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## The potential of bioactive forage legumes for ruminant production in temperate and tropical areas: A One health approach

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Abstract. Nutritional strategies are the most evident and natural methods to integrate ruminant production systems in the One health approach, aiming at the simultaneous protection of the health of humans, animals and the environment. Among these strategies, the use of forage legumes containing some bioactive compounds (mainly phenols, terpenes and alkaloids) is particularly promising as they have shown potential benefits in multiple dimensions throughout the ruminant production chain. In addition to be protein-rich resources, they have been shown to decrease nitrogen fertilization, reduce methane emissions and urinary nitrogen excretion, have anthelmintic and antioxidant activities improving health status of animals, and can improve product quality through their fatty acid profile and oxidative stability. Bioactive legumes are present both in temperate and tropical areas, but their use could be different as the compounds of interest could be of different nature and in different concentrations. Interdisciplinary and transnational research projects could provide a considerable opportunity to share knowledge in this field and to propose global innovative solutions for ruminant nutrition in the future.

Keywords. Bioactive legumes, One health, Ruminant

#### 1. Introduction

The One Health concept is thinking about health at the interface between animal, human and environment, on a local, national and global scale. It highlights the need of interdisciplinarity and cooperation among human and veterinary medicine, agriculture, and science of environment, to address complex health challenges [1]. In the context of ruminant production, the One Health approach is particularly relevant (Figure 1). At the animal-human interface, ruminants can be a source of zoonotic

diseases as for example, for certain strains of bacteria like *Salmonella* and *E. coli* which can cause infections in humans [2]. The excessive use of antimicrobials in ruminants can lead to mechanisms of resistance, which pose a threat to animal and human health, and such resistance is also observed for anthelmintic drugs in ruminants at global scale [3]. Ensuring the safety and the nutritional quality of products derived from ruminants also needs collaboration in monitoring production systems to minimize the risk of foodborne illnesses. At the interface animal-environment, ruminant production has strong implications, including land use, water consumption, and greenhouse gas emissions. In response to climate change, ruminants are particularly vulnerable to heat stress and oxidative stress which cause diverse physiological diseases and impact their productivity [4]. The One Health approach involves considering the environmental impact of farming practices and exploring sustainable production methods that balance the needs of humans, animals, and the environment.

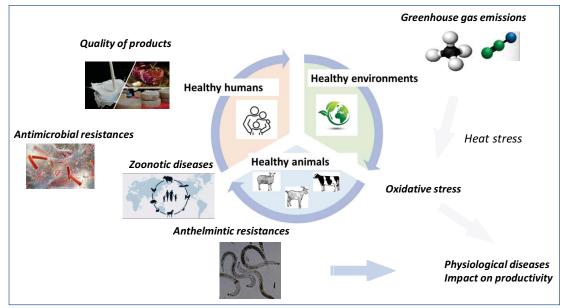


Figure 1. One health approach in ruminant production

Among the strategies relevant in the framework of One health applied to ruminant productions, nutrition can play a crucial role in ensuring the health and productivity of livestock, with a direct impact on human health through the consumption of animal products. And among nutritional strategies, there is a growing recognition of the potential benefits associated with incorporating bioactive forage legumes into the diets [5]. Forage legumes are well known for their high protein content and favorable amino acid profiles reducing the reliance on external protein supplements. They also enhance the soil fertility through nitrogen fixation and reduce the use of nitrogen fertilizers. More specifically, bioactive legumes are characterized by their rich content of bioactive secondary metabolites, including phenolic compounds (flavonoids, tannins), terpenoids and alkaloids, which have been shown to have various physiological effects on ruminants and potential benefits in multiple dimensions of the One health approach.

Bioactive legumes are widely distributed around the world but the specific species grown in each region vary based on local agricultural practices, climate conditions, and the types of livestock raised. In temperate areas, many studies have focused on forage legume containing tannins such as sulla (Hedysarum coronarium), sainfoin (Onobrychis viciifolia) and birdsfoot trefoil (Lotus corniculatus) [6]. In tropical areas, the cultivation of bioactive legumes for ruminants is influenced by the unique climatic conditions of these regions. Species having shown a certain potential include (not exclusively) Acacia spp., peanut (Arachis pintoi) forage, Leucaena leucocephala, Sesbania grandiflora, Albizia falcataria

and *Calliandra calothyrsus*. The main bioactive compounds observed in tropical forage plants include phenolics (flavonoids, tannins), saponins and lignans [7].

This article explores the significance of bioactive legumes in ruminant nutrition from a One Health perspective through three case studies exemplifying the potential of these plants. The first case study illustrates the potential of bioactive legumes to fight gastro-intestinal nematodes (GIN). The second case study illustrates the multiple effects of growing sainfoin to decrease greenhouse gas emissions. The last case study is about the effects of bioactive legumes against oxidative stress and to improve product quality in ruminants.

#### 2. Case study 1

Infection by GIN is a major disease in ruminants and an important obstacle to the development of grazing as an alternative of indoor intensive systems. These parasites belonging to various genera, including *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Cooperia*, can cause inflammation and damage to the gastrointestinal tract, leading to a decrease in voluntary intake and nutrient absorption. Blood-feeding nematodes, such as *Haemonchus contortus*, can cause anemia in ruminants, leading to pale mucous membranes, weakness, and lethargy. Thus, infected animals may experience weight loss, reduced growth rates, and overall poor performances due the diversion of nutrients to combat the infection rather than for muscle development or milk production [8,9]. The economic impact of GIN is substantial, considering the costs associated with decreased productivity, veterinary treatments, and potential losses due to mortality [10].

For decades, the fight against nematodes is only based on chemical molecules with anthelmintic activity from different classes, such as benzimidazoles, macrocyclic lactones (ivermectin, moxidectin), and levamisole. However, the over-use of the different classes of molecules has caused the development of multi-resistance at global scale [3]. The consequence is that it can compromise the effectiveness of deworming treatments and increase the challenges associated with parasite control. In addition, the use of anthelmintic drugs in ruminants can lead to the presence of drug residues in meat and milk as a result of improper use of anthelmintics, including exceeding recommended dosages and inadequate withdrawal periods [11]. Finally, when these drugs are excreted onto pasture during grazing, they can have potential ecotoxicological impacts on grassland entomofauna (insects and other arthropods) [12]. All these elements rise the need to find alternative methods to fight parasitism.

Among the alternatives, some legumes containing bioactive compounds, especially tannins, have been shown to have anthelmintic properties both in temperate and tropical geographical regions [13,14]. In temperate areas, sainfoin was shown to have biological activity against GIN in small (goats and sheep) [15] and large ruminants [16], indicating a wide action spectrum of this plant to fight infection by GIN. Similar results have been observed with birdsfoot trefoil and sulla which also contain condensed tannins. In tropical areas, legume forages such as Sericea lespedeza (*Lespedeza cuneata*) and peanut forage and legume trees such as tzalam (*Lysiloma latisiliquum*) and *Acacia* species that also contain condensed tannins, have been shown to have anthelmintic properties [17,18].

The mechanisms of action can be multifaceted, and different bioactive compounds within the legumes may act synergistically. Additionally, the concentration of these compounds in the diet, the specific species of nematodes, and the overall health and nutritional status of the host animal can influence the effectiveness of bioactive legumes in controlling GIN infections in ruminants. Direct effects are actions against the biology, structure or ultrastructure of the nematodes similarly to a chemical anthelmintic, while indirect effects include modifications in the immune response of the animal host, especially at the level of the gastrointestinal mucosa affecting the biology of GIN. For instance, functional ultrastructural changes have been observed in the GIN larvae after contact with sainfoin extracts [19] and so, the consumption of sainfoin affects inhibit the development of nematode larvae, the fecundity and the fecal egg counts of *Haemonchus contortus*. The tzalam has also been shown to affect the length and fertility of *Haemonchus contortus* females.

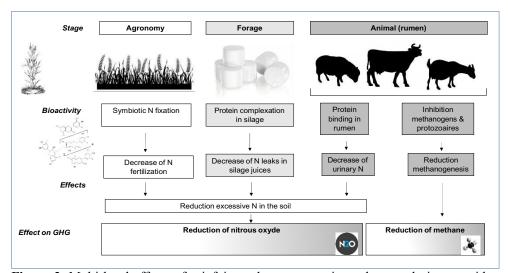
#### 3. Case study 2

Methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) are two greenhouse gases that contribute to climate change, and their emissions from ruminant production play a significant role in the overall greenhouse gas emissions from the agricultural sector. Methane is primarily produced in the rumen through enteric fermentation, while nitrous oxide is mainly produced through processes like nitrification and denitrification, which involve the microbial conversion of nitrogen compounds from excreta and soil. Various strategies are being explored to reduce methane emissions from ruminant production, including dietary interventions, such as the addition of feed supplements that can modify microbial activity in the rumen, and breeding programs to select for animals that produce less methane. Strategies to reduce nitrous oxide emissions from ruminant production include optimizing nitrogen fertilizer use, improving manure management practices, and implementing more efficient grazing and pasture management.

Sainfoin is a traditional temperate forage legume containing condensed tannins. In the last decade, this plant has been used as a common research model for a European interdisciplinary research consortium including plant breeders, agronomists, animal nutritionists, parasitologists and experts in tannin biochemistry [20]. This project has highlighted the potential of sainfoin to decrease greenhouse gas emissions at multiple levels (Figure 2).

In the field, sainfoin showed symbiotic nitrogen fixation similar to the main nitrogen-fixing temperate legumes species (e.g clovers) without rhizobia treatment [21]. Thus, sainfoin appears to be suitable to produce locally high-protein forage while avoiding excessive application of mineral nitrogen fertilizer conducting finally to nitrous oxide emissions. Ensiling grass and sainfoin increases intensity of fermentation and reduces excessive degradation of protein in silage indicating a positive role in the preservation of protein and silage quality [22]. This leads to a reduction of the proportion of soluble nitrogen and ammonia in silage compared with only grass silage, and then a reduction of nitrogen loss in fermentation juices released into the environment.

At the animal level, urinary nitrogen excretion and methane yield (g/kg dry matter intake) decreased when sainfoin is included in the diet and this is consistently observed in sheep [6] and dairy cows [23]. An interesting finding was got in dairy cows in which a diet containing sainfoin led to a reduction of methane emissions and fibre digestibility, while increasing milk yield compared to the same diet without sainfoin, suggesting that it may have redirected metabolism toward protein mass in the body.



**Figure 2.** Multi-level effects of sainfoin to decrease enteric methane and nitrous oxide emissions in systems of ruminant production (sources: Malisch et al. 2017, Copani et al. 2014, Niderkorn et al. 2019, Huyen et al. 2016)

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Similarly, the use of tropical bioactive legumes in ruminant production is a promising strategy for mitigating greenhouse gas emissions. The main bioactive compounds observed in tropical forage plants and relevant for ruminant production are phenolics (flavonoids, tannins), saponins and lignans [24,25,26]. Tropical bioactive legumes with high potential to be served as rumen manipulators include *Coleus amboinicus* [27,28], *Acacia* spp. [29], peanut (*Arachis pintoi*) forage [30,31], *Leucaena leucocephala*, *Albizia falcataria*, *Sesbania grandiflora* and *Calliandra calothyrsus* [32].

#### 4. Case study 3

Oxidative stress is a significant challenge in ruminant livestock, impacting animal health, welfare, and production efficiency [33]. As an alternative approach to alleviate oxidative stress, the incorporation of bioactive legumes in ruminant diets has gained attention due to their potential antioxidant properties mainly attributed to polyphenols and flavonoids [34]. The mechanisms through which these compounds may mitigate oxidative stress in ruminants involve a combination of direct and indirect actions at the cellular and molecular levels. Polyphenols and flavonoids are known for their capacity to counteract highly reactive free radicals that can cause damage cells and biomolecules. Polyphenols act as electron donors, effectively scavenging and neutralizing these free radicals, preventing them from causing cellular damage. Some polyphenols have metal-chelating properties, meaning they can bind and reduce the availability of transition metal ions (such as iron and copper) that are involved in the production of reactive oxygen species [34]. Polyphenols can influence the activity of enzymes involved in antioxidant defense mechanisms. For example, by enhancing the effect of antioxidant endogenous enzymes (e.g. glutathione peroxidase, catalase and superoxide dismutase). This modulation helps to maintain a balanced antioxidant system within cells. Many polyphenols and flavonoids exhibit anti-inflammatory properties. Chronic inflammation is closely linked to oxidative stress. By mitigating inflammation, these compounds indirectly reduce the generation of reactive oxygen species and help in maintaining a redox balance in tissues. Polyphenols may contribute to the preservation of cellular structures, including cell membranes and organelles, by preventing lipid peroxidation and maintaining the integrity of biological membranes. The cumulative effect of these mechanisms helps to mitigate oxidative stress and contributes to the overall health and well-being of ruminant animals.

The bioactive compounds in legumes have also several effects on the quality of ruminant products and oxidative stability [35]. Some bioactive legumes are known to have higher levels of omega-3 fatty acids. This can result in ruminant products with a more favorable fatty acid profile. For instance, the inclusion of bioactive legumes in the diet of dairy cows may lead to higher levels of omega-3 fatty acids and antioxidants in milk which is beneficial for human health. Bioactive legumes often contain higher levels of certain nutrients, such as vitamins, minerals, and antioxidants, which can contribute to the overall nutritional quality of ruminant products [36]. Oxidation processes also impact product quality as lipid peroxidation of meats alter their health value through the loss of fatty acids of interest such as polyunsaturated fatty acids. In addition, the occurrence of undesirable lipid oxidation products during the phases of slaughter, maturation, packaging, processing, cooking and digestion, can increase the risk of cardiovascular disease and colorectal cancer in humans.

In temperate regions, the main bioactive legumes that have shown activity in mitigating oxidative stress are red clover (*Trifolium pratense*) that contains high flavonoid content, but also isoflavones, alfalfa (*Medicago sativa*) known to be rich in saponins associated with antioxidant and anti-inflammatory effects, or the previously evoked tannin-containing legumes sainfoin and birdsfoot trefoil. In tropical areas, some examples of bioactive legumes containing tannins and/or flavonoids with antioxidant activity include *Leucaena leucocephala*, *Gliricidia sepium*, Desmanthus spp., Centrosema spp., pigeon pea (*Cajanus cajan*) or *Mucuna pruriens*.

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#### 5. Issues and challenges

Using bioactive legumes in ruminant diets within a One Health approach involves considering the interactions among animal health, human health, and the environment. While incorporating bioactive legumes can have several benefits, there are also challenges and issues to address:

- The variability in bioactive compounds within a same legume species and among legume species
  needs to be better understood. Bioactive legumes are present in both temperate and tropical areas,
  but their use could be different as the compounds of interest could be of different nature and in
  different concentrations.
- The nutrient content of bioactive legumes can vary depending on factors like species, variety, growing conditions, and harvesting methods. Inconsistencies in nutrient composition may make it challenging to formulate accurate and reliable ruminant diets.
- The bioactive compounds in legumes can influence the digestibility of nutrients and alter the microbial fermentation process in the rumen. Balancing the diet to maintain optimal rumen function is essential for overall ruminant health.
- Depending on their concentration in the whole diet, some bioactive compounds in legumes may act as anti-nutritional agents, impacting nutrient digestion. These compounds can decrease feed intake, absorption of nutrients, and overall animal productivity.
- Certain legumes may contain toxic compounds, such as alkaloids, which can adversely affect ruminant health. Proper selection, processing, and management are crucial to minimize the risk of toxicity.
- Some bioactive legumes may have bitter or unpalatable components, leading to reduced feed intake
  by ruminants. Palatability challenges can be addressed through proper processing or blending with
  other feed ingredients.
- Future research should focus on optimizing legume varieties, understanding their interactions with the rumen microbiome, and developing practical feeding strategies for different livestock systems.

#### 6. Conclusion

One Health highlights the interconnectedness of health at animal, human, and environmental levels, spotting that the health of one is intricately linked to the health of the others. In the context of ruminant production, applying One Health principles helps to address complex challenges and promote sustainable, safe, and responsible farming practices. Addressing issues and challenges requires an interdisciplinary approach involving researchers, veterinarians, agronomists, and policy-makers to promote the sustainable integration of bioactive legumes in ruminant diets within the broader One Health framework. Future research should focus on identifying optimal inclusion levels, exploring synergistic effects with other feed ingredients, and developing strategies to mitigate any adverse effects associated with bioactive legume consumption by ruminants in contrasted geographical areas. Transnational research projects could provide a considerable opportunity to share knowledge in this field and to propose global innovative solutions for ruminant nutrition in the future.

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