	2-3	50	26.0	52.0	23.0

**Cuadro 13:** Composición química del charqui

Componentes		Categoría		
		Entero	Castrado	Media
Humedad	%	7.58	8.95	8.26
Grasa	%	8.77	9.32	9.04
Proteínas	%	73.30	70.50	71.90
Colesterol	mg/100 g	104.4	272.29	188.34
Valor energético	cal/100 g	326.86	318.41	322.63

#### *Nueva metodología del procesamiento de charqui*

Una innovación en el proceso de la elaboración de charqui se ha realizado en túneles de polietileno, reduciendo el tiempo del secado de 15 días a menos de una semana. El uso de este secador solar permite una mejor calidad higiénico-sanitaria para la elaboración del charqui (Figura 16).

#### *Análisis microbiológico en la elaboración del charqui*

Los análisis microbiológicos en unidades formadoras de colonia por gramo, demuestran la calidad sanitaria del producto, donde las cantidades de sal y el tiempo de secado son factores que minimizan el desarrollo de patógenos en la elaboración del charqui (Figura 17). La relación del número de patógenos en carne fresca y charqui es menor al permitido. Los mesófilos en carne fresca se reducen en el proceso de charqui hasta el punto de no observarse desarrollo de colonias. Según las normas de IBNORCA, los valores hallados en carne fresca fueron  $1,00\text{E}+06$  mientras que en charqui fueron  $1,00\text{E}+03$ , en ambos casos el número de bacterias es menor al permitido.

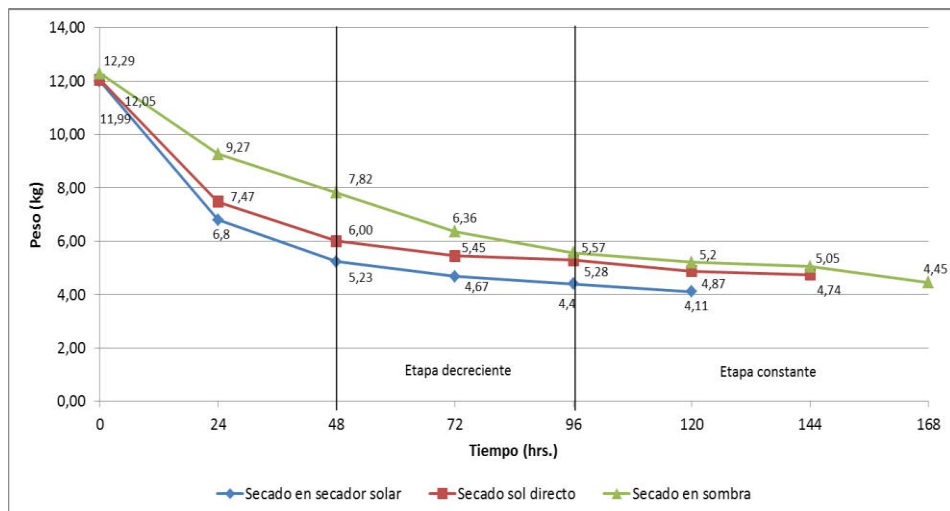


Figura 16: Evolución del peso de carne por tiempo de secado y tratamiento en la elaboración del charqui

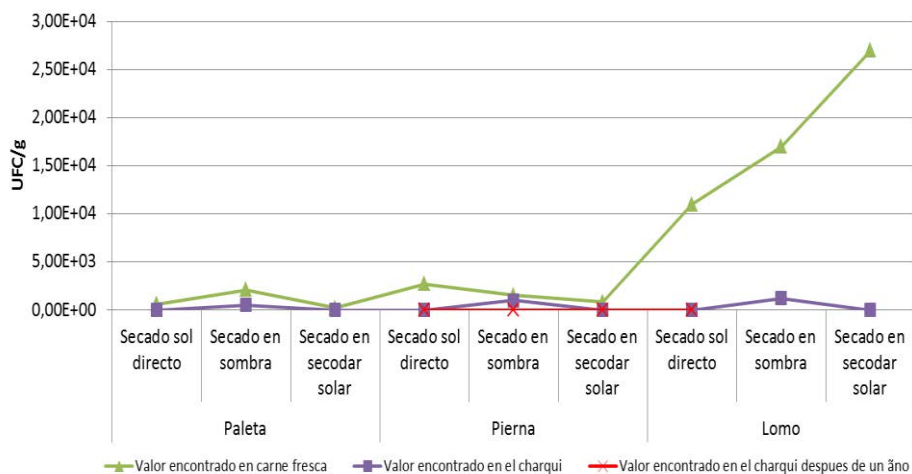
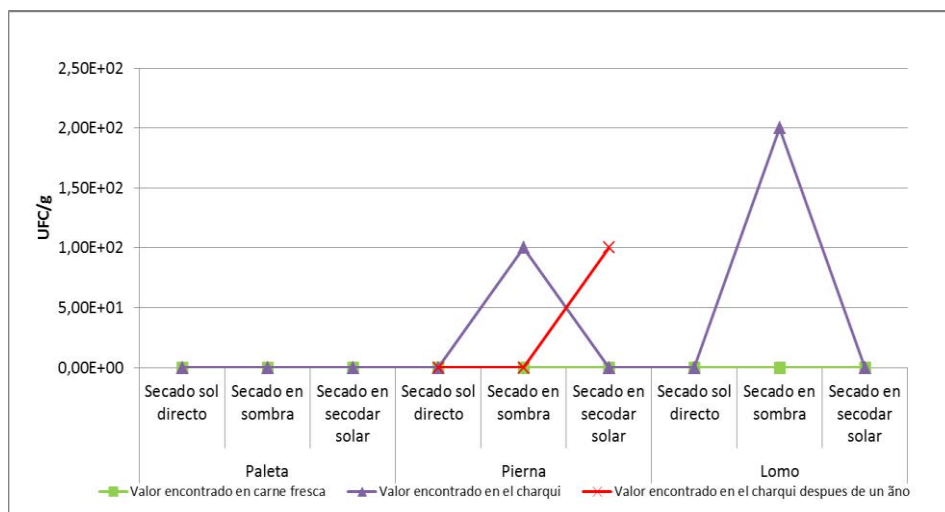


Figura 17: Relación de Mesófilos en carne fresca y charqui

Los causantes de olores y aromas extraños, así como la decoloración de la superficie se deben a la existencia de mohos y levaduras. La exposición solar “neutraliza” la proliferación de colonias de levaduras y mohos (Figura 18). La presencia de *Staphylococcus aureus* fue inapreciable en la carne fresca, mientras que en charqui tuvo una proliferación de  $10^2$  UCF/g, valor que se encuentra en el rango admitido. No se encontraron coliformes totales ni *Clostridium pertringens* ni en carne

fresca ni en charqui. Se observó total ausencia de *Escherichia coli* en carne fresca y charqui, microorganismos que se utilizan como indicadores de contaminación de origen fecal. Igualmente, se observó ausencia de *Salmonella* en todos los casos. El proceso de elaboración de charqui mostró que el producto obtenido era inocuo, sin la presencia de patógenos y dentro de los niveles permitidos para productos elaborados. La técnica elimina todo tipo de patógeno, similar a una liofilización, evitando todo tipo de riesgo de contaminación.



**Figura 18:** Relación de mohos y levaduras en carne fresca y charqui

**Cuadro 14:** Análisis físico-químico de las vísceras en fresco y en charqui

Componentes	Vísceras							
	Hígado		Estómago		Intestinos		Pulmones	
	Charqui	Fresco	Charqui	Fresco	Charqui	Fresco	Charqui	Fresco
Humedad %	10.20	72.83	11.32	66.12	12.67	78.30	14.67	80.13
Grasa %	11.06	3.65	15.26	8.34	12.53	5.27	10.70	3.44
Proteína %	64.23	21.84	61.47	21.20	62.02	20.72	60.03	19.05
Colesterol mg/100 g	135.50	328.02	201.90	147.30	342.50	286.60	325.70	274.90
Valor energético cal/100 g	364.67	105.02	218.60	138.69	405.70	365.86	165.60	95.29

El charqui de vísceras es asimismo muy común en los Andes. Su análisis muestra los siguientes valores: grasas del 10.2 % a 14.67 %, proteínas del 60.03 % a 64.23 %, colesterol de 135.1 a 342.5 mg/100 g, y un aporte energético de 165.6 a 405.7 cal/100 g (Cuadro 14).

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# Wool Scouring in Europe: Urgent and Ecological Solutions

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**Abstract.** Wool scouring is the essential intervening step between agriculture, which provides the fleeces, and the craft and textile industries, which make them into finished products. However, there are many future concerns regarding the problematic situation and recent disappearance of many scouring plants across Europe. If this stage, already fragile in Europe, would break down, the whole value chain may also disappear.

**Resumen.** El lavado de la lana es el paso intermedio esencial entre la agricultura, que proporciona los vellones y las industrias artesanales y textiles que los convierten en productos terminados. Sin embargo, actualmente existe preocupación de cara al futuro por la desaparición en los últimos años de muchas plantas de lavado en Europa, y las dificultades que experimentan otras. Si se interrumpe esta etapa intermedia de lavado, ya debilitada en Europa, toda la cadena de valor podría también desaparecer.

**Keywords:** wool scouring, European wool industry, sheep breeding, wastewater and energy, ecological treatment

## Introduction

For several years already, new initiatives for adding value to wool have been developed by breeders and craft workers. Such initiatives are strongly linked to environmental concerns, especially preservation of threatened breeds. They have worked on creating direct links between breeders and processors, aiming to bring the benefit of value added closer to the wool producer.

In any case, whether it concerns sheep, alpacas, angora or cashmere goats, the first steps of processing are more or less the same: shearing, grading the fleeces, storage and transport to the scouring plant.

### **A weak link**

Wool scouring is the essential intervening step between agricultural processes resulting in the provision of the fleeces and the craft and textiles industries, developing them into finished products. However, there are many future concerns regarding the problematic situation and recent disappearance of many scouring plants across Europe. If this stage, already fragile in Europe, would break down, the whole value chain may also disappear.

### **Wool scouring: a question of scale**

There are numerous techniques for scouring wool, for example:

- wool scouring in hot springs, traditionally used in some regions (Pyrénées, France)
- a trailer with a degreasing bowl heated by solar panel, a washing machine, travelling from one farm to another
- a discontinuous scouring plant set up in Czech Republic by a Romney sheep breeders' association
- a rinsing bowl, using river water in Ariège (France)
- small sized leviathans in regional scouring plants (Saugues and Souvigny, France)
- larger scouring plants (e.g., in Tavares (Portugal) or in Traitex (Belgium))

Bigger plants can be found in the UK (e.g., Haworth), in Italy (e.g., Pettinatura di Verrone) or in Czech Republic (Modiano Nedjeck).

### **The operation of a scouring plant**

The process is always the same: opening the fleeces, scouring with water, heat, sodium carbonate and movements, rinsing, removal of water, and drying.

### **Water, energy and wastewater: an ecological challenge**

Water consumption in industrial scouring plants is generally around 8-20 litres per kg of greasy wool, but sometimes much more in smaller and older plants. The main energy requirement for scouring emerges from heating the water for bowls.

### **50 % impurities**

Depending on the sheep breed and husbandry system used, the wool fibre only represents around a third to two thirds of the total weight of the fleece. The other constituents are wool grease and suint, sands and dust.

### **Wastewater treatments**

Treating wool scour effluent is highly difficult, as wool grease has a high pollution factor. For example, a wool scour processing 1 t of greasy wool a day requires effluent treatment equivalent to the domestic sewage requirement of a town of between 1,250 to 4,000 inhabitants. However, ecological solutions already exist for example by means of spreading to soil, a pre-purification plant for the effluent or methane generation.

### **European meeting in November 2015**

On the initiative of associations ATELIER-Laines d'Europe, Pôle Laine du Pays de Saugues and Lainamac, 150 participants from 15 European countries, representing all stages of the wool processing industry met in Saugues, Haute-Loire, from 4-6 November, 2015 to share ideas and look for solutions.

The proceedings of the meeting include the oral presentations, descriptions of various projects, report of debates, local visits, with an annex including maps, useful addresses and a glossary.

### **Conclusion**

The small scouring plant in Saugues, the town where the meeting took place, was an old one, neither very efficient nor sustainable. The local authorities are now aware of this enterprise playing an important role for about 100 French sheep breeders who bring their fleeces here every year and depend on it. The authorities now support a 3-year plan for improving the whole installation and treatment of wastewater. On the Island of Gotland (Sweden), a new scouring plant is now running since summer 2016. We hope that the dissemination of the book at all levels, farming, crafts and industry, local authorities and EU bodies, will help to maintain the existing installations and even create new ones. It will allow everyone to participate in the development of the European wool industry and consider its future with optimism.

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# Proteomic Method for Determination of Animal Hair Fibres

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**Abstract.** The market of high quality textiles requires suitable analytical methods for the determination of their fiber composition to guarantee that no falsification occurs. The proteomic method, developed in recent years and based on the ultra-performance liquid chromatography coupled with electrospray mass spectrometry (LC/ESI-MS), was successfully used for the identification and quantification of the most commercial Bovidae fibres (wool, cashmere and yak). This is possible because keratin from sheep, cashmere goat and yak are similar but not identical. Indeed, in some specific parts of the protein there are amino acidic variants that can be used as specific peptide-marker to distinguish each animal fibers. In this paper, the proteomic method was applied to other animal hair fibers, such as Camel, Lama, Alpaca, Vicuna and Guanaco, in order to identify new molecular markers to discriminate Camelidae from Bovidae. Preliminary results showed that there are specific molecular markers for Camelidae and for Bovidae, suitable both for qualitative and quantitative analysis.

**Resumen.** El mercado de textiles de alta calidad requiere métodos analíticos adecuados para determinar la composición de la fibra y poder así evitar falsificaciones. El método proteómico, desarrollado en los últimos años y basado en la cromatografía líquida de ultra-rendimiento en acoplamiento con espectrometría de masas por electroespray (LC/ESI-MS), se utilizó con éxito para la identificación y cuantificación de fibras comerciales de la familia Bovidae (lana, cashmere y yak). Esto fue factible porque las queratinas de oveja, cabra cashmere y yak son similares pero no idénticas. De hecho, en algunas partes específicas de la proteína existen variantes aminoacídicas que pueden ser utilizadas como marcadores peptídicos específicos de la fibra de cada especie. En este trabajo, el método proteómico se aplicó a otras fibras animales, como el camello, llama, alpaca, vicuña y guanaco, con el fin de identificar nuevos marcadores moleculares para discriminar la familia Camelidae de la Bovidae. Los resultados preliminares mostraron la existencia de marcadores moleculares específicos para Camelidae y para Bovidae que serían adecuados tanto para el análisis cualitativo como cuantitativo.

**Keywords:** LC/ESI-MS, animal fibers, bovidae, camelidae

## Introduction

Special animal hair fibres, in particular cashmere, yak, mohair, camel and alpaca, are obtained from domestic mammals of the genera *Capra*, *Bos*, *Camelus* and *Llama*. These fibres are valuable natural raw materials used by the fashion industry for manufacturing high quality luxury textiles, and as such are distinct from fibres derived from sheep's wool.

International producers of textiles in cashmere and other specialty fibres require suitable analytical methods for the determination of their fiber composition to guarantee that no falsification occurs, especially when cheaper fibers, like wool and yak, are blended with expensive fibers, like cashmere.

The traditional and most used methods for identifying animal fibres for the textile sector involve microscopy. Light microscopy (LM) allows to observe the internal and external structure of the fibres such as pigmentation, medullation, shape and cuticle morphology (Appleyard, 1978); scanning electron microscopy (SEM) shows the surface morphology and the fine structure of the cuticle at high resolution (Langley et al., 1981).

The traditional microscopic techniques are subjective depending on the expertise of the operator and often are affected by chemical treatments to which the fibers have been submitted during textile processing. Several methods have been studied to improve the objectivity and accuracy of the results of the animal fiber blend identification, like methods based on the extraction and analysis of DNA (Tang et al., 2014) or based on specie-specific monoclonal antibodies (Tonetti et al., 2012). Nevertheless, the results obtained are often affected by chemical treatment such as bleaching, dyeing and depigmentation.

The proteomic method (Paoletta et al., 2013; Vineis et al., 2014), developed in recent years and based on the ultra-performance liquid chromatography coupled with electrospray mass spectrometry (LC/ESI-MS), was successfully used for the identification and quantification of the most commercial Bovidae fibres (wool, cashmere and yak). This is possible because keratin from sheep, cashmere goat and yak are similar but not identical. Indeed, in some specific parts of the protein there are amino acidic variants that can be used as specific peptide-marker to distinguish each animal fiber.

This method has been validated analyzing several samples in different shapes and with different treatments (fibres, slivers, yarns, fabrics, raw materials, dyed, depigmented, bleached, finished), in order to verify the accuracy of the LC/ESI-MS method and the laboratory repeatability (Vineis et al., 2017). Results proved that LC/ESI-MS technique is an objective and accurate method for animal hair fibers analysis, unaffected by chemical treatment and applicable to sample from various stages of textile processing.

The aim of this paper is to apply the proteomic method to other animal hair fibers, such as Camel, Llama, Alpaca, Vicuna and Guanaco, in order to identify new molecular markers to discriminate Camelidae from Bovidae.

## Materials and Methods

### *Samples*

Authentic samples of wool, cashmere, yak, camel and South American camelids (alpaca, llama, vicuna and guanaco) and their blend in different shapes and with different treatments (fibres, slivers, yarns, fabrics, raw materials, dyed, depigmented, bleached, finished) were kindly supplied by the Cashmere and Camel Hair Manufacturers' Institute (CCMI), Boston, MA, USA and by trusted companies that supported the research.

### *Protein-extraction*

Samples were scoured, dehaired, cleaned with petroleum-ether for 2 h in a Soxhlet extractor, rinsed for 1 h in water at room temperature and finally for 1 h in water at 50 °C. The fibres were dried in an oven at 50 °C and then conditioned in standard atmosphere at 20 °C, 65 % RH, for 24 h. The fibres (150 mg) were placed in a test tube and 9.5 ml of extraction buffer (25 mM Tris-HCl, pH 8.5, 2.4 M thiourea, 5 M urea, 5 % dithiothreitol (DTT)) were added. The buffer was left in contact with the fibres for two days at 50 °C under slow stirring. The solution was then filtered on 0.45 µm filter in a centrifuge 5,000 g for 15 min at room temperature.

### *Trypsin digestion*

To 67 µl of the protein solution an equal volume of 100 mM NH<sub>4</sub>HCO<sub>3</sub> was added, mixing by vortex. A 5 µl portion of a 200 mM DTT solution in 100 mM NH<sub>4</sub>HCO<sub>3</sub> was added, followed again by vortex mixing. The solution was incubated for 1 h at room temperature and 4 µl of iodoacetamide 1 M in 100 mM NH<sub>4</sub>HCO<sub>3</sub> was added, followed again by vortex treatment. The solution was further incubated for 1 h at room temperature and the excess iodoacetamide eliminated by adding 20 µl of a 200 mM DTT solution, followed again by treatment with vortex and incubation for 1 h at room temperature. Finally, 818 µl of deionized water and 20 µl of trypsin solution (100 ng/ml in 1 % CH<sub>3</sub>COOH) were added, followed again by vortex treatment. The solution was allowed to stand for 4 hours at 37 °C. The reaction was stopped with 2 µl of HCl (at 37 % or 12N) and the volume was reduced under nitrogen flux.

### *LC/ESI-MS analysis*

The dried samples were dissolved in 100 µl of eluent A, treated with vortex and transferred to vials for UPLC-MS analysis. The analysis was performed using an Acquity UPLC BEH300 C18 1.7 µm column (Waters). Gradient elution was conducted using two eluents: A (H<sub>2</sub>O + 0.2 % CH<sub>3</sub>CN + 0.1 % HCOOH) and B

(CH<sub>3</sub>CN + 0.1 % HCOOH). The instrumental conditions and the acquisition parameters are reported in our previous works (Paolella et al., 2013; Vineis et al., 2014).

## Results and Discussion

The principles of the proteomic method is that keratin sequences, extracted from Bovidae or Camelidae, might be slightly different in the different species, because in some specific parts there are aminoacidic variants. Peptides containing these variants have different molecular weight and different sequences among the species, thus they might be differentially detected by mass spectrometry, acting as species-specific molecular markers. For example the peptide-marker already identified in previous papers for Bovidae like cashmere goat, yak and sheep wool), as reported in Table 1, are specific for the corresponding animal fibres implying that they are absent in the others. Only sheep-goat markers, as the name suggest, can be found in both cashmere and sheep wool, but they are absent in yak.

**Table 1:** Marker peptides identified for Bovidae (wool, cashmere and yak fibres)

Molecular mass (Da)	Retention time (min)	Characteristic ions (m/z)	ID <sup>a</sup>
2634	41.6	879.0	Cashmere 1 (C1)
2691	41.0	898.1	Cashmere 2 (C2)
2607	42.0	870.1	Wool 1 (W1)
2664	41.3	889.0	Wool 2 (W2)
2234	39.0	745.7	Wool-cashmere 1 (WC1)
4738	43.4	948.5 + 1185.6	Wool-cashmere 2 (WC2)
2504	38.9	835.5	Yak 1 (Y1)
2520	39.1	1260.2 + 840.9	Yak 2 (Y2)

Following the same principle, the proteomic method was also applied to other animal hair fibers, such as Camel, Llama, Alpaca, Vicuna and Guanaco, in order to identify new molecular markers to discriminate Camelidae from Bovidae. The pure fiber of Camelidae and Bovidae were analyzed. Comparing the peptides obtained from the analysis in LC/ESI-MS of camel fibers, it was possible to identify several peptides present or only in Camelidae and absent in Bovidae or absent in Camelidae and present in one or more Bovidae. Among these peptides, 5 are present only in Camelidae and 7 only in Bovidae.

The markers have been evaluated for their specificity both qualitatively and quantitatively. All markers were determined in mixed samples by single ion recording (SIR) detection, specifically monitoring only the characteristic ions. Chromatographic areas associated to the markers were integrated in order to estimate the percentage in mixed samples.

In order to verify the correspondence between the calculated and the actual percentages, different calibration curves were produced by preparing two- fiber mixed samples (one Camelidae vs one Bovidae) with different percentage composition: only 3 peptides among those identified can be used as specific and quantitative markers for a generic Camelidae. The limit of detection for Camelidae is 3 %.

In order to confirm UPLC/ESI-MS is an accurate and entirely acceptable method for distinguishing and quantifying the composition of Camelidae from Bovidae, a validation with blind samples in different shapes and with different treatments is necessary. The results already obtained in previous work for wool, cashmere and yak fiber mixtures (the results are reported in Table 2) proved that LC/ESI-MS method is unaffected by chemical treatment and applicable to sample from various stages of textile processing.

**Table 2:** Bovidae samples analysed by UPLC/ESI-MS compared to the actual percentage composition\*

Blind samples	UPLC/ESI-MS			Actual			Aver- aged trueness (%)
	C (%)	W (%)	Y (%)	C (%)	W (%)	Y (%)	
A	23	77	0	20	80	0	2,4
B	56	44	0	50	50	0	4,9
C	85	15	0	80	20	0	4,1
D	26	36	38	20	30	50	8,5
E	33	32	35	30	40	30	5,7
F	0	0	100	0	0	100	0
G	0	0	100	0	0	100	0
H	100	0	0	100	0	0	0
I	70	0	30	75	0	25	4,1
J	20	0	80	15	0	85	4,1
K	100	0	0	100	0	0	0
L	90	0	10	90	0	10	0
M	100	0	0	100	0	0	0
N	43	57	0	50	50	0	5,7
O	0	100	0	0	100	0	0
P	22	10	68	20	10	70	1,6
Q	40	16	44	40	20	40	3,3
R	60	0	40	60	0	40	0
S	100	0	0	100	0	0	0
T	0	0	100	0	0	100	0
U	100	0	0	100	0	0	0
V	100	0	0	100	0	0	0
W	72	0	28	80	0	20	6,5
X	25	45	30	25	50	25	4,1
Y	60	0	40	60	0	40	0
Z	90	0	10	90	0	10	0
a	43	0	57	50	0	50	5,7
b	51	0	49	50	0	50	0,8
c	43	57	0	40	60	0	2,4
d	100	0	0	100	0	0	0
e	0	0	100	0	0	100	0

\*The trueness of the analysis method was calculated as the difference of the mean of the measured value to the actual value. The trueness obtained for the samples was between 0 % (in pure samples) and 8.5 %.

More investigation are necessary but preliminary results of treated samples of mixture Camelidae-Bovidae show that the specific new marker are good candidates to be use in determination of animal hair fibers in all stage of textile processing (data not shown).

Finally, the some approach has been applied in order to distinguish Camel from South American camelids fibres (alpaca, llama, vicuna and guanaco). Between all the peptides identified as potential specific-peptide marker, 8 peptides seem to be specific markers for qualitative analysis (data not shown). The validation as qualitative and quantitative analysis is work in progress.

## Conclusion

The proteomic method based on the use of liquid chromatography coupled with electrospray mass spectrometry was applied to Camelidae animal hair fibers, such as Camel, Llama, Alpaca, Vicuna and Guanaco, in order to identify new molecular markers to discriminate Camelidae from Bovidae. Several peptides were identified and they were evaluated for their specificity both qualitatively and quantitatively. The identification and validation of specific markers suitable to distinguish Camel from South American camelids fibres is in progress. The proteomic method continues to be successful applied to the identification of animal hair fibres (such as Bovidae and Camelidae) in sample of different shapes and with different treatments.

This method showed good repeatability, reproducibility and accuracy, and it was not influenced by different treatments to which the fibers have been subjected or by the presence of other fibers different from animal hairs. The LC/ESI-MS method was tested for qualitative and quantitative analysis and it was already validated for wool, cashmere and yak. The detection limit was of 3 %. According to these results, the UPLC/ESI-MS method is under review for publication as ISO standard method for qualitative and quantitative analysis of wool, cashmere and yak fibres.

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# The Use of Near-Infrared (NIR) Reflectance Spectroscopy to Predict Mohair Quality in Greasy Fleece Samples of Angora Goats

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**Abstract.** One experiment was conducted in order to test the utility of near-infrared spectroscopy (NIRS) to predict mohair quality, i.e. i) clean mohair content and ii) mean fibre diameter (MFD) in Angora goat fleece. A total of 397 mohair fleece samples were collected in 2016 within the framework of the French selection scheme including performance recording, fleece assessment and fibre measurements. Fleece samples were laboratory measured for clean mohair content and MFD by OFDA methodology, then scanned in a Petri dish using a NIRS portable instrument (LabSpec ® 5000; ASD Inc, Boulder, USA) by reflectance in the VIS and NIR regions (350 to 2,500 nm). Partial least square (PLS) regression was used to develop a number of calibration models between the spectral and reference measurements. Different mathematical treatments were used during model development. The methods studied were partial least squares regression (PLS) and first-derivative pretreatment + PLS. Cross validation was used to assess the performance and avoid overfitting of the models. NIR prediction of MFD gave a low  $R^2$  ( $<0.70$ ) whatever the calibration models. By using the first derivative of the raw spectra in the NIR region (800 to 2,500 nm), the calibration models gave a coefficient of determination in calibration ( $R^2$ ) of 0.81 for clean mohair content with a low relative error (3.5 %). It is concluded that NIR reflectance spectroscopy can be used to predict clean mohair content with a good precision but not for determining mean fibre diameter. However the use of OFDA 2000 MFD measurement along the greasy staple can be used as an alternative. Thus NIR spectroscopy to predict clean mohair content and OFDA2000 to measure fibre diameter along greasy staple would be widely used to assist the French Angora goat breeding program and allowing a large reduced cost of fibre measurements.

**Resumen.** Se realizó un experimento para probar la utilidad de la espectroscopia del infrarrojo cercano (NIRS) para predecir la calidad del mohair, es decir 1) limpiar contenido de mohair y 2) diámetro de la fibra media (MFD) en lana de cabra de Angora. Se recolectaron un total de 397 muestras de lana de mohair en 2016 en el marco del esquema

de selección francés incluyendo el registro del rendimiento, evaluación del vellón y mediciones de fibra. Las muestras de lana se analizaron en el laboratorio midiendo el contenido limpio mohair y MFD por metodología OFDA, analizándose luego en una placa Petri con un dispositivo portátil de NIRS (LabSpec® 5000; ASD Inc., Boulder, E.E.U.U.) por reflexión en las regiones VIS y NIR (350 a 2.500 nm). Para desarrollar modelos de calibración entre las mediciones espectrales y las de referencia, se utilizó la regresión de mínimos cuadrados parciales (PLS). Se utilizaron diferentes ajustes matemáticos durante el desarrollo del modelo. Los métodos estudiados fueron la regresión de mínimos cuadrados parciales (PLS) y el pretratamiento de la derivada primera + PLS. Para evaluar la capacidad predictiva de los modelos y evitar sobreajustes, se emplearon técnicas de validación cruzada. La predicción NIR del MFD presentó un  $R^2$  bajo ( $< 0.70$ ) independientemente del modelo de calibración. Usando la primera derivada de los espectros brutos de la región NIR (800 a 2.500 nm), los modelos de calibración dieron un coeficiente de determinación ( $R^2$ ) de 0.81 para contenido mohair limpio con un bajo error relativo (3,5 %). Se concluyó que la espectroscopía de reflectancia NIR puede utilizarse para predecir contenido de mohair limpio con una buena precisión, pero no para determinar el diámetro medio de fibra. Sin embargo, el uso de la medición de la OFDA 2000 MFD sobre la mecha grasa puede utilizarse como alternativa. Por tanto, se podría utilizar ampliamente la espectroscopía NIR para predecir el contenido limpio mohair y el OFDA2000 para medir el diámetro de la fibra sobre la mecha grasa como ayuda en el programa francés de mejora genética de la cabra de Angora, programa de cría y permitiendo una gran reducción del coste de las mediciones de fibra.

**Keywords:** mohair, NIR spectroscopy, clean mohair content, mean fibre diameter

## Introduction

Clean yield of mohair and mean fibre diameter are economically important traits for French producers. The French mohair industry is vertically integrated, where producers are processing the whole production within the textile industry and then sell directly to consumers final products. As clean mohair content ranges from 50 to 90 %, accurate evaluation is required. Standard methods for determining yield are lengthy and time-consuming. Current methods involve scouring or washing in aqueous solution to remove grease, suint and dirt and then drying and weighing the samples prior to analysis by conventional analytical techniques (Connell & Brown, 1978; Connell, 1983; Coleman et al., 1999; Church & O'Neill, 1999). Furthermore, standard methods for determining fibre diameter are based on measurements on clean fibre. However, OFDA2000 methodology allows measurements of fibre diameter along the greasy staple using an automatic correction for greasy factor, but this method has not been tested for mohair.

Near-infrared spectroscopy (NIRS) is an analytical technique based on the absorption of infrared light by organic matter. Since absorption is linked to the chemical composition of samples, it can be predicted after a calibration phase. Near infrared (NIR) reflectance spectroscopy is used for the quality evaluation of

foods and forages from the early 1970s (Murray, 1993; Osborne et al., 1993; Batten 1998). This technique is based on the vibrations of molecular bonds in the NIR electromagnetic region containing H attached to atoms such as N, O and C, which are constituents of the organic matrix (Murray, 1993; Osborne et al., 1993; Batten, 1998). In NIR spectroscopy, calibration is a mathematical process, which generally uses multivariate regression techniques (e.g. partial least squares) to relate absorbance measurements from a NIR spectrophotometer at different wavelengths (e.g. 800 to 2,500 nm) to reference values measured by conventional chemical or physical methods (Osborne et al., 1993; Deaville & Flinn, 2000). The advantages of NIR spectroscopy include the speed of the analysis, simplicity of sample preparation, multiplicity of analysis, and lack of chemicals required (Murray, 1993; Deaville & Flinn, 2000). The NIR technique has been used for both at- and on-line applications in the textile industry for quality control of for example moisture, residual grease and contamination of wool (Slack-Smith et al., 1979; Church & O'Neill, 1999; Hammersley & Townsend, 2004). Previous studies have shown that near-infrared spectroscopy has potential for rapid analysis of raw mohair fleeces to predict clean mohair content (Coleman et al., 1999).

The objective of this study was to explore the potential use of visible (VIS) and NIR spectroscopy coupled with multivariate statistical tools (partial least squares), to predict clean mohair content and fibre diameter of greasy mohair samples and to test fibre diameter measurement along the greasy staple in order to assist the French Angora goat breeding program.

### **Material and methods**

A total of 397 fleece samples from 132 males and 265 females of 18 months to 3 years of age and issued from 38 different herds were collected in 2016 within the framework of the national selection scheme of French Angora goat including performance recording, fleece assessment and fibre measurements (Allain & Roguet, 2003).

#### *Laboratory measurements of clean mohair content and fibre diameter*

All fleece samples were determined for clean mohair content at INRA (GenESI laboratory) using a simplified washing method using sonification for aqueous scoring. Fleece samples remained for at least 24 hours under a controlled ambient environment (20 °C, 60 % relative humidity). Then about 10 g of each fleece samples were weighed, introduced within a fine mesh net and then deposit into an ultrasonic tank filled with a warm soapy-aqueous solution (at 45 °C) for sonification during half an hour. Thereafter fine mesh nets were dried at 85 °C in a drying oven, then put under a controlled ambient environment (20 °C; 60 % relative humidity) for 24 hours and were finally weighed. Clean mohair content was determined as the part of clean mohair to greasy mohair within each fleece sample.

Mean fibre diameter (MFD) measurements were made at INRA (GenESI laboratory) using OFDA 2000 benchtop version on both clean mohair samples on 2 mm fibre snippets cut using a guillotine and greasy samples along the length of greasy mohair staples using the OFDA2000 automatic correction for greasy factor.

#### *NIR measurements*

Fleece samples were scanned using a NIRS portable instrument (LabSpec ® 5000; ASD Inc, Boulder, USA) with a reflectance probe (spot size=10 mm). Fleece samples were remained for at least one week under a controlled ambient environment (20 °C, 60 % relative humidity) before scanning. About 10 g of fleece samples were introduced within a Petri dish. Then 4 spectra were collected from each sample with the reflectance probe in the visible (VIS) and near infrared (NIR) region (350-2,500 nm) at 1-nm intervals, to produce a total of 2,151 data points. A standard reference made of Teflon was scanned after each 20-sample interval to correct for changing conditions such as instrument drift.

#### *Statistical analysis*

Spectral data were transformed into an ASCII text format and exported into SAS (Version 9.2) software for multivariate analysis. The mean spectrum of each sample was estimated by averaging the 4 successive scans and stored as the logarithm of the reciprocal of reflectance ( $\log 1/R$ ) and the first derivative of the spectrum.

Principal component analysis (PCA) was firstly performed before partial least squares (PLS) regression models were developed. PCA was used to derive the first 10 principal components from the spectral data, to examine the possible grouping of samples and secondly to detect possible spectral outliers prior to the development of PLS regression models (Cowe & McNicol, 1985). No mathematical treatments or spectral transformation were applied when PCA was performed.

In order to avoid bias, wavelengths below 400 nm in the VIS regions were discarded from the analysis. Two wavelength regions were analyzed: visible plus near infrared (Vis/NIR, 400 to 2,500 nm), and near infrared (NIR, 800 to 2,500 nm). Calibrating equations were determined either by using the reflectance ( $\log 1/R$ ) raw spectra or by using the first derivative spectra in order to eliminate the uncontrolled variations of the spectra.

Partial least square regression (PLS) calibration models were determined by cross validation according to the root mean square error of cross-validation in order to determine the optimum number of latent variables. Cross validation were performed by splitting at random the calibration samples into 150 subsets of 100 samples where all samples except 100 were used for calibration while the

remaining ones were used for validation test and the process was repeated until all subset samples were used as validation test once.

The coefficient of determination ( $R^2$ ) in cross-validation, root mean square of the standard error of cross validation (RMSECV), the relative error expressed as the ratio of the residual predictive standard deviation to the reference mean and the ratio performance deviation (RPD) calculated as the ratio of standard error of the laboratory result to the standard error of prediction ( $RPD=SD/SEP$ ) were selected to evaluate the PLS calibration equations between the chemical analyses and the NIRS estimates. The higher is the coefficient of determination and the lower is the RMSECV, the better is the prediction performance.

Estimations were considered sufficiently accurate and robust to be applied routinely when the relative error was under 5 % and the coefficient of determination was greater than 0.91 (Coppa et al., 2010). When the relative error ranges from 5 to 10 % and coefficient of determination is greater than 0.82, we advise to use these equations only for analytical purposes. Otherwise equations should be restrained for research and interpreted with caution. An RPD value above 2 indicates good to excellent prediction ability (Moron & Cozzolino, 2004; Nicolai et al., 2007; Pissard et al., 2013).

## Results and discussion

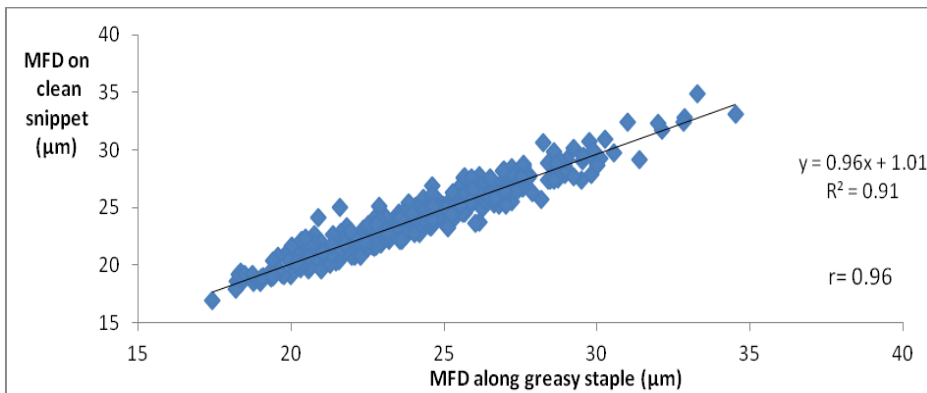
The descriptive statistics for both clean mohair content and fibre diameter of fleece samples are given in Table 1. A wide range of both clean mohair content (range: 52 to 92 %) and fibre diameter (range: 17.83 to 34.89  $\mu\text{m}$ ) was observed.

Mean fibre diameter (MFD) was measured using OFDA methodology on both clean fibre snippets according standard method and along the length of greasy mohair staples using the OFDA2000 automatic correction for greasy factor. Descriptive statistics of MFD measurements reported on table 1 show close similar results between both methods.

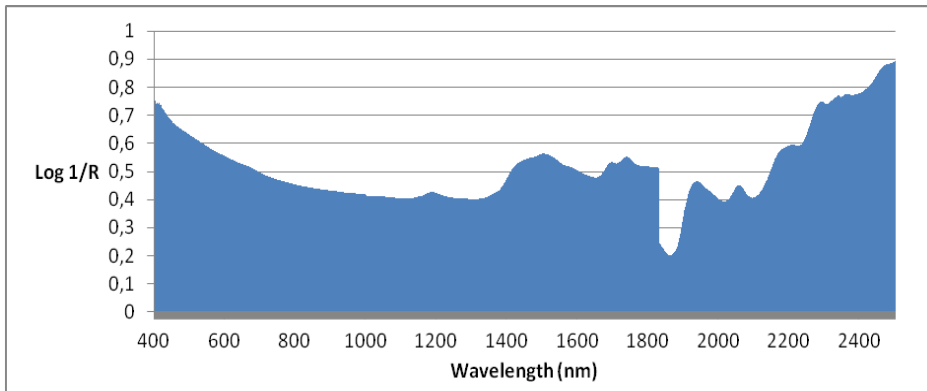
**Table 1:** Descriptive statistics (number of samples (No), mean, standard deviation (SD), coefficient of variation (CV), minimum and maximum) for clean mohair content and mean fibre diameter (MFD) determined using OFDA2000 on clean snippets and along greasy staple of mohair fleece samples

Mohair samples	No	mean	SD	CV (%)	Min	Max
Clean content (%)	397	74.67	5.78	7.74	52.42	91.49
MFD on clean snippet ( $\mu\text{m}$ )	397	24.16	2.91	12.06	17.42	34.53
MFD along greasy staple ( $\mu\text{m}$ )	397	24.09	2.90	12.02	16.93	34.89

Figure 1 shows scatter plot of OFDA2000 MFD measurements on clean fibre snippets and along greasy staples using the OFDA2000 automatic correction of the greasy factor using the formula established by the manufacturer after exhaustive analysis of Australian and New Zealand flocks. The relationship between the 2 MFD measurements determined through a simple linear regression had a high coefficient of determination ( $R^2=0.91$ ) and a low relative error (3.6 %). This result agreed previous studies on wool (Baxter, 2001) and could be extend to mohair. MFD measurement along the greasy staple would be advised to determine fibre diameter without any pre-washing and might have a great interest for screening of potential animals in the breeding programme of the French Angora goat.



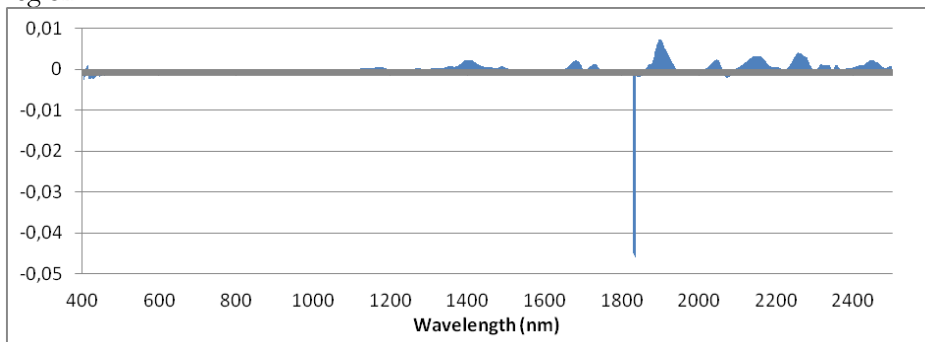
**Figure 1:** Relationship between mean fibre diameter (MFD) measured on clean fibre snippet and along greasy staple



**Figure 2:** VIS and NIR mean spectrum of reflectance ( $\text{Log}1/R$ ) of greasy mohair samples ( $n=397$ )

Figure 2 shows the mean spectrum expressed as the logarithm of the reciprocal of reflectance ( $\text{log } 1/R$ ) in the VIS and NIR region (400 to 2,500 nm) of the greasy

samples. Reflectance values ( $\log 1/R$ ) decreased gradually from 0.7 to 0.45 from the VIS region (400 nm) up to 1,100 nm in the closed NIR region. Thereafter, larger variations were observed up to 2,500 nm with a deep edge in the 1,800-1,900 nm region.



**Figure 3:** First derivative of the VIS and NIR spectrum of reflectance ( $\text{Log}1/R$ ) of greasy mohair samples ( $n=397$ )

Figure 3 shows the mean first-derivative of the spectrum expressed as the logarithm of the reciprocal of reflectance ( $\log 1/R$ ) in the VIS and NIR region (400 to 2,500 nm) of the greasy samples. The use of the first derivative has eliminated the uncontrolled variations of the spectra. The more interested bands of the NIR region were observed from 1,200 to 1,500 nm, at 1,600-1,700 nm, and thereafter from 1,800 to 2,500 nm. The use of the first derivative of  $\log 1/\text{Reflectance}$  is useful to eliminate the uncontrolled variations of the spectra and to resolve overlapping bands (Mika et al., 2003).

In the present study PLS regression analysis were done through cross-validation procedure and not by separating a pool of samples into independent calibration and validation data sets. It could be discussed but several studies showed that the calibration/validation and cross-validation procedures are equally valid (Shenk & Westerhaus 1993; Couâteux et al. 2003; Moron & Cozzolino 2004; Ludwig et al. 2008). Thus we decided to use cross-validation because it was difficult to well split samples into homogenous and representative separate calibration and validation sets. The data set used in the present study was representative of both the French Angora goat population and the sampling made each year within the national breeding program as a fleece sampling collection was made once a year on all candidate animals of farms registered to the national selection scheme. But there were a large variability between samples as they were collected on animals of 1 to 3 years of age, of both sexes and issued from 38 different farms (1-43 samples/farm). It is well known that sex, animal age and herd/farm are important factor influencing clean mohair content and mean fibre diameter (Gifford et al., 1990; Lupton et al., 1996; Allain & Roguet, 2003). Another reason for using cross-validation was the number of samples ( $n=397$ ) selected for our study, which is sufficient for a cross-validation. The cross-validation procedure used in the present

study was done by splitting samples at random into 150 subsets of 100 samples (a quarter of all samples) which were all used as validation test once while other samples were used for calibration.

The statistics of calibration equations for clean mohair content and mean fibre diameter using Vis/NIR and NIR regions are reported in tables 2 and 3 using the raw ( $\log 1/R$ ) and the first derivative of  $\log 1/Reflectance$  spectra respectively. Low coefficient of determination ( $R^2$ ), high RMSECV and low RPD were observed on both predicted clean mohair content and mean fibre diameter by using the raw Vis/NIR or NIR spectra. The  $R^2$ , RMSECV, and RPD were 0.73, 3.18 % and 1.82, and 0.74, 1.6  $\mu\text{m}$  and 1.83 on clean mohair content and mean fibre diameter respectively using raw Vis/NIR spectra (400 to 2,500 nm) and 0.73, 3.21 % and 1.80 and 0.71, 1.66  $\mu\text{m}$  and 1.75 on clean mohair content and mean fibre diameter respectively using raw NIR (800 to 2,500 nm) spectra (Table 2). Relative error of clean mohair content was below 5 % whatever the wavelength regions: 4.07 and 4.11 % in the Vis/NIR and NIR regions respectively while it was above 5 % for mean fiber diameter: 6.30 and 6.60 % respectively in the Vis/NIR and NIR regions. These results observed on greasy mohair samples were not acceptable to predict clean mohair content and mean fibre diameter using the raw ( $\log 1/R$ ) spectra in both Vis/NIR and NIR regions.

**Table 2:** Calibration statistics for clean fleece content and mean fibre diameter in greasy fleece samples ( $n=397$ ) using raw ( $\log 1/Reflectance$ ) Vis/NIR (400 to 2,500 nm) and NIR (800-2,500 nm) spectra

Trait	Wavelength regions <sup>a</sup>	CD ( $R^2$ ) <sup>b</sup>	RMSEV <sup>c</sup>	Relative error (%) <sup>d</sup>	RPD <sup>e</sup>	T <sup>f</sup>
Clean content (%)	Vis/NIR	0.73	3.18	4.07	1.82	15
	NIR	0.73	3.21	4.11	1.80	15
Mean fibre diameter ( $\mu$ )	Vis/NIR	0.74	1.59	6.30	1.83	15
	NIR	0.71	1.66	6.60	1.75	14

<sup>a</sup> Wavelength regions: Vis/NIR (400-2,500 nm) or NIR (800-2,500 nm);

<sup>b</sup> Coefficient of determination ( $R^2$ )

<sup>c</sup> Root means square of residual predictive value;

<sup>d</sup> Relative error as ratio of the residual predictive standard deviation to the reference mean;

<sup>e</sup> Ratio performance deviation: ratio of Standard error of laboratory result to the standard error of prediction

<sup>f</sup> Number of partial least square terms (or latent variables) to develop PLS Cross-validation models;

Higher  $R^2$ , lower RMSECV, higher RDP and lower relative error were observed on clean mohair content in both Vis/NIR and NIR regions by using the first derivative of  $\log (1/Reflectance)$  spectra. The  $R^2$ , RMSECV, and RPD were 0.80, 2.59 % and 2.14 and 0.81, 2.51 % and 2.21 in the Vis/NIR and NIR regions respectively. Relative error of clean mohair content was 3.52 % and 3.40 % in the Vis/NIR and

NIR regions respectively. On the contrary, the  $R^2$ , REMSECV, RPD and relative error were not improved and remained low for mean fibre diameter by using the first derivative spectra (Table 3).

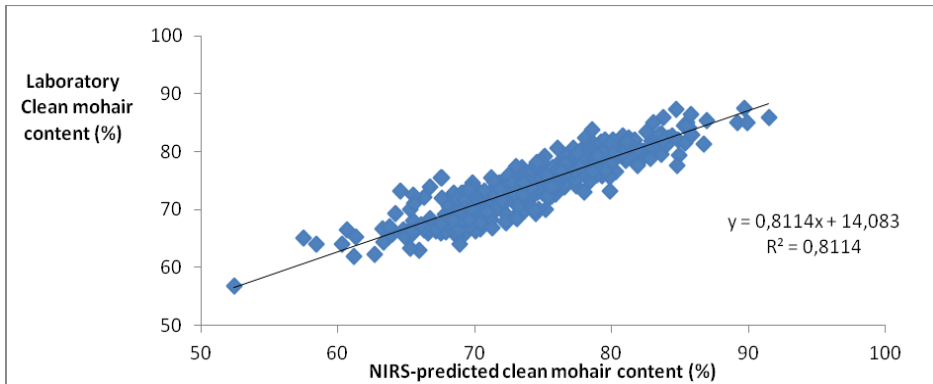
**Table 3:** Calibration statistics for clean fleece content and mean fibre diameter in greasy fleece samples (n=397) using the first derivative of the raw (log 1/Reflectance) Vis/NIR (400 to 2,500 nm) and NIR (800-2,500 nm) spectra

abcdef See footnotes on Table 2

Trait	Wavelength regions <sup>a</sup>	CD ( $R^2$ ) <sup>b</sup>	RMSECV <sup>c</sup>	Relative error <sup>d</sup> (%)	RPD <sup>e</sup>	T <sup>f</sup>
Clean content (%)	Vis/NIR	0.80	2.59	3.52	2.14	10
	NIR	0.81	2.51	3.40	2.21	10
Mean fibre diameter ( $\mu$ )	Vis/NIR	0.76	1.48	5.95	1.97	10
	NIR	0.65	1.77	7.18	1.64	8

By using the first derivative of log (1/R) spectra, the results obtained in this study shows that calibration models would be considered acceptable to predict clean mohair content but not mean fibre diameter in the French Angora goat. Our results agree with those reported earlier about yield of clean mohair (Larsen & Kininon, 1982; Coleman et al., 1999). Contradictory results were reported about the use of NIRS methodology to predict mean fibre diameter. It was reported that NIRS have potential for rapid analysis of fibre diameter in raw mohair fleeces (Coleman et al., 1999) but not in greasy wool (Hammersley, 1992; Hammersley & Townsend, 2004; Cozzolino et al., 2005). However, NIR spectroscopy seems to have good potential for determining fibre diameter in clean wool samples (Cozzolino et al., 2005).

Near-infrared spectroscopy has potential for rapid analysis of raw mohair fleeces in order to determine clean mohair content. As coefficient of determination between NIR spectra and laboratory values were 0.81 in NIR region with an RPD value higher than 2 and a low relative error (3.4 % in NIR). Figure 4 shows scatter plot of clean mohair content measured according laboratory analysis and NIRS predicted using PLS regression. The relationship between the laboratory and NIRS predicted values had a minor bias with a slope close to 1. NIR spectroscopy would be advised to be used for analytical purposes of clean mohair content (Copa et al., 2010) and might have a great interest for screening of potential animals in the breeding programme of the French Angora goat.



**Figure 4:** Relationship of clean mohair content (%) measured in laboratory and near-infrared spectroscopy (NIRS)-predicted clean mohair content

On the contrary, NIR spectroscopy would be unacceptable for measuring mean fiber diameter and should be restrained for research and interpreted with caution. However, according our results MFD measurement along the greasy staple using OFDA2000 methodology allows determination of fibre diameter with a good precision without any pre-washing procedure of fleece samples (Table 1; Figure 1). The use of automatic correction for grease factor using the formula established after exhaustive analysis of Australian and New Zealand flocks could be extended to French Angora goat flocks.

## Conclusion

The objective of this study was to explore the potential use of visible (VIS) and NIR spectroscopy coupled with multivariate statistical tools (partial least squares), to predict mohair quality, i.e. clean mohair content and mean fibre diameter on greasy mohair samples and to test fibre diameter measurement along the greasy staple.

NIR spectroscopy on raw fleece samples is acceptable to predict clean mohair content but not for mean fibre diameter. However OFDA2000 methodology allows determination of fibre diameter along the greasy staple, with a good precision.

It can be concluded that NIR spectroscopy on greasy fleece samples and OFDA mean fibre diameter measurement along the greasy staple, without any pre-washing/cleaning procedure can be widely used to predict mohair quality and to assist the French Angora goat breeding program allowing a large reduced cost of fibre measurements.

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# Variability of Fiber Quality of Chinese Alashan Left Banner White Cashmere Goat

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**Abstract.** The heritability and the phenotypic and genetic correlations of down weight (DW), down fiber diameter (DFD), and coefficient of variation of the down fiber diameter (CVDFD) of Chinese Alashan Left Banner White Cashmere goat were estimated on 1,375 one year old animals, born in 2009, 2011 and 2013 and bred at the Station for Livestock Improvement of Alashan (Left Banner, Inner Mongolia, P.R. China). For all traits, significant effects were for sex, cohort and sex-cohort interaction ( $P < 0.001$ ). The heritability for DFD and CVDFD was high,  $0.41 \pm 0.08$  and  $0.52 \pm 0.06$  respectively. Heritability for the DW was low ( $0.12 \pm 0.03$ ). Phenotypic correlation calculated by Pearson's coefficient showed that DFD is positively correlated with both CVDFD ( $0.29 \pm 0.07$ ) and DW ( $0.20 \pm 0.05$ ). The phenotypic correlation between CVDFD and DW was negative ( $-0.11 \pm 0.06$ ). The genetic correlations between DW and CVDFD and between DFD and CVDFD were both high and positive ( $0.63 \pm 0.16$  and  $0.39 \pm 0.1$ , respectively) while the DW showed a negative genetic correlation with DFD ( $-0.27 \pm 0.2$ ). Our results suggest that the selection for reducing DFD and its CVDFD is possible and a genetic progress can be achieved quickly in the Chinese Alashan Left Banner White Cashmere goat.

**Resumen.** Se estimó la heredabilidad y las correlaciones fenotípicas y genéticas del peso inferior (DW), el diámetro de fibra inferior (DFD) y el coeficiente de variación del diámetro de fibra inferior (CVDFD) en 1.375 cabras Cashmere Alashan Left Banner White de China de un año de edad nacidas en 2009, 2011 y 2013 en la Estación para el Mejoramiento Ganadero de Alashan (Left Banner, Inner Mongolia, R.P.China). Los efectos significativos fueron el sexo, la cohorte y la interacción sexo-cohorte ( $P < 0,001$ ) para todos los caracte-

teres. La heredabilidad de DFD y CVDFD fue alta,  $0,41 \pm 0,08$  y  $0,52 \pm 0,06$  respectivamente. La heredabilidad de DW fue baja ( $0,12 \pm 0,03$ ). El coeficiente de correlación fenotípica de Pearson mostró que DFD está correlacionado positivamente con CVDFD ( $0,29 \pm 0,07$ ) y DW ( $0,20 \pm 0,05$ ). La correlación fenotípica entre CVDFD y DW fue negativa ( $-0,11 \pm 0,06$ ). Las correlaciones genéticas entre DW y CVDFD y entre DFD y CVDFD fueron altas y positivas ( $0,63 \pm 0,16$  y  $0,39 \pm 0,10$ , respectivamente), mientras que DW mostró correlación genética negativa con DFD ( $-0,27 \pm 0,20$ ). Nuestros resultados que es posible seleccionar simultáneamente para reducir DFD y CVDFD, y que se puede lograr un progreso genético rápidamente en la cabra Cashmere Alashan Left Banner White de China.

**Keywords:** Cashmere, Alashan, goat, heritability, genetic correlation

## Introduction

This paper contains the results of the research on the variability of fiber quality on Chinese Alashan Left Banner White Cashmere goat carried out at the Italian University of Camerino in collaboration with the Jilin Agricultural University, the station for Livestock Improvement of Alashan and the Italian University of Perugia. The results are published in Pallotti et al. (2018).

Cashmere is the fine downy-soft winter undercoat found on many goats. Cashmere has a fine texture, and is light yet strong. When it is made into garments, cashmere is extremely warm, much warmer than the equivalent weight in sheep wool.

The U.S. Wool Products Labeling Act of 1939, as amended (U.S.C. 15 Section 68b (a) defines cashmere as the fine (dehaired) undercoat fibers produced by cashmere goat (*Capra hircus* L.). The average diameter of the fibers must not exceed 19 microns and contain no more than 3 % (by weight) of cashmere fibers with average diameters exceeding 30 microns (Cashmere and Camel Hair Manufacturers Institute, 2008).

China is the world's main source of cashmere goats, and has recently become the leading purchaser and processor of cashmere produced in other Asian countries.

In the Domestic Animal Diversity Information System hosted by FAO (<http://dad.fao.org/>) four Chinese cashmere goat breeds are described (Inner Mongolian Cashmere, Liaoning Cashmere, Hexi Cashmere and Shanbei White Cashmere) reared in Liaoning, Inner Mongolia, Xinjiang and Tibet provinces. For the Inner Mongolia cashmere breed many subpopulations are described (Di et al. 2011), which differ in color (pure white or colored) and quality of cashmere (Li et al. 2004). Generally these types are bred without genetic relationship between them in isolated areas.

In the past years, the genetic admixture with breeds like Liaoning and Inner Mongolia Arbas cashmere selected for increased body weight and low weight of the animals, have led to the loss of fineness and homogeneity of fiber.

Nowadays, one of the more genetically preserved populations is the Left Banner Alashan White, which produced very fine cashmere (Bai et al. 2006). Nevertheless, the influence of Liaoning and Arbas goats is now in progress also in Banner, leading to a reduction in fiber quality. In fact, fiber diameter is becoming coarser.

A genetic project coordinated by the Station for Livestock Improvement and supported by the Chinese Agricultural University of Jilin and some Italian public and private institutions (University of Camerino, ENEA and the Loro Piana S.p.A. textile industry) started in 2009 in Alashan Left Banner. The purpose of this project was to estimate the genetic parameters for the production traits of the Left Banner Alashan White Cashmere goat. The traits included are: down weight (DW), down fiber diameter (DFD) and coefficient of variation of down fiber diameter (CVDFD).

### Material and methods

From 2009 to 2013 data were collected from 1,375 one year old goats, born in 2009 (1<sup>st</sup> cohort), 2011 (2<sup>nd</sup> cohort) and 2013 (3<sup>rd</sup> cohort). The structure of the sample is given in table 1.

**Table 1:** Sample structure

Sex	Year	Cohort	Number of animals
Bucks	2009	1	270
	2011	2	185
	2013	3	79
Does	2009	1	341
	2011	2	312
	2013	3	188

The herd was located in the Station for Livestock Improvement of Alashan (latitude 38°24' N and longitude 104°42' E), Left Banner, a semi-desert steppe area of the Inner Mongolia (P.R. China). Fleece samples from the mid side of each animal were collected during the first combing. The samples were dehaired, washed and analyzed in the Loro Piana Metrology Laboratory in Beijing, certified according to the international requirements of the WTO. Analysis of the DFD and the CVDFD were carried out using an OFDA 100, measuring thousands of fiber for each sample. The DW was recorded directly by the laboratory assistants.

Software package SPSS 12.0 was used to determine the effect of sex, cohort and their interaction on the traits. The same software was also used for the computation of phenotypic correlations calculated by Pearson's correlation coefficient.

Heritability and genetic correlation were estimated using the MTDFREML program (Boldman et al. 1995) with a criterion for convergence set at  $10^{-6}$  and sex and age included as fixed effect for each trait. Standard errors of heritability were estimated using the VCE6 package (Neumaier and Groeneveld, 1998).

## Results and Discussion

As showed in the table 2, the average DW values for bucks of the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> cohorts was 501.72 g, 514.49 g and 424.11 g respectively. Lower DW values were recorded for does with a value of 436.69 g, 487.74 g and 404.47 g for the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> cohort respectively.

The average DFD values for the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> cohorts ranged from 14.63  $\mu\text{m}$  to 14.27  $\mu\text{m}$  for the bucks. The does showed slightly thicker fiber with DFD values ranging from 14.49  $\mu\text{m}$  to 15.75  $\mu\text{m}$ . The average CVDFD values for the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> cohorts for the bucks ranged from 27.09 % to 29.39 % showing less variation compared to does were CVDFD ranged from 27.64 % to 41.39 %.

The basic statistical analysis results indicating the potential of the Left Banner Alashan White Cashmere goat showed higher results for bucks in terms of yield, fineness and homogeneity of fleece. The ANOVA showed a significant effect ( $P < 0.001$ ) of sex, cohort and the interaction between sex and cohort on the three traits studied (Table 3).

**Table 2:** Average values for the down weight, the down fiber diameter and the coefficient of variation of down fiber diameter

Trait	Bucks			Does		
	Cohort	Mean	SD	Cohort	Mean	SD
Down weight (g)	1	501.72	120.50	1	436.69	120.41
	2	514.49	132.62	2	487.74	138.04
	3	424.11	73.02	3	404.47	61.74
Down fiber diameter ( $\mu\text{m}$ )	1	14.63	0.93	1	14.49	1.09
	2	14.27	0.75	2	15.01	0.77
	3	14.59	0.67	3	15.75	0.95
Coefficient of variation of down fiber diameter (%)	1	27.09	3.78	1	27.64	5.84
	2	29.39	4.39	2	41.39	8.51
	3	28.52	5.02	3	36.50	9.28

**Table 3:** Anova for the down weight, the down fiber diameter and the coefficient of variation of down fiber diameter

Trait	Source of variation	df	Mean Square	F	P-value
Down weight (g)	Sex	1	373,731.02	26.74	<0.001
	Cohort	2	569,320.45	40.74	<0.001
	Sex x Cohort	2	67,062.43	4.80	<0.001
Down fiber diameter ( $\mu\text{m}$ )	Sex	1	104.26	126.08	<0.001
	Cohort	2	37.35	45.16	<0.001
	Sex x Cohort	2	48.17	58.24	<0.001

As reported by the basic statistical analysis results, bucks showed highest performance in terms of fiber quality. These differences between the two genders may reflect the physiological mechanisms that affect fleece production performance as proposed by Wang et al. (2013).

The differences in weather condition, rainfall and grazing during the five years of the study may explain the effect of cohort in the traits studied. This non genetic factor affected fiber production in other goat breeds as reported for the Inner Mongolia cashmere goat (Zhou et al. 2003) and for the Angora goats (Allain et Roguet, 2003).

As showed in the Table 4 the heritability was low for the DW and high for the DFD with values of 0.12 and 0.41 respectively. Of the three traits studied in this paper the CVDFD had the highest heritability with a value of 0.59. In our study, the low heritability value for DW differs from those reported for the Inner Mongolia cashmere goat (Zhou et al.2002) and for the Arbas strain (Bai et al. 2006) where the heritability was found to be moderate with values of 0.28 and 0.30 respectively. A moderate heritability of DW (0.35) was also found for the Liaoning cashmere goat in a study by Ning et al. (2005). For the DFD we found an heritability value similar to those reported from previous studies on Arbas strain and Liaoning breed (Zhou et al. 2002; Bai et al. 2006; Ning et al. 2005, Wang et al., 2013 and 2015) in which the value was moderate ranging from 0.28 to 0.42.

The DW showed negative genetic correlation with DFD (-0.27) while was highly genetically correlated with CVDFD with a correlation coefficients of 0.63. The genetic correlation between DFD and CVDFD was moderate with a value of 0.39.

The phenotypic correlation of DW was positive for DFD (0.20) and negative and not statistically significant for CVDFD (-0.11) while DFD showed moderate phenotypic correlation with CVDFD with values of 0.29.

The negative genetic and positive phenotypic correlation between DW and DFD from our study are in disagreement with the correlations estimated by Bai et al. (2006) and Wang et al., (2013; 2015), which are both positive.

**Table 4:** Heritability (bold), genetic correlations (above diagonal) and phenotypic correlations (below diagonal) for the fiber traits

Trait	Down weight	Down fiber diameter	CVDFD
Down weight	<b>0.12 ± 0.03</b>	-0.27 ± 0.20	0.63 ± 0.16
Down fiber diameter	0.20 ± 0.05	<b>0.41 ± 0.08</b>	0.39 ± 0.10
CVDFD	-0.11 ± 0.06	0.29 ± 0.07	<b>0.52 ± 0.06</b>

However, the largest differences between our estimates of genetic parameters and those found in other works may be due to the differences in genetic structure between the breeds. Differences in the sample size, the definitions of the traits, the models used for analysis, and the environments to which the populations are subjected may also contribute to these differences (Zhou et al. 2003).

According to our results, in the Chinese Alashan Left Banner White Cashmere goat the selection for reducing DFD and its CVDFD is possible and the genetic progress can be achieved quickly. Both traits have high heritability and high positive genetic and phenotypic correlations, therefore selection based on phenotypic values will lead to a genetic improvement of these two traits. Otherwise, the direct involvement of DW on selection appears not useful due to his low heritability.

## Conclusion

In Alashan Left Banner White Cashmere goat the genetic parameters are favorable for selection of homogeneous and fine fleece while the selection for yield in cashmere would lead to a slow genetic improvement of the trait.

Our data make an addition to understanding the genetics of cashmere fiber production in Alashan Left Banner White Cashmere goat and provide a basis for the development of the efficient genetic selection plan.

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# Effects of Year and Sampling Site on Mean Fibre Diameter of Alashan Cashmere Goat

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**Abstract.** Fourteen female Alashan white cashmere goats were investigated for fibre fineness (diameter) and coefficient of variation of fineness (CV). The investigation focused on the same animals combed at 1, 3 and 4 years of age. Fibre of the animals was collected from 10 different body areas. A statistically significant effect was observed for both, year of collection, and body areas. The present paper suggests that the fibre of one-year old animals should be kept separately from older ones and likewise, fibre from upper parts of the body should be separated from the others.

**Resumen.** Se estudiaron catorce cabras Cashmere Alashan de capa blanca para determinar la finura de su fibra (diámetro) y su coeficiente de variación (CV). Todas las muestras procedían de los mismos animales tomadas a los 1, 3 y 4 años de edad, obteniendo fibras de 10 diferentes áreas corporales. Tanto el año de recolección como el área corporal mostraron efecto estadísticamente significativo. Este trabajo sugiere que deberían tratarse por separado las fibras de los animales de un año de las de animales adultos, así como las fibras obtenidas de la región superior del cuerpo de las del resto.

**Keywords:** Cashmere goat, Alashan, age, body areas

## Introduction

China is the largest producer of cashmere by far, and Alashan white cashmere goats are famous for the quality of their fibre (<http://www.naturalfantasy.it>). Together with homogeneity and length of cashmere fibres (Schneider, 2010), fineness is one of the most important characteristics for spinnability and value of textile products (Allain and Renieri, 2010; McGregor, 2006). In animal fibre producers, the diameter seems to be affected by both, body areas and age (Antonini et al., 2004; Aylan-Parker and McGregor, 2002; McGregor et al., 2011; Tabbaa et al., 2001; Taddeo et al., 2000). Yet, breeders comb cashmere on the whole body of goats, and cashmere from animals of different ages is collected in the same bag, thus reducing the value of cashmere fibre. The aim of this paper is therefore, to promote a new method for fleece collection which may lead to obtaining more fineness and homogeneous cashmere lots.

## Materials and methods

Fourteen female Alashan cashmere goats, born in 2008 at the Animal Improvement Station of Alashan Left Banner, were randomly selected. The farm is located in the territory of Jilantai, in Alashan Left Banner, a semi-desert steppe environment. Selected goats were sampled and weighed at 1, 3 and 4 years of age since May 2009. The cashmere fibre was collected from 10 areas of the goats right body side (Fig. 1) and analysed only for the undercoat. An average of 8,850 fibres for each sample, were analysed by OFDA 100. Investigations were conducted on fibres' diameter and CV. Pearson's correlation coefficients between body weight, diameter and CV, were calculated at different ages. As a significant correlation between the body weight and the other two variables was not observed throughout the whole period of investigation, we disregarded this variable in the "analysis of repeated measures procedure" (Landau and Everitt, 2004), which was aimed at detecting the effects of age and body parts on the fibre parameters investigated. Once it was determined that differences in age and body areas do exist among the means of parameters investigated, pairwise multiple comparisons were used to determine which means differed, as per Hochberg's GT2 significant difference test. Statistical analyses were carried out with SPSS12.0 software.



**Figure 1:** Sampling areas of the goat body

## Results

There were significant differences between fibre diameters of 15.6  $\mu\text{m}$  (sd = 0.64), 17  $\mu\text{m}$  (sd = 0.99) and 16.4  $\mu\text{m}$  (sd = 0.89) for 2009, 2011 and 2012, respectively ( $F = 16.84$ ;  $P < 0.001$ ). Also differences in body weight were found with 20.21 kg (sd = 0.72), 25.68 kg (sd = 0.65) and 27.0 kg (sd = 0.99) for 2009, 2011 and 2012, respectively. Here, body weight did not show homogeneous correlations with the other two characters as presented in table 1.

**Table 1:** Pearson correlation coefficients for body weight (BD), fibre diameter (FD) and the related CV. Coefficients were calculated at 1 (2009), 2 (2011) and 4 (2012) years of age

Year	2009		2011		2012	
	FD	CV	FD	CV	FD	CV
BD	0.351*	-0.123	0.532*	0.687*	-0.003	0.382*

\* Correlation significant at  $p = 0.01$

Significant differences were also observed among the 10 different body areas ( $F=27.01$ ;  $P<0.001$ ) for fiber diameter and CV. The pairwise multiple comparisons method identified three homogeneous groups: the first included the areas 3, 5, 1, 2, 6, 10 and 7, with lower finesses ranging from  $15.7\ \mu\text{m}$  up to  $16.3\ \mu\text{m}$ ; the second included the areas 6, 10, 7, 4 and 9, with intermediate fineness from  $16.2\ \mu\text{m}$  up to  $16.9\ \mu\text{m}$  and the third included the areas 4, 9 and 8 with coarser fibres from  $16.5\ \mu\text{m}$  up to  $17.1\ \mu\text{m}$ .

Correspondingly, the CV with an average of 26.22 % (sd=4.03), 31.16 % (sd=10.79) and 30.71 % (sd=8.26), for 2009, 2011 and 2012 respectively, proved to be significantly different ( $F=18.53$ ;  $P<0.001$ ). Besides, differences were observed also among the 10 different body areas ( $F=5.75$ ;  $P<0.001$ ). The pairwise multiple comparison method identified two homogeneous groups: the first group included the areas 1, 2, 3, 7, 5, 4, 6, 8 and 10 with lower CV ranging from 25.38 % up to 31.76 %, and the second group included the areas 6, 8, 10, and 9, with higher CV, from 30.41 % up to 35.77 %.

## Discussion

The results of this investigation indicated a strong and significant effect of both, the animals' age and its body areas on the diameter of cashmere fibres, thereby partially confirming the results of a previous study (McGregor et al., 2011). Animals combed at one year old showed a better diameter (on average  $< 1.11\ \mu\text{m}$ ) and a major homogeneity (on average  $\text{CV}<4.71\%$ ) when compared to the animals combed at 3 and 4 years of age. The ten different body areas, according to the fibre diameter, can be categorized into three different groups. The first includes areas from the upper part of croup and the thorax (areas: 3, 1, 2, and 5); the second includes neck, shoulder, thigh and anterior legs (areas: 7, 10, 6, and 4) and the third includes hind leg and belly (areas: 8 and 9). The areas of the first group are considered best, as they show on average: i) a diameter of  $15.89\ \mu\text{m}$  (sd=0.14) vs  $16.32\ \mu\text{m}$  (sd=0.14) and  $16.98\ \mu\text{m}$  (sd=0.18) of second and third group, respectively, ii) a CV of 25.58 % (sd=0.29) vs. 28.67 % (sd=2.46) and 32.93 % (sd=4.01) of second and third group, respectively.

## Conclusion

The present results suggest collecting cashmere in a rational way: i) for age, combing first the younger goats and then the older ones and ii) for body areas, keeping the fibre of the upper body parts separately from those of the other parts of the body. Moreover, utilizing this method, the farmers will obtain batches with lower contamination because the first combed areas are generally cleaner and finer than areas that are more contaminated by both vegetable matter and excrements.

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## Abstracts





# **Sustainable Cashmere, Pastoralism, and Coexistence with Predators in Europe**

**N. Kravis**

The creation of a European model of SUSTAINABLE CASHMERE produced by cashmere goats born and raised in Italy. Economic, environmental, energetic, occupational and socio-political sustainability are examined, as well as the fiber characteristics that define the process of genetic selection practiced since 1995. Land management, biodiversity, and animal welfare in the European community are dependent on pastoralism: the essential link between intensive farming and abandonment of marginal agricultural land. The effect of wolf protection and consequent increase of the density of predators in many European countries has had major repercussions on pastoralism and extensive livestock management. The practice of non-lethal predator control (“PREDATOR FRIENDLY FARMING”) is essential to create and maintain a sound ecological equilibrium. But still, much needs to be done at a political level in order to assist the shepherds and to make it viable on a larger scale.

## **Efecto de la precipitación pluvial en la seja de selva y la zona alto andina de la región Puno sobre la producción ganadera de altura**

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El cambio climático se define como una modificación identificable y persistente del estado del clima por variabilidad natural o por efecto de la actividad humana. La zona altoandina se encuentra por encima de los 4,000 msnm (piso puna) abarca el mayor porcentaje de la superficie y se caracteriza por su alta vulnerabilidad a fenómenos climáticos adversos. La precipitación pluvial en la ceja de selva tomando como referencia al mes de agosto en promedio es de 76.3 mm incrementándose en los meses de lluvia y la zona altoandina en promedio es de 18.5 mm y de forma irregular. El año 2016 se caracterizó por una severa sequía que no superó los 350 mm de precipitación pluvial, el seguimiento de 15 unidades productivas de comunidades campesinas nos permitió determinar que el número de afecciones dentro del hato (animales enfermos y muertos) llegó hasta 11.34 % en los adultos y 35.12 % en las crías en comparación con año un normal (2012) y esta se debió a la escases de pastos y agua que afectó a las madres lactantes y en gestación. El porcentaje de abortos en alpacas llegó hasta 33.5 % y en llamas hasta 18.21 % en comparación al promedio general en adultos que no supera el 2 % en un año normal. La aparición de enfermedades infecciosas en alpacas crías llegó hasta un 27.70 % en diarreas y en los adultos hasta un 24.50 % como la fiebre de las alpacas. Se concluye que una baja precipitación pluvial tiene un efecto negativo sobre la producción y productividad de las alpacas y llamas en la zona alto andina, no siendo manifiesto en la zona de ceja de selva.

## Evaluation of Population and Social Composition of Vicunas (*Vicugna vicugna*) in Different Environment Sites of the Laguna Blanca Biosphere Reserve (Catamarca, Argentina)

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The wild vicuna is an important resource for the Laguna Blanca Reserve, where capture and shearing for the sustainable use of its fiber is practiced. The objective of this study was to evaluate population variables of vicunas in eight different vegetation communities of the reserve. Vicuna counts were conducted by direct observation in communities of shrub steppes (2), herbaceous steppes (2), and waterfowl meadows (marshes) (4) during April 2013. Relative abundance (I) (observations/time), percentages of family group (% FG), single males (% SM) and number of offspring (No. Off.) were determined. Regarding FG, we determined Family Group Size (FGS) and Male:Female offspring ratio. The % FG and FGS were assessed on the basis of statistical evidence (Student's T-test) of non-compliance with the FG standard  $\geq 60$  and  $FGS \geq 6$ . MFO<sub>r</sub> was assessed on the basis of statistical evidence ( $\chi^2$  goodness test) of non-compliance with MFO<sub>r</sub> standard  $\neq 1:4:2$ . The largest number of vicunas was recorded in the steppes (shrubs and herbaceous) (113) as well as the No. Off. (19). In the environments of herbaceous steppes, greater % FG was observed, and in the bushes steppes greater % SM. Good values of I in herbaceous and shrub steppes were obtained, but in marsh environments no vicunas were reported. It is concluded that environments shaped by shrubs and herbaceous steppes meet at least two of the three population attributes. In marshes, despite being the best communities, they did not meet any attributes. The absence of vicunas may be due to the presence of domestic livestock on these sites.

## **Animal Welfare Problems in South American Camelids Kept in Europe**

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Since the beginning of the 1980s, llamas and alpacas have been increasingly used as pets, companion and recreational animals in various European countries. They are often kept in smaller herds (2 to 8 animals), which underlines the hobby character. A few breeders are aiming at an agricultural use of the animals. The legal basis for keeping domesticated camelids (llamas and alpacas) is in most countries the Animal Protection Law and in addition, in the case of farming, specific regulations for farm animals. Only in some countries, specific regulations are available for South American Camelids (SACs). SACs are herd animals. Therefore group housing with at least two animals is required. Exceptions are sexually mature males. However even they, if kept alone, must have at least visual contact with the herd. The majority of SACs kept in Europe are on pasture from April to November, which is good from an animal welfare point of view if minimal requirements are fulfilled. SACs older than 6 months, need at least 1000 m<sup>2</sup> area for the first two animals. Each additional animal needs about 100 m<sup>2</sup> more. SACs are “distance animals”. If possible, they avoid direct contact to each other. This must be taken into account, in particular, in the design of stables and shelters. Because of the small herd size and their use as pets, they are in close contact with humans. This may lead to miss behaviour (Berserk-Male syndrome), especially if this is practiced during the early life of the cria. Several typical welfare problems are discussed in the presentation.

## Breeding Objectives for Alpacas of the Highlands Central of Peru

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The aim of this study was to determine the breeding objectives for alpacas. The study was carried out in the communal cooperative San Pedro de Racco located in the region of Pasco-Peru and the records of 2933 alpacas were evaluated during the period 2006-2012. The determination of the breeding objectives, profit equation and economic values was made based on Ponzoni (1986), Tolone et al. (2011), Alfonso et al. (2012) and Borg et al. (2007). The breeding objective was determined assuming the following economic scenario: Sale of white alpaca fiber with flat price, sale of alpacas for meat and sale breeding animals. The profit equation formulated was:

$$EC2 = NM \times TN \times SV (PVL1 \times pf + P1E \times pcb \times 0.43 + (DF1E + DS + RZ + BG) \times pp \times 0.2 - ct) + (NM + NP) (PVL \times pf + PV \times pcb \times 0.2) + NP ((DF + DS + RZ + BG) pp \times 0.1) - NM \times cm - NP \times cp$$

Where the characters were survival at the 1st shear (SV), diameter of fiber at the 1st shear (DF1E), weight of fleece at the 1st shear (PVL1), weight at the 1st shear (P1E), diameter fiber (DF), fleece weight (PVL), live weight (PV). The visual evaluation characters of fiber were general balance (BG), density (DS) and curls (RZ). The economic value of SV, DF1E and P1E was S/. 411.18, S/. 3.86 and S/. 1.10 soles per alpaca mother respectively. The economic value for DS, RZ and BG was S / . 0.24 soles per alpaca mother, being minimal in comparison to the other characters evaluated. The breeding objective determined was:

$$H2 = 411.18 \times GSV + 3.86 \times GDF1E + 4.92 \times GPVL1 + 1.10 \times GP1E + 0.24 \times GDF + 8.26 \times GPVL + 0.86 \times GPV + 0.24 \times GDS + 0.24 \times GRZ + 0.24 \times GBG$$

Where: GSV, GDF1E, GPVL1, GP1E, GDF, GPVL, GPV, GDS, GRZ, GBG, are the genetic additive values of the characters studied. It is concluded, that the production traits SV, PVL, PV, DF, DF1E, P1E y PVL1 are of greater economic importance than the visual evaluation characters BG, DS and RZ. Therefore the production traits must be included in the breeding objectives for the breeding of alpacas.

## Vicugna Pacos As1-Casein: Identification of New Polymorphisms at the Csn1s1 Gene

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Genetic polymorphism in milk proteins is due to gene mutation resulting in either substitution or deletion of amino acids sequence along the peptide chain. South American camelids were genetically poorly investigated so far and little information is available in alpacas (*Vicugna pacos*) regarding the diversity of the caseins. The aim of this study was to investigate the presence of polymorphisms at the CSN1S1 gene in alpacas at protein and DNA level. The analysis of whole alpaca milk by IEF from 20 samples evidenced polymorphic protein patterns corresponding to  $\alpha$ s1-CN migration area. According to the nomenclature in Llamas, the variants were 2 and 3 with 2 being the anodic one. Three variants (2/2, 2/3, 3/3) could be observed. Estimation of frequencies resulted in 0.475 and 0.525 for the variants 2 and 3, respectively. Blood and hair samples were collected from 130 alpacas belonging to different flocks in Germany and Italy. They were used for DNA isolation. The SNP c.366A>G at the exon 12 was successfully genotyped by PCR-RFLP. Two (AA, AG) of the three possible genotypes could be demonstrated resulting in estimated allele frequencies c.366 A 0.91 and c.366G 0.09. Milk samples from IEF showed different genotypes at DNA level for the SNP (c.366Alle>GVal) as the mutation at the exon 12 does not lead to a relevant change in terms of pI of protein and therefore it was not possible to establish a link with IEF results. The polymorphisms found in alpaca have not been described before. The presence of the adenine (c.366A) at the exon 12 of the alpaca CSN1S1 might represent the ancestral condition of the gene because it has been found also in the other camelids. This data adds knowledge to the genetic variability of a species little investigated and opens the opportunity for further investigation in the field of milk protein for South American camelids.

## **Estimación de la heredabilidad de seis caracteres de calidad de fibra de alpacas huacaya del INIA Puno**

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El objetivo fue estimar las heredabilidades de seis caracteres de calidad de fibra de alpacas Huacaya a la primera esquila del banco de germoplasma del Instituto Nacional de Innovación Agraria, ubicado en el departamento de Puno a una altitud de 4,200 metros en la zona agroecológica de Puno seca. La base de datos constó de 9,114 registros de fibra y fueron analizados con el analizador óptico del diámetro de fibra (OFDA, IWTO-47-95) en el laboratorio de fibras del IVITA Marangani. Para estimar los componentes de varianza del diámetro de fibra, desviación estándar del diámetro de fibra, factor de confort, coeficiente de variabilidad del diámetro de fibra, longitud de mecha y peso vellón se utilizó el modelo animal unicaracter  $y = Xb + Zu + e$ , donde  $y$  es el vector de observaciones,  $b$  es el vector de efectos fijos (sexo, color y mes-año de esquila) la edad en días se consideró como covariable lineal,  $u$  es el vector que representa el efecto genético aditivo,  $e$  es el vector de residuales;  $X$  y  $Z$  son las matrices de incidencia de efectos fijos y aleatorios respectivamente. Los componentes de varianza fueron estimados por el método de Máxima Verosimilitud Restringida (REML) utilizando el programa VCE versión 6.0.2. La heredabilidad estimada para el Diámetro de fibra fue de alta magnitud  $0.540 \pm 0.087$ ; para el resto de caracteres fue de media magnitud siendo para la Desviación estándar  $0.311 \pm 0.089$ , Factor de confort  $0.278 \pm 0.077$ , Coeficiente de variabilidad  $0.291 \pm 0.089$ , Peso vellón  $0.158 \pm 0.027$  y Longitud de mecha  $0.268 \pm 0.081$ . Se concluye que es posible obtener una buena respuesta a la selección para los seis caracteres de calidad de fibra en alpacas Huacaya.

## Effect of the Brown Coat-Coding Gene (Typr-1) on Wool and Skin Color of Żelaźnieńska and Wrzosówka Sheep

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The aim of the study was to assess the relationship between frequency of alleles and genotypes of the brown coat-coding gene (TYPR-1) and the color of wool and skin measured objectively. In order to better illustrate the color differences, two breeds were examined: white colored Żelaźnieńska sheep and colorful (mostly grey) Wrzosówka sheep. The study was conducted on randomly chosen ewes (Żelaźnieńska sheep – 93; Wrzosówka sheep – 133) during shearing. Color of wool and skin was examined using a Chroma Mater CR-400 device. Color was measured using the system CIE  $L^*a^*b^*$ .  $L^*$  expresses clarity and varies from 0 (black) to 100 (white).  $a^*$  varies from -60 to +60, where it ranges from - $a^*$  (green) to + $a^*$  (red).  $b^*$  varies from -60 to +60, where it ranges from - $b^*$  (blue) to + $b^*$  (yellow). Color of wool and skin measurements were taken on the right side of sheep at the point of the last rib. DNA was isolated from blood leukocytes. Sample genotyping was performed with the KASPar® system ([www.kbioscience.co.uk](http://www.kbioscience.co.uk)), which uses a single nucleotide polymorphism (SNP) and found three genotypes: CC, CT and TT. Significant and highly significant differences in all color measurements of wool (Żelaźnieńska vs. Wrzosówka:  $L^*$ : 75,64 vs. 46,09  $P \leq 0.01$ ;  $a^*$ : 0,67 vs. 2,33  $P \leq 0.01$ ;  $b^*$ : 5,84 vs. 2,75  $P \leq 0.01$ ) and skin (Żelaźnieńska vs. Wrzosówka:  $L^*$ : 70,03 vs. 54,26  $P \leq 0.01$ ;  $a^*$ : 4,53 vs. 3,98  $P \leq 0.05$ ;  $b^*$ : 6,18 vs. 3,17  $P \leq 0.01$ ) between tested breeds were found, which should be connected to the different wool color in each breed. Differences in color values of wool depending on TYRP-1 gene genotypes were observed only for Wrzosówka sheep. The measurement of the  $L^*$  color parameter made on wool was significantly higher in the case of CC and CT genotypes compared to TT genotype (respectively 47,25 vs 48,27 vs 38,10;  $P \leq 0.01$ ). However, in the measurement of the  $a^*$  color parameter, the situation was reversed and homozygote TT had higher values compared to CC and CT genotypes (3,58 vs 2,17 vs 1,92;  $P \leq 0.01$ , respectively). No differences between all skin color parameters for both breeds were found. The results of studies on wool color, depending on genotype of the TYRP-1 gene in Wrzosówka sheep, opens possibilities to conduct breeding work in order to develop standards for coat color in this breed.

## **Relationship between Classes Assigned by Visual Appraisal and a Selection Index in Function of Live Weight, Fleece Weight and Fiber Diameter in Huacaya Alpacas from Pasco**

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Visual appraisal evaluation is a common practice in highland livestock production of alpacas in Peru. An alternative is using selection indexes based on performance tests. The aim of this study was to evaluate the relationship between the classes assigned by visual appraisal with a selection index based on live weight, fleece weight and fineness in white Huacaya alpaca fiber. 1823 alpacas were evaluated, in which females and males were 1083 and 740 respectively. Index coefficients were estimated for the productive characteristics of live weight (10.41), fleece weight (0.60) and fiber diameter (-11.65), using market prices for alpaca fiber at the producer level of 2014 (MINAGRI 2016). This selection index has an estimated genetic progress for live weight of 1,047 kg, for fleece weight 0.050 kg and fiber diameter -0.71 microns. A linear additive model was used for the comparison of this index with the selective classes, where live weight is included as a covariable. Statistical differences ( $p$ -value  $<0.05$ ) were found between selective class, shear number and source. Selective classes S, A and B show statistically equal means and selective class C shows statistical differences with the other averages for the selection index. When applying a selection index, the weighting to each of these characteristics is governed by the economic value obtained, so it is shown that the selective classes S, A and B have no difference between their averages of the index score.

## Preliminary Comparative Analysis and Localization of *Bos Taurus* SNPs on *Vicugna Pacos* Chromosome 10 (Vpa10)

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Current genomic information on alpaca (*Vicugna pacos*) is incipient. To increase the number of SNPs, we used the Illumina (Bovine Hd Genotyping – 777kSNP) DNA chip to genotype 40 female Huacaya alpacas and identified a total of 10314 potentially similar SNPs between cattle and alpaca. To determine if our strategy has validity and confirm similarity and chromosomal location of SNPs in alpaca, we used the genome database of the *Vicugna\_pacos*-2.0.1 alpaca (ABRR00000000.2; NCBI) and the chromosomal location of 230 molecular markers as well as ZooFISH, generated by Avila et al. (2014) and Balmus et al. (2007), respectively. Positive SNPs in alpaca were compared to sequences from the 4195 scaffolds (KB632434: KB635807; NCBI) with the Megablast software. For preliminary validation of our strategy, we selected all the SNPs located on BTA29 that were positive in the alpaca and compared the sequences of those SNPs with the alpaca scaffolds sequences associated with markers found on chromosomes 10 (VPA10) and 33 (VPA33) (Avila et al., 2014). The latter, supported by ZooFISH analysis, indicated that BTA29 is equivalent to VPA10 and PVA33 according to Balmus et al. (2007). These comparisons identified 42 bovine PNSs co-localized in VPA10 and zero PNSs in VPA33. These preliminary studies indicate that it will be possible to identify an adequate number of conserved SNPs between bovine and alpaca that will allow enriching the genomic information of the alpaca. This strategy demonstrates an alternative to be applied to species with little or no genomic information.

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Balmus G., Trifonov V., Biltueva L., O'Brien E., Fu B., Skidmore J., Allen T., Graphodatsky A., Yang F., Ferguson-Smith M., 2007. Cross-species chromosome painting among camel, cattle, pig and human: further insights into the putative *Cetartiodactyla* ancestral karyotype. *Chromosome Research* (2007) 15:499–514 DOI: 10.1007/s10577-007-1154-x

## Innovative Andrological Evaluation to Optimize the Selection of Fiber Animal

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Breeding soundness evaluation (BSE) is an overall assessment of the ability of a male to mate and to initiate reproduction. It is estimated that sub-fertile males represent the 20 % in an unselected population. Classical BSE includes: libido assessment, physical examination, examination of the reproductive organs and semen evaluation. The development of new diagnostic techniques has introduced new tools to integrate the classical BSE. Trans-scrotal ultrasonography is a non-invasive technique that is ideal in on-farm condition. The echo-doppler approach can increase the accuracy on the testis vascularity evaluation. Ultrasonography applied on the accessory glands can optimize the precision of the BSE in camelids, ram and buck. Testicular fine-needle aspiration cytology reports are available in many species and it has been described to enhance BSE accuracy. The exogenous administration of GnRH has the effect to increase serum levels of LH and testosterone. GnRH challenge test has been associated with the scrotal thermography evaluation. Some studies showed the relation among seminal plasma components with sperm function, stress resistance and cryopreservation success. The *sperm zone pellucida binding test* is based on the concept that spermatozoa need to be able to pass the zone pellucida in order to fertilize an oocyte. The *sperm oviductal cell adhesion test* is based on the evidence, that in-vivo adhesion of spermatozoa to isthmic cells is necessary to develop the ability of fertilization. *In-vitro taxis factors test*, with microfluidics technologies, and *in-vivo sperm test*, based on the use of fibered confocal microscopes, can be able to identify the place reached by sperm after time unit from the insemination. All these new available approaches can increase precision and accuracy of a classical BSE during the selection process of males.

## Use of Seminal Plasma on Interval to Ovulation, Susceptibility of Corpus Luteum to Prostaglandin and Improving of Reproductive Performance in Alpacas (*Vicugna Pacos*) under Peruvian Highland Conditions

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Alpacas, like all camelids, are induced ovulators which means, mating is required to induce ovulation (San Martin et al., 1968). The reproductive efficiency of alpacas under Peruvian highland conditions is low due high embryo mortality, short breeding season and long gestation period and it is one of the main factors that affect the development of breeding programs to produce genetically superior animals with natural mating. The presence of an ovulating inducing factor (OIF) in the seminal plasma of alpacas has been reported by Rios (1989) and later confirmed by Adams et al. (2005). Seminal plasma can, therefore, also be used as an alternative for inducing ovulation with subsequent development of a corpus luteum (CL) capable of maintaining pregnancy and improving the reproductive performance in alpacas. Experiments were performed with the objective to evaluate the effect of seminal plasma (SP) application on: the interval at ovulation (Exp. 1); susceptibility of the corpus luteum to the prostaglandin (PG) induced to ovulation with seminal plasma (Exp. 2); improvement of the pregnancy rate in alpacas under natural mating (Ex. 3); or to evaluate the effect of seminal plasma in females with different times of mating (Exp. 4). Adult, non-pregnant alpacas were used for the different experiments. The animals were evaluated by ultrasound to determine the presence of a dominant follicle ( $\geq 7$  mm) and then assigned to the treatments. Exp. 1: Alpacas were assigned to T1 (n=12) application of 1.0 mL of SP or T2 (n=12) application of 0.04 mg of GnRH (Acetate of busereline) and evaluated by transrectal ultrasonography with a transducer 7.5 MHz (Aloka SSD500) every two hours from 20 to 40 hours or disappearance of dominant follicle previously observed. Exp.2: alpacas were assigned to the following treatments: T1 (n=8): SP + PG D4; T2 (n=8): GnRH+PG D4; T3 (N=8): SP+PG D5; T4 (n=8): GnRH+PG D5; T5 (n=8): SP+PG D6; T6 (n=8): GnRH+PG D6; T7 (n=8): SP+PG D7; T8 (n=9): GnRH+PG D7; T9 (n=8): SP+PG D8;

T10 (n=8): GnRH+PG D8; T11 (n=6): Control: 1mL Saline solution. Animals were evaluated by ultrasonography every twelve hours after application PG (196 µg. Tiaprost). Exp. 3: Non-pregnant alpacas with a follicle  $\geq 7$  mmm were bred by natural mating and then assigned to T1 (n=40): Mating + 1 mL SP; T2 (n=39): Mating + 0.042 mg GnRH and T3 (n=38): Control. Animals were evaluated by ultrasound to pregnancy rate on Day 25 and 62; Exp. 4: Alpacas evaluated by ultrasound were assigned randomly to the following treatments: T1 (n=28): natural mating 5 min; T2 (n=28): natural mating 5 min. + 1.0 mL SP; T3 (n=27): natural mating 10 min.; T4 (n=27): natural mating 10 min + 1.0 mL SP; T5 (n= 26): natural mating  $\geq 15$  min; T6 (n=26): natural mating  $\geq 15$  min + 1.0 mL SP. Results were: EXP.1: Interval to ovulation was  $27.1 \pm 1.9$  and  $26.6 \pm 1.6$  h to SP and GnRH, respectively. EXP. 2: luteolysis was 0.0 %, 0.0 %, 25.0 %, 0.0 %, 100.0 %, 100.0 %, 100.0 %, 100.0 %, 100.0 %, 100.0 %, and 0.0 % for the treatments T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 respectively. EXP. 3: Pregnancy rate was 67.5 %, 51.3 % and 55.3 % to D25 and 92.6 %, 80.0 % and 85.7 % of embryo survival to D62 to T1, T2 and T3 respectively. EXP. 4: Pregnancy rate was 50.0 %, 64.3 %, 62.9 %, 70.3 %, 76.9 % and 80.0 % to D25. The results suggest that SP does not induce differences with GnRH on interval to ovulation and susceptibility to corpus luteum but would be an important alternative to improve the reproductive performance in alpacas with an increase of embryo survival and the use of males to mating a major number of females with similar pregnancy rate.

## Induction of Superovulation in Alpacas According to the Number of Follicles Recruited to the Emergence of Follicular Wave

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Two experiments were designed to evaluate the response to induction of superovulation with equine Chorionic gonadotropin (eCG). EXP. 1: 34 Alpacas were evaluated by ultrasonography to determine the presence of a dominant follicle ( $\geq 7$  mm) and then induced to ovulation with 0.04 mg of GnRH (Acetate of buserelina). They were then evaluated on D2 to determine ovulation on the disappearance of the dominant follicle previously observed and further evaluated by transrectal ultrasonography with a transducer 7.5 MHz (Aloka SSD500) every two days to determine the number of follicles recruited in successive follicular waves. EXP 2: According to the number of follicles emergent  $\geq 3$  mm at Day 4 post induction of ovulation, the alpacas were assigned to T1 (n=13) with  $2.4 \pm 0.5$  follicles and T2 (n=12) with  $6.0 \pm 2.27$  follicles. Alpacas with presence of a dominant follicle  $\geq 7$  mm were induced to ovulation with 0.04 mg of GnRH (Acetate of buserelina). They were then evaluated on D2 to determine ovulation on the disappearance of the dominant follicle previously observed and then induced to superovulation with 700 IU of eCG IM according to the protocol described by Huanca et al. 2009. The alpacas were mounted with fertile males and seven days later were flushed to recover the embryo. The average number of emergent follicles  $\geq 3$  mm at Day 4 was  $3.6 \pm 2.6$  follicles. The repeatability of the number of recruited follicles in successive follicular waves was 0.46. The superovulatory ovarian response in T1 and T2 was  $10.0 \pm 6.9$  and  $10.0 \pm 8.1$  follicles ( $P = 0.68$ ) and  $8.5 \pm 4.3$  and  $7.5 \pm 4.3$  ( $p=0.77$ ) corpus luteum. Embryo recovery rate was 2.6 and 2.2 embryos to T1 and T2, respectively ( $p=0.47$ ), with embryos of good quality. The results suggest that the alpacas present a small number of emerging follicles by follicular wave during their recruitment and that this affects the selection of donors with irregular and low response to superovulatory protocols. This would be linked to intrinsic factors associated with ovarian reserve and genotype.

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## Farmers Wool and Traceability

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Consortium Biella, The Wool Company

Globalisation has been of great benefit for many, but in many cases it has caused unrepairable damage to minor supply lines of agricultural by-products, such as wool. Cheap freight costs to Asia have heavily diminished wools value, especially where the wool is not destined for quality products, but mainly towards the upholstery and furnishings sector. However, new opportunities for the small sheep-breeder are presenting themselves, thanks to a more knowledgeable consumer, who may not be totally in favour of biological labelling (often not quite as transparent as one would hope) but believe in transparency. Wool is natural, renewable and sustainable, and therefore has the perfect characteristics for ethical processing here in Europe, to satisfy this growing demand.

## Feed Intake and Animal Behaviour of Alpaca and Llamas Co-Grazing on Andean Highlands in Peru

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Despite huge interest in raising South American Camelids (SACs) – either as a hobby or for livelihood – and a vast number of scientific publications addressing SACs, relatively few efforts have been made to study feed intake and animal behaviour under grazing conditions. Data on voluntary feed consumption and nutrient requirements are mainly from animals kept in barns or derived from studies involving sheep, goats and cattle (National Research Council, 2007). Therefore, limited information on grazing behaviour of SACs is available (Gauly, 2011). Hence, a comparative study with alpacas and llamas co-grazing on Andean pasture in Peru was conducted with the aim at (i) Exploring group specific differences (i.e. alpaca/llama, male/female) on activity patterns, feed intake, and digestibility; (ii) Relating individual and group behaviour, intake and digestibility parameters with performance indicators and vegetation characteristics. Out of 634 alpacas and 74 llamas co-grazing on an area of 340.6 ha, twelve eight-month-old alpacas (huacaya) and llamas (q'ara) (six females and six males, respectively) were randomly selected for the study. Data was collected during the transition from dry to rainy season between October and December 2016 in the Pasco region (4,350 m.a.s.l.) of Peru. Animal movement was monitored by fitting the animals with global positioning system devices for twelve consecutive days. Simultaneously, visual observation at a 30 seconds interval was performed, with one and a half hours observation per animal. Animal activities monitored were grazing, traveling, resting, drinking, and others. Feed intake and organic matter digestibility were estimated by the combination of internal (Titanium dioxide, 2.5 g/d) and external markers (acid insoluble ash). Faecal grab samples were collected during five days. Diet simulation sampling was performed for four alpacas and four llamas. Vegetation samples for biomass production and botanical composition were taken at two different moments from 14 transects across the grazing area. Live weight of animals was recorded every four weeks. Finally, fibre of alpacas was cut (mid side sampling) at the beginning and at the end of the experiment to estimate clean fibre weight, mean fibre diameter, and fibre length. Differences in grazing behaviour and vegetation utilization between both species and between sexes within a species will be evaluated. Similarly, the relationship between grazing parameters and performance between species and sexes will be assessed. Finally, the existence of a

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preferential grazing, or not, by different species as affected by biomass availability, quality and botanical composition will also be tested.

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## Daily and Seasonal Changes in Body Temperature and Activity Patterns of Llamas in the High Andes of Peru

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Endothermic animals have to invest a substantial amount of energy to keep their body temperature (T<sub>b</sub>) within a narrow limit. Recent studies on wild ungulates, however, revealed that they are able to adjust their T<sub>b</sub> and locomotor activity (LA) according to season and time of the day and thus save energy during adverse environmental conditions. Therefore, the aim of the study was to determine whether the llama, one of the most extensively kept domestic livestock breeds, also exhibits seasonal adjustment mechanisms in terms of T<sub>b</sub> and LA under High Andean conditions. For the present study, 7 female adult non-pregnant llamas were kept within a herd of approx. 300 female llamas under a traditional herding system in the High Andes of Peru. The study site was a semi-arid habitat and located 80 km to the North of the city of Arequipa in Southern Peru at an altitude of 4,400 m. The LA and T<sub>b</sub> (measured in the rumen) were recorded continuously for each animal every 3 min and the location every 30 min for 10 months using a telemetry system. Relative humidity and ambient temperature (T<sub>a</sub>) were recorded every 60 min using miniature data loggers. Daily T<sub>b</sub> varied considerably with daily maximum (mean max.  $\pm$  SD:  $39.47 \pm 0.32$  °C, max. range: 40.55 - 38.97 °C) and minimum (mean min.  $\pm$  SD:  $37.49 \pm 0.36$  °C, min. range: 36.58 – 38.12 °C) T<sub>b</sub>'s occurring around midday and early morning, respectively throughout the study period. The daily T<sub>b</sub> amplitude differed significantly ( $P < 0.001$ ) between seasons and was highest in June-Sep, when T<sub>a</sub> was low (average daily mean  $\pm$  SD:  $1.94 \pm 1.43$  °C, average daily amplitude:  $34.54 \pm 4.79$  °C) and lowest in Dec-March when T<sub>a</sub> was high (average daily mean  $\pm$  SD:  $6.93 \pm 1.22$  °C, average daily amplitude:  $22.34 \pm 3.68$  °C). Average daily T<sub>b</sub> followed the T<sub>a</sub> pattern, i.e. T<sub>b</sub> was correlated with T<sub>a</sub> ( $r = 0.43$ ,  $P < 0.001$ ). Daily distances covered averaged  $5.3 \pm 1.2$  km and ranged from 3.4 - 11.2 km per day. Mean daily LA varied considerably over the study and followed a similar pattern as T<sub>a</sub> with the lowest daily average LA recorded in June ( $19.58 \pm 16.34$  %) and the highest in Jan ( $36.35 \pm 32.9$  %).

The results indicate that llamas in the high Andes adjust their T<sub>b</sub> and LA according to season and time of the day in accordance to T<sub>a</sub>. Throughout the study we found a distinct daily T<sub>b</sub> and LA rhythm. Therefore, despite of the domestica-

tion process, llamas seem to have maintained the ability to adjust their Tb under adverse environmental conditions as has been reported for some wild ungulates.

## Blood Levels of Phosphorus in Pubescent Alpaca (*Vicugna Pacos*) and the Effect of Dietary Phosphorus on Growth of Female Alpacas Post Weaning in Peruvian Andes

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Phosphorus deficiencies have been reported for alpacas in the Peruvian Andes. This is of special relevance because their hidden effects may be affecting some reproductive variables and the growth in one-year-old pubescent alpacas forcing the farmers to start mating at two years with the economic consequences of feeding without producing and delaying genetic progress. This study is performed in an effort to know the status of phosphorus and the effects of this mineral on feed consumption and live weight variables in young female alpacas in two important stages of production such as breeding season (January - February) and weaning (August - September). In the first stage of the study, blood levels of phosphorus were determined in 180 female alpacas of approximately 11-13 months of age located in four regions of greater importance for alpacas breeding in Perú. Levels of  $8.25 \pm 1.63$ ,  $5.25 \pm 1.46$ ,  $6.42 \pm 1.84$ ,  $4.8 \pm 1.61$  mg / dl were found for Cerro de Pasco, Junín, Cuzco and Puno regions, respectively. Significant differences ( $P < 0.05$ ) were found among the four regions, and these data were compared with the expected range for alpacas (Van Saun and Herdt, 2014: 5-11.5 mg/dl). Two regions (one below the mentioned limit and another at the limit) can be considered deficient in phosphorus. This may be related to the deficiencies of this mineral in the high Andean grasslands reported by some authors and may be affecting some reproductive, consumption and growth parameters associated with this mineral. In a second part of the study, a controlled experiment with different concentrations of phosphorus in the diet was performed: 0.16 % (T1), 0.25 % (T2), and 0.34 % (T3). Forty-five female alpacas post weaning ( $25.25 \pm 1.62$  kg) were used, with ad libitum feeding, with 15 animals per treatment housed in individual pens for a period of 4 months. Ages range from six to eight months. Dry matter intake, phosphorus intake and live weight were

evaluated. The average dry matter intake expressed per kg/d was 0.458, 0.562 and 0.652 for T1, T2 and T3 respectively, while the average consumption of alpacas expressed per kilogram of live weight was 1.52 %, 1.73 % and 1.97 % respectively. In both cases there were significant differences between treatments ( $P < 0.05$ ). There was an appreciable reduction in feed consumption when the diet had 0.16 % of phosphorus, while for the diets of 0.25 % and 0.34 % of phosphorus consumption values are expected in camelids for this stage. Finally, the live weight was  $30.58 \pm 1.42$ ,  $32.4 \pm 0.92$  and  $33.3 \pm 2.43$  kg for T1, T2 and T3 respectively, with significant differences ( $P < 0.05$ ) between T1 vs T2 and T3. The effect of lower consumption on T1 may have influenced in weight gain. Effects of phosphorus on reproductive variables to which it is associated are still being evaluated.

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## Digestibility of Bean Pulp Granulated in Rabbits

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Granulated beet pulp is a by-product of the sugar industry and it is used for feeding rabbits. The objective of this work was to determine the digestibility and energy value of granulated beet pulp from different sources in Spain by the method of substitution by total fecal collection in weaned rabbits. Therefore, 87 rabbits were distributed in 6 groups for 6 animal feeds (1 control feed and 5 feeds with 20 % inclusion of the beet pulp), with individualized feeding and consumption ad libitum. The results indicate, that the digestibility of the granulated beet pulp in rabbits was similar ( $p > 0.05$ ) in dry matter ( $76.0 \pm 6.3$  %) and organic matter ( $76.7 \pm 5.8$  %), with a higher tendency for Olmedo pulp. In contrast, the digestibility of the fiber fractions (FDN and FDA) showed difference ( $p < 0.0001$ ), with the best response for the same pulp. Protein digestibility and digestible protein content were similar as well ( $p > 0.05$ ), with  $62.3 \pm 6.3$  % and  $5.2 \pm 0.6$  %, respectively. The digestible energy content, with an average of  $12.8 \pm 0.8$  KJ / g of dry matter and an efficiency of use of  $0.72 \pm 0.05$  in relation to the gross energy was similar. These results confirm that the granulated beet pulp has good digestibility and is an important source of digestible energy for rabbits.

## Correlation between Diameter of Fiber, Medulation and Ancestrality in Alpacas

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The selection in alpacas is based on the fineness of fiber, a characteristic of great importance for the textile industry. However, it has been possible to observe fine animals with the presence of marrow in the fiber. In the present work, we evaluated the correlation between fiber fineness, the percentage of marrow and the correlation with its vicuña ancestor (*Vicugna vicugna*). The complete region was evaluated of the D-loop and the Cytochrome b region in 50 alpacas. The overall average for fiber diameter was  $20.26 \pm 2.69 \mu$ . Between the fiber diameter and the percentage of medulation, the correlation was 0.8322. This positive correlation indicates, that as the percentage of medulation increases, the fiber diameter will also increase. This would reflect the degree of improvement of the herd of alpacas in some way. The median-joining network topology of the D-loop and the Cytochrome b showed two haplogroups to determine ancestry. Regarding the correlation between the percentage of medulation and the vicuña ancestor, we found a negative correlation, whereas there was a positive correlation between the percentage of medulation and the guanaco ancestor. These results allow us to indicate the ancestry of alpacas by evaluating the percentage of medulation and corroborating with molecular genetics in the laboratory.

## **Apelin, a New Adipokine Acting on Hair Follicle: an Immunohistochemical Study on Ovine Skin**

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Apelin (AP) is a novel peptide belonging to the family of adipokines (Pitkin et al., 2010). Adipose tissue is a source of plasma AP and the secretion by this tissue is regulated by several factors including fasting and refeeding. Apelin specific receptor (APJ) shows a widespread tissue distribution and accordingly many physiological roles were described for AP. In this work, the expression of AP and APJ were investigated in the ovine skin by an immunohistochemical technique in order to point out the presence of structures that might be locally responsive to the action of AP. The analyses performed evidenced a clear and intense immunostaining for APJ in the ovine skin, while AP expression could not be observed. The receptor was localized in the hair follicles (HFs), while other structures of the skin appeared to be negative. APJ expression involved the outer root sheath and extended throughout follicular wall, from the infundibulum to the bulb. AP is a recently discovered molecule and, at present, there are not surveys describing it in the skin of any animal species including humans. The strong expression of APJ in the HF, suggests an important role of AP that probably acts on this organ through an endocrine mechanism. The identification of APJ in ovine skin is a preliminary study that introduces the study of AP in the skin and represents the beginning of a comparative survey that could contribute to the improvement of fiber quality.

Pitkin S.L., Maguire J.J., Bonner T.I., Davenport A.P. (2010) *Pharmacological Review* 62:331–342.

## **ICAR – Guideline for the Animal Fibre Production in Alpaca and Cashmere and New Rules for the Organization of the Fibre and Fleece Collection Centers**

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ICAR opened a working group on animal fibre in 2007. The working group first established standards and guidelines on alpaca fibre production and afterwards on cashmere goat production. Further, methods on alpaca and cashmere goat identification as well as guidelines for alpaca and cashmere shearing/combing, management, fibre harvesting and grading were established. The next step of the ICAR animal fibre working group will be, to define guidelines on the identification of the objective and the development of selection criteria in alpaca breeding. Further, the working group will be opening new guidelines for the definition of the animal fibre collection centers. The collection centers will firstly work on plans for the European wool production and subsequently for alpaca production in Latin - America and cashmere production in China.

## Technological Characteristics of White and Colourised Huacaya Alpaca Fibre in Apurímac, Perú

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The objective of this study was to characterize the different technological traits of alpaca fiber produced in the Apurímac region to document its present profile and to provide information for future improvements. Therefore, 145 fiber samples from white and colored alpacas from five communities were analyzed. The effect of sex, age, color and locality on fiber diameter (FD), standard deviation fiber diameter (SDFD), comfort factor (CF) and curvature index (CI) and the relationship between them were analyzed to determine the factors that should be considered when designing a breeding program for Huacaya alpaca in Apurímac, Peru. Statistical difference was found by sex, age, color and locality to the technological characteristics of FD, CF and CI. For SDFD, age was not significant. The FD increases with age ( $p < 0.05$ ) and females are finer than males, with 22.79 and 23.79  $\mu\text{m}$  respectively ( $p < 0.05$ ). There were statistical differences ( $p < 0.05$ ) between dark color fiber (26.69  $\mu\text{m}$ ), the light color (23.81  $\mu\text{m}$ ) and white (22.30  $\mu\text{m}$ ). The CF was 87.41 % in males and 91.23 % in females ( $p < 0.05$ ), decreasing with age ( $p < 0.05$ ). The CF was different among colors ( $p < 0.05$ ), less CF (75.94 %) with dark color. For CI, females were 38.23 % and males 33.76 % ( $p < 0.05$ ), decreasing with age ( $p < 0.05$ ). There were statistical differences ( $p < 0.05$ ) between dark color fiber (29.26 %), light color fiber (34.98 %) and white (38.29 %). There were statistical differences among the five localities ( $p < 0.05$ ) for all the technological traits. We concluded that the quality of white and colored Huacaya alpaca fiber produced in the region of Apurímac has good potential to be improved.

## **The Prickling Issue in Fabrics Made of Camelid Fibres: Possible Mechanical or Genetic Solutions**

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This lecture intends to analyze the physical attributes that determine the comfort of fabrics made of South American Camelid fibers (Lama and Alpaca), the effect on their value and their possible mechanical and/or genetic modifications. While emphasis has always been on mean fibre diameter -in order to determine fibre quality- fibre frequency exceeding 30 microns has a key role in quality. This is essential for light fabrics, where the effect of prickle plays a critical part in consumer's choice. Yet the problem lies in the slow selection response. Dehairing provides an immediate solution, though excessive fiber breakage should be addressed (Wang et al., 2008). It is concluded that the textile fibre quality of South American Camelids is promissory if the presence of objectionable fibres is solved, resulting in a tolerable frequency for consumers (< 3 %). This process could be explored via genetic selection (Frank et al., 2011) or applying dehairing technology (Frank et al., 2017). This implies a true paradigm shift with regard to the classic textile processing of Alpaca and Lama fibres. This would enhance the fibre softness to touch, together with other important features that would render the fibre price more competitive.

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Frank E.N., Frondizi Seghetti D.G., Hick M.V.H., Castillo M.F., Burgos A., Cruz A., 2017. Dehairing of alpaca fibres top with AM2 dehairing technology. This Symposium.

Wang L, Singh A, Wang X., 2008. Dehairing Australian alpaca fibres with a cashmere dehairing machine. Journal of the Textile Institute, vol. 99 (6): 539-544.

## Determination of the Optimal Number of Runs Using AM2 Dehairing Technology in Fibers of Patagonian Goats (Patagonian Cashmere)

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AM2 dehairing technology reduces the objectionable fiber content of shorn fleeces. Nonetheless, it reduces fiber length and the final yield is affected by the successive runs through the dehairing machine, though it allows to specify the number of passes required for each lot (Singh, 2003; Frank et al., 2009). The aim of this work is to establish the number of passes that the machine should make until optimal performance is reached from an objectionable fiber content, fiber length, fiber diameter and yields to dehairing perspective. Work was carried out on fiber sheared from north Patagonian goats, whose fleece was scoured and dehaired until the process seemed final from a visual assessment. From each run, samples were extracted for processing at the laboratory. Measurements comprised: yield to dehairing (% Y), objectionable fiber content (w/w) (FOC), average fiber length (FL) and average fiber diameter (FD). The variables obtained were fitted with a polynomial model and the first derivative of the function was calculated. The value of the variable for the run with the minimum value was estimated, and the value of the variables from an expected optimal value was calculated with the same function. The lowest % Y was obtained in run 10, the minimum FOC in run 6, the lowest FL in run 7 (2.6 cm) and the lowest FD in run 5 (18.1 microns). It is concluded that, if 4 runs are assumed to be optimal to obtain less than 1 % of objectionable fibers, a % Y: 35 - 37 %, FOC: 0.2 %, FL: 3.5 cm and FD: 18.3  $\mu$ m would be obtained.

Frank E.N., Hick M.V.H., Prieto A., Castillo M.F., 2009. Efectos del descordado sobre la calidad de la fibra obtenida de camélidos sudamericanos y cabra criolla patagónica. En: 32° Cong. Arg. Prod. Anim. (resumen). Revista Argentina de Producción Animal Vol 29 Supl. 1: 134-135.

Singh, A. A study on dehairing of Australian cashmere fibres. Ms Ing. Thesis, Deakin, Aust.

## Dehairing of Alpaca Fibres Top with AM2 Dehairing Technology

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Many classes of alpaca fibres contain a certain amount of coarse fibres, which are strong and stiff, and cause discomfort to the end users of alpaca fibre products. It is therefore desirable to separate the coarse alpaca fibres from the fine ones, as it should be done with llama fiber. With the AM2 dehairing technology developed in Argentina, various tests of Llama and Alpaca fiber (Frank et al., 2009) were also performed in Australia (Wang et al., 2008). In all cases, samples of raw fleeces were used. The possibility of using worsted (combed and intersected) arose some time ago. This paper reports trial results on alpaca dehairing using an AM2 technology dehairing machine. The diameters of alpaca fleece, dehaired alpaca fibres and removed alpaca fibres were analyzed; and the fibre lengths before and after dehairing were compared. In this dehairing assay, input included: Alpaca tape top 22 microns average fineness; 30 % CV of fineness; objectionable fiber w/w: 4.88 %; N°/weight: 0.32; Fiber of >30 µm: 9.1 %. Average fiber length (Barbe): 111.8 mm. One dehairing Product/Down (VI) was obtained: average fineness 21.9 µm; 24 % CV of fineness; Objectionable fiber w / w: 2.2 %; N°/weight: 0.16; Fiber of >30 µm: 3.6 % Average fiber length (Barbe): 83.0 mm; Hateur: 75.2 mm (reduction length: 6.9 - 21.2 %). Yield at end dehairing was 83.5%. The product can be processed with the worsted system (combing).

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Wang L, Singh A., Wang X., 2008. Dehairing Australian alpaca fibres with a cashmere dehairing machine. Journal of the Textile Institute, vol. 99 (6): 539-544.

## **Modelación de curvas de crecimiento de llamas q'ara utilizando modelos de crecimiento no lineales**

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El objetivo de este estudio fue describir la curva de crecimiento de llamas Q'ara machos y hembras para lo cual se han utilizado seis modelos de crecimiento no lineales (Brody, Gompertz, Von Bertalanffy, Logístico, Exponencial negativo y Richards). Se analizaron datos de pesos corporales individuales de 15303 y 18085 llamas machos y hembras de la variedad Q'ara. Los datos fueron obtenidos de la estación experimental Quimsachata, del Instituto Nacional de Innovación Agraria (INIA) localizado en el distrito de Santa Lucía, provincia de Lampa, departamento de Puno, Perú. Los parámetros de los modelos fueron estimados por el método iterativo de Gauss Newton por medio del procedimiento NLIN del programa estadístico SAS®. Para saber si un modelo tiene un buen ajuste se usó los siguientes estadísticos: Coeficiente de determinación ajustado ( $R^2_{ajustado}$ ); Cuadrado medio del error (RMS); Raíz del cuadrado medio del error (RMSE); Criterio de información de Akaike (AIC) y el Criterio de información Bayesiana (AIB). Se concluye que el modelo de crecimiento no lineal de Brody es el que mejor describe la curva de crecimiento de llamas Q'ara machos y para las hembras el mejor modelo es el de Richards.

## Genetic Basis of Early Activation of Hair Follicle in Cashmere Goat: An Approach with Candidate Genes

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The cashmere hair follicle perpetually goes through three stages: growth (anagen), involution (catagen) and rest (telogen). Photoperiod is the main proximate factor in the control of seasonal coat change in Cashmere goats. Stem cells play a crucial role in the hair follicle growth. Different types of stem cells are present in hair follicle: bulge stem cells, secondary hair germ, adipose stem cells (ADSCs) and epidermal stem cells. Platelet-derived growth factor (PDGF) is secreted from ADSCs and is involved in hair growth. In this work, we have studied the goat PDGFA and sequenced the full length transcript obtained a long and short isoforms. Using the RT-PCR, we have identified the expression of some molecular signals, including PDGFA, that according to the literature are implicated in the hair growth. Our data may confirm that some genes, especially CD34, BMP2 and PDGFA, can activate hair follicle stem cells, in particular those of the bulge region.

Animal fibres from South American camelids and other fibre or wool bearing species provide important products for use by the human population. The contemporary context includes the competition with petrocarbon-based artificial fibres and concern about excessive persistence of these in the natural environment. Animal fibres present highly valuable characteristics for sustainable production and processing as they are both natural and renewable. On the other hand, their use is recognised to depend on availability of appropriate quality and quantity, the production of which is underpinned by a range of sciences and processes which support development to meet market requirements. This collection of papers combines international experience from South and North America, China and Europe. The focus lies on domestic South American camelids (alpacas, llamas) and also includes research on sheep and goats. It considers latest advances in sustainable development under climate change, breeding and genetics, reproduction and pathology, nutrition, meat and fibre production and fibre metrology.

Publication of this book is supported by the Animal Fibre Working Group of the European Federation of Animal Science (EAAP). 'Advances in Fibre Production Science in South American Camelids and other Fibre Animals' addresses issues of importance to scientists and animal breeders, textile processors and manufacturers, specialised governmental policy makers and students studying veterinary, animal and applied biological sciences.



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