



## Guest Editor introduction - Issue entitled "Methods to face the challenges of ruminant phenotyping"

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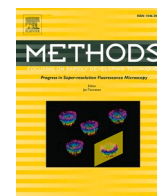
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## Guest Editor's Introduction

## Guest Editor introduction



Ruminants provide multiple benefits to humans: transformation of feed sources inedible for humans into nutrient-rich foods, protein-rich products that contribute to food security, employment and rural economies, recycling of organic matter and nutrients and the associated soil fertility, manure for fuel... At the same time, however, ruminants contribute to greenhouse gas emissions and thus to environmental issues. Ruminant production systems also have to face increasing societal concerns regarding animal welfare and health, as well as human health linked to ruminant meat and dairy consumption, as stated by the last publication from the Food and Agriculture Organization (FAO, IFAD, UNICEF, WFP and WHO. 2020. The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets. Rome, FAO. <https://doi.org/10.4060/ca9692en>).

The recording of traits relative to ruminant health, welfare, meat and milk production is a big challenge for beef and dairy industries, with expected benefits for a range of users, from farmers to consumers. Large efforts have been made by animal scientists to develop methods to quantify traits or proxies relative to ruminants and their rearing practices. The objective of this special issue on *Methods to face the challenges of ruminant phenotyping* is to present a timely collection of articles covering diverse approaches of animal trait phenotyping, including cell biology, high-throughput analysis, image analysis, and mathematical modelling. It includes five reviews providing an overview of different methods to quantify a given trait and six research articles to describe or compare specific methods. It covers four topics: 1) animal health and welfare, 2) digestive processes, which are highly specific in ruminants, 3) body composition and marbling of meat, 4) the use of milk as a matrix and as a source of biomarkers for phenotyping.

Central to the welfare and health of ruminants, the early detection of cases of stress or disease is a key issue for farmers to ensure the wellbeing of their animals in accordance with both ethical and productive performance purposes. Hence, we feature a selection of articles on heat stress, mathematical models to detect changes in circadian rhythm as an early alert of stress or diseases, and the use of immune cells to quantify inflammation and disease. Ruminants have to face the warming climatic conditions. Wijffels et al. reviewed the methods available to detect heat stress in ruminants, from bioclimatic indices to new technologies and computational approaches such as infrared thermography of body surface temperature, automated measures of respiration rate and radiotelemetry of internal body temperature [1]. They highlighted that respiration rate or panting score are useful to assess heat stress in ruminants. They suggest that monitoring of patterns of animal behavior, physiological responses such as the diurnal temperature, or respiratory cycle across day for an animal, may enable accurate assessment of heat stress. These perspectives may be further explored in the research article

by Wagner et al., which reports a new Fourier transform-based method to detect changes in circadian rhythm [2]. Circadian activity rhythms are observed in most vertebrates and are regulated by internal clocks. Stress or disease episodes in animals disrupt their circadian activity rhythm, with potential far-reaching effects on wellbeing, health and performances. The Fourier transform-based method was applied to analyze data provided by a Real-Time Locating System, one type of sensors used in Precision Livestock Farming. The application of the Fourier transform-based method to these data was successful to model circadian rhythm activity and to detect 95% of the rhythm anomalies due to reproductive or disease events, and produced less than 20% of false alerts. The method can detect problems at a very early stage of disease, before clinical signs manifest. At the cellular level, macrophages are among the first cell populations to sense a tissue pathogen or injury and generate the initial molecular signals, alerting the immune system. Monocytes are involved in almost all phases of the inflammation and bridge innate and specific immune responses. Ceciliani et al. [3] reviewed present methods to isolate and characterize bovine monocytes and macrophages, with the perspective to best understand the basic biology and functions of these cell types, as well as their impact on immune response in cows [3]. This knowledge is a prerequisite for advancement in diagnosis and investigation of the pathogenesis of cattle diseases.

Our understanding of the rumen microbiome and its functions are of scientific, industrial, ethical and societal interest, as the rumen microbiome is linked to animal health, nutrition and performances, but at the same time contribute significantly to global greenhouse gas emissions. One of the most promising methods to understand rumen microbiome is the rumen metaproteomics, which has been rarely studied in ruminants. Os Andersen et al. [4] present and discuss current methods of sample handling, protein extraction and data analysis for rumen metaproteomics. This method-based review provides an up-to-date workflow of metagenome-integrated metaproteomics and exemplified how this can increase the protein identification to understand the complex rumen microbiome and its metabolic functions. As an example of the rumen functional particularities, the rumen bacteria produce the B-vitamins that are essential for both the maintenance of metabolic efficiency of the ruminant and B vitamin supply for Humans. While the B-vitamin concentration in dairy and meat ruminant products were reviewed, the link between B-vitamin and ruminant feed efficiency and performance is a novel challenging research topic. In dairy cows, B-vitamin supply is greatly dependent of the activity of the ruminal microbiota. Girard and Graulet [5] have reviewed the methodological approaches used to estimate B vitamin supply and status of ruminants. They propose developments of models based on diet composition to predict vitamin

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supply to cows, and the search of biomarkers in easily accessible specimens to assess B-vitamin status. Lastly, because 46% of the greenhouse gas emissions of the world dairy supply chain (when expressed as carbon dioxide equivalents) result from dairy cow methane (CH<sub>4</sub>) emissions, methods to accurately quantify CH<sub>4</sub> emission is a challenge to overcome for both research and dairy industry. Coppa et al. [6] reports in a research article the repeatability of long-term enteric CH<sub>4</sub> emission from lactating dairy cows using GreenFeed system on farm conditions. This work also tested the capability to rank dairy cows according to their CH<sub>4</sub> emissions across diets and individuals. GreenFeed is reliable in differentiating CH<sub>4</sub> emission in dairy herds across diets. The reliability of long-term measurement of methane emission by the GreenFeed system was also assessed for dairy cows.

Producing ruminants with adequate muscle and adipose masses (i.e. lean-to-fat ratio) is an economic challenge for beef and dairy industries, both for product qualities and adaptive capacities purposes. Furthermore, evaluations of variations in body fat reserves are important to study animal resilience to changing environments. Lerch et al. [7] compare eight methods to estimate the body composition and especially the adipose masses of dairy goat, by benchmarking them against chemical composition (water, lipid, protein, mineral and energy) measured *post-mortem*. Tomography and deuterium oxide dilution space are the most precise methods for in vivo estimation of the mass of lipid in the carcasses. The bioelectrical impedance spectroscopy method was less precise than the two previous methods, (that may be invasive and sensitive to measurement error), but has the advantage of requiring small portable equipment, with no need of sampling and analyses. Many studies on beef nutritional quality require the quantification of intramuscular fat, which is the adipose mass present within the muscle, also called marbling. Moreover, in some countries the amount of intramuscular fat contributes to the pricing of meat cuts. The most used and reliable methods are chemical analysis from approximately 100 g of muscle, with the drawbacks to be solvent- and time-consuming and to be destructive. Two original articles provide alternative methods to quantify the intramuscular fat. Meunier et al. [8] describe an open-access computer image analysis (CIA) method to predict meat and fat content using the image of the 6th rib of bovine captured by a smartphone, and under very few fixed conditions. For one picture, the method provides data for seventeen image features that were produced semi-automatically in less than 5 min. Four to five of these features accurately predict intramuscular fat percent and rib composition. Bardou-Valette et al. [9] compared two alternative methods to the Folch et al. (1957), which is considered the reference method: a miniaturised Folch's method and a near-infrared spectroscopic method. The article provides methods to determine the validity domains and measurement uncertainty for the two alternative methods. The validity domains argue that the alternative methods are well adapted to most commercial bovine meats.

A final section of this special issue is composed of one research article and one review that address the use of milk as a non-invasive and easy to sample fluid for phenotyping ruminants and dairy products. In the recent years, the research was very active to predict new phenotypes from the milk. One of the most applied method to predict phenotype from milk is the mid-infrared (MIR) spectrometry, which allows a fast and cost-effective quantification of milk chemical composition. This is a method of choice to generate phenotypes at large scale as it is already used in dairy industry to quantify fat, protein, and lactose content in milk, in the frame of milk production recording and milk pricing. The research article from Grelet et al. [10] highlights some elements to take into account when working with MIR data in order to predict dairy phenotypes at a large scale. They proposed some guidelines on how to obtain highly precise predictive models, such as the (1) integration of relevant spectral and reference variability in the calibration datasets, (2)

selection of the spectral regions based on spectroscopic information, (3) limitation of the complexity of models (4) the importance of spectral standardization and monitoring of individual instrument stability when using and transferring models on large number of instruments. Milk is also a source of thousands different proteins, which open perspectives for the diagnosis of both milk producing ruminants, and the nutraceutical properties of milk for human consumption. Broadbent et al. [11] reviewed some methodological concepts of liquid chromatography-mass spectrometry (LC-MS), an ideal tool to identify, detect and quantify markers of breeding, production and adaption. However, to date, there is limited application of LC-MS to quantify milk proteins and study cattle phenotypes. Broadbent et al. provide a timely theoretical basis and framework for LC-MS as a rapidly developing technique, and gives examples of its application to measure beta-casein proteoforms in cows' milk, one of the protein components contributing to human allergies to bovine milk.

In summary, this outstanding collection of 11 articles provides the most recent research findings on method development, strategies and tools for the phenotyping of ruminants and their products. We believe that the special issue *Methods to face the challenges of ruminant phenotyping* will lead to new phenotyping methods developed for practical applications. We acknowledge the authors for their contributions and the anonymous reviewers for their evaluation and constructive comments, despite the lockdown due to the COVID-19 pandemic. We also would like to acknowledge the support of Carly Middendorp and her Elsevier's colleagues for their assistance.

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