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Short- and long-term effects of conventional and artificial rearing strategies on the health and performance of growing lambs

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1 **Short- and long-term effects of different rearing strategies on the health and**
2 **performance of growing lambs**

3
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13

14 **Short title:** Effects of artificial rearing of lambs

15 **Abstract**

16 Artificial rearing of young animals represents a challenge in modern ruminant production
17 systems. This work aims to evaluate the short- and long-term effects of the type of
18 rearing on the animal's health, growth, feed utilization and carcass performance.
19 Twenty-four pregnant ewes carrying triplets were used. Within each triplet set, lambs
20 were randomly allocated to one experimental treatment: natural rearing on the ewe
21 (NN); ewe colostrum for 24h followed by artificial rearing with milk replacer (NA); and
22 50g of colostrum alternative supplementation followed by artificial rearing (AA). Milk
23 replacer, ryegrass hay and creep feed were offered *ad libitum* and each experimental
24 group was kept in independent pens until weaning at 45d of age. After weaning all
25 lambs were placed together on the same pasture for fattening for 4 months. Blood
26 samples were taken at 24h after birth, at weaning and at the end of the fattening period
27 (23 weeks). Results showed that no failure in the passive immune transfer was detected
28 across treatments. Although artificially reared lambs at weaning had lower plasma
29 levels of β -hydroxy-butyrate (-62%), HDL (-13%) and amylase (-25%) and higher levels
30 of LDL (+38%) and alkaline phosphatase (+30%), these differences disappeared during
31 the fattening period. Only the greater levels of calcium and the lower levels of
32 haemoglobin and white blood cells detected at weaning in artificially reared lambs
33 (+7.2%, -2.8% and -17.8%) persisted by the end of the fattening period (+4.3%, -3.3%
34 and -9.5%, respectively). Minor diarrheal events from weeks 2 to 5 were recorded with
35 artificial rearing, leading to lower growth rates during the first month. However, these
36 artificially reared lambs caught up towards the end of the milk feeding period and
37 reached similar weaning weights to NN lambs. During the fattening period NN lambs
38 had a greater growth rate (+16%) possibly as a result of their greater early rumen
39 development which allowed a higher feed digestibility during the fattening period in

40 comparison to NA lambs (+5.9%). As a result, NN lambs had heavier final body weights
41 (+7.0%), but tended to have lower dressing percentage (-5.7%) than artificially reared
42 lambs, thus no differences were noted in either carcass weight or in carcass
43 conformation across treatments. In conclusion, the use of a colostrum alternative and
44 milk replacer facilitated the successful rearing of lambs, reaching similar productive
45 parameters; however special care must be taken to maximize the rumen development
46 before weaning.

47

48 **Keywords:** animal performance, colostrum, health, milk replacer, weaning

49 **Implications**

50 This study revealed that artificial rearing of lambs with colostrum alternative and milk
51 replacer represents an appropriate strategy to maximize the number of lambs weaned
52 per ewe with a similar final BW achieved to lambs reared on the ewe. However, direct
53 contact with the ewe provided a competitive advantage in naturally reared lambs
54 allowing them to better develop their immune system and rumen function which led to
55 increased BW gain during the fattening period.

56

57 **Introduction**

58 Two main systems exist for rearing offspring in ruminant production: in commercial dairy
59 systems, or when dam milk is not available in sufficient amount or sanitary condition,
60 newborns are separated from their dams within the first hours after birth and fed either
61 milk replacer or whole milk; in contrast, in meat production systems, newborn animals
62 generally remain with their dams until weaning. A recent study has reported that kid
63 goats reared with their dams had greater rumen development than their twins that were
64 fed on milk replacer and isolated from adult animals, despite both groups having access
65 to the same solid feed (Abecia *et al.*, 2014). However, it remains unknown whether
66 these differences are transitory or if they persist later in life during the fattening period.

67 Lambs are born hypogammaglobulemic due to the complexity of the synepitheliochorial
68 ruminant placenta, which does not allow sufficient transfer of immunoglobulins from the
69 dam to the fetus (Hernández-Castellano *et al.*, 2014, Hernández-Castellano *et al.*,
70 2015), thus IgG transfer from colostrum is vital for the neonatal health (Arguello *et al.*,
71 2004). Insufficient neonatal absorption of colostral immunoglobulins within the first day
72 of life has been associated with failure of passive immunity transfer which is indicated
73 when serum IgG levels are below a certain threshold (generally 10 mg/ml in calves, 12

74 mg/ml in goats and 15 mg/ml in lambs) leading to increased risk for neonatal diseases,
75 mortality and with a negative effect on adult health, longevity and performance (DeNise
76 *et al.*, 1989, Arguello *et al.*, 2004, Faber *et al.*, 2005, Alves *et al.*, 2015). As a result,
77 higher morbidity and mortality rates have been observed in colostrum-deprived lambs
78 (80 and 67%) than colostrum fed lambs (20 and 13%) (Hodgson *et al.*, 1992). In
79 addition, there is increasing evidence showing that nutritional management in the pre-
80 weaning period determines to a great extent the potential for milk production during
81 subsequent lactations: several studies have indicated that those heifers fed with a
82 greater volume of the same high quality colostrum (Faber *et al.*, 2005) and those with a
83 greater plasma concentration of IgG shortly after birth (DeNise *et al.*, 1989) had higher
84 milk yield than their counterpart control animals during their productive life. Moreover it
85 has been noted that increased growth rate before weaning results in positive effects on
86 milk yield in cattle (Soberon *et al.*, 2012). Thus, the general recommendation is to
87 actively feed lambs with colostrum from a freshly lambed ewe in order to maximize
88 passive immunity transfer. However, when ewe colostrum is scarce the
89 supplementation of lambs with colostrum alternatives may represent a strategy to
90 maximize the number of lambs weaned. Nevertheless, it remains unknown whether
91 these early life interventions in lambs could have similar long-lasting consequences to
92 those described in cattle.

93 In this study we hypothesized that nutritional interventions early in the life of the lambs
94 could have immediate effects on the animal's health and performance, with some
95 effects persistent later in life under conventional production systems. These nutritional
96 interventions during the pre-weaning period consisted of 1) lambs remained with the
97 ewe (natural rearing) (NN), 2) ewe colostrum followed by artificial rearing with milk
98 replacer (NA), and 3) colostrum alternative supplementation and artificial rearing (AA).

100 **Material and methods**

101 *Animals and diets*

102 All animal procedures were carried out according to the Home Office Scientific
103 Procedures, Act 1986 (PLL 40/3653; PIL 40/9798). Triplet lambs were used to provide
104 similar genetic background, gestation environment and ewe colostrum in order to
105 minimize the inter-animal variation across treatments. Thus, after pregnancy scanning,
106 twenty-four pregnant Aberdale ewes carrying triplets were selected from the
107 Aberystwyth University commercial flock. A total of 72 Aberdale-texel crossbreed lambs
108 were born within an 8-day period (14th to the 22nd April). At birth umbilical cords were
109 disinfected with iodine and lambs were weighed. One animal of each triplet set was
110 randomly allocated to 1 of 3 experimental treatments. During this allocation process sex
111 and initial body weight of the lambs was considered resulting in similar sex distribution
112 (average 13 males and 11 females per group) and birth weights (3.8 ± 0.8 kg) across
113 treatments. All three lambs were kept with their mother in an individual pen during the
114 first 24h after birth. Two lambs (NN and NA) were encouraged to suckle ewe colostrum
115 by connecting them to a ewe's teat four times over the first 24h (1, 2, 4 and 6 h after
116 birth) until the gut filling was evident in order to ensure a high colostrum intake. Then,
117 one of those lambs (NN) remained with its mother suckling ewe milk from birth to
118 weaning, while the second lamb (NA) was separated from its dam after 24h and
119 artificially reared with milk replacer. On the contrary, the third lamb (AA) was not
120 encouraged to suckle ewe colostrum, instead it was immediately fed with 50g of
121 colostrum alternative divided in two equal doses at 1h and 6h after birth followed by
122 artificial rearing with milk replacer. In this latter group, no obvious signs of gut filling with
123 ewe colostrum were noted suggesting a minimal intake of ewe colostrum. Colostrum

124 alternative was freshly prepared by mixing 25g of product (Lamb Volostrum, Volac Ltd.)
125 in 50ml of water at 30°C and provided by a stomach tube at each time (1h and 6h after
126 birth). Milk replacer was prepared by mixing 200g of milk powder (Lamlac Instant,
127 Volac Ltd.) with water to make up 1 litre of reconstituted milk following the manufacturer
128 instructions. During their first week of life all lambs had access to heat lamps and warm
129 milk replacer (39°C) offered *ad libitum* using temperature controlled feeders (Ewe 2
130 Feeder, Volac Ltd, UK). Lambs that did not suckle were stomach tubed and trained to
131 suck from a teat connected to the milk feeder. After one week of age all lambs were
132 able to suckle and milk replacer was offered *ad libitum* at room temperature (average
133 12°C) using two buckets connected to four teats for each experimental group. These
134 milk buckets were emptied twice a day and thoroughly cleaned and rinsed, using soap
135 and hot water.

136 At 24h after birth, blood was sampled (see below), and all animals were tagged and
137 intramuscularly injected with 1 ml of AD₃E (NAPHA Veterinary, UK) to prevent vitamin
138 deficiency. Then, all lambs from the same treatment were placed together in a single
139 pen (10m×12m) with clean and dry barley straw bedding and *ad libitum* access to creep
140 feed (NuGro CCF, UK), ryegrass hay and water (chemical composition described in
141 Supplementary Table S1). During the milk feeding stage all three groups of animals
142 were physically separated from each other (by approximately 1 m) but kept in the same
143 building (average temperature of 12°C, relative humidity of 86%, and an average of 10
144 hours of day light). Treatments NA and AA also had free access to milk replacer which
145 was freshly prepared twice a day at 09:00h and 17:00h. Lambs from treatment NN
146 shared a pen with their mothers that were fed twice a day with the same ryegrass hay
147 and commercial concentrate (Wynnstay, High Production Ewes, UK). Ewes were
148 physically separated from the NN lambs for 10 minutes during the concentrate feeding.

149 Group intakes of milk replacer and creep feed were recorded daily until weaning.
150 Animals were inspected daily for signs of disease. The incidence of diarrheal events
151 was recorded, and the severity was assessed based on a score from 1 (absence) to 4
152 (severe). Animals with a score above 3 received an intramuscular antibiotic treatment
153 (Pen-Strep, Norbrook, UK). Lambs were weekly weighed using a digital balance to
154 determine their growth during the entire duration of the experiment.
155 Animals were weaned at 45d of age by abrupt weaning and kept in the same building
156 with the same solid feed for a further one week. When lambs were on average 8 weeks
157 of age, all experimental lambs were grouped together on the same ryegrass pasture
158 (*Lolium perenne*) with free access to creep feed until 10 weeks of age but not thereafter.
159 Thus all lambs grazed the same pasture over 5 months (from June to November). When
160 the average body weight (BW) of a given set of triplets reached the optimum slaughter
161 weight (approximately 40kg and between 23 to 31 weeks of age), all three lambs were
162 slaughtered in a commercial abattoir. Carcass weight and performance was assessed
163 at an official abattoir according to the EUROP classification (Johansen *et al.*, 2006).

164

165 *Sampling and analyses*

166 Blood samples (5ml) were collected from the jugular vein at 24h after birth, at weaning
167 (45d) and at the end of the fattening period (23wks). One blood subsample (2ml) was
168 placed in a tube with anticoagulant (K₃-EDTA) mixed by inversion 10 times, kept at 4°C
169 and immediately analysed for haematology using a Mythic 18 Vet Haematology
170 Analyser (Woodley Equipment Company Ltd., UK). This analysis determined levels of
171 the main blood cells and their morphotypes (see below). A second subsample (3ml) was
172 placed in a tube without anticoagulant; serum was harvested by centrifugation at

173 2,000×g for 15min and stored at -20°C until analysis. Serum metabolites were
174 determined using RX Daytona⁺ equipment (Randox Laboratories Ltd. UK).
175 Colostrum (10ml) and milk (50ml) samples were obtained by hand milking from each
176 ewe at 24h after the birth of the first lamb and at 45d post-partum, respectively.
177 Samples were kept frozen and milk and colostrum composition (Table 1) was
178 determined using a milk analyser (LactoScope Advance FTIR, Delta Instruments,
179 Netherlands). Concentration of IgG in serum and colostrum was determined using the
180 Sheep IgG ELISA 96 well plate kit (Gen Way, USA, reference GWB-OVI374) after
181 dilution (4×10^{-4} and 4×10^{-6} for serum and colostrum respectively) and absorbance at
182 450nm was measured using a plate reader (PowerWave XS2, BioTek, UK).
183 Temperature corrected density (nD_{TC}) in serum samples (100µL) was measured in
184 triplicate using an automatic digital refractometer (Reichert AR200 Ver 1.8, Ametek,
185 Germany) and concentration of IgG in was estimated based on the regression
186 equations described by Morrill (2011): $IgG \text{ (mg/ml)} = 5919.1 \times nD_{TC} - 7946.1$
187 (Table 1 here)

188

189 *Faecal analysis*

190 At 23wks of age faecal grab samples were collected from each animal on two non-
191 consecutive days, frozen and pooled by animal (30g DM approximately). On the same
192 days as faecal sampling, ryegrass pasture was cut to 5 cm above soil level from 4
193 different locations of the field and immediately frozen for further analysis. The effect of
194 the experimental treatments on pasture digestibility was estimated using the acid
195 insoluble ash as an internal marker (Thonney *et al.*, 1979). For feed and faeces
196 analyses, dry matter (DM) content was determined by drying in an oven at 105°C for
197 24h. Organic matter (OM) concentration was determined by heating at 550°C for 6h in a

198 muffle furnace. Nitrogen and carbon concentration was measured by the Dumas
199 combustion method (Elementar analyser, Vario MAX cube, Germany). Neutral-
200 detergent (NDF) and acid-detergent fibre (ADF) were determined using an Automated
201 Fiber Analyzer (ANKOM 2000, USA) using heat stable amylase and sodium sulphide.
202 For faecal fingerprint analysis, samples were analysed as previously reported (Belanche
203 *et al.*, 2017). Briefly, freeze dry samples were ground to a fine powder (IKA Analytical
204 Mill, Stauffer, Germany) and analysed by attenuated total reflectance (ATR) from 4000
205 to 600cm⁻¹ using an Equinox 55 Fourier Transformed Infrared Spectrophotometer
206 (Bruker Ltd, Coventry, UK)), and scanned using the Golden Gate ATR accessory
207 (Specac Ltd., Slough, UK). Infrared settings and data collection were conducted as
208 previously reported (Belanche *et al.*, 2014). Fourier transformed infra-red (FTIR) spectra
209 were imported into Matlab (version 2007b, The MathWorks Inc., Natick, USA),
210 averaged, transformed to the first Savitsky-Golay derivative to smooth baseline noise
211 and improve spectral resolution using a 13-point window, and then mean centre
212 normalized (mean=1, Standard Deviation=1). Data were then analysed by non-
213 parametric permutational multivariate analysis of variance using PRIMER-6 software
214 (PRIMER-E Ltd., Plymouth, UK). Statistical signification was calculated after 999
215 random permutations of residuals under a reduced model using the Monte Carlo test.
216 For graphical interpretation, principal component analysis was conducted and a
217 Canonical variate analysis was performed based on the data compiled in the main
218 principal components (Genstat 18th Edition, VSN International, Hemel Hempstead, UK).

219
220 *Calculations and statistical analysis*
221 Haematological analysis determined the levels of red blood cells, haemoglobin,
222 haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH),

223 mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width
224 (RBCDW), white blood cells and its morphotype percentages, platelets, mean platelets
225 volume (MPV), thrombocrit and platelet distribution width (PDW). While the plasma
226 metabolic analysis measured: calcium, glucose, β -hydroxybutyrate (BHB), cholesterol,
227 triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL), albumin,
228 creatinine, urea, ammonia, L-lactate dehydrogenase and alkaline phosphatase levels.
229 Globulins and LDL concentrations in plasma were mathematically calculated:

$$230 \quad \text{Globulins} = \text{Total proteins} - \text{Albumin}$$

$$231 \quad \text{LDL} = \text{Cholesterol} - \text{HDL} - (\text{Triglycerides} / 5)$$

232 To evaluate the effect of experimental treatments on blood parameters, data were
233 analysed using an ANOVA as follows:

$$234 \quad Y_{ijk} = \mu + R_i + T_j + RT_{ij} + T_k + A_l + e_{ijkl}$$

235 where Y_{ijk} is the dependent, continuous variable ($n = 24$), μ is the overall mean, M_i is the
236 fixed effect of the type of rearing ($i = \text{NN vs NA vs AA}$), T_j is the fixed effect of the animal
237 age ($j = \text{weaning vs fattening}$), FV_{ij} is their interaction, S_k is the random effect of the
238 triplet set used as a block ($k = 1$ to 24), A_l is the random effect of the animal ($j = 1$ to 72)
239 and e_{ijkl} is the residual error. For animal weight, growth and carcass performance data,
240 the term sex (male vs females) was also included as a fixed effect. When significant
241 effects were detected across treatments, means were compared by Fisher's protected
242 LSD-test (Genstat 18th Edition, VSN International, Hemel Hempstead, UK). Significant
243 effects were declared at $P < 0.05$.

244

245 **Results**

246 *Animal health*

247 At 24h after birth all animals remained in good health and neither haematological nor
248 plasma IgG differences were observed across treatments (Table 2). Animals artificially
249 reared (NA and AA) suffered a greater incidence of diarrhoea episodes than NN lambs
250 from 2 to 5 weeks of age ($P=0.001$) but this effect disappeared thereafter. Antibiotic
251 usage was also higher for NA and AA lambs than for NN lambs ($P<0.001$) and the
252 number of animals with recurrent diarrhoea which required more than 2 antibiotic doses
253 were 0, 6 and 9 for NN, NA and AA lambs, respectively.

254 (Table 2 here)

255 The age of the lambs exerted a major effect on the blood cell distribution (Table 3) and
256 the concentration of most plasma metabolites (Table 4). At weaning animals had a
257 greater concentration of red blood cells, haemoglobin, RBCDW, lymphocytes, platelets,
258 thrombocrit and plasma levels of calcium, glucose, cholesterol, triglycerides, HDL, LDL,
259 albumin, creatinine, amylase and alkaline phosphatase than animals at fattening
260 ($P<0.001$). On the contrary, at fattening animals had a greater concentration of white
261 blood cells, monocytes, granulocytes, MPV, PDW and plasma levels of BHB, total
262 proteins, globulins and urea ($P<0.001$). However, artificial rearing also had a small mid-
263 and long-term effect on the animals' health (Table 3). NN lambs had a greater
264 haemoglobin ($P=0.012$), haematocrit ($P=0.044$), white blood cells ($P=0.007$) and
265 calcium levels than NA and AA lambs, independently of the age considered. Moreover a
266 significant interaction was observed for several metabolites and haematological
267 parameters: at weaning NN lambs had greater RBCDW ($P>0.001$), BHB ($P<0.001$),
268 HDL ($P=0.001$) and amylase plasma levels ($P<0.001$), as well as lower MCHC
269 ($P=0.014$), PDW ($P=0.004$), LDL ($P=0.009$) and alkaline phosphatase ($P<0.001$) were
270 observed in NN lambs than in NA or AA but no such differences were observed at
271 fattening.

272 (Table 3 and Table 4 here)

273

274

275 *Animal performance*

276 Average group intake of milk replacer remained constant until week 3 (300 g DM/d per
277 lamb) and linearly increased thereafter reaching 550 g DM/d at weaning for AA and NA
278 groups while milk intake in NN lambs was not recorded. Group intake of creep feed also
279 remained low and constant until week 4 across treatments, and increased linearly
280 thereafter reaching an average of 256, 137 and 96 g DM/d at weaning for treatments
281 NN, NA and AA, respectively. No differences in body weight (BW) were observed at
282 birth across treatments, but NN lambs had a greater BW than NA and AA lambs from
283 week 2 to 5, these differences disappeared during the weaning stage and reappeared
284 from week 11 onwards (Figure 1). No differences in the average daily gain (ADG) were
285 observed before weaning (Table 5), but NN lambs had a greater ADG during the
286 fattening period calculated from weaning to 23 weeks of age ($P<0.001$). In terms of
287 carcass composition, NN lambs had a higher slaughter weight than NA and AA lambs
288 ($P<0.001$), but NN lambs tend to have a lower dressing percentage resulting in similar
289 carcass weight and conformation across treatments. Male lambs tended to have a
290 greater BW at birth and ADG during the fattening period ($P=0.047$) however no
291 differences were observed in carcass conformation.

292 (Figure 1 and Table 5 here)

293

294 *Pasture utilization*

295 The chemical structure of the faecal samples tended to differ ($P=0.079$) between
296 treatments based on the PERMANOVA analysis of the FTIR spectral data

297 (Supplemental Table 2). Canonical variate analysis (Figure 2) compiling the information
298 of the first 15 principal components (representing 98.1% of the total variance) showed
299 that these differences were more obvious between NA and the other two experimental
300 groups. In terms of pasture digestibility (Table 6), values were always highest for NN
301 lambs: NN and AA lambs had higher digestibility for DM ($P<0.001$), C ($P<0.001$) and N
302 ($P=0.003$) than NA lambs, while no differences were observed in NDF and ADF
303 digestibility.

304 (Figure 2 and Table 5 and Table 6 here)

305

306 **Discussion**

307 *Effect of colostrum alternative*

308 Colostrum products have been shown to provide a degree of passive immunity transfer
309 (Seymour *et al.*, 1995, Castro *et al.*, 2007), although the results vary greatly depending
310 on the product used, colostrum preservation methods, dosage techniques and inter
311 animal variation (Arguello *et al.*, 2004). As a result, colostrum products that typically
312 contain lacteal-derived or plasma-derived IgG are classified as either colostrum
313 replacers or colostrum supplements depending on their ability to raise serum IgG
314 concentration above a certain threshold (typically 15 mg/ml in lambs) (Alves *et al.*,
315 2015). Colostrum supplements (as in our study) can be used to increase the amount of
316 IgG fed to lambs when only low or medium quality / quantity colostrum is available.
317 However, supplements cannot replace high quality colostrum which is still considered
318 the gold standard for feeding newborn lambs (Jones *et al.*, 2004). Our study aimed to
319 simulate two real scenarios in the artificial rearing of lambs: one (NN and NA lambs)
320 consisting of maximizing colostrum intake by encouraging lambs to suckle for at least 4
321 times from the ewe; and an alternative strategy (AA lambs) based on colostrum

322 alternative supplementation of lambs with an insufficient intake of ewe colostrum. To
323 achieve this situation, AA lambs were not encouraged to suckle and had to compete
324 with their two siblings for the remaining ewe colostrum. A rapid change in the colostrum
325 composition to transitional milk has been described during the post-partum period
326 (Alves *et al.*, 2015). In our study, despite the late sampling of ewe colostrum (24h after
327 the first lamb was born), the IgG concentrations (average 42.2 g/l) were comparable to
328 published literature (from 15.7 to 65 g/l) in which the samples were collected just after
329 parturition (Vatankhah, 2013, Alves *et al.*, 2015, Hernández-Castellano *et al.*, 2015),
330 possibly as a result of a higher colostrum production in high prolific ewes. As a result,
331 only one lamb had an IgG concentration below 15 mg/ml at 24h after birth suggesting
332 effective overall passive immunity transfer across treatments (Alves *et al.*, 2015). This
333 may explain the lack of differences in terms of growth, haematology parameters and
334 blood metabolites levels between NA and AA lambs, as well as, the absence of deaths
335 before weaning. Moreover, the high level of easily digestible energy and protein in the
336 colostrum alternative also seems to represent an important source of nutrients for the
337 lambs during its first hours of life to maintain body temperature and good health (Jones
338 *et al.*, 2004). Thus, the supply of colostrum alternative after birth can be considered an
339 appropriate strategy to prevent health problems and maximize the number of lambs
340 weaned per ewe when ewe colostrum is insufficient.

341

342 *Effect of artificial rearing on lamb's health*

343 This study does not attempt a direct comparison of the effects of milk replacer vs
344 maternal milk since artificial rearing involves the replacement of the contributions made
345 by the ewe which are essential to the growth and development of the lamb. This not
346 only includes the feed supply but also the warmth, shelter and “mothering” normally

347 provided by the ewe. Our experiment showed a greater incidence of diarrhoea events in
348 artificially reared lambs than those reared on the ewe. These diarrhoea episodes
349 appeared from week 2 to week 5; they were very mild (<2.0 scored) and required an
350 average of 1.2 antibiotic doses per lamb, whilst antibiotic usage in NN lambs was
351 negligible. Although these diarrheal events did not trigger any deaths, they could explain
352 the lower ADG for NA and AA lambs during the first 5 weeks. Similar diarrhoea events
353 starting at 2 weeks of age have been described in calves and various pathogens
354 compatible with enteric infections have been identified in the necropsy (i.e. *Salmonella*,
355 *Cryptosporidium parvum*, *Escherichia coli* and coronavirus) (Quigley *et al.*, 2006).
356 Various studies have investigated the effect of different artificial milk feeding strategies
357 to prevent diarrheal events and to improve animal performance: Jasper and Weary
358 (2002) concluded that *ad libitum* nipple feeding of whole milk to dairy calves vs
359 restricted can increase weight gain with no diarrheal problems nor detrimental effects on
360 feed intake after weaning. While Quigley (2006) observed that calves fed a variable
361 amount of milk replacer (peaking at 3 weeks of age with 908 g/d) had greater ADG but
362 also increased incidence of diarrhoea that required added veterinary treatment in
363 comparison to those fed a fixed amount (454 g/d). Thus, it seems that our artificial
364 rearing strategy based on the *ad libitum* access to milk replacer might explain the
365 incidence of moderate diarrhoea but did help to prevent feed competition between
366 lambs, since lambs in contrast to calves tend to be reared in groups with a large number
367 of animals. More research is needed to assess whether these diarrheal events could be
368 minimized by using alternative rearing systems such as automatic feeding machines.
369 Although most lambs remained in good health from birth to slaughter, the
370 haematological analysis revealed that NN lambs had higher levels of white blood cell at
371 weaning in comparison to artificially reared lambs (+21.6%), and those differences

372 persisted during the fattening period (+10.5%). It has been shown that colostrum and
373 milk have viable cells, including neutrophils and macrophages, which secrete a range of
374 immune-related components (Stelwagen *et al.*, 2009). Our findings are in line with this
375 observation and suggest that direct contact with adult animals in NN may also represent
376 an important exposure to antigens which may help in the immune system development
377 of young lambs with long-lasting effects on the levels of white blood cells. Moreover,
378 artificially reared lambs had lower haemoglobin levels (-2.8%) and haematocrit (-5.3%)
379 at weaning in comparison to NN lambs. The variation in the size of red cells
380 (anisocytosis) provided an insight of the potential reasons of slight signs of anaemia.
381 Since neither the size of the red blood cells (MCV) nor the amount of haemoglobin per
382 cell (MCH) were affected, it seems that the normocytic anaemia was very mild and
383 partially compensated by a greater amount of haemoglobin per unit of volume (MCHC
384 +2.6%). Despite this lack of severity, artificially reared lambs still had lower levels of
385 haemoglobin (-3.3%) and haematocrit (-0.4%) during the fattening period suggesting a
386 small but long term effect of the type of rearing strategy on the animals health. On the
387 contrary, NN lambs had a higher coefficient of variation in red blood cell distribution
388 width (RBCDW, +20.0%) which is compatible with early stages of iron deficiency at
389 weaning in animals having limited amounts of milk (Blaxter *et al.*, 1957), possibly as a
390 result of a lower milk intake and lower iron content in the ewe milk in comparison to
391 lambs fed milk replacer *ad libitum*. This observation was supported by the lower blood
392 calcium concentration in NN lambs at weaning (-6.7%) and fattening (-4.2%). Increases
393 in plasma glucose and urea concentrations have been associated with higher artificial
394 milk intake in calves (Quigley *et al.*, 2006). However, in our study all experimental
395 treatments had similar glucose, urea and total protein levels at weaning, possibly
396 because a lower milk intake in NN lambs during late milk feeding period in comparison

397 to those fed milk replacer was compensated by a greater creep feed intake (256 vs 116
398 g/d). Our experiment indicates that protein and energy sources included in the milk
399 replacer were highly digestible since no differences in the plasma concentration of
400 metabolites related with the protein (total proteins, albumin, globulin, creatinine, urea
401 and ammonia) and energy (glucose) metabolism were detected across treatments.
402 These findings agree with the similar content of urea nitrogen, total protein, albumin and
403 globulin in the serum of lambs fed milk replacers made up of milk protein or other
404 protein sources (Huang *et al.*, 2015). Most of the milk bypasses the rumen through the
405 oesophageal groove, thus high milk intake in artificially reared lambs may increase the
406 amino acid flow to the small intestine leading to an increase in the deamination
407 processes occurring in the liver as was reflected by increased levels of alkaline
408 phosphatase (+30%) as an indicator of the liver stress (Reichling & Kaplan, 1988). On
409 the contrary, solid feed (carbohydrates and proteins) is fermented in the rumen
410 producing volatile fatty acids and ammonia as the main fermentation end product. Thus,
411 the increased levels of β -hydroxy-butyrate in NN at weaning (+2.6-fold times) suggest a
412 greater physiological and fermentative development of the rumen. Although cholesterol
413 and triglyceride concentrations were unaffected by the experimental treatments,
414 artificially reared lambs had lower levels of HDL (-13%) and higher levels of LDL (+38%)
415 at weaning than NN lambs. Increased blood levels of LDL is considered a circulatory
416 risk factor which is mainly determined by diet, physical activity, genetics, sex and age
417 (Sigurdardottir *et al.*, 2002). Overall, our data also showed that most of the
418 haematological and metabolite differences observed at weaning were transient and
419 tended to disappear later in life with no further effects on the animal's health.

420

421 *Effect of artificial rearing on productive performance*

422 This study revealed that in comparison with artificially reared lambs, NN lambs had a
423 higher neonatal growth suggesting that the ewe mothering instinct helps lambs to suckle
424 more efficiently during the first days of life. Moreover this competitive advantage was
425 maintained until 3 weeks after birth, when NN lambs reached the greatest differences in
426 BW (+10.5%), corresponding with the peak in the lactation curve described for
427 crossbred ewes rearing lambs (Cardellino & Benson, 2002). However, these differences
428 tended to disappear as weaning approached , possibly due to the increased milk intake
429 recorded for the artificially reared lambs (average 2.9 L/d), resulting in similar BW at
430 weaning across treatments. This observation agrees with the lack of differences in
431 weaning weights reported for Comisana lambs reared artificially or conventionally
432 (Napolitano *et al.*, 2002).

433 However, differences in BW gain reappeared after weaning despite all lambs being
434 grazed together on the same pasture. As a result, NN lambs had a greater growth
435 during the fattening period (+16%) and higher BW from week 13 onwards. Several
436 reasons could explain these findings: i) The greater solid feed intake observed in NN
437 lambs at weaning (256 vs 116 g DM/d) has been described as a key factor which
438 promotes the rumen physiological development in calves and facilitates a smooth
439 transition to the solid diet (Khan *et al.*, 2011). ii) The direct contact with adult animals
440 represents a source of microbes (i.e. bacteria, protozoa, methanogens, anaerobic fungi)
441 which are crucial for the development of the symbiotic rumen microbiota (Belanche *et*
442 *al.*, 2010, Belanche *et al.*, 2011). iii) Adult animals teach young animals in terms of
443 feeding behaviour since the presence of adult companions has been reported to
444 increase solid feed intake and performance of calves before and after weaning (Vieira *et*
445 *al.*, 2012) as was noted in our experiment.

446 Our findings also suggest that the greater BW gain in NN lambs during the fattening
447 period may in part be explained by greater feed DM digestibility (+5.9%) in comparison
448 to NA lambs, although differences were less obvious (+2.0%) when compared with AA
449 lambs. These differences in forage utilization were also observed based on the
450 fingerprint analysis of faecal samples using FTIR spectroscopy. As a result, NN lambs
451 reached a greater final BW (+7.0%) at slaughter but they performed substantially worse
452 in dressing percentage (-5.7%) leading to similar carcass weight, carcass confirmation
453 and fatness. This observation indicates that NN lambs may have a greater rumen size,
454 slower rumen transit time or greater wool yield all of which could reduce the killing out
455 percentage. These findings support previous observations which suggest that rearing
456 lambs on the ewe, and the early intake of solid feed are important drivers not only for
457 the rumen anatomical enlargement, but also for the physiological and microbiological
458 development (Yáñez-Ruiz *et al.*, 2015). Thus, more research is needed based on a
459 better description of the rumen dynamics of feed utilization, rumen microbiota and
460 animal behavioural studies to elucidate which factor plays a greater role on animal
461 resilience and productivity during the post-weaning processes as well as later in life.

462

463 **Acknowledgements**

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559 fermentation through interventions during early life: a review. *Frontiers in microbiology* 6, 1-

560 12

561 **Table 1.** Colostrum and milk composition

	Colostrum		Milk	
	Natural	Alternative ¹	Natural	Replacer ²
CP, %	22.6	22.1	4.35	4.77
Fat, %	15.5	4.52	5.51	4.84
Lactose, %	2.79	2.82	4.90	7.33
Solids, %	40.9	30.5	15.4	17.4
Solids non-fat, %	27.2	27.9	10.4	13.1
IgG, g/l	42.2	32.1		

562 ¹Values after mixing 25g of colostrum alternative (Lamb Volostrum, Volac Ltd.) with 50
 563 ml of water

564 ²After mixing 200g of milk replacer (Lamlac Instant, Volac Ltd.) with water to make up 1
 565 litre of reconstituted milk

566 **Table 2.** Effect of colostrum alternative and artificial rearing on plasma IgG levels and
 567 haematology at 24h after birth and incidence of diarrhoea before weaning.

Type of rearing	NN	NA	AA	SED ¹	P-value
Red blood cells (10 ⁶ /μl)	8.20	7.77	8.01	0.221	0.152
White blood cells (10 ³ /μl)	6.48	5.82	6.01	0.539	0.461
Platelets (10 ³ /μl)	630 ^a	502 ^b	575 ^{ab}	48.8	0.041
Haematocrit (%)	38.0	36.2	37.3	1.14	0.276
ELISA IgG (mg/ml)	40.1	45.6	37.1	4.19	0.135
Refractometer IgG (mg/ml)	38.3	38.3	32.5	2.88	0.075
Diarrhoea score ²					
Week 2	1.13 ^b	1.83 ^a	2.04 ^a	0.229	<0.001
Week 3	1.29 ^b	1.96 ^a	2.33 ^a	0.269	0.001
Week 4	1.08 ^b	1.96 ^a	1.92 ^a	0.252	0.001
Week 5	1.04 ^b	1.58 ^a	1.96 ^a	0.227	<0.001
Week 6	1.04	1.08	1.25	0.121	0.201
Week 7	1.04	1.04	1.17	0.108	0.415
Antibiotic usage (doses/lamb) ³	0.08 ^b	0.96 ^a	1.42 ^a	0.333	<0.001

568 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum
 569 alternative and artificial milk feeding.

570 ¹Standard error of the difference among means. Within a row means without a common
 571 superscript differ ($P<0.05$).

572 ²Diarrhoea score: 1 absence, 2 very mild, 3 moderate and 4 severe.

573 ³Intramuscular Penicillin-Streptomycin

Table 3. Effect of colostrum alternative and artificial rearing on haematology and blood metabolites in lambs.

Type of rearing ¹	Weaning (45 days)			Fattening (23 wks)				<i>P</i> -value		
	NN	NA	AA	NN	NA	AA	SED ¹	Rearing	Age	RxA
Red blood cells (10 ⁶ /μl)	11.4	11.4	11.7	11.3	10.9	11.1	0.237	0.148	<0.001	0.178
Haemoglobin (g/dl)	11.5	11.0	11.3	11.1	10.6	10.8	0.236	0.012	<0.001	0.940
Haematocrit (%)	38.3	35.8	36.7	36.2	34.6	37.5	1.422	0.044	0.210	0.320
MCV (fL)	33.6	31.5	32.1	32.1	31.9	34.0	1.435	0.384	0.799	0.240
MCH, (pg)	10.1	9.72	9.77	9.83	9.74	9.78	0.201	0.397	0.472	0.415
MCHC (%)	30.0 ^b	30.8 ^a	30.8 ^a	30.6 ^a	30.6 ^a	30.7 ^a	0.208	0.005	0.374	0.014
RBCDW (%)	25.4 ^a	20.0 ^b	20.7 ^b	17.9 ^c	18.2 ^c	17.9 ^c	0.508	<0.001	<0.001	<0.001
White blood cells (10 ³ /μl)	7.95	6.31	6.76	8.91	8.40	7.73	0.557	0.007	<0.001	0.369
Lymphocytes (%)	56.5	56.9	54.4	53.2	47.6	51.1	2.365	0.38	<0.001	0.121
Monocytes (%)	11.6	11.9	10.9	13.7	14.7	14.4	0.621	0.349	<0.001	0.149
Granulocytes (%)	31.9	31.2	34.7	33.1	37.6	34.5	2.091	0.432	0.023	0.057
Platelets (10 ³ /μl)	1982 ^a	1419 ^b	1695 ^{ab}	548 ^c	616 ^c	639 ^c	179.0	0.145	<0.001	0.055
MPV (fl)	5.20	4.90	4.71	5.71	5.98	5.75	0.364	0.641	<0.001	0.400
Thrombocrit	1.10	0.72	0.82	0.29	0.33	0.62	0.189	0.301	<0.001	0.078
PDW (%)	30.1 ^d	36.0 ^c	34.9 ^c	46.0 ^a	42.1 ^b	44.0 ^{ab}	2.453	0.694	<0.001	0.004

575 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative and artificial milk feeding.

576 MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration;

577 RBCDW, red blood cell distribution width; MPV, mean platelets volume; PDW, platelet distribution width

578 ¹Standard error of the difference among means. Within a row means without a common superscript differ (*P*<0.05).

579 **Table 4.** Effect of colostrum alternative and artificial rearing on blood metabolites in lambs.

Item ¹	Weaning (45 days)			Fattening (23 wks)			SED ¹	P-value		
	NN	NA	AA	NN	NA	AA		Rearing	Age	RxA
Calcium (mM)	2.33	2.52	2.48	1.84	1.92	1.93	0.116	0.075	<0.001	0.854
Energy										
Glucose (mM)	5.47	5.89	5.75	3.50	3.56	3.56	0.306	0.277	<0.001	0.881
BHB (µM)	265 ^b	100 ^c	100 ^c	342 ^a	372 ^a	394 ^a	29.57	0.006	<0.001	<0.001
Lipids (mM)										
Cholesterol	2.82	2.91	2.79	1.27	1.31	1.23	0.184	0.596	<0.001	0.822
Triglycerides ²	0.78	0.72	0.75	0.24	0.21	0.24	0.061	0.481	<0.001	0.885
HDL	1.91 ^a	1.65 ^b	1.66 ^b	0.62 ^c	0.65 ^c	0.61 ^c	0.104	0.489	<0.001	0.001
LDL ³	0.76 ^b	1.11 ^a	0.98 ^a	0.60 ^c	0.61 ^c	0.57 ^c	0.098	0.061	<0.001	0.009
Proteins, (g/l)										
Total Proteins	45.4	46.7	46.3	65.3	67.3	66.6	2.534	0.619	<0.001	0.984
Albumin	32.9	33.5	33.3	30.2	31.1	31.2	1.019	0.551	<0.001	0.952
Globulin ²	12.5	13.3	13.0	35.1	36.2	35.4	1.730	0.732	<0.001	0.994
Creatinine (µM)	83.0	85.8	87.9	78.3	80.4	78.5	4.640	0.583	0.051	0.656
Urea, mM	3.85	3.95	3.82	9.98	9.85	10.1	0.342	0.933	<0.001	0.803
Ammonia (µM)	83.6	81.9	85.2	84.9	86.0	89.7	5.690	0.505	0.548	0.747
Enzymes (U/l)										
Amylase	25.7 ^a	20.3 ^b	18.3 ^b	12.5 ^c	10.8 ^c	12.6 ^c	1.721	0.037	<0.001	<0.001
L-lactate dehydrogenase	1171	1238	1112	1163	1093	1098	64.40	0.178	0.262	0.501
Alkaline Phosphatase	637 ^b	841 ^a	819 ^a	177 ^c	184 ^c	183 ^c	44.00	0.005	<0.001	<0.001

580 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative and artificial milk feeding.

581 ¹Standard error of the difference among means. Within a row means without a common superscript differ ($P < 0.05$).

582 ²BHB, beta-hydroxybutyrate; HDL, high density lipoproteins; LDL, low density lipoproteins.

583 ³Mathematically calculated: LDL = Cholesterol – HDL – (Triglycerides / 5); Globulin = Total Proteins – Albumin

584 **Table 5.** Effect of colostrum alternative and artificial rearing on animal and carcass performances in lambs.

Item ²	Type of lactation			Sex		SED ¹	P-value	
	NN	NA	AA	Males	Females		Rearing	Sex
Animal performance								
BW at birth (kg)	3.81	3.89	3.88	4.07	3.56	0.124	0.794	0.005
BW at weaning 45d (kg)	18.5	18.9	18.3	19.1	18.0	0.572	0.583	0.001
BW at fattening, 23 weeks (kg)	38.6 ^a	37.2 ^b	35.3 ^b	38.7	35.2	1.022	0.004	0.035
ADG from 0 to 45 days (g/d)	325	332	318	332	319	5.110	0.568	0.444
ADG from 45d to 23 weeks (g/d)	176 ^a	153 ^b	150 ^b	170	150	5.050	<0.001	0.047
Carcass performance								
Final BW (kg)	42.3 ^a	40.4 ^b	38.7 ^b	41.4	39.5	0.754	<0.001	0.155
Warm carcass weight (kg)	18.3	18.2	17.6	18.6	17.4	0.532	0.624	0.490
Dressing percentage (%)	43.1 ^b	45.3 ^a	46.2 ^a	45.3	44.2	1.390	0.052	0.311
Conformation ³	3.78	3.63	3.61	3.82	3.52	0.167	0.750	0.853
Fatness ³	2.72	2.74	2.76	2.76	2.76	0.166	0.971	0.495

585 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative and artificial milk feeding.

586 ¹Standard error of the difference among means. Within a row means without a common superscript differ ($P<0.05$).

587 ²BW, body weight; ADG, average daily gain.

588 ³EUROP classification: Conformation. E=5. U=4. R=3. O=2. P=1. Fatness: 1=1. 2=2. 3L=3. 3H=3.5. 4L=4. 4H=4.5. 5=5.

589 **Table 6.** Effect of artificial rearing on total tract digestibility (% in DM basis) in grazing
590 lambs (23 weeks of age).

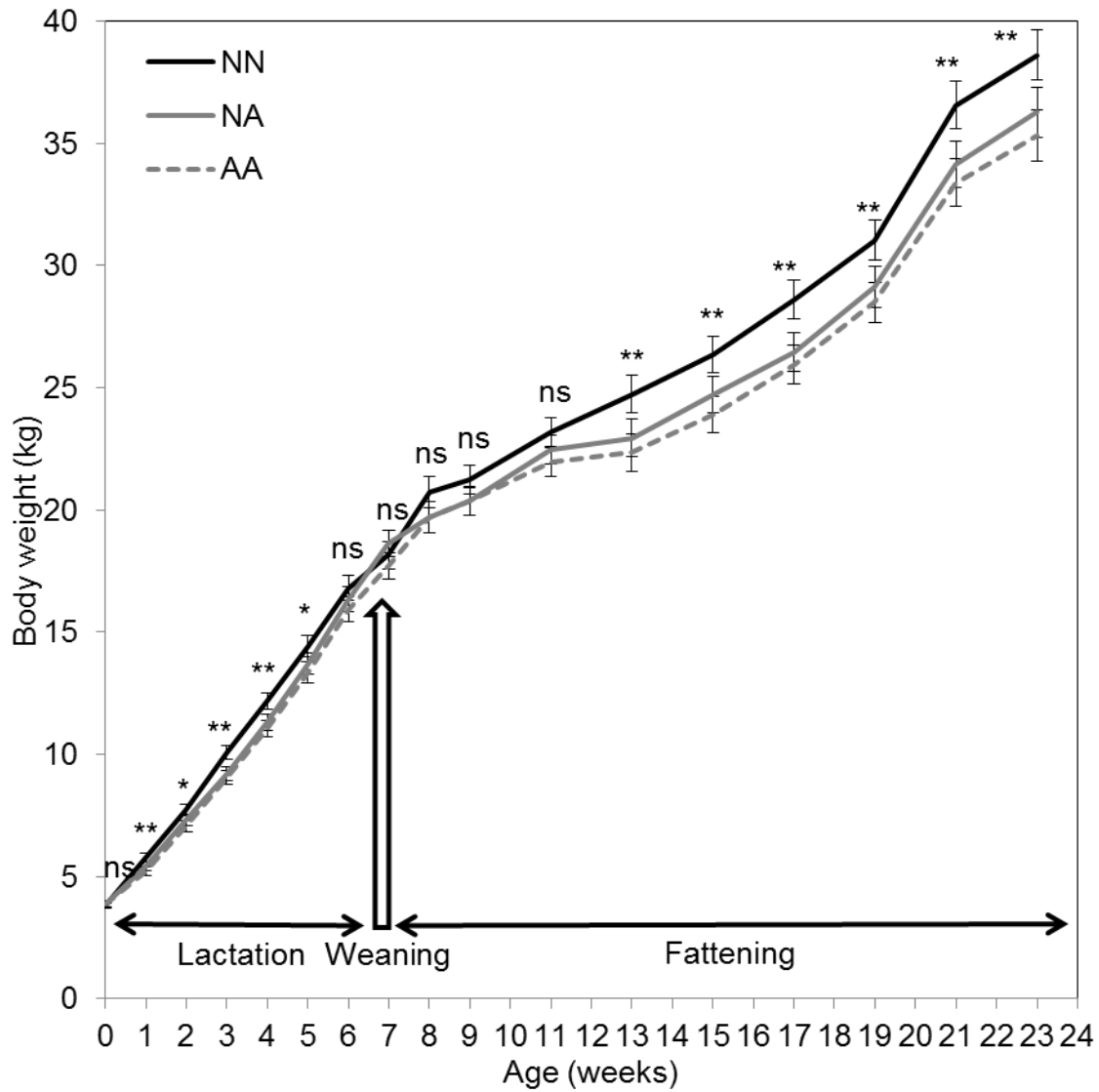
Item ²	NN	NA	AA	SED ¹	P-value
DM	66.3 ^a	62.6 ^b	65.0 ^a	0.83	<0.001
C	61.7 ^a	56.8 ^b	60.3 ^a	1.02	<0.001
N	75.5 ^a	73.2 ^b	75.1 ^a	0.69	0.003
NDF	51.7	50.7	53.8	1.36	0.143
ADF	38.4	34.2	36.5	2.47	0.327

591 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum
592 alternative and artificial milk feeding.

593 ¹Standard error of the difference among means. Within a row means without a common
594 superscript differ ($P<0.05$).

595 ²DM, dry matter; C, carbon, N, nitrogen, NDF, neutral detergent fibre; ADF, acid
596 detergent fibre.

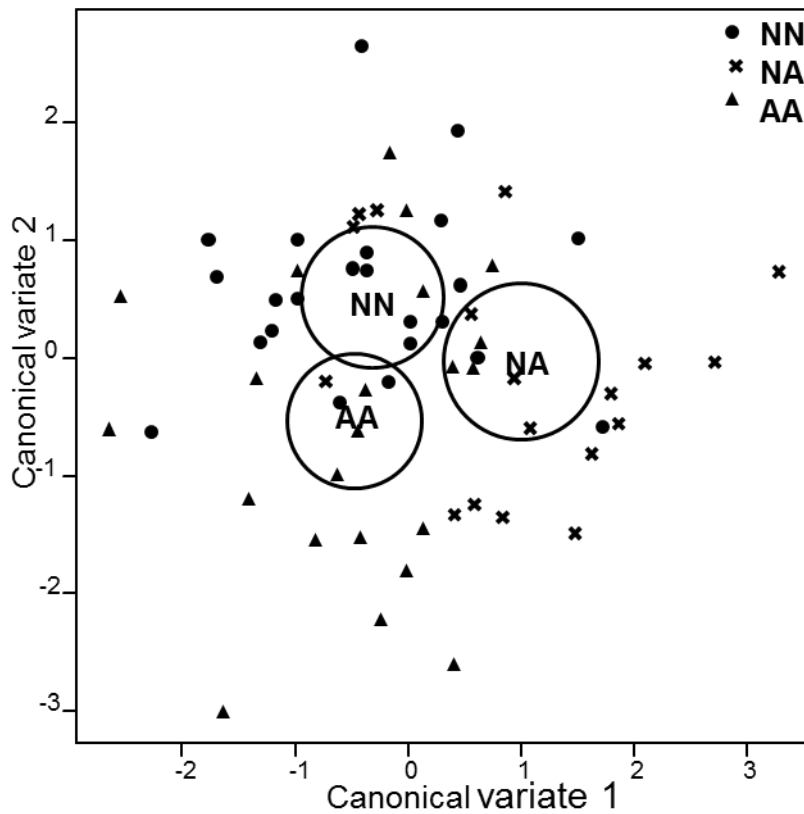
597 **Figure 1.** Effect of colostrum alternative and artificial rearing on lamb's growth. NN,
 598 natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative
 599 and artificial milk feeding. Standard error of the mean level of signification is depicted:
 600 ns, not significant, * P<0.05, ** P<0.01



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603 **Figure 2.** Canonical variate analysis illustrating the impact of nutritional intervention in
604 early life on the faecal FTIR spectra from lambs of 23 weeks of age. NN, natural rearing
605 (circles); NA, ewe colostrum and artificial milk feeding (crosses); AA, colostrum
606 alternative and artificial milk feeding (triangles). Big circles indicate the 99% confidential
607 interval of the mean for each treatment.



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620 **Short- and long-term effects of different colostrum and milk feeding strategies on**
621 **health and performance in growing lambs**

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623 Alejandro Belanche, Jessica Cooke, Eleanor Jones, Hilary J. Worgan and Charles J.
624 Newbold

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626

627 **Table S1.** Chemical composition (g/kg DM) of the main experimental feeds.

Items ¹	Creep-feed	Hay	Pasture
OM	926	936	904
CP	183	61	114
NDF	528	644	510
ADF	139	346	221
C/N ratio	15.0	45.4	24.0

628 ¹DM, dry matter; CP, crude protein, NDF, neutral detergent fibre; ADF, acid detergent
629 fibre; C/N ratio, carbon to nitrogen ratio.

630

631 **Table S2.** PERMANOVA illustrating the effect of colostrum alternative and artificial
632 lactation on the faecal FTIR spectra in grazing lambs (23 weeks old).

Type of lactation ¹	Pseudo-F	P-value
Treatment effect	1.79	0.079
Pair-wise comparisons		
NN vs NA	1.39	0.135
NN vs AA	1.31	0.130
NA vs AA	1.56	0.099

633 NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum
634 alternative and artificial milk feeding. Greater Psuedo-F and lower P-values indicates
635 differences between treatments.

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637