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

Cheng, Long; Cantalapiedra-Hijar, Gonzalo; Meale, Sarah; Rugoho, Innocent; Jonker, Arjan; Khan, M.A.; Al-Marashdeh, Omar; Dewhurst, RJ

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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**

3 L. Cheng[a*], G. Cantalapiedra-Hijar[b], S. J. Meale[c], I. Rugoho[d,] A. Jonker[e], M. A. Khan[e], O.

4 Al-Marashdeh[f], R. J. Dewhurst[g]

5

6 [a]*Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Dookie*

7 *Campus, 3647, Victoria, Australia;*

8 [b]*Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, F-63122 Saint-*

9 *Genès-Champanelle, France;*

10 [c]*School of Agriculture and Food Sciences, Faculty of Science, University of Queensland,*

11 *Gatton, 4343 Queensland Australia;*

12 [d]*Lely Australia Pty Ltd, 84 Agar Drive, Truganina, 3029, Victoria, Australia;*

13 [e]*AgResearch Limited, Grasslands Research Centre, Private Bag 11008, Palmerston*

14 *North 4410, New Zealand;*

15 [f]*Faculty of Agriculture and Life Sciences, Lincoln University, P.O. Box 85084, Lincoln,*

16 *New Zealand;*

17 [g]*Scotland's Rural College, King's Buildings, West Mains Road, Edinburgh EH9 3JG,*

18 *United Kingdom.*

19

20

21 Corresponding author: Long Cheng.

22 *Email: long.cheng@unimelb.edu.au

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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

136   **Markers and proxies to monitor ruminal function in young ruminants**

137   At birth, the rumen is sterile, and physically and metabolically underdeveloped. Initiation

138   of solid feed consumption, acquisition of anaerobic microbes, the establishment of

139   ruminal fermentation, growth of papillae, maturation of salivary function, and physical

140   expansion of the rumen are achieved during the first ~four months of life (Khan et al.,

141   2011; Khan et al., 2016) in response to nutritional inputs and management practices

142   (Yáñez-Ruiz et al., 2015; Steele et al., 2016; Meale et al., 2017a). For example, the

143   provision of forage vs. concentrate to young ruminants provide a strong physical

144   stimulus for the expansion of the rumen to increase its volume, physical development,

145   and motility (Castells Domingo, 2013) and promote the development of rumination

146   behavior, saliva flow, and buffering capacity (Laarman and Oba, 2011; Khan et al.,

147   2016). In summary, there is emerging evidence that early life nutritional interventions

148   influence ruminal development in young ruminants (Khan et al., 2016), with lifelong

149   consequences on their welfare and performance (Khan et al., 2011; Soberon and Van

150   Amburgh, 2013). There is renewed interest in finding mechanisms by which the host

151   and diet can influence these developments, not least because there may be the longer-

152   term setting of the interaction between the host, rumen and microbiome (Yáñez-Ruiz et

153   al., 2015). However, there are serious constraints to the ability to use existing sampling

154   techniques and limitations associated with the low ruminal volume and smaller microbial

155   population. A number of approaches have been developed to study ruminal function

156   less invasively and these have often been applied in studies with adult ruminants (see

157   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.

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

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

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

426

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

427

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

428

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

429

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

430

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

431

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

432

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

433

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

434

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

435

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

436

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

437

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

438

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

439

both ruminal function and host FE.

440

Conclusion

441

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

442

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

443

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

444

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

445

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

446

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

447

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

448

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

450 mature ruminants.

Ethics approval

452 Not applicable

Data and model availability statement

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

Author ORCIDs

456 L. Cheng: 0000-0002-8483-0495

457 G. Cantalapiedra-Hijar: 0000-0001-9486-8238

458 S. J. Meale: 0000-0002-8417-1186

459 I. Rugoho: 0000-0002-3792-3584

460 A. Jonker: 0000-0002-6756-8616

461 M. A. Khan: 0000-0001-7590-5128

462 O. Al-Marashdeh: 0000-0001-6493-0395

463 R. J. Dewhurst: 0000-0002-9357-7372

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.

Declaration of interest

468 No competing interests to report.

Declaration of interest

21

470 None

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480

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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

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==