Review: Markers and proxies to monitor ruminal function and feed efficiency in young ruminants

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Abstract:	Developing the rumen's capacity to utilize recalcitrant and low-value feed resources is important for ruminant production systems. Early life nutrition and management practices have been shown to influence development of the rumen in young animals with long-term consequences on their performance. Therefore, there has been increasing interest to understand ruminal development and function in young ruminants to improve feed efficiency, health, welfare, and performance of both young and adult ruminants. However, due to the small size, rapid morphological changes and low initial microbial populations of the rumen, it is difficult to study ruminal function in young ruminants without major invasive approaches or slaughter studies. In this review we discuss the usefulness of a range of proxies and markers to monitor ruminal function and nitrogen use efficiency (a major part of feed efficiency) in young ruminants. Breath sulphide and methane emissions showed the greatest potential as simple markers of a developing microbiota in young ruminants. However, there is only limited evidence for robust indicators of feed efficiency at this stage. The use of nitrogen isotopic discrimination based on plasma samples appeared to be the most promising proxy for feed efficiency in young ruminants. More research is needed to explore and refine potential proxies and markers to indicate ruminal function and feed efficiency in young ruminants, particularly for neonatal ruminants.

Review: Markers and proxies to monitor ruminal function and feed efficiency in young ruminants

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Abstract

Developing the rumen's capacity to utilize recalcitrant and low-value feed resources is important for ruminant production systems. Early life nutrition and management practices have been shown to influence development of the rumen in young animals with long-term consequences on their performance. Therefore, there has been increasing interest to understand ruminal development and function in young ruminants to improve feed efficiency, health, welfare, and performance of both young and adult ruminants. However, due to the small size, rapid morphological changes and low initial microbial populations of the rumen, it is difficult to study ruminal function in young ruminants without major invasive approaches or slaughter studies. In this review we discuss the usefulness of a range of proxies and markers to monitor ruminal function and nitrogen use efficiency (a major part of feed efficiency) in young ruminants. Breath sulphide and methane emissions showed the greatest potential as simple markers of a developing microbiota in young ruminants. However, there is only limited evidence for robust indicators of feed efficiency at this stage. The use of nitrogen isotopic discrimination based on plasma samples appeared to be the most promising proxy for feed efficiency in young ruminants. More research is needed to explore and refine potential proxies and markers to indicate ruminal function and feed efficiency in young ruminants, particularly for neonatal ruminants.

Keywords: purine derivatives; faecal lipids; breath sulphide and methane; body measures; nitrogen isotopes

Implications

Simple measurements of sulphides and methane in breath could be used to provide a practical and non-invasive tool to monitor the developing microbiota of young ruminants. Plasma nitrogen isotopic discrimination is a promising proxy for feed efficiency in young ruminants and could be applied through a simple blood testing programme. However, the review indicated a lack of published international literature on the development of markers and proxies for ruminal function and feed efficiency in young ruminants, which would complement the much larger body of research on husbandry of young ruminants.

Importance of the rumen in digesting forages

The rumen and its microorganisms (bacteria, protozoa, fungi and archaea) facilitate the utilisation of substrates that are not available to mammalian enzymes (Van Nevel and Demeyer, 1996; Liem et al., 2001), producing absorbable substrates for the host ruminant (Bergman, 1990). Physical breakdown during ingestion and rumination, as well as in the rumen make feeds more accessible for microbial colonisation (Cheng et al., 1980; McAllister et al., 1994). The simple sugars formed are used by the microbes to produce volatile fatty acids (**VFAs**), mainly acetate (used for fatty acid synthesis), propionate (used for glucose synthesis), and butyrate which are largely used as energy sources in the ruminant body. Proportions of these VFAs in the rumen are influenced by microbial community composition (Henderson et al., 2015; Seshadri et al., 2018) and ruminal conditions. The ruminal conditions are influenced by intake rate, dietary forage to concentrate ratio, and nature (e.g., degradation rate, molecular structure) of the diet. In general, forage dominated diets stimulate acetate formation whereas, concentrate dominated diets promote propionate formation. Feeds with higher levels of starch and

protein levels promote propionate production, yet simple sugars and hemicellulose promote butyrate production, and cellulose promotes acetate production (Bannink et al., 2006).

Feed must be retained in the reticulo-rumen for long enough to allow the microorganisms to effectively ferment and break down plant fiber (Liem et al., 2001). Ruminants have a filter system between the reticulum and omasum to extend the ruminal retention time for slow fermenting neutral detergent fiber, which is a major component of forage (Van Soest, 1996). The rate and amount of microbial protein synthesis is determined by the availability of energy and protein in the rumen (Tedeschi et al., 2000). Carbohydrates are the main energy source for bacteria, although they can also be used as carbon skeletons for protein synthesis in combination with ammonia, amino acids or small peptides (Bach et al., 2005; Lanzas et al., 2008). Degradation of proteins yields peptides and amino acids, which are utilised by the microbes (transamination) or deaminated to yield VFAs, carbon dioxide and ammonia (Tamminga, 1979; Bach et al., 2005). The ammonia that exceeds the capacity of microbial growth is absorbed through the ruminal wall, converted into urea and circulated back into the rumen via saliva or excreted in the urine.

Morphological development of the rumen in young ruminants

The development of the rumen and microbial colonization is a two-way interaction between the host and microbial community. Morphological development of the rumen is promoted by the consumption of solid feed. The associated production and absorption of VFAs as fermentation end products stimulates the development of ruminal papillae, enabling their absorption and facilitating further epithelial metabolism (Sander et al.,

1959; Suárez et al., 2006). Butyrate is the greatest stimulator of epithelial length and function, followed by propionate. Conversely, it is the physical structure of substrates like roughages, which expand ruminal volume, contribute to muscular development (Tamate et al., 1962; Stobo et al., 1966), and stimulate rumination and flow of saliva to the rumen (Hodgson, 1971).

The main enzymatic activities (fibrolysis, amylolysis, proteolysis, and ureolysis) of ruminal microbiota have been observed in the rumen from four (Sahoo et al., 2005) or ten (Kmet et al., 1986) days of age. Over 60 glycoside hydrolase microbial genes have been observed in the rumen during the early stages of life, suggesting great potential for plant carbohydrate metabolism even in the absence of regular plant cell wall intake (Li et al., 2012). As a calf grows, the ketogenic capacity of the rumen must develop to that of a mature rumen, as 60 to 80% of all VFAs are absorbed across the ruminal wall, with 75 to 90% of absorbed butyrate being metabolized by the ruminal epithelium (Allen, 1997).

Microbial development in the rumen of young ruminants

Microbial inoculation of the rumen was considered to begin immediately after birth, through contact with the vaginal canal, fecal material, colostrum, skin and saliva of the dam. Yet recently, methanogens, fibrolytic bacteria, and Proteobacteria were detected in the rumen of calves less than 20 minutes after birth (Guzman et al., 2015). Quantification of bacterial and archaeal RNA (Malmuthuge et al., 2015), suggests that inoculation may in fact occur prior to birth, with rapid shifts occurring in the first days of life as primo-colonizing aerobic or facultative anaerobic bacteria shape the biotype for the strictly anaerobic microbes which sequentially establish thereafter (Jami et al., 2013). Similarities

between establishment of the rumen and epimural microbial communities have been identified, as Proteobacteria were also found to be present at >90% of sequences from goat kids at birth (Rieu et al., 1990; Jiao et al., 2015; Wang et al., 2017). This is potentially due to their role in scavenging oxygen diffusing from the capillary network (Cheng et al., 1979), facilitating the establishment of anaerobic communities.

Recent studies (Li et al., 2012; Jami et al., 2013; Meale et al., 2016) suggest the pre-weaned rumen contains the same dominant phyla, Bacteroidetes, Firmicutes and Proteobacteria, as the more mature post-weaned rumen, although relative abundance varies with age. Firmicutes increase after weaning (Jami et al., 2013; Meale et al., 2016; Meale et al., 2017a and 2017b), whilst Bacteroidetes, and specifically Prevotella, appear more dependent on solid food intake, than the removal of milk from the diet, reaching a stable abundance after 7 weeks of age (Meale et al., 2017a and 2017b), and once solid food consumption rises above 100 g per d, respectively (Rey et al., 2014; Meale et al., 2016; Meale et al., 2017a and 2017b). This indicates that the earlier a calf begins to consume solid feed, the sooner a ruminal bacterial community that is more representative of a mature ruminal develops. Furman et al. (2020) showed the effects of delivery method (spontaneous vs. caesarean) and diet, as well as random effects in early life on the development of the ruminal microbiome. Others (Roehe et al., 2016; Wallace et al., 2019) have identified host genetic effects on the ruminal microbiome. All of these genetic and early life effects reinforce the need to quantify ruminal function in young ruminants – before, during and after weaning.

Markers and proxies to monitor ruminal function in young ruminants

At birth, the rumen is sterile, and physically and metabolically underdeveloped. Initiation of solid feed consumption, acquisition of anaerobic microbes, the establishment of ruminal fermentation, growth of papillae, maturation of salivary function, and physical expansion of the rumen are achieved during the first ~four months of life (Khan et al., 2011; Khan et al., 2016) in response to nutritional inputs and management practices (Yáñez-Ruiz et al., 2015; Steele et al., 2016; Meale et al., 2017a). For example, the provision of forage vs. concentrate to young ruminants provide a strong physical stimulus for the expansion of the rumen to increase its volume, physical development, and motility (Castells Domingo, 2013) and promote the development of rumination behavior, saliva flow, and buffering capacity (Laarman and Oba, 2011; Khan et al., 2016). In summary, there is emerging evidence that early life nutritional interventions influence ruminal development in young ruminants (Khan et al., 2016), with lifelong consequences on their welfare and performance (Khan et al., 2011; Soberon and Van Amburgh, 2013). There is renewed interest in finding mechanisms by which the host and diet can influence these developments, not least because there may be the longer-term setting of the interaction between the host, rumen and microbiome (Yáñez-Ruiz et al., 2015). However, there are serious constraints to the ability to use existing sampling techniques and limitations associated with the low ruminal volume and smaller microbial population. A number of approaches have been developed to study ruminal function less invasively and these have often been applied in studies with adult ruminants (see review by (Dewhurst et al., 2000)). The purpose of the next section is to review these

158 methods for prospects to be used with young ruminants; Table 1 provides a summary
159 overview.

160 ***Urinary purine derivatives***

161 Urinary excretion of purine derivatives (**PD**) has been used as an index of microbial
162 protein supply in ruminants post-weaning (Chen et al., 1990; Funaba et al., 1997). The
163 basis of this approach is that nucleic acids leaving the rumen are of microbial origin
164 (McAllan, 1980). Purines are important components of nucleic acids (the bases adenine
165 and guanine) and absorbed purines are metabolised and excreted in urine as their end-
166 products, which include allantoin, uric acid, xanthine, and hypoxanthine (Chen and
167 Ørskov, 2004). Thus, urinary excretion of PD is quantitatively related to the mass of
168 microbial protein supply to the host. However, this estimation is generally associated
169 with error due to several factors including the endogenous contribution to urinary PD,
170 variation in the purine to N ratio in the bacteria used as a reference (e.g., differences
171 between solid- and liquid-associated bacteria (Bates et al., 1985)) and losses of PD
172 through routes other than urinary excretion.

173 Firstly, saliva contains high levels of uric acid and allantoin, which are recycled to the
174 rumen and degraded by the ruminal microbes (Chen et al., 1990 and 1992) and
175 estimates of microbial protein supply from urinary excretion of PD need to be corrected
176 for such losses (Chen et al., 1992). However, because the development of salivary
177 glands in calves varies and relates to time since weaning, there is a variable ratio of
178 urinary PD to the intestinal flow of PD, and subsequent estimation of microbial protein
179 (Funaba et al., 1997). Secondly, the endogenous PD contribution might also be variable
180 during the period immediately after weaning. While endogenous PD production has

been reported to be independent of the age (Funaba et al., 1997),Chen et al. (1992) showed that the abrupt removal of dietary protein supply increased endogenous allantoin production in sheep. More research is required to confirm the effect of endogenous production of PD on the accuracy of microbial protein prediction from urinary excretion of PD in young ruminants. Lastly, DMI increases with age in young ruminants after weaning, which means that a greater amount of feed PD may escape ruminal degradation and contribute to the total urinary excretion of PD (Shingfield, 2000), resulting in an overestimation of microbial protein. In conclusion, it appears that urinary excretion of PD is suitable to rank treatments based on relative differences in microbial protein synthesis, but not to give quantitative reference measurements for the individual animal, as suggested previously for adult ruminants (Shingfield, 2000).

Faecal ether lipids (archaeol)

Recently, researchers have looked for distinctive components of methanogens (archaea) which could be measured directly in biological samples, either intact or following metabolism. The cell membranes of methanogenic archaea include unusual lipids, such as archaeol and caldarchaeol, which contain distinctive ether linkages. Archaeol is present in faeces from ruminants, but has not been detected in faeces from other herbivores (Gill et al., 2011). Therefore, faecal archaeol was proposed as a biomarker for methane (**CH_4**) emissions from growing and adult ruminants. However, the relationship between CH_4 emissions and faecal archaeol concentrations is weak (Gill et al., 2011; McCartney et al., 2013; Schwarm et al., 2015), most likely due to differences in the passage rate of methanogens from the rumen (McCartney et al., 2014) suggesting that faecal archaeol has limited potential as a marker for

methanogenesis. Whilst the ability to distinguish ruminant faeces from non-ruminant faeces suggests that faecal archaeol might be a useful marker for the development and function of the rumen and methanogenesis, we are not aware of any published literature where such effects are measured during pre- and post-weaning periods in young ruminants.

Breath sulphide

Techniques to estimate the degradation of proteins by ruminal microbes have depended on the use of fistulated cows, so identifying markers of protein breakdown that can be accomplished in accessible samples is crucial to better understanding this aspect of ruminal function. Ruminal degradation of sulphur compounds, such as sulphates, methionine and cysteine, results in generation of hydrogen sulphide in the ruminal headspace gas (Dewhurst et al., 2007a). Some of the hydrogen sulphide is absorbed through the lungs (Dougherty et al., 1962) and subsequently metabolised to dimethyl sulphide, which is a distinctive component of cow's breath (Elliott-Martin et al., 1997). The multiple origins of sulphides in ruminal gases or breath makes its measurement limited as a potential marker of protein degredation, but it does seem a viable option to monitor the establishment of the ruminal microbiota.

Much higher levels of hydrogen sulphide are generated when cattle consume water or feed contaminated with high levels of sulphate and this leads to a serious condition called polioencephalomalacia, which often results in death. A functioning ruminal microbial population is required for production of hydrogen sulphide and adaptation to high sulphate intakes (proliferation of sulphate-utilising microorganisms) can take several days (Lutnicki et al., 2014). Despite this adaptation, polioencephalomalacia was

induced in 6-week old lambs offered a high sulphur diet (Gooneratne et al., 1989) demonstrating the potential to use sulphides as a marker for the activity of rumen microorganisms in young ruminants.

Methane emissions

Anaerobic fermentation of feed in the rumen into acetate and butyrate also generates hydrogen (Janssen, 2010). This hydrogen is largely utilized by methanogens in the rumen, together with carbon dioxide, to form CH_4, which is emitted by the ruminants. Whilst hydrogenotrophic methanogensis is the main pathway to CH_4 in the rumen, some diets can promote increased levels of other pathways, including from methyl-containing compounds (e.g., Neill et al. (1978)). Both hydrogenotrophic and methylotrophic methanogenesis are microbial (archaeal) processes, so the CH_4 emitted is a quantitative proxy indicating ruminal fermentation. The DMI is the main driver of CH_4 production in post-weaning growing cattle (Jiao et al., 2014; Jonker et al., 2016) and sheep (Muetzel and Clark, 2015) offered forage-based diets with CH_4 yields typically between 18 and 26 g/kg DMI. To our knowledge, there is little information available on CH_4 emissions from pre-weaned calves. One study with veal calves of over 15 weeks of age and 136 kg BW fed exclusively milk replacer found that negligible amounts of CH_4 were produced (<2 g/kg DMI) (Van den Borne et al., 2006). Dairy calves in two studies offered solid calf starter meal from three days of age (and total mixed ration with 50% hay from four weeks of age) already produced between 5 and 26 g CH_4/kg DMI before weaning (when between two and eight weeks of age), while consuming 0.2 to 1.2 kg of solid feed (Muetzel, 2015). Up to week five of age, the CH_4 yield was lower (<16 g/kg DMI) than post-weaning (21 to 26 g/kg DMI), and this appeared to be associated with

low ruminal acetate/propionate ratio and high propionate concentration pre-weaning. Fermentation of feed into propionate leads to less hydrogen formation in the rumen and therefore less CH_4 formation (Van Nevel and Demeyer, 1996; Janssen, 2010). A meta-analysis indicated that the acetate/propionate ratio was higher in calves with access to forage pre-weaning and higher ruminal pH pre- and post-weaning (Imani et al., 2017). The higher CH_4 yield in calves from week six to ten of age in the second study compared to the first study by Muetzel and Clark (2015) could be due to higher forage intake, though this was not specified.

Most respiration chamber facilities are designed for work with larger growing and adult cattle and are often not suited for work with pre-weaning and young ruminants, which may have extremely low CH_4 emissions. However, it might be possible to operate these large chambers at a lower air flow rate to enable measurement of low CH_4 emissions (Muetzel, 2015), and there are also respiration chambers for small ruminants and facilities where chamber size is adjustable allowing quantification of CH_4 in young calves (e.g., Van den Borne et al., 2016). The laser CH_4 detector is highly sensitive to CH_4 concentrations in gas samples and has been used to estimate CH_4 emissions from cattle based on the frequency and CH_4 concentrations in eructed ruminal gases (Chagunda, 2013). Preliminary studies at Scotland's Rural College have demonstrated that the use of the laser CH_4 detector to monitor the onset and development of a functioning ruminant in calves pre- and post-weaning (Dewhurst; personal communication). Future studies are needed to validate if the laser CH_4 detector can provide usefull CH_4 data, which in turn reflects ruminal function in young ruminants.

Fatty acids

Over the years there has been an increasing interest in the use of odd- and branched-chain fatty acids (**OBCFA**) as potential biomarkers to monitor ruminal function in mature ruminants (Vlaeminck et al., 2006a). This approach is also based on looking for components of ruminal microbes which are present (intact or as metabolites) in accessible samples; OBCFA are hardly found in feedstuffs, but are present at higher levels in microbial lipids. For example, Kim et al. (2005) showed that OBCFA can be useful markers to study ruminal microbial colonization, but the patterns of OBCFA did not identify the types of bacteria colonising herbage. Vlaeminck et al. (2005) noted that OBCFA can be used as markers for the duodenal flow of microbial matter in dairy cows, especially where feed intake data are not available. In addition, other studies (Vlaeminck et al., (2006b), Dewhurst et al., (2007a and 2007b); Bhagwat et al., (2012)) evaluated the potential of OBCFA in milk to predict ruminal proportions of VFA and showed a strong relationship between milk OBCFA and molar proportions of individual VFA in the rumen. However, the past studies document the use of OBCFA as potential markers to monitor ruminal function used mature ruminants and many used milk samples. The potential of OBCFA to monitor ruminal function in young ruminants has not been explored.

Importance of feed efficiency for ruminant production

Feed is a major and variable input cost in ruminant production systems. Improvement in feed utilization and conversion into products (feed efficiency; **FE**) is crucial, as it can lead to a substantial increase in productivity, profitability and potential gains in sustainability. While there are different ways to define FE depending on the production

system, stock class and type of saleable products, most of the literature refers to FE as feed conversion efficiency (**FCE**), which is the product output per unit of feed intake, such as BW gain/DMI in growing sheep. Further, residual feed intake (**RFI**) defines the difference between actual and expected feed intake, based on BW and growth of the animal, and it measures FE that is independent of BW gain and mature body size (Crews, 2005). The RFI is increasingly used by animal breeders as a way to avoid selection for FE leading to correlated increases in animal size.

Challenges to quantify feed efficiency in young ruminants

There are many challenges when seeking to quantify FE in young ruminants, particularly in a grazing system or neonatal stage, where it is difficult to measure intake accurately. Ruminant FE measurements involve two components: product output (e.g., BW gain and milk production) and feed intake. The optimum test duration to accurately measure individual FE in growing ruminants ranges from 42 (Wang et al., 2006) to 100 days (Archer and Bergh, 2000). The International Committee for Animal Recording recommends a minimum period of 60 days, together with an adjustment period of at least 21 days, in which both individual animal feed intake and routine recording of animal BW are applied to remove as much of the non-genetic variation as possible. The recommended length for FE recording is a compromise between accuracy and minimum cost. In general, the duration for measuring an animal trait depends on its repeatability (i.e., time-consistency or reliability), together with the frequency of the measurement (e.g., weekly vs. monthly). Repeatability or intra-class correlation coefficient is a measure of the tendency of animals to maintain their ranking over time and gives information about the magnitude of measurement errors (within-animal

variance) compared to phenotypic variability (between-animal variance). Therefore, more repeatable animal traits subjected to less errors need less time to be accurately measured compared to those that are less repeatable.

In growing beef cattle, repeatability of DMI was reported to range between 0.51 and 0.70, whereas that for BW gain ranges between -0.03 and 0.21 (Kelly et al., 2010; Coyle et al., 2016). Thus, the measurement of DMI is not a critical trait determining the duration of the FE test (Archer et al., 1997). Repeatability of milk yield is higher than BW gain, ranging between 0.32 and 0.53 according to different estimates (7 studies summarized by Roman et al.~~,~~ (2000). Thus, the time required to rank lactating cows according to their FE could be expected to be significantly shorter than the time required to rank growing ruminants. The exploration of markers and proxies of FE is needed to overcome the issues associated with the length and cost of measuring these traits, and will be essential in most field conditions where a reliable direct measurement of intake and performance is not possible or at least extremely challenging.

Markers and proxies to monitor feed efficiency

The use of markers and proxies to monitor FE has focused on growing or lactating animals, particularly the later stage of growing and finishing cattle. The next section reviews markers and proxies for prospects to be used with young ruminants (Table 2 provides a summary overview).

Body condition score and body weight

The use of BW and BCS as proxies for FE is relatively simple, inexpensive and easy to implement on-farm (Negussie et al., 2017). Talebi (2012) showed a positive correlation

(r^2 = 62%) between final BW and FCE in lambs. Similarly, several studies with young ruminants (Arthur et al., 1996; Basarab et al., 2003; Herd et al., 2016) noted a positive correlation between BCS and RFI, but the accuracy of the prediction varies. Other studies using young beef cattle reported a weak correlation between RFI and BW (Herd and Bishop, 2000; Schenkel et al., 2004). Similarly, studies have shown no relationship between RFI and BW in growing bulls (Arthur et al., 2001a; Arthur et al., 2001b), steers (Nkrumah et al., 2004), beef heifers (Kelly et al., 2010) and growing dairy heifers (Green et al., 2013). Current literature shows that although there are overall low to moderate relationships between RFI and BW, BW cannot be confidently used as a proxy for RFI in young ruminants. The use of both BW and BCS as proxies to predict FCE in young ruminants requires further investigation.

Methane emissions

Emissions of CH_4 are a loss of energy for the animal and reduced CH_4 emissions might therefore be associated with improved FE. However, the relationship between FE (RFI in most cases) and CH_4 yield has been inconsistent with relationships having been neutral (Hegarty et al., 2007; Waghorn and Hegarty, 2011; Freetly and Brown-Brandl, 2013; Alemu et al., 2017), positive (Nkrumah et al., 2006) and negative (Mercadante et al., 2015; Herd et al., 2016; McDonnell et al., 2016) in post-weaned growing cattle. Whilst CH_4 is an important loss of energy that can range from 2 to 12% of gross energy (**GE**) intake (Johnson and Johnson, 1995), it is more usually in the range 4 to 8% of GE intake with even less variation between animals offered the same diet. The use of CH_4 as a proxy for FE in young ruminants needs more study.

Blood- and milk-based markers

Since major mechanisms underlying the between-animal variability in FE are related to animal metabolism (Cantalapiedra-Hijar et al., 2018b), it can be argued that markers at the metabolic level may be more reliable than those at the digestive level to detect differences in FE across individuals. The potential of several hormones, such as leptin, insulin and insulin-like growth factor-1, aspartate aminotransferase and albumin as markers of between-animal variation in FE has been proposed by several authors (Johnson and Johnson, 1995; Richardson and Herd, 2004; Kelly et al., 2010), though a recent review found inconsistent results (Cantalapiedra-Hijar et al., 2018a and 2018b). This could be due to interactions among plasma hormones and diet, physiological stage, age of animals or even the sampling procedures (Cantalapiedra-Hijar et al., 2018a and 2018b), which precludes their use as reliable markers of FE at the individual animal level.

Recently, there has been an increasing interest in studying metabolites, proteins and genes potentially related to between-animal variability in FE. A recent study by Duarte et al. (2019) identified a common pathway related to branch-chain amino acid degradation through a meta-analysis of genome-wide association studies on RFI. Branch-chain amino acids have an important role in protein synthesis and turnover, energy-consuming metabolic processes, and their degradation can contribute to gluconeogenesis. Cantalapiedra-Hijar et al. (2018b) also identified protein turnover rate as one of the main determinants of animal variability in FE, and metabolites related to amino acid metabolism and protein turnover have already been proposed as indicators

384 of FE (Richardson and Herd, 2004; Karisa et al., 2014; Meale et al., 2017a; Meale et al.,
385 2017b).

386 A promising marker of FE is based on the ^{15}N natural enrichment of animal proteins
387 over the diet (nitrogen isotopic discrimination; $\Delta^{15}N_{animal\text{-}diet}$). Nitrogen exists as two
388 stable isotopes in nature: the more abundant light ^{14}N, and the heavy ^{15}N. The
389 $\Delta^{15}N_{animal\text{-}diet}$ originates from the isotopic selectivity of enzymes, leading to different ^{15}N
390 natural abundance between substrates and products during metabolic reactions
391 (Gannes et al., 1998). Transaminase and deaminase in the animal liver are involved in
392 major amino acid catabolism, and they have been suggested as key factors in the origin
393 of $\Delta^{15}N_{animal\text{-}diet}$ (Macko et al., 1986). Therefore, ruminants $\Delta^{15}N$ biologically links to
394 protein metabolism (e.g., protein- or nitrogen-use efficiency) (Cantalapiedra-Hijar et al.,
395 2018a) and indirectly links to FE, as nitrogen-use efficiency (**NUE**) is a major
396 component of FE (Nasrollahi et al., 2020).

397 This new biomarker seems to reflect changes in NUE or FCE across dietary conditions
398 (Cheng et al., 2013; Cantalapiedra-Hijar et al., 2018b), but also across individuals under
399 the same diet and condition (Wheadon et al., 2014; Cantalapiedra-Hijar et al., 2018a).
400 More studies are warranted to explore the potential and limitation of this new isotopic
401 biomarker of between-animal variation in FE as well as to assess how heritable it is.
402 Several studies have demonstrated the significant negative relationship between FCE
403 and $\Delta^{15}N_{plasma\text{-}diet}$, and between FCE and $\Delta^{15}N_{blood\text{-}diet}$ (Cantalapiedra-Hijar et al., 2018a),
404 with the fact that it is driven by the protein component of growth (i.e., NUE) suggested
405 by the improvement in relationships when ultrasound-based estimates of body

composition, such as fat deposition are included in the relationship (Meale et al., 2018). However, it is unclear how this pertains to neonatal ruminants.

It is worth noting that although urea nitrogen content of blood or milk has also been proposed as a moderately heritable marker of NUE, it may not be able to capture the animal variability properly in NUE (Huhtanen et al., 2015). Furthermore, several studies have also proposed blood urea nitrogen as a marker for RFI, but its inconsistency across time (Richardson and Herd, 2004) precludes its use as a universal marker of FE. The fact that blood or milk urea nitrogen describes digestive rather than metabolic variations in the NUE (Hof et al., 1997), could parly explain why it fails to reflect the between-animal variability in NUE or FE.

Wool and hair-based marker

While $\Delta^{15}N_{plasma\text{-}diet}$ showed its potential use as a biomarker to indicate FE in both large and small ruminants. A recent study showed that $\Delta^{15}N_{wool\text{-}diet}$ was negatively correlated with FCE of growing sheep (Cheng et al., 2015). Though the study is a preliminary analysis, it highlights the potential to use a marker from non-invasive and easy to obtain samples, such as wool, to predict FE. Wool is known to provide a cumulative ^{15}N signature, which may be used to indicate a cumulative change in FE over a longer period of time than blood. However, it is unclear how this pertains to neonatal ruminants. Further, hair from cattle demonstrated a similar potential to indicate FE (Meale et al., 2017b).

Bringing ruminal function and feed efficiency together, and what comes next?

Unifying the two areas of this review, there is a current interest in the relationship between ruminal function and FE in the context of selection for increased FE, as well as the possibility to manipulate it through persistent effects of early-life interventions. Richardson and Herd (2004) analysed the FE trait in beef cattle and suggested that digestion contributes a relatively small proportion of the trait in comparison with metabolic effects. At the same time, the ruminal metagenomics work of Roehe et al. (2016) suggests that ruminal processes have a strong relationship with FE. Clearly, there is still a lot to learn about the interactions between the host and microbiome in the rumen. Given the interest in genetic effects on FE and the long-term effects of early-life development of ruminal function, there is a real need to make measurements of ruminal function and FE on larger numbers of animals (genetic studies) offered with different diets (diet studies). Detailed simultaneous analysis of microbiomes and metabolomes in such large studies will help identify new markers or proxies that can be used to optimise both ruminal function and host FE.

Conclusion

The use of breath sulphide and CH_4 emissions show potential as non-invasive markers for the establishment of the rumen microbiota in young ruminants, however their use as robust indicators of FE is limited at this stage. The use of ^{15}N discrimination from plasma samples appears the most promising proxy for FE, which maintains its strong negative relationship with NUE and FCE across varying animal ages, indicating its potential as a marker to facilitate larger scale studies with growing ruminants. More research is needed to explore potential proxies and markers to indicate ruminal function

and FE in young ruminants, as the current available indicators are mostly developed for mature ruminants.

Ethics approval

Not applicable

Data and model availability statement

Data mentioned in this publication can be found from cited literature

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LC and RD conceptualized and designed the review, LC, GC, SM, IR, AJ, MK, OA and RJ drafted different sections according to their expertise and revised the article.

Declaration of interest

No competing interests to report.

Declaration of interest

None

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845

846 **Table 1**

847 Markers/proxies to monitor rumen function in young ruminants.

Biomarker/proxy	Target use	Connection to physiology	Major factors affecting accuracy	Key references	Potential use
Urinary excretion of PD	Predicts rumen microbial (protein) synthesis	Nucleic acids leaving the rumen are essentially of microbial origin. Purines are important components of nucleic acids (the bases adenine and guanine) and absorbed purines are metabolised and excreted in urine as their end-products, which include allantoin, uric acid, xanthine and hypoxanthine.	1. variable purine to nitrogen ratio in bacteria. 2. losing PD through other excretion routes (e.g., uric acid and allantoin in saliva). 3. endogenous PD contributions.	Bates et al. (1985); Chen et al. (1992); Gonzalez-Ronquillo et al. (2003); Chen and Ørskov (2004)	Yes
Faecal ether lipids (archaeol)	Predicts enteric CH_4 emissions	Unusual faecal lipids which originate from the membrane lipids of methanogens and so are related to methanogenesis.	1. passage rate of methanogens from the rumen (selective retention). 2. distribution and kinetics of methanogens in the rumen may contribute to genetic variation in CH_4 production.	Gill et al. (2011); McCartney et al. (2013); Schwarm et al. (2015)	No

Breath sulphide	Indicates ruminal microbiota development	Rumen degradation of sulphur compounds generates hydrogen sulphide, which is absorbed and metabolised to sulphides, some of which are exhaled.	1. water or feed contamination with sulphate. 2. variable proportion excreted in urine. 3. a functioning rumen microbial population and adaptation is required to establish relevant microbes.	Raisbeck et al. (2008); Cammack et al. (2010); Lutnicki et al. (2014)	Yes
CH_4 emissions	Indicates ruminal microbiota development	Anaerobic fermentation of feed in the rumen generates hydrogen, which is largely utilized by methanogens to form CH_4.	1. pre-weaning and young ruminants has low CH_4 emissions. 2. different methods (e.g., laser CH_4 detector vs. chambers) to measure CH_4 have very different accuracies.	Chagunda (2013); Muetzel (2015)	Yes
OBCFA	Monitors rumen function, including microbial synthesis and volatile fatty acid proportions	OBCFA are hardly found in feedstuffs but are present at higher levels in rumen microbial lipids; these appear in animal lipids, including blood and milk.	1. limited studies in young animals. 2. some post-ruminal synthesis or modification can affect OBCFA levels.	Westreicher-Kristen et al. (2020)	No

848 PD = purine derivatives; CH_4 = methane; OBCFA = odd- and branched-chain fatty acids

849 **Table 2**

850 Markers/proxies to monitor feed efficiency in young ruminants.

Biomarker/proxy	Target use	Connection to physiology	Factors affecting accuracy	Key references	Potential use
BCS and BW gain	Indicates FE	BCS is related to body fat deposition, which is a major contributor to BW gain. BW gain contributes to the calculation of FE.	1. age 2. genetics 3. nutrition 4. measurement duration	Herd and Bishop (2000); Schenkel et al. (2004); Talebi (2012)	BCS- No BW gain- may be useful for feed conversion efficiency, but not for residual feed intake
CH_4 emissions	Indicates FE	Methanogenesis is an energy loss during fermentation of feed in the rumen, thus CH_4emissions contribute to inefficient use of feed.	1. between animal variation in energy losses from the same diets. 2. difficulties to measure CH_4 emissions accurately.	Johnson and Johnson (1995); Hegarty et al. (2007)	May be
Blood- and milk-based markers	Indicates FE /nitrogen	Biomarkers like insulin-like growth factor-1, aspartate aminotransferase, urea	1. genetics 2. nutrition	Richardson and Herd (2004); Huhtanen et al.	$\Delta^{15}N$ – Yes Others – May be

	use efficiency	nitrogen, plasma nitrogen isotopic fractionation ($\Delta^{15}N$) are related to either energy or protein metabolism or both.	3. protein turnover rate 4. sampling time	(2015); Cantalapiedra-Hijar et al. (2018a)	
Wool and hair-based marker	Indicates FE	Wool/hair $\Delta^{15}N$ is related to protein turnover and deamination and transamination in liver, which in turn reflects nitrogen use efficiency. Nitrogen use efficiency is a major component of FE.	1. genetics 2. nutrition 3. protein turnover rate 4. sampling techniques	Cheng et al. (2015); Meale et al. (2017b)	May be

851 CH_4 = methane; FE = feed efficiency

Dear editor

We revised the manuscript per technical editor suggestion:

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

https://www.editorialmanager.com/animal/l.asp?i=376549&l=X4YVYS1T

We went through the doc and revised the manuscript accordingly.

There is 1 section had less than 8 lines, but we need to keep it as it provides introduction to a specific section of this review paper.

There are a few references we cannot find all information needed per reference template, but we tried to provide as much as possible info that we can find.

Additional Comments from Editor/Reviewer(s) to Author, if any:

There are some minor typographical errors and corrections to wording in the revised sections that must be addressed prior to publication. A thorough proof-read of the paper is essential.

Done by native speaker of a co author.

p. 2 line 34-35 Should read 'In this review we discuss the usefulness of a range of proxies ...'

Changed

p. 10 line 223 Should read 'The multiple origins of sulphides in ruminal gases or breath makes its measurement limited as a potential marker of protein degredation, but it does seem a viable option to monitor the establishment of the rumen microbiota.

Changed

p.12 line 263 should read 'Most respiration chamber facilities are designed for work with larger growing and adult cattle ...'

Changed

line 266 should be chambers

Changed