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Physiological biomarkers for precision feed formulation a critical review of blood, milk, and fecal biomarkers used for real-time monitoring of real-time nutrient assimilation and metabolic health in dairy cattle

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Abstract

In order to maximise productivity and cattle health, precision feeds for dairy cows have evolved from end-user-designed nutrient-based formulations to information-driven cattle feeds based on physiological responses. The potential of blood, milk, and faecal physiological indicators for in situ monitoring of food absorption and metabolic condition in dairy cows is critically assessed in this study. Various blood indicators, including glucose, urea nitrogen, beta-hydroxybutyrate (BHBA), and non-esterified fatty acids (NEFA), indicate systemic physiological changes, protein metabolism, and energy balance. Milk urea nitrogen, fat protein ratio, and somatic cell count are non-invasive markers of nutritional efficiency, metabolic health, and udders state. Undigested nutrients, microbial metabolites, and fibre fraction are examples of faecal nutrients that are helpful indicators of digestive efficiency and nutrition utilisation in the colon and rumen. Feed management is becoming more dynamic and adaptable due to the use of these biomarkers and new technologies like metabolomics, genomics, sensor-based monitoring systems, and precision livestock farming tools. These reduce production losses, increase feed efficiency, and aid in the early detection of metabolic disorders. The goal of this paper is to provide an overview of the categorisation, application, and function of biomarkers and how they might revolutionise precision nutrition for efficient and sustainable dairy production systems.

Keywords: Physiological biomarkers, precision feed formulation, dairy cattle, metabolic health, blood biomarkers, milk biomarkers, fecal biomarkers, precision livestock farming

Introduction

An adaptable, data-driven, and animal-specific precision nutrition strategy is needed for dairy farming in order to meet the growing demands for high production and efficiency. dietary utilisation inefficiencies and heightened vulnerability to metabolic illnesses are caused by the conventional strategy to eating, which primarily emphasises overall dietary needs and ignores individual variability in metabolism, health state, and environmental influences (Antunes-Fernandes, E.C. *et al.*, 2016) ^[1]. In this regard, a physiological type biomarker has shown itself to be a useful tool for evaluating the metabolic state and real-time nutrition absorption of dairy cows. The biomarkers can be identified from biological samples such as blood, milk, manure and others that reflects the dynamic physiologic and biochemical status of the animal, enabling more effective and timely approaches to feeding them.

In addition to providing milk information on metabolism and production efficiency, milk biomarkers can provide information on the health of the udder; whilst blood parameters provide information on metabolism and energy balance, protein metabolism, and the regulation of hormones in the system. Addition of faecal biomarkers further enhances this evaluation as they give information on nutritional losses, rumen function and digestive efficiency. By enabling real-time monitoring and analysis of animal health, this can offer

farmers insights into potential metabolic issues at an early stage, enhancing overall precision livestock management systems (Bicalho, M.L.S. *et al.*, 2017) ^[2]. This will allow for prompt nutritional management. Moreover, many developments in sensor technology, data processing, and omics techniques have enabled the improvement of monitoring system accuracy and utility of biomarkers. This changing requirement is helping to facilitate sustainable dairy production, animal health and optimal feed utilisation. It is important to understand the role and application of physiological biomarker in modern farm situations to come up with next-generation precision feed formulation.

Concept and Classification of Physiological Biomarkers

Indicators of how well a functioning living organism performs in terms of metabolism, nutritional utilization, health status and physiological reactions in dairy cattle are physiological biomarkers, which can be measured to evaluate functioning of a live organism. In precision feed formulation, biomarkers are tools that can be used to measure digestibility, bioavailability and metabolic rates of nutrients and can be used in real-time decision making of feeding strategies. Physiological biomarkers provide an earlier and more sensitive measurement of metabolic derangements before clinical signs or symptoms of illness appear, compared with traditional measures of disease such as milk production or body weight (Cardoso, F.C. *et al.*, 2020) ^[3]. The biomarkers, indeed, are typically obtained from biological samples, which combine numerous components of the animal's body, including blood, milk, faeces, urine and saliva.

Physiological biomarkers can be approximately classified into two groups according to their biological relevance and their biological function. Indicators of human nutrition, such as blood glucose, urea nitrogen and milk urea nitrogen (MUN) represent the intake, digestion and absorption of nutrients. Second, Metabolic biomarkers include insulin, non-esterified fatty acids (NEFA), and beta-hydroxybutyrate (BHBA) information on metabolic pathways, and energy status. This information plays an important role in assessing metabolic health and the diagnosis of metabolic disorders such as ketosis (Dijkstra, J. *et al.*, 2012) ^[4]. The physiological response to a stress, and the immune response to environmental and/or dietary stresses, is demonstrated using cortisol, haptoglobin and inflammatory cytokines as bio-parameters. Finally, the feasibility of quantifying rumen function and feed efficiency is offered by biomarkers of digestive events, including faecal markers of rumen fibre fractions, undigested starch and microbial metabolites, and efficiency biomarkers.

Further, Biomarkers can be segregated based on the usage of it, diagnostic, prognostic, and monitoring are all of the distinct roles they play in diagnosis, prognosis and treatment of disease respectively. As more and more technology in the field of omics and sensors for monitoring, this categorization of biomarkers are becoming more complex, including all types of genomic, proteomic, and metabolomic biomarkers. In general, understanding and categorising physiological biomarkers are crucial factors to take into account when integrating them into precision feeding systems with the goal of optimising dairy cattle production, health, and sustainability.

Blood Biomarkers for Assessing Nutrient Assimilation

Blood markers are closely linked to the body's absorption, transportation, and metabolic use of nutrients, and they offer reliable and dynamic indications of dairy cattle's nutritional assimilation. Unlike exterior performance features, blood measurements would provide real-time information on how the various food components are used into useable metabolic substrates. One of the most crucial markers is blood glucose, which represents the metabolism of carbohydrates and relates to the balance between the body's energy needs and supplies. As gluconeogenesis in ruminants results in the production of important amounts of glucose, glucose levels are important for regulating the fermentation efficiency in the rumen and available energy (Doelman, J. *et al.*, 2021) ^[5]. Blood urea nitrogen (BUN) is also commonly used as an indicator of protein metabolism – much of the BUN is in equilibrium with the level of dietary protein and the ability of the animal's tissues and rumen microbes to utilize the protein provided; elevated BUN may indicate high dietary protein or low capacity to use protein stored in the rumen by the animal.

Two blood lipid parameters, non-esterified fatty acids (NEFA) and blood β -hydroxybutyrate (BHBA) are important parameters which can be used to assess energy balance and fat mobilisation. Higher BHBA levels helps diagnose incomplete oxidation of fatty acids, which may represent “subclinical ketosis”, but abnormal higher NEFA is a sign of negative energy balance (dietary energy deficiency). Also, the plasma amino acid profile can be useful for detecting amino acid imbalances or deficiencies and provide all the data with respect to the efficiency of protein digestion and absorption. The level of minerals, such as calcium, phosphorus and magnesium, also show the level of mineral nutrition and its involvement in metabolic processes.

Blood Biomarkers and Metabolic Health Monitoring

Blood biomarkers are crucial for closely monitoring the metabolic health of dairy cows and they are very sensitive and direct measure of the physiological state and an early warning indicator for metabolic diseases. Metabolic stress in cattle occurs at various times during their production during high energy demands and inconsistent feed intake, such as transition, early lactation and optimum production period. To prevent these imbalances, or to help rectify herd management practices where they occur, blood parameters can help identify these imbalances before clinical signs are present (Duffield, T.F. *et al.*, 2009) ^[6]. Two of the most relevant variables to assess an energy balance are beta-hydroxybutyrate (BHBA) levels (when excessive, associated with incomplete oxidation of fatty acids, subclinical/clinical ketosis) and non-esterified fatty acids (NEFA) levels (when high, associated with mobilisation of BFT, negative energy balance).

These measurements allow the investigation of information that could be gathered from the hormones insulin and glucose, since these hormones are responsible for the regulation of the animal's metabolism, and they can provide information about the way in which the animal meets its energy needs when the physiological state and dietary composition varies. Furthermore, blood urea nitrogen (BUN) is an indicator to evaluate nitrogen utilization efficiency and a protein metabolism problem; a high or low BUN could indicate a

metabolic issue and/or a protein imbalance in the diet. Two significant markers that help determine liver function, the aspartate aminotransferase (AST) and the gamma-glutamyl transferase (GGT) are markers that point towards fatty liver diseases or liver stresses.

Plus, the minerals, such as calcium, phosphorus and magnesium, are important indicators of metabolic health – especially during the periparturient period – when a cow could be vulnerable to developing metabolic problems like hypocalcaemia. Cytokines and haptoglobin are markers for inflammatory variables such as immune health and overall inflammation while cortisol is a marker for physical stress (Ferlizza, E. *et al.*, 2020) ^[7]. As a whole, these blood profiles give a complete picture of dairy cow metabolic health, enabling them to be closely monitored and managed. These offer multiple benefits to routine herd health plans, including early detection of problems, increased level of treatment success and effective, sustainable dairy systems.

Milk Biomarkers as Indicators of Nutritional Efficiency

Since milk composition is directly related to the relationship between nutrition, metabolism, and condition, it is a simple, non-invasive, and affordable method of assessing energy efficiency in dairy cows. Unlike blood sampling, milk collection doesn't upset the animal, and the samples may be collected on a regular basis, giving it the perfect matrix for continuous monitoring in precision feeding. One of the most popular tests that measures the effectiveness of protein utilisation and is helpful in comprehending protein metabolism is milk urea nitrogen (MUN). While high MUN levels are associated with high protein intakes or poor digestibility/utilization leading to health loss (economic) and excessive N excretion in the environment, ideal MUN levels represent the balance of dietary protein and energy intake and, when reached, do not result in economic loss (Grelet, C. *et al.*, 2016) ^[8].

The ratio of milk fat to milk protein is another crucial indicator for evaluating energy balance and rumen health. A lower feed to ration ratio may indicate ruminal acidosis or insufficient fibre intake, whereas a greater feed to ration ratio may indicate negative energy balance and accelerated mobilisation of body fat reserves (normal situation during early lactation). Additionally, because lactose synthesis is directly linked to the energy of glucose in the bloodstream and plays a significant role in the regulation of milk volume, the concentration of milk lactose reflects the energy status and activities of the mammary gland. Hence, the difference in lactose content may signal some issues with energy

metabolism or health in general (Hook, S.E. *et al.*, 2010) ^[9]. Another critical milk biomarker is somatic cell count (SCC) that is mainly a reflection on udder health and immune response. Elevated SCC can negatively affect both overall output and nutritional utilisation. It is an indication of mastitis or an inflammatory condition. Furthermore, the most recent advancements in milk metabolomics research have assisted in identifying metabolites linked to certain milk units, such as the metabolite content of fatty acid groups and ketone bodies, which can provide additional insights into metabolic efficiency and health state. When combined, these milk biomarkers will offer useful information for evaluating the effectiveness of dietary approaches, directing the use of nutrients in the diet, and helping to maximise milk production and characteristics.

Milk-Based Indicators of Metabolic and Physiological Status

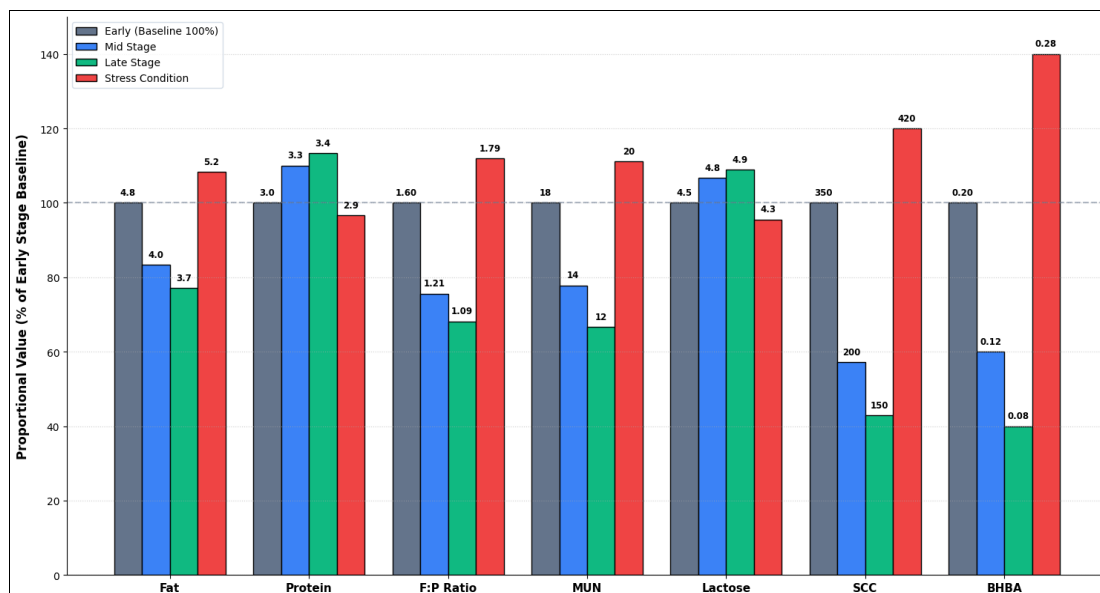
A useful non-invasive method for evaluating dairy cow biochemical and endocrine physiology is milk composition, which is a dynamic indication. Changes in energy balances, nutritional status, and health status are intimately linked to changes in milk ingredients, particularly during transitional and early lactation periods. The fat: protein ratio has long been recognised as a useful measure of metabolic health; high ratios are linked to greater body fat mobilisation and negative energy balance, whereas low ratios are linked to ruminal acidosis or an imbalance in energy and protein intake. Lower levels of milk urea nitrogen (MUN) may suggest a protein deficit in the diet, whereas higher levels may suggest an excess of protein in the food or inefficient microbial protein production (Li, S. *et al.*, 2012) ^[10].

The amount of lactose in milk is also an important factor associated with the availability of glucose and the efficiency of synthesis of mammary gland, which in turn has effect on energy status of the mammary gland and its synthesis efficiency. If less lactose is left, it is a sign of stress or change in patient's health. Since high somatic cell count (SCC) may be indicative of inflammation or mastitis and thus indirectly affect metabolic capacity, SCC remains one of the key markers of the immune response and udder state. Additionally, the presence of milk ketone molecules, such as beta-hydroxybutyrate (BHBA), is a reliable marker of both subclinical ketosis and energy metabolism. All of these milk molecules are useful indicators of metabolic changes and physiological processes, and they will give a complete picture of the nutritional and health conditions of dairy-intensive agricultural systems.

Table 1: Numerical Representation of Milk Biomarkers Across Lactation Stages

Stage Code	Lactation Stage	Fat (%)	Protein (%)	F:P Ratio	MUN (mg/dL)	Lactose (%)	SCC ($\times 10^3/\text{mL}$)	BHBA (mmol/L)
1	Early (0–30 days)	4.8	3.0	1.60	18	4.5	350	0.20
2	Mid (60–120 days)	4.0	3.3	1.21	14	4.8	200	0.12
3	Late (150–250 days)	3.7	3.4	1.09	12	4.9	150	0.08
4	Stress Condition	5.2	2.9	1.79	20	4.3	420	0.28

Source: Adapted from dairy biomarker studies including de Koster&Opsomer (2013), Overton *et al.* (2004), and ISO-IDF milk composition reports.



Graph 1: Proportional Shifts in Milk Biomarkers Across Lactation Stages & Stress Conditions

Fecal Biomarkers for Digestive Efficiency and Nutrient Utilization

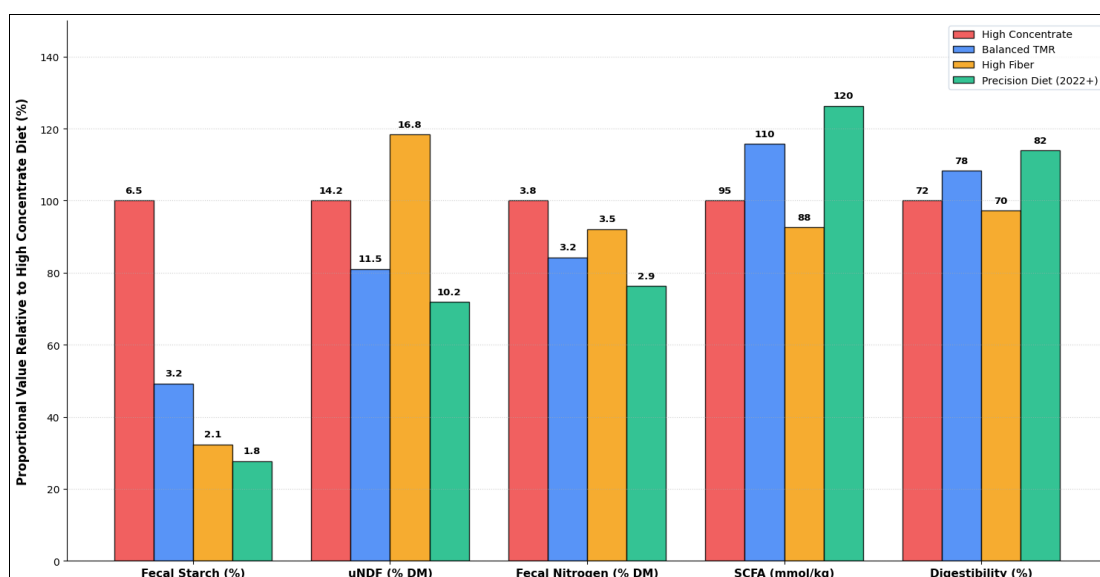
Because they display the degree of feed digestion, microbial activity, and nutrient losses inside the gastrointestinal tract (GIT), faecal biomarkers provide important information on digestive efficiency and nutrient utilisation in dairy calves. Faecal parameters differ from the blood or milk parameters because they add the indigestible/unabsorbed portion of the food and they can be very useful in assessing the efficiency of feed utilization and rumen health. The faecal biomarkers are; undigested neutral detergent fibre (uNDF), faecal starch concentration, nitrogen excretion, and microbial metabolites (such as short-chain fatty acids) (Matthews, C. *et al.*, 2019)^[11]. High uNDF levels result in low fibre digestibility/low number of rumen microorganisms, high faecal starch levels

result in poor fermentation efficiency in the rumen/feed pass quickly. Moreover, faecal nitrogen is related to the efficiency of protein utilization and high nitrogen losses may suggest imbalance of protein levels in the ration, or insufficient amount of microbial protein production.

The accuracy and speed of evaluating faecal biomarkers in real time in precision feeding systems have been enhanced by new analytical technologies as metabolomics and near-infrared spectroscopy (NIRS). These indicators can minimise the environmental effect associated with nutrient excretion rates, optimise feed conversion efficiency, and help formulate rations. A comprehensive understanding of how nutrients are used and digestive efficiency in dairy cows may be obtained by combining faecal biomarkers with other physiological data.

Table 2: Recent Fecal Biomarker Data for Digestive Efficiency in Dairy Cattle

Diet Type Code	Diet Description	Fecal Starch (%)	uNDF (% DM)	Fecal Nitrogen (% DM)	SCFA (mmol/kg)	Digestibility (%)
1	High Concentrate Diet	6.5	14.2	3.8	95	72
2	Balanced TMR	3.2	11.5	3.2	110	78
3	High Fiber Diet	2.1	16.8	3.5	88	70
4	Precision Diet (2022+)	1.8	10.2	2.9	120	82



Graph 2: Digestive Efficiency Profiles & Fecal Biomarker Composition Across Dietary Regimes

Emerging Omics-Based and Sensor-Derived Biomarkers

In fact, biomarkers derived from new omics technologies and sensors are revolutionising precision nutrition for dairy cows by enabling real-time monitoring at a much greater resolution. Using omics technologies (genomics, transcriptomics, proteomics, and metabolomics) to research nutrition provides comprehensive knowledge on the molecular mechanisms underlying health status, metabolic control, and nutrient absorption (Nozière, P. *et al.*, 2006) [12]. Among these, metabolomics has become a very specialised field that reflects the dynamic changes seen in the presence of small molecules (metabolites) found in biological fluids, such as blood, milk, and rumen fluid, among others, that provide direct access to the metabolic response in relation to diet. These metabolites aid in the understanding of early indicators of metabolic diseases, feed nutritional shortages, and feed utilisation inefficiencies. Similarly, genomics and transcriptomics may be used to look for hints of nutrition metabolism, differences in feed efficiency, and gene expression, while proteomic investigations reveal changes in protein expression that contribute to stress, immunity, and metabolic adaptability. Concurrently, animal physiology and behaviour are continually and indirectly monitored using sensor technology. These technologies, which offer real-time data on body temperature, rumen activity, eating habits, milk composition, and activity, include wearable sensors, rumen bolus, infrared thermography, and automated milking systems. The findings from these biomarkers, collected from the sensors, are an immediate reflection of the changes in food's utilization, the onset of disease and the metabolic stress (Paixão, M.G. *et al.*, 2017) [13]. Combined omics data and sensor output variables are used together to create predictive modelling and decision support systems for precision feed formulation, using sophisticated data analysis, artificial intelligence and machine learning. It is possible to be able to derive metabolic abnormalities, produce feed optimisation plans, and also make real-time make adjustments to feeding plan tactics by combining these technologies. This comprehensive system serves not just animal welfare, sustainability and better efficiency of milk production, but makes strides toward "smart dairies and data-driven systems of dairy production.

Integration of Biomarkers into Precision Feed Formulation Systems

A major step forward in dairy nutrition has been the introduction of Physiological Indicators within a feed formulation system for precision nutrition, allowing management decisions to be made for each individual animal. Dynamic feeding strategies can now be designed that replicate and benefit from the physiological and metabolic changes experienced by individual dairy cows as they change in real-time, with the use of blood, milk, faecal and sensors based markers. This allows the ration formulation to be changed back from a "static" ration to a ration that adapts according to the actual requirements of the animal, thus improving feed efficiency, resulting in less loss of nutrients. The level of energy supplementation for example might be indicated by blood measurements of NEFA and glucose, the level of protein supplementation might be indicated by milk urea nitrogen and the properties of the feed may be optimised using faecal measures of Marker Substance for digestibility and fibre (for example Roehe, R. *et al.*, 2021) [14]. The more advanced precision feeding systems include platforms integrating biomarkers with automatic sensor feeds and add more prediction tools and herd management software.

These systems allow for dietary interventions to prevent or treat metabolic abnormalities such as ketosis, acidosis, and protein inefficiency, if they are identified early in life (Tedeschi, L.O. *et al.*, 2021) [15]. Furthermore, the integration of AI and machine learning algorithms in the application further improves decision-making processes, helping in trend identification and forecasting nutritional requirements in different physiological and environmental conditions. This can reduce nutrient loss, have beneficial impacts on animal health, production, and the environment.

Conclusion and Future Perspectives

Precision feed formulation with physiological biometrics is an exciting dairy nutrition revolution and will allow more individualised, precise and adaptive management of animal production and health from a nutritional perspective. Blood, milk and faecal matrices have a lot of information about digestibility, metabolic condition and nutrient absorption with their respective biomarkers. This helps in the early detection of nutritional imbalance/start of subclinical diseases. Integrated in precision livestock systems, they can control real-time autonomic and nutrition readings to maximize use of feed, minimize losses in production, and assure animal welfare. This study emphasises that a multi-dimensional perspective based on many indications offers the best assessment of the physiological and metabolic health of the dairy farmer's cattle, and that no one sign can be employed alone. Precision nutrition has a bright future because to advanced omics technologies, sensor-based monitoring, and artificial intelligence analytics. Such advancements will allow constant monitoring and predictive modelling, leading to the ability to proactively manage nutrition in a more informed manner. Also, this technology or a similar one will have a much stronger chance of being implemented widely if there were reasonably priced on-farm diagnostics that would be available, especially in resource poor areas. Needs include biomarker threshold standardisation, better data interchange and validation between production systems for improved practical application. In summary, biomarker research and digital technologies will ultimately support the farmers in future to optimise the efficiency of nutrient use, reduce farmers' environmental footprint, and ensure production of dairy products over time and across new challenges.

Conflict of Interest

Not available

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