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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.

1 **Review: Markers and proxies to monitor ruminal function and feed efficiency in**

2 **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.

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

69   protein levels promote propionate production, yet simple sugars and hemicellulose

70   promote butyrate production, and cellulose promotes acetate production (Bannink et al.,

71   2006).

72   Feed must be retained in the reticulo-rumen for long enough to allow the

73   microorganisms to effectively ferment and break down plant fiber (Liem et al., 2001).

74   Ruminants have a filter system between the reticulum and omasum to extend the

75   ruminal retention time for slow fermenting neutral detergent fiber, which is a major

76   component of forage (Van Soest, 1996). The rate and amount of microbial protein

77   synthesis is determined by the availability of energy and protein in the rumen (Tedeschi

78   et al., 2000). Carbohydrates are the main energy source for bacteria, although they can

79   also be used as carbon skeletons for protein synthesis in combination with ammonia,

80   amino acids or small peptides (Bach et al., 2005; Lanzas et al., 2008). Degradation of

81   proteins yields peptides and amino acids, which are utilised by the microbes

82   (transamination) or deaminated to yield VFAs, carbon dioxide and ammonia

83   (Tamminga, 1979; Bach et al., 2005). The ammonia that exceeds the capacity of

84   microbial growth is absorbed through the ruminal wall, converted into urea and

85   circulated back into the rumen via saliva or excreted in the urine.

86   **Morphological development of the rumen in young ruminants**

87   The development of the rumen and microbial colonization is a two-way interaction

88   between the host and microbial community. Morphological development of the rumen is

89   promoted by the consumption of solid feed. The associated production and absorption

90   of VFAs as fermentation end products stimulates the development of ruminal papillae,

91   enabling their absorption and facilitating further epithelial metabolism (Sander et al.,

92   1959; Suárez et al., 2006). Butyrate is the greatest stimulator of epithelial length and

93   function, followed by propionate. Conversely, it is the physical structure of substrates

94   like roughages, which expand ruminal volume, contribute to muscular development

95   (Tamate et al., 1962; Stobo et al., 1966), and stimulate rumination and flow of saliva to

96   the rumen (Hodgson, 1971).

97   The main enzymatic activities (fibrolysis, amylolysis, proteolysis, and ureolysis) of

98   ruminal microbiota have been observed in the rumen from four (Sahoo et al., 2005) or

99   ten (Kmet et al., 1986) days of age. Over 60 glycoside hydrolase microbial genes have

100   been observed in the rumen during the early stages of life, suggesting great potential for

101   plant carbohydrate metabolism even in the absence of regular plant cell wall intake (Li

102   et al., 2012). As a calf grows, the ketogenic capacity of the rumen must develop to that

103   of a mature rumen, as 60 to 80% of all VFAs are absorbed across the ruminal wall, with

104   75 to 90% of absorbed butyrate being metabolized by the ruminal epithelium (Allen,

105   1997).

106   **Microbial development in the rumen of young ruminants**

107   Microbial inoculation of the rumen was considered to begin immediately after birth,

108   through contact with the vaginal canal, fecal material, colostrum, skin and saliva of the

109   dam. Yet recently, methanogens, fibrolytic bacteria, and Proteobacteria were detected in

110   the rumen of calves less than 20 minutes after birth (Guzman et al., 2015). Quantification

111   of bacterial and archaeal RNA (Malmuthuge et al., 2015), suggests that inoculation may

112   in fact occur prior to birth, with rapid shifts occurring in the first days of life as primo-

113   colonizing aerobic or facultative anaerobic bacteria shape the biotype for the strictly

114   anaerobic microbes which sequentially establish thereafter (Jami et al., 2013). Similarities

115   between establishment of the rumen and epimural microbial communities have been

116   identified, as Proteobacteria were also found to be present at >90% of sequences from

117   goat kids at birth (Rieu et al., 1990; Jiao et al., 2015; Wang et al., 2017). This is potentially

118   due to their role in scavenging oxygen diffusing from the capillary network (Cheng et al.,

119   1979), facilitating the establishment of anaerobic communities.

120   Recent studies (Li et al., 2012; Jami et al., 2013; Meale et al., 2016) suggest the pre-

121   weaned rumen contains the same dominant phyla, Bacteroidetes, Firmicutes and

122   Proteobacteria, as the more mature post-weaned rumen, although relative abundance

123   varies with age. Firmicutes increase after weaning (Jami et al., 2013; Meale et al., 2016;

124   Meale et al., 2017a and 2017b), whilst Bacteroidetes, and specifically Prevotella,

125   appear more dependent on solid food intake, than the removal of milk from the diet,

126   reaching a stable abundance after 7 weeks of age (Meale et al., 2017a and 2017b), and

127   once solid food consumption rises above 100 g per d, respectively (Rey et al., 2014;

128   Meale et al., 2016; Meale et al., 2017a and 2017b). This indicates that the earlier a calf

129   begins to consume solid feed, the sooner a ruminal bacterial community that is more

130   representative of a mature ruminal develops. Furman et al. (2020) showed the effects of

131   delivery method (spontaneous vs. caesarean) and diet, as well as random effects in

132   early life on the development of the ruminal microbiome. Others (Roehe et al., 2016;

133   Wallace et al., 2019) have identified host genetic effects on the ruminal microbiome. All

134   of these genetic and early life effects reinforce the need to quantify ruminal function in

135   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

181 been reported to be independent of the age (Funaba et al., 1997),Chen et al. (1992)

182 showed that the abrupt removal of dietary protein supply increased endogenous

183 allantoin production in sheep. More research is required to confirm the effect of

184 endogenous production of PD on the accuracy of microbial protein prediction from

185 urinary excretion of PD in young ruminants. Lastly, DMI increases with age in young

186 ruminants after weaning, which means that a greater amount of feed PD may escape

187 ruminal degradation and contribute to the total urinary excretion of PD (Shingfield,

188 2000), resulting in an overestimation of microbial protein. In conclusion, it appears that

189 urinary excretion of PD is suitable to rank treatments based on relative differences in

190 microbial protein synthesis, but not to give quantitative reference measurements for the

191 individual animal, as suggested previously for adult ruminants (Shingfield, 2000).

192 ***Faecal ether lipids (archaeol)***

193 Recently, researchers have looked for distinctive components of methanogens

194 (archaea) which could be measured directly in biological samples, either intact or

195 following metabolism. The cell membranes of methanogenic archaea include unusual

196 lipids, such as archaeol and caldarchaeol, which contain distinctive ether linkages.

197 Archaeol is present in faeces from ruminants, but has not been detected in faeces from

198 other herbivores (Gill et al., 2011). Therefore, faecal archaeol was proposed as a

199 biomarker for methane (**CH_4**) emissions from growing and adult ruminants. However,

200 the relationship between CH_4 emissions and faecal archaeol concentrations is weak

201 (Gill et al., 2011; McCartney et al., 2013; Schwarm et al., 2015), most likely due to

202 differences in the passage rate of methanogens from the rumen (McCartney et al.,

203 2014) suggesting that faecal archaeol has limited potential as a marker for

204   methanogenesis. Whilst the ability to distinguish ruminant faeces from non-ruminant

205   faeces suggests that faecal archaeol might be a useful marker for the development and

206   function of the rumen and methanogenesis, we are not aware of any published literature

207   where such effects are measured during pre- and post-weaning periods in young

208   ruminants.

209   ***Breath sulphide***

210   Techniques to estimate the degradation of proteins by ruminal microbes have depended

211   on the use of fistulated cows, so identifying markers of protein breakdown that can be

212   accomplished in accessible samples is crucial to better understanding this aspect of

213   ruminal function. Ruminal degradation of sulphur compounds, such as sulphates,

214   methionine and cysteine, results in generation of hydrogen sulphide in the ruminal

215   headspace gas (Dewhurst et al., 2007a). Some of the hydrogen sulphide is absorbed

216   through the lungs (Dougherty et al., 1962) and subsequently metabolised to dimethyl

217   sulphide, which is a distinctive component of cow's breath (Elliott-Martin et al., 1997).

218   The multiple origins of sulphides in ruminal gases or breath makes its measurement

219   limited as a potential marker of protein degradation, but it does seem a viable option to

220   monitor the establishment of the ruminal microbiota.

221   Much higher levels of hydrogen sulphide are generated when cattle consume water or

222   feed contaminated with high levels of sulphate and this leads to a serious condition

223   called polioencephalomalacia, which often results in death. A functioning ruminal

224   microbial population is required for production of hydrogen sulphide and adaptation to

225   high sulphate intakes (proliferation of sulphate-utilising microorganisms) can take

226   several days (Lutnicki et al., 2014). Despite this adaptation, polioencephalomalacia was

227   induced in 6-week old lambs offered a high sulphur diet (Gooneratne et al., 1989)

228   demonstrating the potential to use sulphides as a marker for the activity of rumen

229   microorganisms in young ruminants.

230   ***Methane emissions***

231   Anaerobic fermentation of feed in the rumen into acetate and butyrate also generates

232   hydrogen (Janssen, 2010). This hydrogen is largely utilized by methanogens in the

233   rumen, together with carbon dioxide, to form CH_4, which is emitted by the ruminants.

234   Whilst hydrogenotrophic methanogensis is the main pathway to CH_4 in the rumen, some

235   diets can promote increased levels of other pathways, including from methyl-containing

236   compounds (e.g., Neill et al. (1978)). Both hydrogenotrophic and methylotrophic

237   methanogenesis are microbial (archaeal) processes, so the CH_4 emitted is a

238   quantitative proxy indicating ruminal fermentation. The DMI is the main driver of CH_4

239   production in post-weaning growing cattle (Jiao et al., 2014; Jonker et al., 2016) and

240   sheep (Muetzel and Clark, 2015) offered forage-based diets with CH_4 yields typically

241   between 18 and 26 g/kg DMI. To our knowledge, there is little information available on

242   CH_4 emissions from pre-weaned calves. One study with veal calves of over 15 weeks of

243   age and 136 kg BW fed exclusively milk replacer found that negligible amounts of CH_4

244   were produced (<2 g/kg DMI) (Van den Borne et al., 2006). Dairy calves in two studies

245   offered solid calf starter meal from three days of age (and total mixed ration with 50%

246   hay from four weeks of age) already produced between 5 and 26 g CH_4/kg DMI before

247   weaning (when between two and eight weeks of age), while consuming 0.2 to 1.2 kg of

248   solid feed (Muetzel, 2015). Up to week five of age, the CH_4 yield was lower (<16 g/kg

249   DMI) than post-weaning (21 to 26 g/kg DMI), and this appeared to be associated with

250 low ruminal acetate/propionate ratio and high propionate concentration pre-weaning.

251 Fermentation of feed into propionate leads to less hydrogen formation in the rumen and

252 therefore less CH_4 formation (Van Nevel and Demeyer, 1996; Janssen, 2010). A meta-

253 analysis indicated that the acetate/propionate ratio was higher in calves with access to

254 forage pre-weaning and higher ruminal pH pre- and post-weaning (Imani et al., 2017).

255 The higher CH_4 yield in calves from week six to ten of age in the second study

256 compared to the first study by Muetzel and Clark (2015) could be due to higher forage

257 intake, though this was not specified.

258 Most respiration chamber facilities are designed for work with larger growing and adult

259 cattle and are often not suited for work with pre-weaning and young ruminants, which

260 may have extremely low CH_4 emissions. However, it might be possible to operate these

261 large chambers at a lower air flow rate to enable measurement of low CH_4 emissions

262 (Muetzel, 2015), and there are also respiration chambers for small ruminants and

263 facilities where chamber size is adjustable allowing quantification of CH_4 in young

264 calves (e.g., Van den Borne et al., 2016). The laser CH_4 detector is highly sensitive to

265 CH_4 concentrations in gas samples and has been used to estimate CH_4 emissions from

266 cattle based on the frequency and CH_4 concentrations in eructed ruminal gases

267 (Chagunda, 2013). Preliminary studies at Scotland's Rural College have demonstrated

268 that the use of the laser CH_4 detector to monitor the onset and development of a

269 functioning ruminant in calves pre- and post-weaning (Dewhurst; personal

270 communication). Future studies are needed to validate if the laser CH_4 detector can

271 provide usefull CH_4 data, which in turn reflects ruminal function in young ruminants.

272   *Fatty acids*

273   Over the years there has been an increasing interest in the use of odd- and branched-

274   chain fatty acids (**OBCFA**) as potential biomarkers to monitor ruminal function in mature

275   ruminants (Vlaeminck et al., 2006a). This approach is also based on looking for

276   components of ruminal microbes which are present (intact or as metabolites) in

277   accessible samples; OBCFA are hardly found in feedstuffs, but are present at higher

278   levels in microbial lipids. For example, Kim et al. (2005) showed that OBCFA can be

279   useful markers to study ruminal microbial colonization, but the patterns of OBCFA did

280   not identify the types of bacteria colonising herbage. Vlaeminck et al. (2005) noted that

281   OBCFA can be used as markers for the duodenal flow of microbial matter in dairy cows,

282   especially where feed intake data are not available. In addition, other studies

283   (Vlaeminck et al., (2006b), Dewhurst et al., (2007a and 2007b); Bhagwat et al., (2012))

284   evaluated the potential of OBCFA in milk to predict ruminal proportions of VFA and

285   showed a strong relationship between milk OBCFA and molar proportions of individual

286   VFA in the rumen. However, the past studies document the use of OBCFA as potential

287   markers to monitor ruminal function used mature ruminants and many used milk

288   samples. The potential of OBCFA to monitor ruminal function in young ruminants has

289   not been explored.

290   **Importance of feed efficiency for ruminant production**

291   Feed is a major and variable input cost in ruminant production systems. Improvement in

292   feed utilization and conversion into products (feed efficiency; **FE**) is crucial, as it can

293   lead to a substantial increase in productivity, profitability and potential gains in

294   sustainability. While there are different ways to define FE depending on the production

295 system, stock class and type of saleable products, most of the literature refers to FE as

296 feed conversion efficiency (**FCE**), which is the product output per unit of feed intake,

297 such as BW gain/DMI in growing sheep. Further, residual feed intake (**RFI**) defines the

298 difference between actual and expected feed intake, based on BW and growth of the

299 animal, and it measures FE that is independent of BW gain and mature body size

300 (Crews, 2005). The RFI is increasingly used by animal breeders as a way to avoid

301 selection for FE leading to correlated increases in animal size.

302 **Challenges to quantify feed efficiency in young ruminants**

303 There are many challenges when seeking to quantify FE in young ruminants,

304 particularly in a grazing system or neonatal stage, where it is difficult to measure intake

305 accurately. Ruminant FE measurements involve two components: product output (e.g.,

306 BW gain and milk production) and feed intake. The optimum test duration to accurately

307 measure individual FE in growing ruminants ranges from 42 (Wang et al., 2006) to 100

308 days (Archer and Bergh, 2000). The International Committee for Animal Recording

309 recommends a minimum period of 60 days, together with an adjustment period of at

310 least 21 days, in which both individual animal feed intake and routine recording of

311 animal BW are applied to remove as much of the non-genetic variation as possible. The

312 recommended length for FE recording is a compromise between accuracy and

313 minimum cost. In general, the duration for measuring an animal trait depends on its

314 repeatability (i.e., time-consistency or reliability), together with the frequency of the

315 measurement (e.g., weekly vs. monthly). Repeatability or intra-class correlation

316 coefficient is a measure of the tendency of animals to maintain their ranking over time

317 and gives information about the magnitude of measurement errors (within-animal

318   variance) compared to phenotypic variability (between-animal variance). Therefore,

319   more repeatable animal traits subjected to less errors need less time to be accurately

320   measured compared to those that are less repeatable.

321   In growing beef cattle, repeatability of DMI was reported to range between 0.51 and

322   0.70, whereas that for BW gain ranges between -0.03 and 0.21 (Kelly et al., 2010; Coyle

323   et al., 2016). Thus, the measurement of DMI is not a critical trait determining the

324   duration of the FE test (Archer et al., 1997). Repeatability of milk yield is higher than BW

325   gain, ranging between 0.32 and 0.53 according to different estimates (7 studies

326   summarized by Roman et al., (2000). Thus, the time required to rank lactating cows

327   according to their FE could be expected to be significantly shorter than the time required

328   to rank growing ruminants. The exploration of markers and proxies of FE is needed to

329   overcome the issues associated with the length and cost of measuring these traits, and

330   will be essential in most field conditions where a reliable direct measurement of intake

331   and performance is not possible or at least extremely challenging.

332   **Markers and proxies to monitor feed efficiency**

333   The use of markers and proxies to monitor FE has focused on growing or lactating

334   animals, particularly the later stage of growing and finishing cattle. The next section

335   reviews markers and proxies for prospects to be used with young ruminants (Table 2

336   provides a summary overview).

337   *Body condition score and body weight*

338   The use of BW and BCS as proxies for FE is relatively simple, inexpensive and easy to

339   implement on-farm (Negussie et al., 2017). Talebi (2012) showed a positive correlation

340 (r^2 = 62%) between final BW and FCE in lambs. Similarly, several studies with young

341 ruminants (Arthur et al., 1996; Basarab et al., 2003; Herd et al., 2016) noted a positive

342 correlation between BCS and RFI, but the accuracy of the prediction varies. Other

343 studies using young beef cattle reported a weak correlation between RFI and BW (Herd

344 and Bishop, 2000; Schenkel et al., 2004). Similarly, studies have shown no relationship

345 between RFI and BW in growing bulls (Arthur et al., 2001a; Arthur et al., 2001b), steers

346 (Nkrumah et al., 2004), beef heifers (Kelly et al., 2010) and growing dairy heifers (Green

347 et al., 2013). Current literature shows that although there are overall low to moderate

348 relationships between RFI and BW, BW cannot be confidently used as a proxy for RFI

349 in young ruminants. The use of both BW and BCS as proxies to predict FCE in young

350 ruminants requires further investigation.

351 ***Methane emissions***

352 Emissions of CH_4 are a loss of energy for the animal and reduced CH_4 emissions might

353 therefore be associated with improved FE. However, the relationship between FE (RFI

354 in most cases) and CH_4 yield has been inconsistent with relationships having been

355 neutral (Hegarty et al., 2007; Waghorn and Hegarty, 2011; Freetly and Brown-Brandl,

356 2013; Alemu et al., 2017), positive (Nkrumah et al., 2006) and negative (Mercadante et

357 al., 2015; Herd et al., 2016; McDonnell et al., 2016) in post-weaned growing cattle.

358 Whilst CH_4 is an important loss of energy that can range from 2 to 12% of gross energy

359 (**GE**) intake (Johnson and Johnson, 1995), it is more usually in the range 4 to 8% of GE

360 intake with even less variation between animals offered the same diet. The use of CH_4

361 as a proxy for FE in young ruminants needs more study.

362   ***Blood- and milk-based markers***

363   Since major mechanisms underlying the between-animal variability in FE are related to

364   animal metabolism (Cantalapiedra-Hijar et al., 2018b), it can be argued that markers at

365   the metabolic level may be more reliable than those at the digestive level to detect

366   differences in FE across individuals. The potential of several hormones, such as leptin,

367   insulin and insulin-like growth factor-1, aspartate aminotransferase and albumin as

368   markers of between-animal variation in FE has been proposed by several authors

369   (Johnson and Johnson, 1995; Richardson and Herd, 2004; Kelly et al., 2010), though a

370   recent review found inconsistent results (Cantalapiedra-Hijar et al., 2018a and 2018b).

371   This could be due to interactions among plasma hormones and diet, physiological

372   stage, age of animals or even the sampling procedures (Cantalapiedra-Hijar et al.,

373   2018a and 2018b), which precludes their use as reliable markers of FE at the individual

374   animal level.

375   Recently, there has been an increasing interest in studying metabolites, proteins and

376   genes potentially related to between-animal variability in FE. A recent study by Duarte

377   et al. (2019) identified a common pathway related to branch-chain amino acid

378   degradation through a meta-analysis of genome-wide association studies on RFI.

379   Branch-chain amino acids have an important role in protein synthesis and turnover,

380   energy-consuming metabolic processes, and their degradation can contribute to

381   gluconeogenesis. Cantalapiedra-Hijar et al. (2018b) also identified protein turnover rate

382   as one of the main determinants of animal variability in FE, and metabolites related to

383   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

406　composition, such as fat deposition are included in the relationship (Meale et al., 2018).

407　However, it is unclear how this pertains to neonatal ruminants.

408　It is worth noting that although urea nitrogen content of blood or milk has also been

409　proposed as a moderately heritable marker of NUE, it may not be able to capture the

410　animal variability properly in NUE (Huhtanen et al., 2015). Furthermore, several studies

411　have also proposed blood urea nitrogen as a marker for RFI, but its inconsistency

412　across time (Richardson and Herd, 2004) precludes its use as a universal marker of FE.

413　The fact that blood or milk urea nitrogen describes digestive rather than metabolic

414　variations in the NUE (Hof et al., 1997), could parly explain why it fails to reflect the

415　between-animal variability in NUE or FE.

416　***Wool and hair-based marker***

417　While $\Delta^{15}N_{plasma-diet}$ showed its potential use as a biomarker to indicate FE in both large

418　and small ruminants. A recent study showed that $\Delta^{15}N_{wool-diet}$ was negatively correlated

419　with FCE of growing sheep (Cheng et al., 2015). Though the study is a preliminary

420　analysis, it highlights the potential to use a marker from non-invasive and easy to obtain

421　samples, such as wool, to predict FE. Wool is known to provide a cumulative ^{15}N

422　signature, which may be used to indicate a cumulative change in FE over a longer

423　period of time than blood. However, it is unclear how this pertains to neonatal

424　ruminants. Further, hair from cattle demonstrated a similar potential to indicate FE

425　(Meale et al., 2017b).

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

427 Unifying the two areas of this review, there is a current interest in the relationship

428 between ruminal function and FE in the context of selection for increased FE, as well as

429 the possibility to manipulate it through persistent effects of early-life interventions.

430 Richardson and Herd (2004) analysed the FE trait in beef cattle and suggested that

431 digestion contributes a relatively small proportion of the trait in comparison with

432 metabolic effects. At the same time, the ruminal metagenomics work of Roehe et al.

433 (2016) suggests that ruminal processes have a strong relationship with FE. Clearly,

434 there is still a lot to learn about the interactions between the host and microbiome in the

435 rumen. Given the interest in genetic effects on FE and the long-term effects of early-life

436 development of ruminal function, there is a real need to make measurements of ruminal

437 function and FE on larger numbers of animals (genetic studies) offered with different

438 diets (diet studies). Detailed simultaneous analysis of microbiomes and metabolomes in

439 such large studies will help identify new markers or proxies that can be used to optimise

440 both ruminal function and host FE.

441 **Conclusion**

442 The use of breath sulphide and CH_4 emissions show potential as non-invasive markers

443 for the establishment of the rumen microbiota in young ruminants, however their use as

444 robust indicators of FE is limited at this stage. The use of ^{15}N discrimination from

445 plasma samples appears the most promising proxy for FE, which maintains its strong

446 negative relationship with NUE and FCE across varying animal ages, indicating its

447 potential as a marker to facilitate larger scale studies with growing ruminants. More

448 research is needed to explore potential proxies and markers to indicate ruminal function

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

450   mature ruminants.

451   **Ethics approval**

452   Not applicable

453   **Data and model availability statement**

454   Data mentioned in this publication can be found from cited literature

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464   **Author contributions**

465   LC and RD conceptualized and designed the review, LC, GC, SM, IR, AJ, MK, OA and

466   RJ drafted different sections according to their expertise and revised the article.

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468   No competing interests to report.

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480

481 Alemu, A., D. Vyas, G. Manafiazar, J. Basarab, and K. Beauchemin, 2017. Enteric methane

482   emissions from low–and high–residual feed intake beef heifers measured using

483   GreenFeed and respiration chamber techniques. Journal of Animal Science 95, 3727-

484   3737.

485 Allen, M. S, 1997. Relationship between fermentation acid production in the rumen and the

486   requirement for physically effective fiber. Journal of Dairy Science 80, 1447-1462.

487 Archer, J., P. Arthur, R. Herd, P. Parnell, and W. Pitchford, 1997. Optimum postweaning test for

488   measurement of growth rate, feed intake, and feed efficiency in British breed cattle.

489   Journal of Animal Science 75, 2024-2032.

490 Archer, J., and L. Bergh, 2000. Duration of performance tests for growth rate, feed intake and

491   feed efficiency in four biological types of beef cattle. Livestock Production Science 65,

492   47-55.

493   Arthur, P., J. Archer, D. Johnston, R. Herd, E. Richardson, and P. Parnell, 2001a. Genetic and

494   phenotypic variance and covariance components for feed intake, feed efficiency, and

495   other postweaning traits in Angus cattle. Journal of Animal Science 79, 2805-2811.

496   Arthur, P.F., Herd, R.M., Wright, J., Xu, G., Dibley, K. and Richardson, E.C., 1996. Net feed

497   conversion efficiency and its relationship with other traits in beef cattle. In Proceedings-

498   Australian Society of Animal Production Conference 1996, 21, 107-110

499   Arthur, P., G. Renand, and D. Krauss, 2001b. Genetic and phenotypic relationships among

500   different measures of growth and feed efficiency in young Charolais bulls. Livestock

501   Production Science 68, 131-139.

502   Bach, A., S. Calsamiglia, and M. D. Stern, 2005. Nitrogen metabolism in the rumen. Journal of

503   Dairy Science 88, E9-E21.

504   Bannink, A., J. Kogut, J. Dijkstra, J. France, E. Kebreab, A. Van Vuuren, and S. Tamminga,

505   2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of

506   lactating cows. Journal of Theoretical Biology 238, 36-51.

507   Basarab, J., M. Price, J. Aalhus, E. Okine, W. Snelling, and K. Lyle, 2003. Residual feed intake

508   and body composition in young growing cattle. Canadian Journal of Animal Science 83,

509   189-204.

510   Bates, D. B., J. A. Gillett, S. A. Barao, and W. G. Bergen, 1985. The effect of specific growth

511   rate and stage of growth on nucleic acid-protein values of pure cultures and mixed

512   ruminal bacteria. Journal of Animal Science 61, 713-724.

513   Bergman, E, 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in

514   various species. Physiological Reviews 70, 567-590.

515   Bhagwat, A., B. De Baets, A. Steen, B. Vlaeminck, and V. Fievez, 2012. Prediction of ruminal

516   volatile fatty acid proportions of lactating dairy cows based on milk odd-and branched-

517   chain fatty acid profiles: New models, better predictions. Journal of Dairy Science 95,

518   3926-3937.

519   Cammack, K. M., C. L. Wright, K. Austin, P. Johnson, R. Cockrum, K. Kessler, and K. Olson,

520   2010. Effects of high-sulfur water and clinoptilolite on health and growth performance of

521   steers fed forage-based diets. Journal of Animal Science 88,1777-1785.

522   Cantalapiedra-Hijar, G., M. Abo-Ismail, G. Carstens, L. Guan, R. Hegarty, D. Kenny, M. McGee,

523   G. Plastow, A. Relling, and I. Ortigues-Marty, 2018a. Biological determinants of

524   between-animal variation in feed efficiency of growing beef cattle. Animal 12 (suppl. 2),

525   321-335.

526   Cantalapiedra-Hijar, G., R. Dewhurst, L. Cheng, A. Cabrita, A. Fonseca, P. Nozière, D.

527   Makowski, H. Fouillet, and I. Ortigues-Marty, 2018b. Nitrogen isotopic fractionation as a

528   biomarker for nitrogen use efficiency in ruminants: a meta-analysis. Animal 12,1827-

529   1837.

530   Castells D., 2013. Effect of different fiber sources on the digestive function and development of

531   calves. 1 recurs electrònic (141 p.). ISBN 9788449037818. PhD Thesis, The

532   Autonomous University of Barcelona, Barcelona, Spain,

533   https://ddd.uab.cat/record/126826

534   Chagunda, M., 2013. Opportunities and challenges in the use of the Laser Methane Detector to

535   monitor enteric methane emissions from ruminants. Animal 7(suppl. 2), 394-400.

536   Chen, X., F. D. Hovell, and E. Ørskov, 1990. Excretion of purine derivatives by ruminants:

537   recycling of allantoin into the rumen via saliva and its fate in the gut. British Journal of

538   Nutrition 63,197-205.

539   Chen X.B., E.R. Ørskov, 2004 Research on urinary excretion of purine derivatives in ruminants:

540   past, present and future. In: Makkar H.P.S., Chen X.B. (eds) Estimation of microbial

541   protein supply in ruminants using urinary purine derivatives. Springer, Dordrecht. 180-

542   210. https://doi.org/10.1007/978-1-4020-2844-1_21

543   Chen, X. B., S. A. Chowdhury, F. D. D. Hovell, E. R. Ørskov, and D. J. Kyle, 1992. Endogenous

544   allantoin excretion in response to changes in protein supply in sheep. The Journal of

545   Nutrition 122, 2226-2232.

546   Cheng K. J., J. P. Fay, R. E. Howarth, J. W. Costerton, 1980. Sequence of events in the

547   digestion of fresh legume leaves by rumen bacteria. Applied and Environmental

548   Microbiology. 40, 613-25.

549   Cheng, K., R. McCowan, and J. Costerton, 1979. Adherent epithelial bacteria in ruminants and

550   their roles in digestive tract function. The American Journal of Clinical Nutrition 32,139-

551   148.

552   Cheng, L., C. M. Logan, R. J. Dewhurst, S. Hodge, H. Zhou, and G. Edwards, 2015. Effects of

553   feed intake and genetics on tissue nitrogen-15 enrichment and feed conversion

554   efficiency in sheep. Journal of Animal Science 93, 5849-5855.

555   Cheng, L., A. Sheahan, S. Gibbs, A. Rius, J. Kay, S. Meier, G. Edwards, R. Dewhurst, and J.

556   Roche, 2013. Nitrogen isotopic fractionation can be used to predict nitrogen-use

557   efficiency in dairy cows fed temperate pasture. Journal of Animal Science 91, 5785-

558   5788.

559   Coyle, S., C. Fitzsimons, D. Kenny, A. Kelly, and M. McGee, 2016. Repeatability of feed

560   efficiency in steers offered a high-concentrate diet. Journal of Animal Science 94, 719-

561   719.

562   Crews, J. D., 2005. Genetics of efficient feed utilization and national cattle evaluation: a review.

563   Genetics and Molecular Research: GMR 4, 152-165.

564   Dewhurst, R., D. Davies, and R. Merry, 2000. Microbial protein supply from the rumen. Animal

565   Feed Science and Technology 85,1-21.

566   Dewhurst, R., E. Kim, R. Evans, A. Cabrita, and A. Fonseca, 2007a. Effects of dietary sulphur

567   sources on concentrations of hydrogen sulphide in the rumen head-space gas of dairy

568   cows. Animal 1, 531-535.

569 Dewhurst, R., J. Moorby, B. Vlaeminck, and V. Fievez, 2007b. Apparent recovery of duodenal

570   odd-and branched-chain fatty acids in milk of dairy cows. Journal of Dairy Science 90,

571   1775-1780.

572 Dougherty, R., W. Stewart, M. Nold, I. Lindahl, C. Mullenax, and B. Leek, 1962. Pulmonary

573   absorption of eructated gas in ruminants. American Journal of Veterinary Research 23,

574   205-212.

575 Duarte, D., C. J. Newbold, E. Detmann, F. Silva, P. Freitas, R. Veroneze, and M. Duarte, 2019.

576   Genome-wide association studies pathway-based meta-analysis for residual feed intake

577   in beef cattle. Animal Genetics 50, 150-153.

578 Elliott-Martin, R., T. Mottram, J. Gardner, P. Hobbs, and P. Bartlett, 1997. Preliminary

579   investigation of breath sampling as a monitor of health in dairy cattle. Journal of

580   Agricultural Engineering Research 67, 267-275.

581 Freetly, H., and T. Brown-Brandl, 2013. Enteric methane production from beef cattle that vary in

582   feed efficiency. Journal of Animal Science 91, 4826-4831.

583 Funaba, M., K. Kagiyama, T. Iriki, and M. Abe, 1997. Duodenal flow of microbial nitrogen

584   estimated from urinary excretion of purine derivatives in calves after early weaning.

585   Journal of Animal Science 75,1965-1973.

586 Furman, O., L. Shenhav, G. Sasson, F. Kokou, H. Honig, S. Jacoby, T. Hertz, O. X. Cordero, E.

587   Halperin, and I. Mizrahi, 2020. Stochasticity constrained by deterministic effects of diet

588   and age drive rumen microbiome assembly dynamics. Nature Communications 11, 1-13.

589 Gannes, L. Z., C. M. Del Rio, and P. Koch, 1998. Natural abundance variations in stable

590   isotopes and their potential uses in animal physiological ecology. Comparative

591   Biochemistry and Physiology Part A: Molecular & Integrative Physiology 119, 725-737.

592 Gill, F., R. Dewhurst, R. Evershed, E. McGeough, P. O'Kiely, R. Pancost, and I. Bull, 2011.

593   Analysis of archaeal ether lipids in bovine faeces. Animal Feed Science and Technology

594   166, 87-92.

595 Gonzalez-Ronquillo, M., J. Balcells, J. Guada, and F. Vicente, 2003. Purine derivative excretion

596   in dairy cows: Endogenous excretion and the effect of exogenous nucleic acid supply.

597   Journal of Dairy Science 86, 1282-1291.

598 Gooneratne, S., A. Olkowski, and D. Christensen, 1989. Sulfur-induced polioencephalomalacia

599   in sheep: some biochemical changes. Canadian Journal of Veterinary Research 53, 462.

600 Green, T., J. Jago, K. Macdonald, and G. Waghorn, 2013. Relationships between residual feed

601   intake, average daily gain, and feeding behavior in growing dairy heifers. Journal of

602   Dairy Science 96, 3098-3107.

603 Guzman C.E., L.T. Bereza-Malcolm, B. De Groef, A.E. Franks, 2015. Presence of selected

604   methanogens, fibrolytic bacteria, and proteobacteria in the gastrointestinal tract of

605   neonatal dairy calves from birth to 72 hours. PLOS One 10, e0133048.

606   https://doi.org/10.1371/journal.pone.0133048

607 Hegarty, R., J. P. Goopy, R. Herd, and B. McCorkell, 2007. Cattle selected for lower residual

608   feed intake have reduced daily methane production. Journal of Animal Science 85,1479-

609   1486.

610 Henderson, G., F. Cox, S. Ganesh, A. Jonker, W. Young, L. Abecia, E. Angarita, P. Aravena, G.

611   N. Arenas, and C. Ariza, 2015. Rumen microbial community composition varies with diet

612   and host, but a core microbiome is found across a wide geographical range. Scientific

613   Reports 5, 14567.

614 Herd, R., and S. Bishop, 2000. Genetic variation in residual feed intake and its association with

615   other production traits in British Hereford cattle. Livestock Production Science 63, 111-

616   119.

617 Herd, R. M., J. Velazco, P. Arthur, and R. Hegarty, 2016. Associations among methane

618   emission traits measured in the feedlot and in respiration chambers in Angus cattle bred

619   to vary in feed efficiency. Journal of Animal Science 94, 4882-4891.

620 Hodgson, J., 1971. The development of solid food intake in calves 4. The effect of the addition

621   of material to the rumen, or its removal from the rumen, on voluntary food intake. Animal

622   Science 13, 581-592.

623 Hof, G., M. Vervoorn, P. Lenaers, and S. Tamminga, 1997. Milk urea nitrogen as a tool to

624   monitor the protein nutrition of dairy cows. Journal of Dairy Science 80, 3333-3340.

625 Huhtanen, P., E. Cabezas-Garcia, S. Krizsan, and K. Shingfield, 2015. Evaluation of between-

626   cow variation in milk urea and rumen ammonia nitrogen concentrations and the

627   association with nitrogen utilization and diet digestibility in lactating cows. Journal of

628   Dairy Science 98, 3182-3196.

629 Imani, M., M. Mirzaei, B. Baghbanzadeh-Nobari, and M. Ghaffari, 2017. Effects of forage

630   provision to dairy calves on growth performance and rumen fermentation: A meta-

631   analysis and meta-regression. Journal of Dairy Science 100, 1136-1150.

632 Jami, E., A. Israel, A. Kotser, and I. Mizrahi, 2013. Exploring the bovine rumen bacterial

633   community from birth to adulthood. The ISME Journal 7, 1069-1079.

634 Janssen, P. H., 2010. Influence of hydrogen on rumen methane formation and fermentation

635   balances through microbial growth kinetics and fermentation thermodynamics. Animal

636   Feed Science and Technology 160, 1-22.

637 Jiao, H., T. Yan, D. A. Wills, A. F. Carson, and D. A. McDowell, 2014. Development of prediction

638   models for quantification of total methane emission from enteric fermentation of young

639   Holstein cattle at various ages. Agriculture, Ecosystems & Environment 183,160-166.

640 Jiao, J., J. Huang, C. Zhou, and Z. Tan, 2015. Taxonomic identification of ruminal epithelial

641   bacterial diversity during rumen development in goats. Applied and Environmental

642   Microbiology 81, 3502-3509.

643 Johnson, K. A., and D. E. Johnson, 1995. Methane emissions from cattle. Journal of Animal

644   Science 73, 2483-2492.

645 Jonker, A., S. Muetzel, G. Molano, and D. Pacheco, 2016. Effect of fresh pasture forage quality,

646   feeding level and supplementation on methane emissions from growing beef cattle.

647   Animal Production Science 56,1714-1721.

648 Karisa, B., J. Thomson, Z. Wang, C. Li, Y. Montanholi, S. Miller, S. Moore, and G. Plastow,

649   2014. Plasma metabolites associated with residual feed intake and other productivity

650   performance traits in beef cattle. Livestock Science 165, 200-211.

651 Kelly, A., M. McGee, D. Crews Jr, A. Fahey, A. Wylie, and D. Kenny, 2010. Effect of divergence

652   in residual feed intake on feeding behavior, blood metabolic variables, and body

653   composition traits in growing beef heifers. Journal of Animal Science 88, 109-123.

654 Khan, M., A. Bach, D. Weary, and M. Von Keyserlingk, 2016. Invited review: Transitioning from

655   milk to solid feed in dairy heifers. Journal of Dairy Science 99, 885-902.

656 Khan, M., D. Weary, and M. Von Keyserlingk, 2011. Invited review: Effects of milk ration on

657   solid feed intake, weaning, and performance in dairy heifers. Journal of Dairy Science

658   94,1071-1081.

659 Kim, E.J., R. Sanderson, M.S. Dhanoa, and R.J. Dewhurst, 2005. Fatty acid profiles associated

660   with microbial colonization of freshly ingested grass and rumen biohydrogenation. Journal

661   of Dairy Science, 88, 3220-3230.

662 Kmet, V., K. Boda a, P. Javorský, and R. Nemcová, 1986. The enzymatic activity of rumen

663   microflora in calves. Journal of Animal Physiology and Animal Nutrition 56, 73-77.

664 Laarman, A., and M. Oba, 2011. Effect of calf starter on rumen pH of Holstein dairy calves at

665   weaning. Journal of Dairy Science 94, 5661-5664.

666 Lanzas, C., G. Broderick, and D. Fox, 2008. Improved feed protein fractionation schemes for

667   formulating rations with the Cornell Net Carbohydrate and Protein System. Journal of

668   Dairy Science 91, 4881-4891.

669   Li, R. W., E. E. Connor, C. Li, V. Baldwin, Ransom L, and M. E. Sparks, 2012. Characterization

670   of the rumen microbiota of pre-ruminant calves using metagenomic tools. Environmental

671   Microbiology 14, 129-139.

672   Liem, K. F., W. E. Bemis, W. F. Walker, L. Grande, and W. F. Walker Jr. The University of

673   California, 2001. Functional anatomy of the vertebrates: an evolutionary perspective,

674   Volume 1, Edition 3, Harcourt College Publishers, San Diego, California, USA

675   Lutnicki, K., E. Madej, T. Riha, and L. Kurek, 2014. Polioencephalomalacia in ruminants caused

676   by excessive amount of sulphur-a review. Bulletin of the Veterinary Institute in Pulawy

677   58, 321-326.

678   Macko, S. A., M. L. F. Estep, M. H. Engel, and P. Hare, 1986. Kinetic fractionation of stable

679   nitrogen isotopes during amino acid transamination. Geochimica et Cosmochimica Acta

680   50, 2143-2146.

681   Malmuthuge, N., P. J. Griebel, and L. L. Guan, 2015. The gut microbiome and its potential role

682   in the development and function of newborn calf gastrointestinal tract. Frontiers in

683   Veterinary Science 2, 36.

684   McAllan, A., 1980. The degradation of nucleic acids in, and the removal of breakdown products

685   from the small intestines of steers. British Journal of Nutrition 44, 99-112.

686   McAllister, T., H. Bae, G. Jones, and K.-J. Cheng, 1994. Microbial attachment and feed

687   digestion in the rumen. Journal of Animal Science 72, 3004-3018.

688   McCartney, C., I. Bull, and R. J. Dewhurst, 2014. Using archaeol to investigate the location of

689   methanogens in the ruminant digestive tract. Livestock Science 164,39-45.

690   McCartney, C., I. Bull, T. Yan, and R. J Dewhurst, 2013. Assessment of archaeol as a molecular

691   proxy for methane production in cattle. Journal of Dairy Science 96, 1211-1217.

692   McDonnell, R., K. Hart, T. Boland, A. Kelly, M. McGee, and D. Kenny, 2016. Effect of

693   divergence in phenotypic residual feed intake on methane emissions, ruminal

694   fermentation, and apparent whole-tract digestibility of beef heifers across three

695   contrasting diets. Journal of Animal Science 94, 1179-1193.

696   Meale, S. J., M. D. Auffret, M. Watson, D. P. Morgavi, G. Cantalapiedra-Hijar, C.-A. Duthie, R.

697   Roehe, and R. J. Dewhurst, 2018. Fat accretion measurements strengthen the

698   relationship between feed conversion efficiency and nitrogen isotopic discrimination

699   while rumen microbial genes contribute little. Scientific Reports 8, 1-7.

700   Meale, S. J., S. Li, P. Azevedo, H. Derakhshani, T. DeVries, J. Plaizier, M. Steele, and E.

701   Khafipour, 2017a. Weaning age influences the severity of gastrointestinal microbiome

702   shifts in dairy calves. Scientific Reports 7, 1-13.

703   Meale, S. J., S. Li, P. Azevedo, H. Derakhshani, J. C. Plaizier, E. Khafipour, and M. A. Steele,

704   2016. Development of ruminal and fecal microbiomes are affected by weaning but not

705   weaning strategy in dairy calves. Frontiers in Microbiology 7, 582.

706   Meale, S. J., D. P. Morgavi, I. Cassar-Malek, D. Andueza, I. Ortigues-Marty, R. J. Robins, A.-M.

707   Schiphorst, S. Laverroux, B. Graulet, and H. Boudra, 2017b. Exploration of biological

708   markers of feed efficiency in young bulls. Journal of Agricultural and Food Chemistry 65,

709   9817-9827.

710   Mercadante, M. E. Z., A. P. d. M. Caliman, R. C. Canesin, S. F. M. Bonilha, A. Berndt, R. T. S.

711   Frighetto, E. Magnani, and R. H. Branco, 2015. Relationship between residual feed

712   intake and enteric methane emission in Nellore cattle. Revista Brasileira de Zootecnia

713   44, 255-262.

714   Muetzel, S., 2015. Framework & Milestone report–H2 management in the rumen, Final Report,

715   MPI Technical Paper No: 2018/30, Ministry for Primary Industries, NewZealand

716   Government, Wellington 6140, NewZealand. http://www.mpi.govt.nz/news-

717   resources/publications.aspx

718   Muetzel, S., and H. Clark, 2015. Methane emissions from sheep fed fresh pasture. New

719   Zealand Journal of Agricultural Research 58, 472-489.

720   Nasrollahi, S. M., S. J. Meale, D. P. Morgavi, A. M. Schiphorst, R. J. Robins, I. Ortigues-Marty,

721   and G. Cantalapiedra-Hijar, 2020. The origin of N isotopic discrimination and its

722   relationship with feed efficiency in fattening yearling bulls is diet-dependent. PLOS One

723   15, e0234344.

724   Negussie, E., Y. de Haas, F. Dehareng, R. J. Dewhurst, J. Dijkstra, N. Gengler, D. Morgavi, H.

725   Soyeurt, S. van Gastelen, and T. Yan, 2017. Invited review: Large-scale indirect

726   measurements for enteric methane emissions in dairy cattle: A review of proxies and

727   their potential for use in management and breeding decisions. Journal of Dairy Science

728   100, 2433-2453.

729   Neill, A. R., D. W. Grime, and R. Dawson, 1978. Conversion of choline methyl groups through

730   trimethylamine into methane in the rumen. Biochemical Journal 170, 529-535.

731   Nkrumah, J., J. Basarab, M. Price, E. Okine, A. Ammoura, S. Guercio, C. Hansen, C. Li, B.

732   Benkel, and B. Murdoch, 2004. Different measures of energetic efficiency and their

733   phenotypic relationships with growth, feed intake, and ultrasound and carcass merit in

734   hybrid cattle. Journal of Animal Science 82, 2451-2459.

735   Nkrumah, J., E. Okine, G. Mathison, K. Schmid, C. Li, J. Basarab, M. Price, Z. Wang, and S.

736   Moore, 2006. Relationships of feedlot feed efficiency, performance, and feeding

737   behavior with metabolic rate, methane production, and energy partitioning in beef cattle.

738   Journal of Animal Science 84, 145-153.

739   Raisbeck, M., S. Riker, C. Tate, R. Jackson, M. Smith, K. Reddy, and J. Zygmunt, 2008. Water

740   quality for Wyoming livestock & wildlife: a review of the literature pertaining to health

741   effects of inorganic contaminants, B-1183, Wyoming Game and Fish Department,

742   University of Wyoming, Cheyenne, Wyoming 82006, Western United State.

743   Rey, M., F. Enjalbert, S. Combes, L. Cauquil, O. Bouchez, and V. Monteils, 2014. Establishment

744   of ruminal bacterial community in dairy calves from birth to weaning is sequential.

745   Journal of Applied Microbiology 116, 245-257.

746   Richardson, E., and R. Herd, 2004. Biological basis for variation in residual feed intake in beef

747   cattle. 2. Synthesis of results following divergent selection. Australian Journal of

748   Experimental Agriculture 44, 431-440.

749   Rieu, F., G. Fonty, B. Gaillard, and P. Gouet, 1990. Electron microscopy study of the bacteria

750   adherent to the rumen wall in young conventional lambs. Canadian Journal of

751   Microbiology 36, 140-144.

752   Roehe, R., R. J. Dewhurst, C. A. Duthie, J. A. Rooke, N. McKain, D. W. Ross, J. J. Hyslop, A.

753   Waterhouse, T. C. Freeman, and M. Watson, 2016, Bovine host genetic variation

754   influences rumen microbial methane production with best selection criterion for low

755   methane emitting and efficiently feed converting hosts based on metagenomic gene

756   abundance. PLOS Genetics 12, e1005846. https://doi.org/10.1371/journal.pgen.1005846

757   Roman, R., C. Wilcox, and F. Martin, 2000. Estimates of repeatability and heritability of

758   productive and reproductive traits in a herd of Jersey cattle. Genetics and Molecular

759   Biology 23, 113-119.

760   Sahoo, A., D. Kamra, and N. Pathak, 2005. Pre-and postweaning attributes in faunated and

761   ciliate-free calves fed calf starter with or without fish meal. Journal of Dairy Science 88,

762   2027-2036.

763   Sander, E., R. Warner, H. Harrison, and J. Loosli, 1959. The stimulatory effect of sodium

764   butyrate and sodium propionate on the development of rumen mucosa in the young calf.

765   Journal of Dairy Science 42,1600-1605.

766   Schenkel, F., S. Miller, and J. Wilton, 2004. Genetic parameters and breed differences for feed

767   efficiency, growth, and body composition traits of young beef bulls. Canadian Journal of

768   Animal Science 84, 177-185.

769   Schwarm, A., M. Schweigel-Röntgen, M. Kreuzer, S. Ortmann, F. Gill, B. Kuhla, U. Meyer, M.

770   Lohölter, and M. Derno, 2015. Methane emission, digestive characteristics and faecal

771   archaeol in heifers fed diets based on silage from brown midrib maize as compared to

772   conventional maize. Archives of Animal Nutrition 69, 159-176.

773 Seshadri, R., S. C. Leahy, G. T. Attwood, K. H. Teh, S. C. Lambie, A. L. Cookson, E. A. Eloe-

774   Fadrosh, G. A. Pavlopoulos, M. Hadjithomas, and N. J. Varghese, 2018. Cultivation and

775   sequencing of rumen microbiome members from the Hungate1000 Collection. Nature

776   Biotechnology 36, 359.

777 Shingfield, K., 2000. Estimation of microbial protein supply in ruminant animals based on renal

778   and mammary purine metabolite excretion. A review. Journal of Animal and Feed

779   Sciences 9, 169-212.

780 Soberon, F., and M. Van Amburgh, 2013. Lactation Biology Symposium: The effect of nutrient

781   intake from milk or milk replacer of preweaned dairy calves on lactation milk yield as

782   adults: a meta-analysis of current data. Journal of Animal Science 91, 706-712.

783 Steele, M. A., G. B. Penner, and F. Chaucheyras-Durand, 2016. Development and physiology of

784   the rumen and the lower gut: Targets for improving gut health. Journal of Dairy Science

785   99, 4955-4966.

786 Stobo, I., J. Roy, and H. J. Gaston, 1966. Rumen development in the calf: 1. The effect of diets

787   containing different proportions of concentrates to hay on rumen development. British

788   Journal of Nutrition 20, 171-188.

789 Suárez, B., C. Van Reenen, G. Beldman, J. Van Delen, J. Dijkstra, and W. Gerrits, 2006. Effects

790   of supplementing concentrates differing in carbohydrate composition in veal calf diets: I.

791   Animal performance and rumen fermentation characteristics. Journal of Dairy Science

792   89, 4365-4375.

793 Talebi, M., 2012. Feed intake, feed efficiency, growth and their relationship with Kleiber ratio in

794   Lori-Bakhtiari lambs. Archiva Zootechnica 15, 33.

795 Tamate, H., A. McGilliard, N. Jacobson, and R. Getty, 1962. Effect of various dietaries on the

796   anatomical development of the stomach in the calf. Journal of Dairy Science 45, 408-

797   420.

798 Tamminga, S., 1979. Protein degradation in the forestomachs of ruminants. Journal of Animal

799   Science 49, 1615-1630.

800 Tedeschi, L., D. Fox, and J. Russell, 2000. Accounting for ruminal deficiencies of nitrogen and

801   branched-chain amino acids in the structure of the Cornell net carbohydrate and protein

802   system. Proceedings of the Cornell Nutrition Conference for Feed Manufacturers.

803   October 24-26, 2000, Rochester Marriott Thruway Hotel, Rochester, New York, Article

804   number 19, 224-238.

805 Van den Borne, J., M. Verstegen, S. Alferink, R. Giebels, and W. Gerrits, 2006. Effects of

806   feeding frequency and feeding level on nutrient utilization in heavy preruminant calves.

807   Journal of Dairy Science 89, 3578-3586.

808 Van Nevel, C., and D. Demeyer, 1996. Control of rumen methanogenesis. Environmental

809   Monitoring and Assessment 42, 73-97.

810 Van Soest, P. J., 1996. Allometry and ecology of feeding behavior and digestive capacity in

811   herbivores: a review. Zoo Biology: Published in affiliation with the American Zoo and

812   Aquarium Association 15, 455-479.

813 Vlaeminck, B., C. Dufour, A. Van Vuuren, A. Cabrita, R. J. Dewhurst, D. Demeyer, and V.

814   Fievez, 2005. Use of odd and branched-chain fatty acids in rumen contents and milk as

815   a potential microbial marker. Journal of Dairy Science 88, 1031-1042.

816 Vlaeminck, B., V. Fievez, A. Cabrita, A. Fonseca, and R. Dewhurst, 2006a. Factors affecting

817   odd-and branched-chain fatty acids in milk: A review. Animal Feed Science and

818   Technology 131, 389-417.

819   Vlaeminck, B., V. Fievez, D. Demeyer, and R. J. Dewhurst, 2006b. Effect of forage: concentrate

820   ratio on fatty acid composition of rumen bacteria isolated from ruminal and duodenal

821   digesta. Journal of Dairy Science 89, 2668-2678.

822   Waghorn, G., and R. Hegarty, 2011. Lowering ruminant methane emissions through improved

823   feed conversion efficiency. Animal Feed Science and Technology 166, 291-301.

824   Wallace, R. J., G. Sasson, P. C. Garnsworthy, I. Tapio, E. Gregson, P. Bani, P. Huhtanen, A. R.

825   Bayat, F. Strozzi, and F. Biscarini, 2019. A heritable subset of the core rumen

826   microbiome dictates dairy cow productivity and emissions. Science Advances 5,

827   eaav8391.

828   Wang, Z., J. D. Nkrumah, C. Li, J.A. Basarab, L.A. Goonewardene, E.K. Okine, D.H.Crews Jr,

829   and S.S. Moore, 2006. Test duration for growth, feed intake, and feed efficiency in beef

830   cattle using the GrowSafe System. Journal of Animal Science, 84, 2289-2298.

831   Wang, Z., C. Elekwachi, J. Jiao, M. Wang, S. Tang, C. Zhou, Z. Tan, and R. J. Forster, 2017.

832   Changes in metabolically active bacterial community during rumen development, and

833   their alteration by rhubarb root powder revealed by 16S rRNA amplicon sequencing.

834   Frontiers in Microbiology 8,159.

835   Westreicher-Kristen, E., J. Castro-Montoya, M. Hasler, and A. Susenbeth, 2020. Relationship of

836   milk odd-and branched-chain fatty acids with urine parameters and ruminal microbial

837   protein synthesis in dairy cows fed different proportions of maize silage and red clover

838   silage. Animals 10, 316.

839   Wheadon, N., M. McGee, G. Edwards, and R. Dewhurst, 2014. Plasma nitrogen isotopic

840   fractionation and feed efficiency in growing beef heifers. British Journal of Nutrition 111,

841   1705-1711.

842   Yáñez-Ruiz, D. R., L. Abecia, and C. J. Newbold, 2015. Manipulating rumen microbiome and

843   fermentation through interventions during early life: a review. Frontiers in Microbiology 6,

844   1133.

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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_4 emissions 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 degradation, 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