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Animal health and greenhouse gas intensity: the paradox of periparturient parasitism

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1 **Animal health and greenhouse gas intensity: the paradox of**
2 **periparturient parasitism**

3

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13

14

15 **Abstract**

16 Here we provide the first direct measurements of pathogen challenge
17 impacts on greenhouse gas (GHG) production, yield and intensity. Twin-
18 rearing ewes were *ad libitum* fed pelleted lucerne from day₋₃₂ to day₃₆ (day₀ is
19 parturition), and repeatedly infected with 10,000 *Teladorsagia circumcincta*
20 infective larvae (n=16), or sham-dosed with water (n=16). A third group of 16
21 ewes were fed at 80% of uninfected ewes' feed intake during lactation.
22 Methane emissions were measured in respiration chambers (day₃₀ to day₃₆)
23 whilst total tract apparent nutrient digestibility around day₂₈ informed
24 calculated manure methane and nitrous oxide emissions estimates.
25 Periparturient parasitism reduced feed intake (-9%) and litter weight gain (-
26 7%) and doubled maternal body weight loss. Parasitism reduced daily enteric
27 methane production by 10%, did not affect methane yield per unit dry matter
28 intake but increased yield per unit digestible organic matter intake by 14%.
29 Parasitism did not affect daily calculated manure methane and nitrous oxide
30 production, but increased manure methane and nitrous oxide yields per unit
31 dry matter intake by 16% and 4%, respectively, and per unit digestible organic
32 matter intake by 46% and 31%, respectively. Accounting for increased lucerne
33 input for delayed weaning and maternal body weight loss compensation,
34 parasitism increased calculated GHG intensity per kg lamb weight gain for
35 enteric methane (+11%), manure methane (+32%) and nitrous oxide (+30%).
36 Supplemented with the global warming potential (GWP) associated with
37 production of pelleted lucerne, we demonstrated that parasitism increased
38 calculated GWP per kg lamb weight gain by 16%, which was similar to the
39 measured impact of parasitism on feed conversion ratio. Thus, arising from

40 pathogen-induced feed efficiency reduction and modified GHG emissions, we
41 demonstrated that ovine periparturient parasitism increases GHG intensity.
42 This implies that ewe worm control can not only improve production efficiency
43 but also reduce the environmental footprint of sheep production systems.

44

45 **Key words:** disease, parasitism, environmental footprint, methane, nitrous
46 oxide, sheep

47

48

49 **1. Introduction**

50 It is well recognized that pathogen exposure often results in anorexia,
51 i.e. reduction in feed intake. In the case of sub-clinical gastrointestinal
52 nematode parasitism, feed intake is typically reduced by up to 20-25% in e.g.
53 growing and periparturient sheep, though wide ranges of parasitism-induced
54 anorexia and associated production losses have been reported across
55 different species (Sykes, 1994; Kyriazakis et al., 1998; Zaralis et al., 2008).
56 Variation in feed intake can be expected to correlate with variation in
57 greenhouse gas (GHG) production from both respiration and manure
58 emission. This implies that pathogen challenge would be expected to result in
59 reduced daily production of methane (CH₄), carbon dioxide (CO₂) and nitrous
60 oxide (N₂O), provided that GHG yield, defined as the amount of GHG
61 produced per unit feed intake, is not affected. However, since pathogen
62 challenge reduces productivity, arising from a combination of anorexia and
63 reduced efficiency of resource use for production purposes (Sykes, 1994;
64 Coop and Kyriazakis, 1999), challenged animals would be expected to take
65 longer and require more resource input to achieve the same productive
66 output. GHG production associated with this extra resource input required
67 would effectively be the consequence of pathogen challenge on resource
68 efficiency, and thus increase GHG intensity.

69 Here, we provide the first direct assessment of the impact of pathogen
70 challenge on GHG emission in livestock. We have assessed effects of
71 gastrointestinal parasitism on performance, digestibility, CH₄ and CO₂
72 production and yield, and on feed efficiency, in lactating ewes. Furthermore,
73 we used IPCC (2006) assumptions and literature data where data were not

74 derived from the experiment carried out to extend the above to include
75 estimates of manure CH₄ and N₂O production and yield. These estimates are
76 used to test the hypothesis that periparturient parasitism increases ewe GHG
77 intensity and global warming potential (GWP) for lamb production.

78

79 **2. Materials and Methods**

80 **2.1 Animals and housing**

81 Twelve 4-5 year old Mule ewes (Bluefaced Leicester × Scottish
82 Blackface) were recruited from each of four larger mating group approximately
83 45 days before observed mean parturition date (day₀), with mean expected
84 parturition dates separated by a week. Ewe body weight (BW) and body
85 condition score (CS) recorded on day₋₃₉ for the total of 48 ewes used
86 averaged (±se) 68.2±0.79 kg and 2.5±0.06, respectively. Ewes were served
87 by Suffolk rams and confirmed to be bearing twins by ultrasonic scanning
88 prior to the experiment and were housed individually, in pens sized 1.30 m ×
89 2.15 m with adjacent creep area of the same size for their lambs. From day₋₄₅
90 until day₃₀, the ewes were housed in a naturally ventilated and illuminated
91 shed, with additional low-level lighting at night times during lambing. From
92 day₃₀ until day₃₆, ewes and their lambs were housed in respiration chambers
93 (see below) in similar sized pens. Fresh wood shavings were used as bedding
94 and added daily, and fresh water was *ad libitum* available. A small amount of
95 shavings were also used daily in the respiration chambers.

96

97 **2.2. Experimental treatments and feeding**

98 The twelve ewes within each of the four mating groups were divided
99 into three groups of four ewes based on initial BW, which resulted in three
100 groups of 16 ewes with similar mean initial BW, CS and faecal egg counts
101 (FEC). From day⁻⁴⁵ to day⁻³², the ewes received *ad libitum* medium quality hay
102 and approximately 300 g/day/head of a commercial ewe nut. From day⁻³² until
103 day⁻²⁵, allowances of hay and commercial ewe nuts were gradually reduced
104 and completely replaced with increasing amounts of pelleted lucerne. From
105 day⁻²⁴ onwards, two groups of ewes were fed lucerne *ad libitum* and either
106 uninfected (CON) or dosed with parasites (PAR). Details of the experimental
107 infection are provided below. A third group of ewes were managed as CON
108 ewes during pregnancy but fed restrictedly at 80% of intakes achieved by
109 CON ewes during lactation (RES). The RES group was included to assess to
110 what extent GHG production, yield and intensity would be affected by reduced
111 feed intake per se. Ewes were fed at 07.30 and 1500 h. The experiment was
112 approved by SRUC's Ethical Review Committee (ED AE 03/2011) and carried
113 under Home Office authorization (PPL 60/3782).

114

115 **2.3. Experimental infection**

116 Because the ewes were 4 to 5 years old and had previously grazed
117 natural pastures infested predominantly with *Teladorsagia circumcincta*, they
118 were expected to have had substantial prior exposure to this parasite, an
119 abomasal nematode of particular concern in temperate regions. The ewes
120 were orally treated on day⁻³⁸ with levamisole (Levacide, Norbrook, Newry, UK)
121 and ivermectin (Oramec, Merial, Harlow, UK) at the rate of 7.5 and 0.2 mg/kg
122 BW, respectively, to remove worm burdens. A subsequent FEC taken on day.

123 22 averaged 0 (0-1) eggs per g fresh faeces (epg), suggesting that the drench
124 was effective. The PAR ewes were then trickle infected with 10,000 infective
125 *T. circumcincta*, suspended in 10 ml of water and administered every Monday,
126 Wednesday and Friday from day-21 onwards until the end of the experiment.
127 The CON and RES ewes were sham-infected with 10 ml of water on the same
128 days. The *T. circumcincta* strain used was the Moredun Ovine Susceptible
129 Isolate that has been maintained in the laboratory for several years. This
130 infection model has repeatedly been used in our lab to induce sub-clinical
131 parasitism in periparturient ewes (Houdijk et al., 2003, 2006, Zaralis et al.,
132 2009; Kidane et al., 2010).

133 **2.4. Measurements and calculations**

134 **Performance.** The ewes were weighed on day-39 and then weekly
135 from day-31 onwards, as well as within 12 h of parturition to assess daily
136 weight gain during late pregnancy and during lactation through linear
137 regression of BW on time. The lambs were weighed within 12 h after birth and
138 weekly afterwards to assess daily litter weight gain in the same way. Since the
139 lambs did not receive creep feed, lamb BW and daily weight gain were used
140 to calculate milk production (Robinson et al., 1969). Ewe CS was taken
141 approximately fortnightly, by lumbar palpation on a zero to five point scale,
142 and to an accuracy of a quarter (Russel et al., 1969), where 0 is emaciated
143 and 5 is obese. Feed samples were collected every day during the experiment
144 during feeding and were pooled for chemical analyses (Table 1) as per
145 standard protocols (Ministry of Agriculture Fisheries and Food, 1992). Feed
146 refusals were recorded twice weekly (Mon and Thu) and analysed for dry

147 matter (DM) only. This allowed for the calculation of achieved mean daily dry
148 matter intake (DMI).

149 **Parasitism.** The level of parasitism was monitored through regular
150 faecal sampling for FEC, according to a modified flotation method (Christie
151 and Jackson, 1982), with a sensitivity of 1 epg. This was done for all ewes at
152 housing, day-22 and day-11 and at parturition, and then weekly thereafter (for
153 PAR ewes only).

154 **Digestibility.** Apparent total tract DM, organic matter (OM) and
155 nitrogen (N) digestibility were assessed through using acid insoluble ash (AIA)
156 as an internal, indigestible marker. Feed samples collected daily during
157 feeding were pooled for DM, N, ash and AIA analyses, with OM calculated as
158 DM minus ash. Faeces were collected directly from the rectum of all ewes for
159 three consecutive days (day₂₇ to day₂₉) and were pooled per individual ewe
160 and kept frozen at -20 °C before analysis of DM, ash, AIA and N, and
161 calculation of OM as above. Feed and faecal AIA were analysed using the 2
162 M HCl procedure described by van Keulen and Young (1977). The above
163 analyses allowed us to calculate faecal OM and N output, as well as digestible
164 OM intake (dOMI).

165 Furthermore, daily fresh faeces production was calculated using mean
166 achieved DM intake, faeces DM contents and total tract DM digestibility in
167 order to estimate worm egg output (eggs/day). The latter was estimated by
168 multiplying mean FEC during lactation (eggs/g) with the calculated fresh
169 faeces production (g/day), under the assumption that since ewes were fed the
170 same diet throughout lactation, total tract DM digestibility measured from

171 day₂₃ to day₂₆ can be extrapolated over the whole lactation period (Kidane et
172 al. 2009).

173 ***Enteric methane and carbon dioxide emissions.*** Staggered lambing
174 arising from the four mating groups used allowed for four rounds of housing in
175 one of six indirect open-circuit respiration chambers (No Pollution Industrial
176 Systems Ltd., Edinburgh, UK) for six days from day₃₀ to day₃₆ with two ewes
177 per treatment per chamber, individually housed with their lambs to achieve
178 two treatment replicates per round, and thus n=8 in total. Experimental
179 treatments and DMI determination were maintained in chamber. Daily CH₄
180 and CO₂ production was measured as described in detail elsewhere (Rooke
181 et al., 2014). Briefly, each chamber has an area of 25.4 m² with appropriate
182 penning for two ewes and their four lambs. Air was removed from the
183 chambers by exhaust fans set at 50 litre/s and temperature and humidity were
184 set at 15 ± 1°C and 60 ± 5% relative humidity, respectively. Exhaust air was
185 sampled for gas analysis sequentially for 45 s from each chamber, and
186 methane and carbon dioxide concentrations were measured by infrared
187 absorption spectroscopy. Animals remained in the chambers for 6 days,
188 where the first four days were used for adaptation, and the last two days were
189 used to quantify CH₄ and CO₂ production. Measurements of CH₄ and CO₂
190 concentrations were made every 6 minutes in the mechanically ventilated air
191 entering and leaving each chamber and exhaust air flow rate (every 30 min)
192 corrected to standard temperature and pressure. Due to missing data arising
193 from operational issues in Round 2, a full data set for each room was
194 available for only the last 24 h, which was the dataset used for the present

195 analysis. Daily CH₄ and CO₂ production was divided by mean daily DMI and
196 dOMI to obtain CH₄ and CO₂ yield.

197 **Manure methane emissions.** Methane emissions from manure were
198 estimated from the volume of volatile solids produced, defined as the sum of
199 faecal and urine OM output (IPCC, 2006). Faecal OM output was derived from
200 mean daily in chamber DMI, feed OM content and total tract OM digestibility,
201 whilst total urine OM output was calculated under the assumption that energy
202 excretion in urine is $0.04 \times \text{GE intake}$ (IPCC, 2006) and that energy
203 concentration in urine is 18.75 MJ/kg DM (IPCC, 2006). We further assumed
204 that the manure is directly deposited on pasture in a cool climate (i.e. not
205 stored), and thus a methane conversion factor of 1% and a maximum
206 methane-producing capacity of 0.19 m³ per kg volatile solids (IPCC, 2006).
207 This resulted in a methane yield of 1.273 g per kg volatile solids. Daily manure
208 CH₄ production was divided by mean daily in chamber DMI and dOMI to
209 obtain manure CH₄ yield estimates.

210 **Manure N₂O emissions.** The N₂O emission from manure was
211 estimated from total N excreted, i.e. the sum of faecal N and urine N outputs.
212 Faecal N output is derived from mean daily in chamber DMI, feed N content,
213 and apparent total tract N digestibility. Urine N output is estimated from the
214 assumption that urine N output equals $0.46 \times \text{N apparently absorbed during}$
215 lactation. This coefficient was derived from a series of then N balance
216 estimates in lactating ewes (Lynch et al., 1988; Malik et al., 1999; Pappas,
217 1977; Maamouri et al., 2011), whilst N absorbed was derived from apparent
218 total tract N digestibility carried out. As above, we assume that the manure is
219 deposited directly on the pasture in a cool environment, and 1% of manure N

220 is directly volatilised into nitrous oxide N, 20% of manure N is indirectly
221 volatilised through ammonia with an efficiency factor of 0.01 for conversion
222 into nitrous oxide N and 30% of manure N is leached through nitrate with an
223 efficiency factor of 0.0075 for conversion of manure N into nitrous oxide N
224 (IPCC, 2006). This resulted in a N₂O yield estimate of 22.393 g per kg N
225 excreted. Resulting daily N₂O production was divided by mean daily DMI and
226 dOMI to obtain N₂O yield.

227 ***Greenhouse gas emission intensity calculations.*** The GHG yields
228 derived as above were used to calculate GHG emission intensity per
229 functional unit, which was one kg lamb BW gain (BWG) from parturition until
230 weaning. We used the observed DMI, ewe BW loss, lamb birth weight and
231 lamb BW gain until day₃₆, to calculate through extrapolation the number of
232 days and the amount of DMI needed, as well as the total ewe BW loss
233 incurred, to reach a target weaning weight of 25 kg live weight for each of two
234 lambs reared (Kidane et al., 2010). This DMI was multiplied with GHG yields
235 as measured for CON, PAR and RES ewes.

236 We also calculated DMI needed to restore final ewe BW to initial ewe
237 BW (day-39), to account for BW loss incurred as a resource used to rear
238 lambs to weaning. We assumed that the metabolizable energy (ME)
239 requirement for restoring ewe BW was 39.75 MJ/kg for CON and RES ewes
240 (AFRC, 1993) but 49.16 MJ/kg for PAR ewes for reasons outlined below (see
241 'sensitivity analysis'). Since any influence of PAR or RES treatment on
242 digestibility and efficiency of resource utilization for weight gain post weaning
243 would unlikely remain present post weaning, as ewes return to full immunity to
244 parasites when lactation ceases (Houdijk, 2008), DMI to restore ewe BW was

245 multiplied with mean GHG yields for CON ewes. Total DMI was divided over
246 total lamb weight gain to calculate feed conversion ratio. Furthermore, post
247 weaning urine N output was estimated from the assumption that urine N
248 output equals $0.76 \times N$ apparently absorbed; this coefficient was derived from
249 a series of then N balance estimates in growing sheep (Akinbamijo et al.,
250 1994; Lima et al., 2011; Gorniak et al., 2014; Kimambo et al., 1988).

251 GHG emission intensity data from the above described CH₄ and N₂O
252 sources were combined into one GWP figure after converting CH₄ and N₂O
253 into their CO₂ equivalents. Conversion factors used were 25 and 298 CO₂-eq
254 for methane and nitrous oxide, respectively (IPCC, 2006). Data from CH₄ and
255 N₂O were supplemented with a GWP of 320 g CO₂-eq/kg for the production,
256 dehydration and transport of lucerne with 11% residual moisture (Gallego et
257 al., 2011), to derive at total GWP (kg CO₂-eq/kg BWG). Thus, breath CO₂ was
258 not included in these calculations, as GWP assessments focus on net
259 contributors to global warming, i.e. fossil-derived CO₂ only. We also used
260 lamb BWG to estimate milk production (Robinson et al., 1969), in order to
261 estimate GHG intensity per kg milk.

262 **Sensitivity analysis.** It was deemed appropriate to use a greater
263 requirement of ME per kg BW gain to restore BW for PAR ewes than for CON
264 and RES ewes, as PAR ewes likely lost relatively more fat than did CON and
265 RES ewes (see results on CS). The 49.16 MJ/kg BW gain was arbitrarily
266 chosen as the average of 39.75 MJ/kg (AFRC, 1993) and 58.57 MJ/kg
267 (Olthoff et al. 1989), which would be requirements for fat deposition only. A
268 sensitivity analysis was undertaken for the impact of this assumption by

269 comparing GWP intensity for PAR ewes to that of CON and RES ewes for ME
270 requirements per kg BW gain taken as 39.75, 49.16 and 58.57 MJ/kg.

271 Since there are no data on the effect of parasitism in general, and of *T.*
272 *circumcincta* challenge in particular, on urine N excretion in lactating sheep,
273 we assumed that the aforementioned urine N as a proportion of apparent N
274 absorbed is similar between CON, PAR and RES ewes. However, earlier
275 studies showed that *T. circumcincta* infections in young lambs increased
276 urinary N output as a proportion of apparent N absorbed by 30 to 40% relative
277 to non-infected control lambs (Parkins et al., 1973; Sykes and Coop, 1977).
278 We therefore undertook a second sensitivity analysis by comparing GWP
279 intensity for urine N output by increasing the value of $0.46 \times N$ absorbed to
280 0.65 (40% increase) during lactation.

281

282 **2.5. Statistical analysis**

283 Data obtained during late pregnancy and early lactation were analysed
284 separately, whilst the effect of parasitism during late pregnancy was analysed
285 through comparing PAR ewes with combined CON/RES ewes. During late
286 pregnancy, parameters that were repeatedly taken, i.e. feed intake, ewe BW
287 and CS were analysed via repeated measures ANOVA. Parameters that were
288 measured once, i.e. ewe and litter BW at birth, were analysed through
289 ANOVA. Likewise, during lactation, ewe feed intake, BW, CS and hourly
290 methane and carbon dioxide production were analysed via repeated
291 measures ANOVA, whilst ewe and litter BW gain, digestibility figures, daily
292 GHG production, yield and intensity data were analysed through ANOVA.

293 Models included mating group as block and day₋₃₉ observations as
294 covariate where appropriate. The ewe was the experimental unit for
295 production, parasitological and digestibility observations (n=16). However, for
296 any parameter related to emission production, yields and intensities, the
297 respiration chamber with paired ewes was used as experimental unit (n=8).
298 Means were separated through the use of Fisher's protected LSD test at
299 $P < 0.05$.

300

301 **3. Results**

302 **3.1. Performance during late pregnancy**

303 Figure 1 shows mean food intake over time during both late pregnancy
304 and early lactation. Treatment did not interact with time for DM intake during
305 late pregnancy ($P=0.33$), which averaged 3.02 and 2.77 kg/day for the
306 combined CON/RES ewes and PAR ewes, respectively (s.e.d. 0.13 kg/day;
307 $P=0.05$). Time and treatment did not interact for BW during late pregnancy
308 ($P=0.70$), reflected in an numerically greater BW gain for the combined
309 CON/RES ewes than for PAR ewes at 272 and 228 g/day, respectively (s.e.d.
310 31 g/day, $P=0.11$). CON/RES ewes tended to be heavier at parturition than
311 PAR ewes, averaging 70.1 and 68.0 kg, respectively (s.e.d. 1.19 kg; $P=0.08$),
312 though litter BW at birth did not differ, averaging at 9.07 and 9.30 kg,
313 respectively (s.e.d. 0.44 kg; $P=0.61$). Treatment did not interact with time for
314 CS ($P=0.47$) during late pregnancy, which gradually increased from 2.51 ± 0.06
315 on day₋₃₉ to 2.72 ± 0.06 on day₋₆ ($P=0.017$).

316

317 **3.2. Total tract apparent digestibility and performance during**
318 **early lactation**

319 Table 2 shows that the total tract DM, OM and CP digestibilities were
320 smaller in PAR ewes than in both CON and RES ewes ($P<0.05$). As for late
321 pregnancy, feed intake and time did not interact during early lactation (Figure
322 1; $P=0.33$). Table 2 shows that mean DM intake was greater for CON ewes
323 than for PAR ewes, which was in turn greater than for RES ewes. However,
324 PAR and RES ewes achieved similar levels of dOM and digestible CP intake,
325 which were both smaller than those for the CON ewes. Ewe BW loss was less
326 for CON ewes than for PAR ewes, which in turn was less than for RES ewes.
327 However, litter BW gain and calculated milk production were greater for CON
328 ewes than for PAR and for RES ewes, whilst both did not differ between PAR
329 and RES ewes (Table 2).

330 Treatment tended to interact with time for CS ($P=0.07$) during lactation;
331 CS averaged 2.46 ± 0.06 across treatments on day₆ and 2.08, 1.84 and 2.02
332 on day₂₉ for CON, PAR and RES, respectively (se 0.07), suggesting that CS
333 reduced for all ewes ($P<0.01$) but at a higher rate for PAR from ewes than for
334 CON and RES ewes during lactation.

335

336 **3.3. Parasitism**

337 Ewe FEC averaged 101 (86 - 119) epg on day₋₃₉ and 0 (0 - 1) epg at
338 day₋₂₂. Following infection from day₋₂₁ onwards, FEC of CON/RES and PAR
339 ewes averaged 1 (1-2) and 0 (0-1) epg on day₋₁₁, respectively, and 3 (2-4) and
340 26 (15-43) epg at parturition, respectively ($P<0.001$). The FEC of PAR ewes
341 then gradually increased to 71 (48 to 106) epg by day₂₈. Combined with

342 calculated fresh faeces production, the latter translated into 962 (675-1371) x
343 10³ worm eggs per day.

344

345 **3.4. GHG production and yield**

346 Figure 2 displays the average hourly CH₄ (Fig 2a) and CO₂ (Fig 2b)
347 production of sets of two CON, PAR and RES ewes. Experimental treatment
348 interacted with time for both GHG (P<0.001), arising from the larger diurnal
349 variation in the RES ewes. Table 3 shows enteric and manure GHG
350 production and yield per kg dry matter intake (DMI) as well as per kg
351 digestible organic matter intake (dOMI), expressed both as CH₄ and N₂O and
352 converted to CO₂ equivalents. We observed that CON ewes produced more
353 enteric CH₄ than PAR ewes, which in turn produced more enteric CH₄ than
354 RES ewes. However, whilst enteric CH₄ yield per kg DMI did not differ,
355 averaging 10.23 g per kg DMI, enteric CH₄ yield per kg dOMI was ~14%
356 greater for PAR ewes than for CON and RES ewes (P<0.05). We observed
357 similar patterns for CO₂ emissions, though CO₂ production did not differ
358 between CON and PAR ewes (P=0.15), CO₂ yield per kg DMI did not differ,
359 averaging 690 g, whilst CO₂ yield per kg dOMI for RES ewes was
360 intermediate to that of CON and PAR ewes, whose CO₂ yields differed
361 (P<0.05).

362 The pattern in manure GHG emissions differed from enteric GHG
363 emissions. CON and PAR ewes produced similar volumes of manure volatile
364 solids, and thus CH₄, per day though manure CH₄ yield was greater for PAR
365 ewes than for CON ewes, both per kg DMI (P<0.05) and per kg dOMI
366 (P<0.01). Likewise, CON and PAR ewes produced similar amount of manure

367 N, and thus N₂O, per day though N₂O yields were greater for PAR ewes than
368 for CON ewes, both per kg DMI (P=0.082) and per kg dOMI (P<0.05).

369

370 **3.5. GHG intensity and sensitivity analysis**

371 Table 4 shows the outcomes of the underlying calculations towards the
372 treatment effects on GHG intensity. PAR and RES ewes required on
373 averaged ~5 days longer than CON ewes to reach the target lamb weaning
374 BW. This was associated with the same total DMI for CON and PAR ewes,
375 but a significantly smaller DMI for RES ewes (P<0.01). Total BW loss was
376 greater for PAR ewes than for CON ewes (P<0.05), and in turn greater for
377 RES ewes than for PAR ewes (P<0.05). The estimated amount of DMI
378 needed to restore BW lost was smaller in CON ewes than in PAR and RES
379 ewes (P<0.01). However, the two pools of DMI combined were very similar for
380 CON and RES ewes but greater for PAR ewes (P<0.05). The latter was
381 reflected in a greater feed conversion ratio (P<0.05). Manure volatile solids
382 and N output were greater for PAR ewes than for CON and RES ewes.

383 Table 4 also shows that the resulting GHG intensity for each of the
384 underlying sources was greater for PAR ewes than for CON and RES ewes.
385 Combined with the GWP for lucerne production, our calculations show that
386 the GWPs for lamb production of CON, PAR and RES ewes were 5.09, 5.91
387 and 5.28 kg CO₂-eq per kg lamb weight gain, respectively, which was
388 calculated to correspond with 1.04, 1.24 or 1.10 kg CO₂-eq per kg milk,
389 respectively (s.e.d. 0.04 kg CO₂-eq per kg; P<0.05).

390 The sensitivity analysis indicated that GWP per kg lamb BWG was not
391 very sensitive to variation in urine N excretion; an increase in urine N from

392 0.46 to 0.60 × N absorbed during lactation increased GWP by less than 1.4%
393 from 5.91 to 5.99 CO₂-eq per kg lamb weight gain. However, GWP was more
394 sensitive to variation in ME requirement for ewe BW gain; GWP for PAR ewes
395 varied from 5.69 to 5.91 to 6.13 kg CO₂-eq/kg BWG for ME requirements per
396 kg BW gain taken as 39.75, 49.16 and 58.57 MJ/kg, respectively.
397 Nevertheless, each of these figures were significantly greater than the 5.09 kg
398 CO₂-eq/kg BWG for the CON ewes.

399

400 **4. Discussion**

401

402 Here we propose a framework that accounts for pathogen-induced
403 variation in GHG yield and reduction in feed efficiency in order to
404 experimentally test the hypothesis that periparturient parasitism increases
405 ewe GHG intensity for lamb production. To our knowledge, this is the first set
406 of data that empirically addresses the consequences of impaired animal
407 health on GHG intensity. The data obtained support the view that whilst ovine
408 periparturient gastrointestinal nematode parasitism reduced GHG production
409 per day, it paradoxically resulted in an increased GHG intensity for their lamb
410 BWG by ~16%. Given that the latter was of similar magnitude to the impact of
411 ewe parasitism on feed conversion ratio, the calculated increased GHG
412 intensity largely came from accounting for the impact on production losses
413 rather than on GHG yield per kg dry matter intake (DMI). As such, impact of
414 animal health on GHG intensity is driven by a combination of a reduction in
415 feed intake and feed nutritive value, the latter illustrated by ewe parasitism
416 increasing GHG yield per kg digestible organic matter intake (dOMI).

417 Periparturient parasitism evoked anorexia in our ewes, especially
418 during lactation, but also reduced milk production and accelerated ewe BW
419 loss, which is in support of earlier observations (Leyva et al., 1982; Zaralis et
420 al., 2009). Although the degree of anorexia was relatively small at 9%, its
421 effect on litter weight gain (milk production) and ewe BW loss would have
422 been exacerbated by the negative impact of parasite challenge on total tract
423 nutrient digestibility, which agrees with other studies (Parkins et al., 1973;
424 Sykes and Coop, 1977). The impact on OM digestibility, which can be seen as
425 a proxy for energy digestibility, resulted in a reduction in dOMI of ~26% for
426 PAR ewes relative to CON ewes. The PAR ewes minimised the impact of the
427 latter on ME availability by mobilising more body fat, as the rate and extent of
428 body condition score loss tended to be greater than in CON ewes.
429 Consequently, it was assumed that the ME requirements per kg BW gain for
430 replenishment of body reserves for PAR ewes upon weaning was also greater
431 than that for CON ewes, due to the higher energy contents of fat (AFRC,
432 1993). The sensitivity analysis for the arbitrarily taken ME requirement for BW
433 gain of 49.16 MJ/kg as the average of 39.75 MJ/kg for average body
434 composition (AFRC (1993), and 58.57 MJ/kg for fat deposition only (Olthoff et
435 al., 1989), indicated that the impact of ovine parasitism on calculated GHG
436 intensity for lamb weight gain would reduce to 12% if standard assumptions
437 are taken but remains significant, with the largest contribution coming from
438 N₂O. Calculated GHG intensity for lamb weight gain was not very sensitive to
439 urinary N excretion; increasing the latter by up to 40%, i.e. the effect of
440 parasitism on N balance in growing lambs (Parkins et al., 1973; Sykes and
441 Coop, 1977), increased GHG intensity for lamb weight gain by less than 2%.

442 This is reflective of the relatively small proportion of nitrogen intake that is
443 excreted with the urine in lactating ewes, where a significant proportion of
444 dietary nitrogen is excreted with the milk.

445 Compared to our previous studies where similar infection protocols
446 were used in periparturient ewes (Houdijk et al., 2003, Houdijk et al., 2006;
447 Kidane et al., 2010), FEC of the PAR ewes were relatively low with levels
448 below 100 epg. In addition, achieved DMI was relatively high, typically ~60%
449 greater than that of ewes that were fed at 90% of assumed ME requirements
450 (Kidane et al., 2010). The low FEC observed may have been the outcome of
451 concurring high intake levels of metabolizable protein (MP), which is known to
452 reduce ewe FEC through improved host resistance to nematodes (Houdijk et
453 al., 2012). Since *in vitro* OM digestibility (Table 1) was very similar to *in vivo*
454 OM digestibility (Table 2) for CON ewes, we can assume that the reduction in
455 total tract OM and CP digestibility (Table 2) for PAR ewes proportionally
456 reduced book values of fermentable ME and by-pass protein content
457 (Hazzledine, 2014). Consequently, consumed lucerne pellets may have
458 yielded ~70 g MP per kg DMI for PAR ewes, compared to ~80 g MP per kg
459 DMI in CON and RES ewes. This suggests that MP intake for PAR ewes was
460 likely around 330 g per day during lactation. Whilst a similar level of calculated
461 MP intake significantly reduced worm egg output in ewes fed rations based on
462 xylose-treated soya bean meal compared to a low MP control, the same level
463 of calculated MP intake failed to reduce worm egg output in ewes fed rations
464 based on faba beans (Sakkas et al., 2012). Furthermore, the high level of
465 feed intake achieved in our ewes would have resulted in a large weight of
466 faeces, which all else being equal would reduce the FEC due to its expression

467 as number of eggs per gram and thus its sensitivity to faecal dilution (Houdijk,
468 2008). Indeed, the calculated total worm egg output of approximately one
469 million eggs per day around day₂₈ of lactation was in agreement with that
470 observed when MP supply was scarce, even though FEC were about four
471 times greater than in our PAR ewes (Kidane et al., 2010). Taken together, the
472 above would support the view that immunity to parasites was at least to some
473 extent compromised in our PAR ewes, in contrast to what the low FEC we
474 reported here would intuitively indicate.

475 Ruminants lose 2 to 12% of their gross energy intake in the form of
476 CH₄ (Johnson and Johnson, 1995), which accounts for a significant proportion
477 of GHG emissions from livestock production systems. In our data, observed
478 CH₄ yield averaged 10.23 g/kg DMI, equivalent to 3.3% of gross energy,
479 which is relatively low compared to CH₄ energy losses on lucerne reported
480 elsewhere, e.g. 4.7% on lucerne hay fed *ad libitum* (Pinares-Patinõ et al.,
481 2003), 5.1% on pelleted lucerne fed restrictedly (Pinares-Patinõ et al., 2013;
482 Waghorn et al., 2002), 5.9% on silage fed *ad libitum* (Bouchard et al., 2013),
483 or 6.6% on freshly-cut and fed *ad libitum* (Waghorn et al., 2002). Furthermore,
484 it was also lower than the 6.3% loss of CH₄ energy and out of the range of
485 3.7% to 13.3% reported from 61 studies in housed and grazing sheep fed a
486 large variety of forage-based rations (McBride et al., 2013). There may be
487 several reasons why CH₄ yield observed in our study was low. The high level
488 of feed intake achieved in our ewes was likely a major contributor, since it is
489 well established that CH₄ yield decreases with increased feed intake (Johnson
490 and Johnson, 1995; Jentsch et al., 2007), which reduces rumen retention time
491 (AFRC, 1993), and thus reduces CH₄ emissions (Goopy et al., 2014). Intake

492 was likely high since lucerne was offered as dried pellets rather than as long
493 forage. In agreement with this observation, it has been reported that CH₄ yield
494 on grass nuts was ~35% less than on fresh grass or grass silage in lowland
495 replacement ewes (Aubry et al., 2014). In addition, subject to variety,
496 environment and harvest date (Pecetti et al., 2006), lucerne is known to
497 contain variable amounts of saponins, which may not only mitigate
498 methanogenesis (Jayanegara et al., 2010; Cieslak et al., 2013), but may also
499 be anthelmintic (Ali et al., 2011), and it can therefore not be excluded that this
500 may also have contributed to the relatively low FEC observed.

501 In our experiment, observed CO₂ yield averaged 690 g per kg DMI,
502 which agrees with the earlier respiration and carbon balance measurements
503 using sheep, fed restricted amounts of dried grass (Blaxter and Graham,
504 1955; Midwood et al., 1994). Tables 3 and 4 show that breath CO₂ could be
505 considered proportionally the largest component of GHG production, yields
506 and intensities measured. However, since breath CO₂ is an inevitable
507 consequence of metabolism, CO₂ contributions to GWP calculations are those
508 arising from fossil fuel use only (Cederberg et al., 2013). However, even if we
509 include breath CO₂ in our GWP calculations, the relative effect of parasitism
510 on GHG intensity remains 16%. Within the animal derived GHG contributions,
511 enteric CH₄, manure N₂O and manure CH₄ comprise 67, 30 and 3%,
512 respectively for CON and RES ewes and 64, 32 and 4% respectively for PAR
513 ewes in the final calculated GWP per kg lamb BWG figures. However, the
514 relative contribution of these GHG sources to the increase in calculated GHG
515 intensity averaged at 47, 43 and 7%, respectively. Whilst this indicates that
516 enteric CH₄ remains the largest contributor of GWP per kg lamb BWG, enteric

517 CH₄ and manure N₂O contributed almost equally to the parasitism-increased
518 GHG intensity.

519 We identified that the impact of parasitism on observed and calculated
520 yields per kg dOMI was more pronounced than per kg DMI (Table 3).
521 Although this was the direct consequence of impact of parasitism on total tract
522 OM digestibility (Table 2), it raises the question what would be the most
523 appropriate unit to express GHG yield. It might be argued that most studies
524 resort to yield per unit DMI since data on total tract OM digestibility are not
525 generally available. Since it has been suggested that animals aim to optimise
526 ME intake through their feeding behaviour (Tolkamp, 2010), and ME is directly
527 proportional to the digestible organic matter component of the ration, dOMI is
528 an excellent proxy in the absence of respiration data. Furthermore, variation in
529 GHG production is better explained per unit dOMI rather than DMI, as the
530 former better accounts for differences in diet quality (Muetzel, 2009). In this
531 respect, it might be argued that the 23% relative impact of parasitism on GHG
532 intensity on the basis of yield per kg dOMI is not dissimilar to the 16%
533 increase on the basis of GHG intensity per kg lamb BWG. Hence, when OM
534 digestibility data are available, expression of GHG yield per unit dOMI may be
535 preferred over GHG yield per unit DMI, as it would also be more reflective of
536 the most preferred expression, i.e. GHG per unit product (Hristov et al., 2013).

537 The functional unit in our study was one kg of lamb BW gain between
538 birth and weaning, and we used this to estimate the level of milk production
539 (Robinson et al., 1969). Although the aim of our work was not to develop a full
540 life cycle assessment for ewe milk production, the averaged calculated GHG
541 intensity of 1.13±0.02 kg CO₂-eq per kg milk fitted in the wide range of

542 intensities reported for sheep milk production from earlier life cycle
543 assessments, i.e. from 0.9-1.7 kg CO₂-eq per kg milk (Haas et al., 2001) but
544 was lower than more recent estimates, i.e. 1.8-4.5 kg CO₂-eq per kg milk
545 (Batalla et al., 2004) and 8.4 CO₂-eq per kg milk (Opio et al., 2013). This likely
546 reflects the development in life cycle assessment methodologies over the last
547 few years, and an increased understanding of GHG sources that require to be
548 taken into account (Cederberg et al., 2013). In the context of controlling
549 parasitism, this would need to include GHG emissions arising from the
550 intervention.

551 Using the reported impacts of parasitism on FCR and assuming no
552 impact on GHG yields, it can be calculated that lamb parasitism may increase
553 GHG intensities to greater magnitudes than observed here in ewes, by up to
554 21% under housed conditions (Kyriazakis et al., 1994; Zaralis et al., 2008)
555 and up to 139% under field conditions (Thamsborg and Agergaard, 2002).
556 These figures could increase significantly if lamb parasitism does increase
557 GHG yield, which has yet to be established. Thus, improvement of animal
558 health has great potential to contribute to climate change mitigation strategies
559 (Shields and Orme-Evans, 2015). Indeed, a 10% reduction in calculated GHG
560 intensity has been estimated from suppressive anthelmintic treatments in
561 continuously naturally infected, growing lambs (Keynon et al., 2013), though
562 in agreement with our observations, reduced exposure can be expected to
563 result in an even greater performance benefit (Coop et al., 1982) and thus
564 reduction in GHG intensity. However, studies that have examined implications
565 of improving animal health on GHG emission intensity are scarce, despite that
566 improving feed efficiency has been recognised as a major driver to reduce

567 GHG emissions (Basarab et al., 2013), and connections among animal health
568 and resource efficiency are obvious (Hristov et al., 2013). Perhaps as a
569 consequence, improving animal health as a climate change mitigation option
570 is not often considered amongst other more technical options, including
571 reducing undernourishment (Eckard et al., 2010; Gerber et al., 2013). The
572 framework developed here and the results for the specific hypothesis tested,
573 may provide a tool and impetus for further studies in this field, including
574 assessing different nutritional and challenge environments. Furthermore, they
575 may assist to ensure that improvement of animal health is an increasingly
576 recognised and integrated component of efforts to reduce the environmental
577 footprint of animal production systems.

578

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588

589 **References**

590 AFRC. 1993. Energy and protein requirements of ruminants. An Advisory
591 Manual Prepared by the AFRC Technical Committee on Responses to
592 Nutrients, CAB International, Wallingford.

593 Akinbamijo, O.O., Lahlou-Kassi, I.A., Tembely, S., 1994. Fascioliasis and
594 nutrient metabolism in pregnant and non-pregnant sheep. In: Lebbie
595 S.H.B., Kagwini E. (Eds.), Small Ruminant Research and Development
596 in Africa. Proceedings of the 3rd Biennial Conference of the African Small
597 Ruminant Research Network. ILRI, Nairobi.
598 (<http://www.fao.org/wairdocs/ilri/x5473b/x5473b15.htm>)

599 Ali, N., Shah, S.W.A., Shah, I., Ahmed, G., Ghias, M., Khan, I. 2011. Cytotoxic
600 and anthelmintic potential of crude saponins isolated from *Achillea*
601 *Wilhelmsii* C. Koch and *Teucrium Stocksianum* boiss. BMC
602 Complement. Altern. Med. 11, 106.

603 Aubry, A., Annett, R., Yan, T., 2014. Effects of breed and forage types on
604 methane emission factors for lowland replacement ewes aged between
605 8 and 19 months. Adv. Anim. Biosci. 5, 25.

606 Basarab, J.A., Beauchemin, K.A., Baron, V.S., Ominski, K.H., Guan, L.L.,
607 Miller, S.P., Crowley, J.J., 2013. Reducing GHG emissions through
608 genetic improvement for feed efficiency: effects on economically
609 important traits and enteric methane production. Animal 7 (S2), 303–
610 315.

611 Batalla, I., Pinto, M., Unamunzaga, O., Besga G., del Hierro, Ó., 2004.
612 Integrating social and economic criteria in the carbon footprint analysis in
613 sheep dairy farms. In: Schenck, R., Huizenga, D. (Eds.), Proceedings of

614 the 9th International Conference on Life Cycle Assessment in the Agri-
615 Food Sector. ACLCA, Vashon, pp. 88–95.

616 Blaxter, K. L., Graham, N.McC., 1955. Plane of nutrition and starch
617 equivalents. J. Agric. Sci. 46, 292–306.

618 Bouchard, K., Wittenberg, K.M., Legesse, G., Krause, D.O., Khafipour, E.,
619 Buckley, K.E., Ominski, K.H., 2013. Comparison of feed intake, body
620 weight gain, enteric methane emission and relative abundance of rumen
621 microbes in steers fed sainfoin and lucerne silages under western
622 Canadian conditions. Grass Forage Sci. 70, 116–129.

623 Cederberg, C., Henriksson, M., Berglund, M., An LCA researcher's wish list –
624 data and emission models needed to improve LCA studies of animal
625 production. Anim. 7 (S2), 212–219.

626 Christie, M., Jackson, F., 1982. Specific identification of strongyle eggs in
627 small samples of sheep faeces. Res. Vet. Sci. 32, 113–117.

628 Cieslak, A., Szumacher-Strabel, M., Stochmal, A., Oleszek, W., 2013. Plant
629 components with specific activities against rumen methanogens. Anim. 7
630 (S2), 253–265.

631 Coop, R.L., Kyriazakis, I., 1999. Nutrition-parasite interaction. Vet. Parasitol.
632 84, 187–204.

633 Coop, R.L., Sykes, A.R., Angus, K.W., 1982. The effect of three levels of
634 intake of *Ostertagia circumcincta* larvae on growth rate, food intake and
635 body composition of growing lambs. J. Agricul. Sci. 98, 247–255.

636 Eckard, R.J., Grainger, C., de Klein, C.A.M., 2010. Options for the abatement
637 of methane and nitrous oxide from ruminant production: A review. Livest.
638 Sci. 130, 47–56.

639 Gallego, A., Hospido, A., Moreira, M.T., Feijoo, G., 2011. Environmental
640 assessment of dehydrated alfalfa production in Spain. *Resour. Conserv.*
641 *Recy.* 55, 1005–1012.

642 Gerber, P.J., Hristov, A.N., Henderson, B., Makkar, H., Oh, J., Lee, C.,
643 Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz, A., Dell, C., Adesogan,
644 A.T., Yang, W.Z., Tricarico, J.M., Kebreab, E., Waghorn, G., Dijkstra, J.,
645 Oosting, S. 2013. Technical options for the mitigation of direct methane
646 and nitrous oxide emissions from livestock: a review. *Anim.* 7 (S2), 220-
647 234.

648 Goopy, J.P., Donaldson, A., Hegarty, R.S., Vercoe, P.E., Haynes, F., Barnett,
649 M., Oddy, V.H., 2014. Low-methane yield sheep have smaller rumens
650 and shorter rumen retention time. *Br. J. Nutr.* 111, 578-585.

651 Gorniak, T., Meyer, U., Südekum, K.-H., Dänicke, S., 2014. Effect of ambient
652 temperature on nutrient digestibility and nitrogen balance in sheep fed
653 brown-midrib maize silage. *Arch. Anim. Nutr.* 68, 336–344.

654 Haas, G., Wetterich, F., Köpke, U., 2001. Comparing intensive, extensified
655 and organic grassland farming in southern Germany by process life
656 cycle assessment. *Agricul. Ecosys. Environ.* 83, 43–53.

657 Hazzledine, M., 2014. Premier Atlas Ingredients Matrix. Premier Nutrition
658 Products LTD, Staffs, UK.

659 Houdijk, J.G.M., Kyriazakis, I., Jackson, F., Huntley, J.F. and Coop, R.L.,
660 2003. Is the allocation of metabolisable protein prioritised to milk
661 production rather than to immune functions in *Teladorsagia circumcincta*
662 infected lactating ewes? *Int. J. Parasitol.* 33, 327–338.

663 Houdijk, J.G.M., Jackson, F., Coop, R.L., Kyriazakis, I., 2006. Rapid
664 improvement of immunity to *Teladorsagia circumcincta* is achieved
665 through a reduction in the demand for protein in lactating ewes. *Int. J.*
666 *Parasitol.* 36, 219–227.

667 Houdijk, J.G.M., Kyriazakis, I., Kidane, A., Athanasiadou, S., 2012.
668 Manipulating small ruminant parasite epidemiology through the
669 combination of nutritional strategies. *Vet. Parasitol.* 186, 38–50.

670 Houdijk, J.G.M., 2008. Influence of periparturient nutritional demand on
671 resistance to parasites in livestock. *Par. Immunol.* 30, 113–121.

672 Hristov, A.N., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz,
673 A., Dell, C., Adesogan, A., Yang, W., Tricarico, J., Kebreab, E.,
674 Waghorn, G., Dijkstra, J., Oosting, S., 2013. Mitigation of greenhouse
675 gas emissions in livestock production – A review of technical options for
676 non-CO₂ emissions. In: Gerber, P.J., Henderson B., Makkar, H.P.S.,
677 (Eds.), *FAO Animal Production and Health Paper No. 177*. FAO, Rome.

678 IPCC, 2006. *Guidelines for National Greenhouse Gas Inventories*.
679 Intergovernmental panel on Climate Change.

680 Jayanegara, A., Goel, G., Makkar, H.P.S., Becker, K., 2010. Reduction in
681 methane emissions from ruminants by plant secondary metabolites:
682 effects of polyphenols and saponins. In: Odongo, N.E., Garcia, M.,
683 Viljoen, G.J., (Eds.), *Sustainable Improvement of Animal Production and*
684 *Health*. FAO, Rome, pp. 151–157.

685 Jentsch W., Schweigel M., Weissbach F., Scholze H., Pitroff W., Derno M.,
686 2007. Methane production in cattle calculated by the nutrient
687 composition of the diet. *Arch. Anim. Nutr.* 61, 10–19.

688 Johnson, K.A., Johnson, D.E., 1995. Methane emissions from cattle. *J. Anim.*
689 *Sci.* 73, 2483–2492.

690 Kenyon, F., Dick, J.M., Smith, R.I., Coulter, D.G., McBean, D., Skuce, P.J.,
691 2013. Reduction in greenhouse gas emissions associated with worm
692 control in lambs. *Agric.* 3, 271–284.

693 Kidane, A., Houdijk, J.G.M., Athanasiadou, S., Tolkamp, B.J., Kyriazakis, I.,
694 2010. Nutritional sensitivity of periparturient resistance to nematode
695 parasites in two breeds of sheep with different nutrient demands. *Br. J.*
696 *Nutr.* 104, 1477–1486.

697 Kidane, A., Houdijk, J.G.M., Tolkamp, B.J., Athanasiadou, S., Kyriazakis, I.,
698 2009. Consequences of infection pressure and protein nutrition on
699 periparturient resistance to *Teladorsagia circumcincta* and performance
700 in ewes. *Vet. Parasitol.* 165, 78–87.

701 Kimambo, A.E., Macrae, J., C., Dewey, P.J.S., 1988. The effect of daily
702 challenge with *Trichostrongylus colubriformis* larvae on the nutrition and
703 performance of immunologically-resistant sheep. *Vet. Parasitol.* 28,
704 205–212.

705 Kyriazakis, I., Oldham, J.D., Coop, R.L., Jackson, F., 1994. The effect of
706 subclinical intestinal nematode infection on the diet selection of growing
707 sheep. *Br. J. Nutr.* 72, 665–677.

708 Kyriazakis, I., Tolkamp, B.J., Hutchings, M.R., 1998. Towards a functional
709 explanation for the occurrence of anorexia during parasitic infections.
710 *Anim. Behav.* 56, 265–274.

- 711 Leyva, V., Henderson, A.E., Sykes, A.R., 1982. Effect of daily infection with
712 *Ostertagia circumcincta* larvae on food intake, milk production and wool
713 growth in sheep. J. Agric. Sci. 99, 249–259.
- 714 Lima, R., Díaz, R.F., Castro, A., Fievez, V., 2011. Digestibility, methane
715 production and nitrogen balance in sheep fed ensiled or fresh mixtures
716 of sorghum–soybean forage. Livest. Sci. 141, 36–46.
- 717 Lynch, G.P., Elsasser, T.H., Rumsey, T.S., Jackson Jr, C. and Douglass, L.W.
718 1988. Nitrogen metabolism by lactating ewes and their lambs. J. Anim.
719 Sci. 66, 3285–3294.
- 720 Maâmouri, O., Atti, N., Mahouachi, M. and Kraeim, K., 2011. Effect of grass
721 nitrogen protection by *Acacia cyanophylla* on nitrogen balance and
722 sheep milk production. In: Bouche, R., Derkimba, A., Casabianca, F.
723 (Eds.), New trends for innovation in the Mediterranean animal
724 production. Wageningen Academic Publishers, Wageningen, pp. 153–
725 156.
- 726 Malik, B., Nicol A.M., van Houtert, M., 1999. Source of excess nitrogen affects
727 nutrient partitioning in lactating ewes. Proc. New Zeal. Soc. Anim. Prod.
728 59, 158–161.
- 729 McBride, J., Morrison, S.J., Yan, T., 2013. Review of enteric methane
730 emissions of cattle and sheep fed diets relevant to UK farming
731 conditions. Adv. Anim. Biosci. 4 (2), 299.
- 732 Midwood, A.J., Haggarty, P., McGaw, B.A., Mollison, G.S., Milne, E., Duncan,
733 G.J., 1994. Validation in sheep of the doubly labelled water method for
734 estimating CO₂ production. Am. J. Physiol. 266, R169–R179.

- 735 Ministry of Agriculture Fisheries and Food, 1992. Analysis of Agricultural
736 Materials, 2nd Ed. Her Majesty's Stationary Office, London.
- 737 Muetzel, S., 2009. Effect of level of intake on methane production per kg of
738 dry matter intake. MAF Technical Paper No: 2011/95.
- 739 Olthoff, J.C., Dickerson, G.E., Nienaber, J.A., 1989. Energy utilization in
740 mature ewes from seven breeds with diverse production potentials. J.
741 Anim. Sci. 67, 2550-2564.
- 742 Opio, C., Gerber, P., Mottet, A., Falcucci, A., Tempio, G., MacLeod, M.,
743 Vellinga, T., Henderson, B., Steinfeld, H., 2013. Greenhouse gas
744 emissions from ruminant supply chains. A global life cycle assessment.
745 FAO, Rome.
- 746 Pappas, A., 1977. Protein requirements of lactating Chios ewes. J. Anim. Sci.
747 44, 672–679.
- 748 Parkins, J.J., Holmes, P.H., Bremner, K.C., 1973. The pathophysiology of
749 ovine ostertagiasis: some nitrogen balance and digestibility studies. Res.
750 Vet. Sci. 14, 21–28.
- 751 Pecetti, L., Tava, A., Romani, M., De Benedetto, M.G., Corsi, P., 2006.
752 Variety and environment effects on the dynamics of saponins in lucerne
753 (*Medicago sativa* L.). Eur. J. Agron. 25, 187–192.
- 754 Pinares-Patinõ, C.S., Hickey, S.M., Young, E.A., Dodds, K.G., MacLean, S.,
755 Molano, G., Sandoval, E., Kjestrup, H., Harland, R., Hunt, C., Pickering
756 N.K., McEwan J.C. 2013. Heritability estimates of methane emissions
757 from sheep. Anim. 7(s2), 316–321.
- 758 Pinares-Patinõ, C.S., Ulyatt, M.J., Waghorn, G.C., Lassey, K.R., Barry, T.N.,
759 Holmes, C.W., Johnson, D.E. 2003. Methane emission by alpaca and

760 sheep fed on lucerne hay or grazed on pastures of perennial
761 ryegrass/white clover or birdsfoot trefoil. J. Agric. Sci. 140, 215–226.

762 Robinson, J.J., Foster, W.H., Forbes, T.J., 1969. The estimation of the milk
763 yield of a ewe from body weight data on the suckling lamb. J. Agric. Sci.
764 Camb. 72, 103–107.

765 Rooke, J.A., Wallace, R.J., Duthie, C.-A., McKain, N., Motta de Souza, S.,
766 Hyslop, J.J., Ross, D.W., Waterhouse, T., Roehe, R., 2014. Hydrogen
767 and methane emissions from beef cattle and their rumen microbial
768 community vary with diet, time after feeding and genotype. Br. J. Nutr.
769 112, 398–407.

770 Russel, A.J.F., Doney, J.M., Gunn, R.G., 1969. Subjective assessment of
771 body fat in live sheep. J. Agric. Sci. Camb. 72, 451–454.

772 Sakkas, P., Houdijk, J.G.M., Athanasiadou, S., Kyriazakis, I., 2012. Sensitivity
773 of periparturient breakdown of immunity to parasites to dietary protein
774 source. J. Anim. Sci. 90, 3954–3962.

775 Shields, S., Orme-Evans, G., 2015. The impacts of climate change mitigation
776 strategies on animal welfare. Anim. 5, 361–394.

777 Sykes, A.R., Coop, R.L., 1977. Intake and utilization of food by growing sheep
778 with abomasal damage caused by daily dosing with *Ostertagia*
779 *circumcincta* larvae. J. Agric. Sci., Camb. 88, 671–677.

780 Sykes, A.R., 1994. Parasitism and production in farm animals. Anim. Prod.
781 59, 155–172.

782 Thamsborg, S.M., Agergaard, N., 2002. Anorexia and food utilization in
783 nematode infected lambs on pasture. Anim. Sci. 75, 303–313.

784 Thomas, P.C., Roberston, S., Chamberlain, D.G., Livingstone, R.M.,
785 Garthwaite, P.H., Dewey, P.J.S., Smart, R., Whyte, C., 1988. Predicting
786 the metabolizable energy content of compounded feeds for ruminants.
787 In: Haresign, W., Cole, D.J.A., (Eds.), Recent Advances in Animal
788 Nutrition. Butterworths, London, pp. 127–146.

789 Tolkamp, B.J., 2010. Efficiency of energy utilisation and voluntary feed intake
790 in ruminants. *Anim.* 4, 1084–1092.

791 van Keulen, J., Young, B.A., 1977. Evaluation of the acid-insoluble ash as a
792 natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44, 282–
793 287.

794 Waghorn, G.C., Tavendale, M.H., Woodfield, D.R., 2002. Methanogenesis
795 from forages fed to sheep. *Proc. New Zeal. Grassl. Assoc.* 64, 167–171.

796 Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M, Wylie, A.R.G., Kyriazakis, I. 2008.
797 Food intake and plasma leptin concentrations during gastrointestinal
798 parasitism in lambs of two breeds. *J. Anim. Sci.* 86, 1891–1903.

799 Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M, Wylie, A.R.G., Kyriazakis, I., 2009.
800 Consequences of protein supplementation on anorexia, expression of
801 immunity and plasma leptin concentrations in parasitised ewes of two
802 breeds. *Br. J. Nutr.* 101, 499–509.

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805 **Legends to figures**

806

807 Figure 1. Dry matter intake of twin-rearing ewes fed pelleted lucerne,
808 either sham-infected and fed *ad libitum* (○), or trickle-infected
809 with *Teladorsagia circumcincta* and fed *ad libitum* (●), or sham-
810 infected and fed restrictedly during lactation at 80% of intake by
811 ewes fed *ad libitum* (◇).

812 Figure 2. Hourly methane and carbon dioxide production of pairs of
813 pelleted lucerne fed twin-rearing ewes at 5 wks into lactation,
814 either sham-infected and fed *ad libitum* (○), or trickle-infected
815 with *Teladorsagia circumcincta* and fed *ad libitum* (●), or sham-
816 infected and fed restrictedly during lactation at 80% of intake by
817 ewes fed *ad libitum* (◇). The arrow indicates time of feeding.

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819

820 Table 1. Analysed composition of lucerne.

821

Analysis	
Dry matter (g/kg)	974
Neutral detergent fibre (g/kg DM)	448
Acid detergent fibre (g/kg DM)	362
Crude protein (6.25×N, g/kg DM)	163
Ash (g/kg DM)	103
Acid hydrolysis ether extract (AH-EE, g/kg DM)	19.4
Acid insoluble ash (g/kg DM)	13.7
<i>In vitro</i> organic matter digestibility (NCGD ¹ , %)	57.2
Gross energy (MJ/kg DM)	17.9
Digestible energy ² (MJ/kg DM)	10.2
Metabolizable energy ³ (MJ/kg DM)	8.3

822

823 ¹Neutral cellulose and gammanase digestibility

824 ²Calculated as ME/0.81 (AFRC, 1993)

825 ³Calculated from AH-EE and NCGD (Thomas et al., 1988).

826

827 Table 2. Apparent feed digestibility and ewe performance during lactation of
 828 twin-rearing ewes fed pelleted lucerne, either sham-infected and fed *ad*
 829 *libitum* (CON), or trickle-infected with *Teladorsagia circumcincta* and fed *ad*
 830 *libitum* (PAR), or sham-infected and fed restrictedly during lactation at 80% of
 831 intake by ewes fed *ad libitum* (RES).

832

	Treatments ¹			s.e.d.
	CON	PAR	RES	
Apparent digestibility				
Dry matter (DM, %)	53.8 ^a	45.6 ^b	55.3 ^a	2.98
Organic matter (OM, %)	54.6 ^a	46.4 ^b	55.8 ^a	2.99
Crude protein (CP, %)	58.8 ^a	53.4 ^b	61.9 ^a	2.87
Performance				
DM intake (kg/day)	4.58 ^a	4.29 ^b	3.55 ^c	0.10
Digestible OM intake (kg/day)	2.24 ^a	1.78 ^b	1.77 ^b	0.13
Digestible CP intake (kg/day)	0.44 ^a	0.37 ^b	0.36 ^b	0.02
Ewe body weight gain (g/day)	-69 ^a	-162 ^b	-252 ^c	37
Litter body weight gain (g/day)	718 ^a	669 ^b	657 ^b	24
Estimated milk production (g/day)	3524 ^a	3223 ^b	3142 ^b	149

833

834 ¹Data within the same row with different superscripts differ (P<0.05).

835 Table 3. Observed and calculated greenhouse gas production and yield
 836 during lactation of twin-rearing ewes fed pelleted lucerne, either sham-
 837 infected and fed *ad libitum* (CON), or trickle-infected with *Teladorsagia*
 838 *circumcincta* and fed *ad libitum* (PAR), or sham-infected and fed restrictedly
 839 during lactation at 80% of intake by ewes fed *ad libitum* (RES).

	Treatments ¹			s.e.d.
	CON	PAR	RES	
Enteric methane (observed)				
CH ₄ production (g/day/ewe)	55 ^a	49 ^b	41 ^c	2.8
(CO ₂ -eq)	1375 ^a	1225 ^b	1025 ^c	70
CH ₄ yield (g/kg DMI)	10.6	10.3	10.3	0.56
(CO ₂ -eq)	265	258	258	14
CH ₄ yield (g/kg dOMI)	22.0 ^a	25.2 ^b	20.9 ^a	1.54
(CO ₂ -eq)	550 ^a	630 ^b	523 ^a	39
Manure methane (calculated)				
Volatile solids production (kg/day)	2.24 ^a	2.44 ^a	1.69 ^b	0.12
CH ₄ production (g/day/ewe)	2.86 ^a	3.10 ^a	2.15 ^b	0.14
(CO ₂ -eq)	72 ^a	78 ^a	54 ^b	4
CH ₄ yield (g/kg DMI)	0.56 ^a	0.65 ^b	0.54 ^a	0.03
(CO ₂ -eq)	14 ^a	16 ^b	14 ^a	1
CH ₄ yield (g/kg dOMI)	1.22 ^a	1.79 ^b	1.15 ^a	0.24
(CO ₂ -eq)	30 ^a	45 ^b	29 ^a	6
Manure nitrous oxide (calculated)				
N excretion (g/day)	91.6 ^a	89.6 ^a	68.7 ^b	2.77
N ₂ O production (g/day/ewe)	2.05 ^a	2.01 ^a	1.54 ^b	0.06
(CO ₂ -eq)	611	599	459	18
N ₂ O yield (g/kg DMI)	0.40 ^{ab}	0.42 ^a	0.39 ^b	0.01
(CO ₂ -eq)	119 ^{ab}	125 ^a	116 ^b	3
N ₂ O yield (g/kg dOMI)	0.85 ^a	1.12 ^b	0.81 ^a	0.12
(CO ₂ -eq)	253 ^a	334 ^b	241 ^a	36
Methane and nitrous oxide combined as CO ₂ -eq (calculated)				
GHG production (g/day/ewe)	2044 ^{a,x}	1909 ^{a,y}	1524 ^b	72
GHG yield (g/kg DMI)	399	397	386	14
GHG yield (g/kg dOMI)	844 ^a	1039 ^b	798 ^a	83
Breath carbon dioxide (observed)				
CO ₂ production (g/day/ewe)	3463 ^a	3241 ^a	2844 ^b	147
CO ₂ yield (g/kg DMI)	676	674	721	26
CO ₂ yield (g/kg dOMI)	1413 ^a	1664 ^b	1470 ^{ab}	111

840 ¹Data with different superscripts differ (^{a,b,c}P<0.05; ^{x,y}P<0.10).

841 Table 4. Effects of maternal parasitism and restricted feeding on time and dry
 842 matter intake (DMI) required to wean two lambs at 25 kg each and
 843 compensate for loss of body weight (BW), and its effect on calculated ewe
 844 global warming potential (GWP) of twin-rearing ewes fed pelleted lucerne,
 845 either sham-infected and fed *ad libitum* (CON), or trickle-infected with
 846 *Teladorsagia circumcincta* and fed *ad libitum* (PAR), or sham-infected and fed
 847 restrictedly during lactation at 80% of intake by ewes fed *ad libitum* (RES).
 848

		Treatments ¹			
		CON	PAR	RES	s.e.d.
Calculated performance and manure production parameters					
Days to target (n)		57.1 ^a	61.8 ^b	62.7 ^b	2.39
Total BW lost (kg)		3.4 ^a	10.0 ^b	14.4 ^c	1.85
DMI to target (kg)		253 ^a	257 ^a	214 ^b	9.5
DMI to compensate BW loss (kg)		16 ^a	60 ^b	68 ^b	9.5
Total DMI (kg)		270 ^a	316 ^b	283 ^a	11.6
Feed conversion ratio ²		6.7 ^a	7.8 ^b	7.0 ^a	0.31
Manure volatile solids (kg)		115 ^a	151 ^b	118 ^a	10.2
Manure N output (kg)		4.75 ^a	6.11 ^b	5.10 ^a	0.28
Calculated GWP (kg CO ₂ -eq/kg lamb body weight gain)					
Enteric	CH ₄	1.79 ^a	2.00 ^b	1.80 ^a	0.10
Manure	N ₂ O	0.80 ^a	1.00 ^b	0.87 ^a	0.05
	CH ₄	0.09 ^a	0.12 ^b	0.10 ^a	0.01
Feed	CO ₂ -eq	2.41 ^a	2.79 ^b	2.51 ^a	0.11
Total GWP		5.09 ^a	5.91 ^b	5.28 ^a	0.21
Calculated breath CO ₂ (g/kg lamb body weight gain)					
		4.52 ^a	5.21 ^b	4.96 ^b	0.19

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¹Data within the same row with different superscripts differ (^{a,b,c}P<0.05).

²Feed conversion ratio is calculated as total DMI divided over total lamb BW gain.