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

Alejandro Belanche[1, 2,*], Jessica Cooke[3], Eleanor Jones[1], Hilary J. Worgan[1] and Charles J. Newbold[1,4]

[1]IBERS, Aberystwyth University, SY23 3DA, Aberystwyth, UK.

[2]Estacion Experimental del Zaidín (CSIC), Profesor Albareda, 1, 18008, Granada, Spain

[3]Volac International Ltd., Orwell, Cambridgeshire, SG8 5QX, Royston, UK

[4]SRUC, Peter Wilson Building, King's Buildings, EH9 3JG.Edinburgh, UK

*Corresponding author: Alejandro Belanche a.belanche@csic.es

Short title: Effects of artificial rearing of lambs

Abstract

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

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

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

Implications

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

Introduction

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

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

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

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

Material and methods

Animals and diets

Triplet sibling lambs were used to provide similar genetic background, gestation environment and ewe colostrum in order to minimize the inter-animal variation across treatments. Thus, after pregnancy scanning, twenty-four pregnant Aberdale ewes carrying triplets were selected from the Aberystwyth University commercial flock. A total of 72 Aberdale-texel crossbreed lambs were born within an 8-day period (14th to the 22nd April). At birth umbilical cords were disinfected with iodine and lambs were weighed. One sibling of each triplet set was randomly allocated to 1 of 3 experimental treatments. During this allocation process sex and initial body weight of the lambs was considered resulting in similar sex distribution (average 13 males and 11 females per group) and birth weights (3.8±0.8 kg) across treatments. All three sibling lambs were kept with their mother in an individual pen during the first 24h after birth. Two siblings (NN and NA) were encouraged to suckle ewe colostrum by connecting them to a ewe´s teat four times over the first 24h (1, 2, 4 and 6 h after birth) until the gut filling was evident in order to ensure a high colostrum intake. Then, one of those siblings (NN) remained with its mother suckling ewe milk from birth to weaning, while the second sibling (NA) was separated from its dam after 24h and artificially reared with milk replacer. On the contrary, the third sibling (AA) was not encouraged to suckle ewe colostrum, instead it was immediately fed with 50g of colostrum alternative divided in two equal doses at 1h and 6h after birth followed by artificial rearing with milk replacer. In this latter group, no obvious signs of gut filling with ewe colostrum were noted suggesting a minimal intake of it. Colostrum alternative was freshly prepared by mixing 25g of product (Lamb Volostrum, Volac Ltd.) in 50ml of water at 30ºC and provided by a stomach tube at each time (1h and 6h after birth). Milk replacer was prepared by mixing

200g of milk powder (Lamlac Instant, Volac Ltd.) with water to make up 1 litre of reconstituted milk following the manufacturer instructions. During their first week of life all lambs had access to heat lamps and warm milk replacer (39ºC) offered *ad libitum* using temperature controlled feeders (Ewe 2 Feeder, Volac Ltd, UK). Lambs that did not suckle were stomach tubed and trained to suck from a teat connected to the milk feeder. After one week of age all lambs were able to suckle and milk replacer was offered *ad libitum* at room temperature (average 12ºC) using two buckets connected to four teats for each experimental group. These milk buckets were emptied twice a day and thoroughly cleaned and rinsed, using soap and hot water.

At 24h after birth, blood was sampled (see below), and all animals were tagged and intramuscularly injected with 1 ml of AD_3E (NAPHA Veterinary, UK) to prevent vitamin deficiency. Then, all lambs from the same treatment were placed together in a single pen (10m×12m) with clean and dry barley straw bedding and *ad libitum* access to creep feed (NuGro CCF, UK), ryegrass hay and water (chemical composition described in Supplementary Table S1). During the milk feeding stage all three groups of animals were physically separated from each other (1 m gap) but kept in the same building with an average temperature of 12ºC, relative humidity of 86% and an average of 10 hours of day light. Treatments NA and AA also had free access to milk replacer which was freshly prepared twice a day at 09:00h and 17:00h. Lambs from treatment NN shared a pen with their mothers that were fed twice a day with the same ryegrass hay and commercial concentrate (Wynnstay, High Production Ewes, UK). Ewes were physically separated from the NN lambs for 10 minutes during the concentrate feeding. Group intakes of milk replacer and creep feed were recorded daily until weaning. Animals were inspected daily for signs of disease. The severity of diarrheal events was recorded based on the following score index (Bentounsi et al., 2012): 1 corresponds to normal

lamb faeces in pellets, 2 corresponds to “soft” faeces (similar to cow pat), 3 corresponds to mild diarrhoea with semi-liquid faeces and 4 corresponds to profuse diarrhoea with liquid faeces. Animals with a score equal or above 3 received a single dose of intramuscular antibiotic treatment (Pen-Strep, Norbrook, UK). Lambs were weekly weighed using a digital balance to determine their growth during the entire duration of the experiment.

Animals were weaned at 45d of age by abrupt weaning and kept in the same building with the same solid feed for a further week. When lambs were on average 8 weeks of age, all experimental lambs were grouped together on the same ryegrass pasture (*Lolium perenne*) with free access to creep feed until 10 weeks of age but not thereafter. Thus all lambs grazed the same pasture over 5 months (from June to November). Animals belonging to a same sibling set were always sampled, weighed at the same time. Moreover, when the average body weight (BW) of a given set of siblings reached the optimum slaughter weight (approximately 40kg and between 23 to 31 weeks of age), all three lambs were slaughtered in a commercial abattoir. Carcass weight and performance was assessed at an official abattoir according to the EUROP classification (Johansen et al., 2006).

Sampling and analyses

Blood samples (5ml) were collected from the jugular vein at 24h after birth for IgG and blood cells measurements. Moreover, blood samples were also taken when animals reached 45d of age (weaning) and at 23wk of age (near the end of the fattening period). One blood subsample (2ml) was placed in a tube with anticoagulant (K_3-EDTA) mixed by inversion 10 times, kept at 4ºC and immediately analysed for haematology using a Mythic 18 Vet Haematology Analyser (Woodley Equipment Company Ltd., UK). This

analysis determined levels of the main blood cells and their morphotypes (see below). A second subsample (3ml) was placed in a tube without anticoagulant; serum was harvested by centrifugation at 2,000×*g* for 15min and stored at -20ºC until analysis. Serum metabolites were determined using RX Daytona^{+} equipment (Randox Laboratories Ltd. UK).

Colostrum (10ml) and milk (50ml) samples were obtained by hand milking from each ewe at 24h after the birth of the first lamb and at 45d post-partum, respectively. Samples were kept frozen and milk and colostrum composition (Table 1) was determined using a milk analyser (LactoScope Advance FTIR, Delta Instruments, Netherlands). Concentration of IgG in serum and colostrum was determined using the Sheep IgG ELISA 96 well plate kit (Gen Way, USA, reference GWB-OVI374) after dilution (4×10^{-4} and 4×10^{-6} for serum and colostrum respectively) and absorbance determination at 450nm using a plate reader (PowerWave XS2, BioTek, UK). Concentration of IgG was also estimated based on the serum density: Temperature corrected density (nD_{TC}) in serum samples (100µL) was measured in triplicate using an automatic digital refractometer (Reichert AR200 Ver 1.8, Ametek, Germany) and the estimated serum IgG concentration was obtained base on the regression equations described by Morril (2011): IgG (mg/ml) = $5919.1 \times nD_{TC} - 7946.1$

(Table 1 here)

Faecal analysis

At 23wk of age faecal grab samples were collected from each animal on two non-consecutive days, frozen and pooled by animal (30g DM approximately). On the same days as faecal sampling, ryegrass pasture was cut to 5 cm above soil level from 4 different locations of the field and immediately frozen for further analysis. The effect of

the experimental treatments on pasture digestibility was estimated using the acid insoluble ash as an internal marker (Thonney et al., 1979). For feed and faeces analyses, dry matter (DM) content was determined by drying in an oven at 105ºC for 24h. Organic matter (OM) concentration was determined by heating at 550ºC for 6h in a muffle furnace. Nitrogen and carbon concentration was measured by the Dumas combustion method (Elementar analyser, Vario MAX cube, Germany). Neutral-detergent (NDF) and acid-detergent fibre (ADF) were determined using an Automated Fiber Analyzer (ANKOM 2000, USA) using heat stable amylase and sodium sulphide.

For faecal fingerprint analysis, samples were analysed as previously reported (Belanche et al., 2017). Briefly, freeze dry samples were ground to a fine powder (IKA Analytical Mill, Staufer, Germany) and analysed by attenuated total reflectance (ATR) from 4000 to 600cm^{-1} using an Equinox 55 Fourier Transformed Infrared Spectrophotometer (Bruker Ltd, Coventry, UK)), and scanned using the Golden Gate ATR accessory (Specac Ltd., Slough, UK). Infrared settings and data collection were conducted as previously reported (Belanche et al., 2014). Fourier transformed infra-red (FTIR) spectra were imported into Matlab (version 2007b, The MathWorks Inc., Natick, USA), averaged, transformed to the first Savitsky-Golay derivative to smooth baseline noise and improve spectral resolution using a 13-point window, and then mean centre normalized (mean=1, Standard Deviation=1). Data were then analysed by non-parametric permutational multivariate analysis of variance using PRIMER-6 software (PRIMER-E Ltd., Plymouth, UK). Statistical signification was calculated after 999 random permutations of residuals under a reduced model using the Monte Carlo test. For graphical interpretation, principal component analysis was conducted and a Canonical variate analysis was performed based on the data compiled in the main principal components (Genstat 18th Edition, VSN International, Hemel Hempstead, UK).

Calculations and statistical analysis

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

Globulins = Total proteins – Albumin

LDL = Cholesterol – HDL – (Triglycerides / 5)

To evaluate the effect of experimental treatments on blood parameters, data were analysed using an repeated-measures procedure (REML) using Genstat 18th Edition (VSN International, Hemel Hempstead, UK) as follows:

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

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

Results

Animal health

At 24h after birth all animals remained in good health and no haematological differences were observed across treatments (Table 2). AA lambs tended to have a lower plasma IgG concentrations at 24h of age in comparison to NN and NA lambs when measured by refractometry (P=0.075) but did not reach statistical significance when measured with ELISA (P=0.135). Animals artificially reared (NA and AA) suffered a greater incidence of diarrhoea episodes than NN lambs from 2 to 5 weeks of age but this effect disappeared thereafter. Antibiotic usage was also higher for NA and AA lambs than for NN lambs (P<0.001) and the number of animals with recurrent diarrhoea which required more than 2 antibiotic doses were 0, 6 and 9 for NN, NA and AA lambs, respectively. No antibiotic treatment was required for any lamb from week 5 onwards.

(Table 2 here)

The age of the lambs exerted a major effect on the blood cell distribution (Table 3) and the concentration of most plasma metabolites (Table 4). At weaning animals had a greater concentration of red blood cells, haemoglobin, RBCDW, lymphocytes, platelets, thrombocrit and plasma levels of calcium, glucose, cholesterol, triglycerides, HDL, LDL, albumin, creatinine, amylase and alkaline phosphatase than animals at fattening (P<0.01). On the contrary, at fattening animals had a greater concentration of white blood cells, monocytes, granulocytes, MPV, PDW and plasma levels of BHB, total proteins, globulins and urea (P<0.001). However, artificial rearing also had some mid- and long-term effects on the animals´ health (Table 3). NN lambs had a greater haemoglobin (P=0.029), haematocrit (P=0.070), white blood cells (P=0.009) and calcium levels than NA and AA lambs, independently of the age considered. Moreover a

274 significant interaction was observed for several metabolites and haematological
275 parameters: at weaning NN lambs had greater RBCDW (P>0.001), BHB (P<0.001),
276 HDL (P=0.025) and amylase plasma levels (P<0.001), as well as lower MCHC
277 (P=0.012), PDW (P=0.013), LDL (P=0.009) and alkaline phosphatase (P=0.002) were
278 observed in NN lambs than in NA or AA but no such differences were observed at
279 fattening.
280 (Table 3 and Table 4 here)
281
282 *Animal performance*
283 Average group intake of milk replacer remained constant until week 3 (300 g DM/d per
284 lamb) and linearly increased thereafter reaching 550 g DM/d at weaning for AA and NA
285 groups while milk intake in NN lambs was not recorded. Group intake of creep feed also
286 remained low and constant until week 4 across treatments, and increased linearly
287 thereafter reaching an average of 256, 137 and 96 g DM/d at weaning for treatments
288 NN, NA and AA, respectively. No differences in body weight (BW) were observed at
289 birth across treatments, but NN lambs had a greater BW than NA and AA lambs from
290 week 2 to 5, these differences disappeared during the weaning stage and reappeared
291 from week 11 onwards (Figure 1). No differences in the average daily gain (ADG) were
292 observed before weaning (Table 5), but NN lambs had a greater ADG during the
293 fattening period calculated from weaning to 23 weeks of age (P<0.001). In terms of
294 carcass composition, NN lambs had a higher slaughter weight than NA and AA lambs
295 (P<0.001), but NN lambs tend to have a lower dressing percentage resulting in similar
296 carcass weight and conformation across treatments. Male lambs tended to have a
297 greater BW at birth and ADG during the fattening period (P=0.047) however no
298 differences were observed in carcass conformation.

(Figure 1 and Table 5 here)

Pasture utilization

The chemical structure of the faecal samples tended to differ (P=0.079) between treatments based on the PERMANOVA analysis of the FTIR spectral data (Supplementary Table 2). Canonical variate analysis (Figure 2) compiling the information of the first 15 principal components (representing 98.1% of the total variance) showed that these differences were more obvious between NA and the other two experimental groups. In terms of pasture digestibility (Table 6), values were always highest for NN lambs: NN and AA lambs had higher digestibility for DM (P<0.001), C (P<0.001) and *N* (P=0.003) than NA lambs, while no differences were observed in NDF and ADF digestibility.

(Figure 2 and Table 5 and Table 6 here)

Discussion

Effect of colostrum alternative

Colostrum products have been shown to provide a degree of passive immunity transfer (Seymour et al., 1995, Castro et al., 2007), although the results vary greatly depending on the product used, colostrum preservation methods, dosage techniques and inter animal variation (Arguello et al., 2004b). As a result, colostrum products that typically contain lacteal-derived or plasma-derived IgG are classified as either colostrum replacers or colostrum supplements depending on their ability to raise serum IgG concentration above a certain threshold (typically 15 mg/ml in lambs) (Alves et al., 2015). Colostrum supplements (as in our study) can be used to increase the amount of IgG fed to lambs when only low or medium quality / quantity colostrum is available.

However, supplements cannot replace high quality colostrum which is still considered the gold standard for feeding newborn lambs (Jones et al., 2004). Our study aimed to simulate two real scenarios in the artificial rearing of lambs: one (NN and NA lambs) consisting of maximizing colostrum intake by encouraging lambs to suckle for at least 4 times from the ewe; and an alternative strategy (AA lambs) based on colostrum alternative supplementation of lambs with an insufficient intake of ewe colostrum. To achieve this later situation, AA lambs were not encouraged to suckle and had to compete with their two siblings for the remaining ewe colostrum.

A rapid change in the colostrum composition to transitional milk has been described during the post-partum period (Alves et al., 2015). In our study, despite the late sampling of ewe colostrum (24h after the first lamb was born), the IgG concentrations (average 42.2 g/l) were comparable to published literature (from 15.7 to 65 g/l) in which the samples were collected just after parturition (Vatankhah, 2013, Alves et al., 2015, Hernández-Castellano et al., 2015), possibly as a result of a higher colostrum production in high prolific ewes. As a result, only one lamb had an IgG concentration below 15 mg/ml at 24h after birth suggesting effective overall passive immunity transfer across treatments (Alves et al., 2015). This may explain the lack of differences in terms of growth, haematology parameters and blood metabolites levels between NA and AA lambs, as well as, the absence of deaths before weaning. Moreover, the high level of easily digestible energy and protein in the colostrum alternative also seems to represent an important source of nutrients for the lambs during its first hours of life to maintain body temperature and good health (Jones et al., 2004). Thus, the supply of colostrum alternative after birth can be considered an appropriate strategy to prevent health problems and maximize the number of lambs weaned per ewe when ewe colostrum is insufficient.

Effect of artificial rearing on lamb´s health

This study does not attempt a direct comparison of the effects of milk replacer vs maternal milk since artificial rearing involves the replacement of the contributions made by the ewe which are essential to the growth and development of the lamb. This not only includes the feed supply but also the warmth, shelter and “mothering” normally provided by the ewe. Our experiment showed a greater incidence of diarrhoea events in artificially reared lambs than those reared on the ewe. These diarrhoea episodes appeared from week 2 to week 5; they were very mild (<2.0 scored) and required an average of 1.2 antibiotic doses per lamb, whilst antibiotic usage in NN lambs was negligible. Although these diarrheal events did not trigger any deaths, they could explain the lower ADG for NA and AA lambs during the first 5 weeks. Similar diarrhoea events starting at 2 weeks of age have been described in calves and various pathogens compatible with enteric infections have been identified in the necropsy (i.e. *Salmonella*, *Cryptosporidium parvum*, *Escherichia coli* and coronavirus) (Quigley et al., 2006). None of the lambs required the use of antibiotics from week 5 onwards, and the study of the rumen microbial community showed no residual antibiotic effects at 45d and 23wks of age (data not shown). Thus the potential long-term effect of antibiotics on blood metabolites and animal performance seems to be negligible under our experimental conditions.

Various studies have investigated the effect of different artificial milk feeding strategies to prevent diarrheal events and to improve animal performance: Jasper and Weary (2002) concluded that *ad libitum* nipple feeding of whole milk to dairy calves vs restricted can increase weight gain with no diarrheal problems nor detrimental effects on feed intake after weaning. While Quigley (2006) observed that calves fed a variable

amount of milk replacer (peaking at 3 weeks of age with 908 g/d) had greater ADG but also increased incidence of diarrhoea that required added veterinary treatment in comparison to those fed a fixed amount (454 g/d). Thus, it seems that our artificial rearing strategy based on the *ad libitum* access to milk replacer might explain the incidence of moderate diarrhoea but did help to prevent feed competition between lambs, since lambs in contrast to calves tend to be reared in groups with a large number of animals. More research is needed to assess whether these diarrheal events could be minimized by using alternative rearing systems such as automatic feeding machines.

Although most lambs remained in good health from birth to slaughter, the haematological analysis revealed that NN lambs had higher levels of white blood cell at weaning in comparison to artificially reared lambs (+21.6%), and those differences persisted during the fattening period (+10.5%). It has been shown that colostrum and milk have viable cells, including neutrophils and macrophages, which secrete a range of immune-related components (Stelwagen et al., 2009). Our findings are in line with this observation and suggest that direct contact with adult animals in NN may also represent an important exposure to antigens which may help in the immune system development of young lambs with long-lasting effects on the levels of white blood cells. Moreover, artificially reared lambs had lower haemoglobin levels (-2.8%) and haematocrit (-5.3%) at weaning in comparison to NN lambs. The variation in the size of red cells (anisocytosis) provided an insight of the potential reasons of slight signs of anaemia. Since neither the size of the red blood cells (MCV) nor the amount of haemoglobin per cell (MCH) were affected, it seems that the normocytic anaemia was very mild and partially compensated by a greater amount of haemoglobin per unit of volume (MCHC +2.6%). Despite this lack of severity, artificially reared lambs still had lower levels of haemoglobin (-3.3%) and haematocrit (-0.4%) during the fattening period suggesting a

399 small but long term effect of the type of rearing strategy on the animals health. On the
400 contrary, NN lambs had a higher coefficient of variation in red blood cell distribution
401 width (RBCDW, +20.0%) which is compatible with early stages of iron deficiency at
402 weaning in animals having limited amounts of milk (Blaxter et al., 1957), possibly as a
403 result of a lower milk intake and lower iron content in the ewe milk in comparison to
404 lambs fed milk replacer *ad libitum*. This observation was supported by the lower blood
405 calcium concentration in NN lambs at weaning (-6.7%) and fattening (-4.2%). Increases
406 in plasma glucose and urea concentrations have been associated with higher artificial
407 milk intake in calves (Quigley et al., 2006). However, in our study all experimental
408 treatments had similar glucose, urea and total protein levels at weaning, possibly
409 because a lower milk intake in NN lambs during late milk feeding period in comparison
410 to those fed milk replacer was compensated by a greater creep feed intake (256 vs 116
411 g/d). Our experiment indicates that protein and energy sources included in the milk
412 replacer were highly digestible since no differences in the plasma concentration of
413 metabolites related with the protein (total proteins, albumin, globulin, creatinine, urea
414 and ammonia) and energy (glucose) metabolism were detected across treatments.
415 These findings agree with the similar content of urea nitrogen, total protein, albumin and
416 globulin in the serum of lambs fed milk replacers made up of milk protein or other
417 protein sources (Huang et al., 2015). Most of the milk bypasses the rumen through the
418 oesophageal groove, thus high milk intake in artificially reared lambs may increase the
419 amino acid flow to the small intestine leading to an increase in the deamination
420 processes occurring in the liver as was reflected by increased levels of alkaline
421 phosphatase (+30%) as an indicator of the liver stress (Reichling and Kaplan, 1988). On
422 the contrary, solid feed (carbohydrates and proteins) is fermented in the rumen
423 producing volatile fatty acids and ammonia as the main fermentation end product. Thus,

the increased levels of β-hydroxy-butyrate in NN at weaning (+2.6-fold times) suggest a greater physiological and fermentative development of the rumen. Although cholesterol and triglyceride concentrations were unaffected by the experimental treatments, artificially reared lambs had lower levels of HDL (-13%) and higher levels of LDL (+38%) at weaning than NN lambs. Increased blood levels of LDL is considered a circulatory risk factor which is mainly determined by diet, physical activity, genetics, sex and age (Sigurdardottir et al., 2002). Overall, our data also showed that most of the haematological and metabolite differences observed at weaning were transient and tended to disappear later in life with no further effects on the animal's health.

Effect of artificial rearing on productive performance

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

However, differences in BW gain reappeared after weaning despite all lambs being grazed together on the same pasture. As a result, NN lambs had a greater growth during the fattening period (+16%) and higher BW from week 13 onwards. Several

reasons could explain these findings: i) The greater solid feed intake observed in NN lambs at weaning (256 vs 116 g DM/d) has been described as a key factor which promotes the rumen physiological development in calves and facilitates a smooth transition to the solid diet (Khan et al., 2011). ii) The direct contact with adult animals represents a source of microbes (i.e. bacteria, protozoa, methanogens, anaerobic fungi) which are crucial for the development of the symbiotic rumen microbiota (Belanche et al., 2010, Belanche et al., 2011). iii) Adult animals teach young animals in terms of feeding behaviour since the presence of adult companions has been reported to increase solid feed intake and performance of calves before and after weaning (Vieira et al., 2012) as was noted in our experiment.

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

474 plays a greater role on animal resilience and productivity during the post-weaning
475 processes as well as later in life.
476
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481
482 **Declaration of interest**
483 There is not conflict of interests.
484
485 **Ethics committee**
486 All animal procedures were carried out according to the Home Office Scientific
487 Procedures, Act 1986 and protocols were approved by the Aberystwyth University
488 Ethics committee (PLL 40/3653; PIL 40/9798).
489
490 **Software and data repository resources**
491 None of the data were deposited in an official repository
492
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600 **Table 1.** Colostrum and milk composition from sheep

	Colostrum		Milk	
	Natural[1]	Alternative[2]	Natural[1]	Replacer[3]
Crude protein, %	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
Immunoglobulin G, g/l	42.2	32.1		

601 [1]Natural colostrum and milk sampled at 24h and 45d after parturition, respectively

602 [2]Values after mixing 25g of colostrum alternative with 50 ml of water

603 [3]After mixing 200g of milk replacer with water to make up 1 lire of reconstituted milk

604 [2-3]Figures obtained experimentally which may differ from the declared composition

605 **Table 2**. Effect of colostrum alternative and artificial rearing on plasma IgG levels and
606 haematology at 24h after birth and incidence of diarrhoea in lambs.

Type of rearing	NN	NA	AA	SED	*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 IgG2 (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[1]					
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)[2]	0.08[b]	0.96[a]	1.42[a]	0.333	<0.001

607 Abbreviations: NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA,
608 colostrum alternative and artificial milk feeding; IgG, Immunoglobulin G; SED, Standard
609 error of the difference among means. Within a row means without a common
610 superscript differ (*P*<0.05).
611 [1]Diarrhoea score: 1 absence, 2 very mild, 3 moderate and 4 severe.
612 [2]Intramuscular Penicillin-Streptomycin

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

	Weaning (45 days)			Fattening (23 weeks)				*P*-value		
Type of rearing[1]	NN	NA	AA	NN	NA	AA	SED	Rearing	Age	R×A
Red blood cells (10^6/µl)	11.4	11.4	11.7	11.3	10.9	11.1	0.233	0.357	0.002	0.323
Haemoglobin (g/dl)	11.5	11.0	11.3	11.1	10.6	10.8	0.176	0.029	0.002	0.982
Haematocrit (%)	38.3	35.8	36.7	36.2	34.6	37.5	1.441	0.07	0.401	0.417
MCV (fL)	33.6	31.5	32.1	32.1	31.9	34.0	1.448	0.305	0.723	0.299
MCH, (pg)	10.1	9.72	9.77	9.83	9.74	9.78	0.201	0.241	0.555	0.577
MCHC (%)	30.0^{b}	30.8^{a}	30.8^{a}	30.6^{a}	30.6^{a}	30.7^{a}	0.211	0.006	0.448	0.012
RBCDW (%)	25.4^{a}	20.0^{b}	20.7^{b}	17.9^{c}	18.2^{c}	17.9^{c}	0.523	<0.001	<0.001	<0.001
White blood cells (10^3/µl)	7.95	6.31	6.76	8.91	8.40	7.73	0.571	0.009	<0.001	0.273
Lymphocytes (%)	56.5	56.9	54.4	53.2	47.6	51.1	2.398	0.355	<0.001	0.121
Monocytes (%)	11.6	11.9	10.9	13.7	14.7	14.4	0.620	0.371	<0.001	0.181
Granulocytes (%)	31.9	31.2	34.7	33.1	37.6	34.5	2.114	0.352	0.043	0.066
Platelets (10^3/µl)	1982^{a}	1419^{b}	1695^{ab}	548^{c}	616^{c}	639^{c}	182.7	0.129	<0.001	0.054
MPV (fl)	5.20	4.90	4.71	5.71	5.98	5.75	0.363	0.662	<0.001	0.419
Thrombocrit	1.10	0.72	0.82	0.29	0.33	0.62	0.197	0.265	<0.001	0.078
PDW (%)	30.1^{d}	36.0^{c}	34.9^{c}	46.0^{a}	42.1^{b}	44.0^{ab}	2.437	0.678	<0.001	0.013

Abbreviations: NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative and artificial milk feeding; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RBCDW, red blood cell distribution width; MPV, mean platelets volume; PDW, platelet distribution width; SED, Standard error of the difference among means; R×A , Interaction rearing system and age. Within a row means without a common superscript differ (P<0.05).

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

	Weaning (45 days)			Fattening (23 weeks)				*P*-value		
Item[1]	NN	NA	AA	NN	NA	AA	SED[1]	Rearing	Age	R×A
Calcium (mM)	2.33	2.52	2.48	1.84	1.92	1.93	0.122	0.067	<0.001	0.832
Energy										
Glucose (mM)	5.47	5.89	5.75	3.50	3.56	3.56	0.319	0.438	<0.001	0.732
BHB (µM)	265[b]	100[c]	100[c]	342[a]	372[a]	394[a]	30.56	<0.001	<0.001	<0.001
Lipids (mM)										
Cholesterol	2.82	2.91	2.79	1.27	1.31	1.23	0.163	0.683	<0.001	1
Triglycerides[2]	0.78	0.72	0.75	0.24	0.21	0.24	0.063	0.512	<0.001	0.909
HDL	1.91[a]	1.65[b]	1.66[b]	0.62[c]	0.65[c]	0.61[c]	0.092	0.16	<0.001	0.025
LDL[1]	0.76[b]	1.11[a]	0.98[a]	0.60[c]	0.61[c]	0.57[c]	0.090	0.032	<0.001	0.009
Proteins, (g/l)										
Total Proteins	45.4	46.7	46.3	65.3	67.3	66.6	2.646	0.659	<0.001	0.986
Albumin	32.9	33.5	33.3	30.2	31.1	31.2	1.061	0.55	<0.001	0.919
Globulin[1]	12.5	13.3	13.0	35.1	36.2	35.4	1.803	0.771	<0.001	0.985
Creatinine (µM)	83.0	85.8	87.9	78.3	80.4	78.5	4.802	0.597	0.029	0.811
Urea (mM)	3.85	3.95	3.82	9.98	9.85	10.1	0.357	0.96	<0.001	0.756
Ammonia (µM)	83.6	81.9	85.2	84.9	86.0	89.7	5.704	0.593	0.306	0.928
Enzymes (U/l)										
Amylase	25.7[a]	20.3[b]	18.3[b]	12.5[c]	10.8[c]	12.6[c]	1.676	0.021	<0.001	<0.001
L-lactate dehydrogenase	1171	1238	1112	1163	1093	1098	64.69	0.302	0.136	0.248
Alkaline Phosphatase	637[b]	841[a]	819[a]	177[c]	184[c]	183[c]	43.39	0.002	<0.001	0.002

619 Abbreviations: NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative and artificial milk feeding;
620 BHB, beta-hydroxybutyrate; HDL, high density lipoproteins; LDL, low density lipoproteins; SED, Standard error of the difference among
621 means; R×A , Interaction rearing system and age. Within a row means without a common superscript differ (P<0.05).
622 [1]Mathematically calculated: LDL= Cholesterol – HDL – (Triglycerides / 5); Globulin = Total Proteins – Albumin

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

	Type of lactation			Sex			P-value	
Item[2]	NN	NA	AA	Males	Females	SED[1]	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, 45 days (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[1]	3.78	3.63	3.61	3.82	3.52	0.167	0.750	0.853
Fatness[1]	2.72	2.74	2.76	2.76	2.76	0.166	0.971	0.495

Abbreviations: NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA, colostrum alternative and artificial milk feeding; BW, body weight; ADG, average daily gain; SED, Standard error of the difference among means. Within a row means without a common superscript differ ($P<0.05$).

[1]EUROP 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.

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

Item	NN	NA	AA	SED[1]	P-value
Dry mater	66.3[a]	62.6[b]	65.0[a]	0.83	<0.001
Carbon	61.7[a]	56.8[b]	60.3[a]	1.02	<0.001
Nitrogen	75.5[a]	73.2[b]	75.1[a]	0.69	0.003
Neutral detergent fibre	51.7	50.7	53.8	1.36	0.143
Acid detergent fibre	38.4	34.2	36.5	2.47	0.327

630 Abbreviations; NN, natural rearing; NA, ewe colostrum and artificial milk feeding; AA,
631 colostrum alternative and artificial milk feeding; SED, Standard error of the difference
632 among means. Within a row means without a common superscript differ (P<0.05).

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

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

Figure 1 hi-resolution

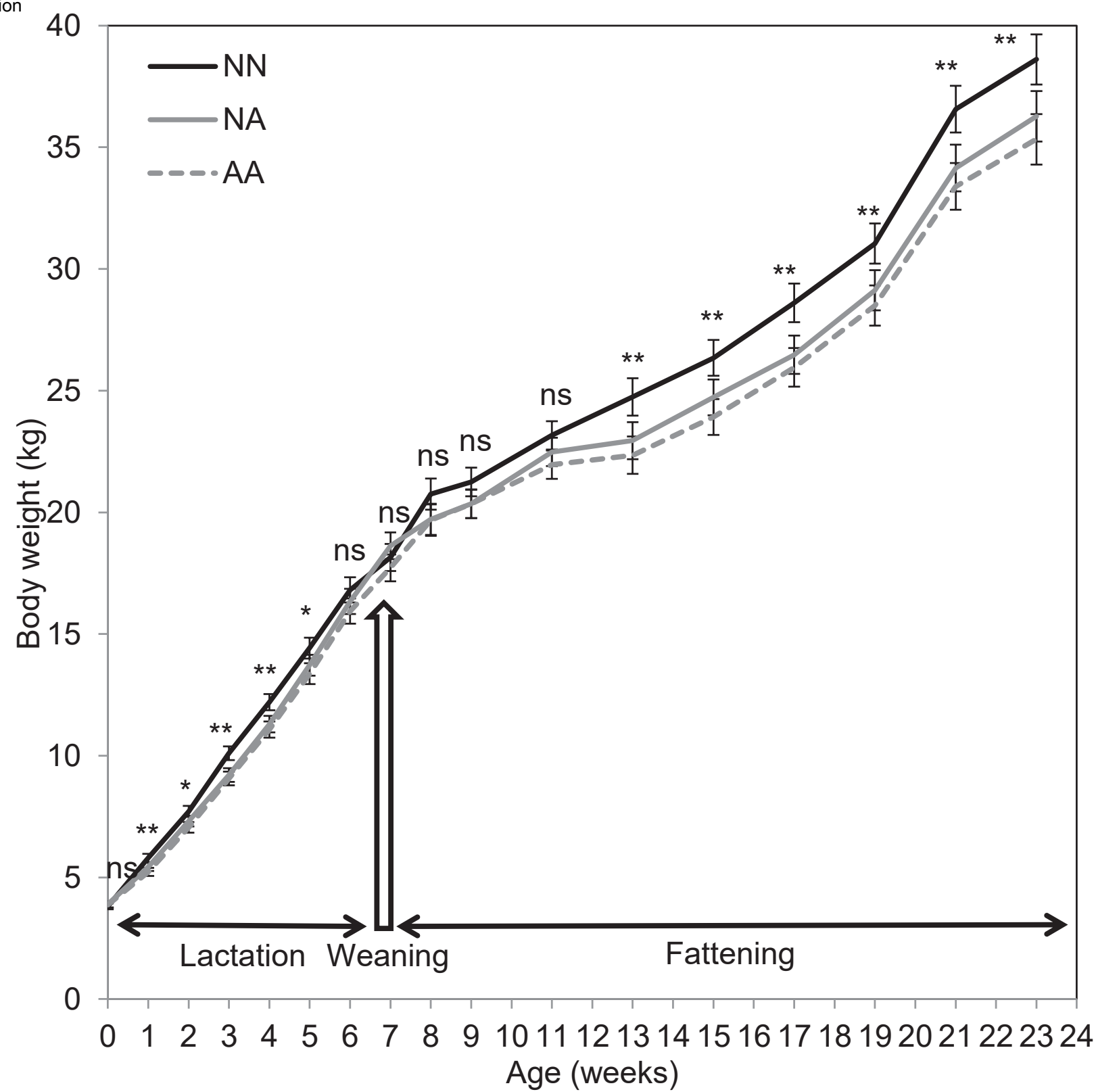
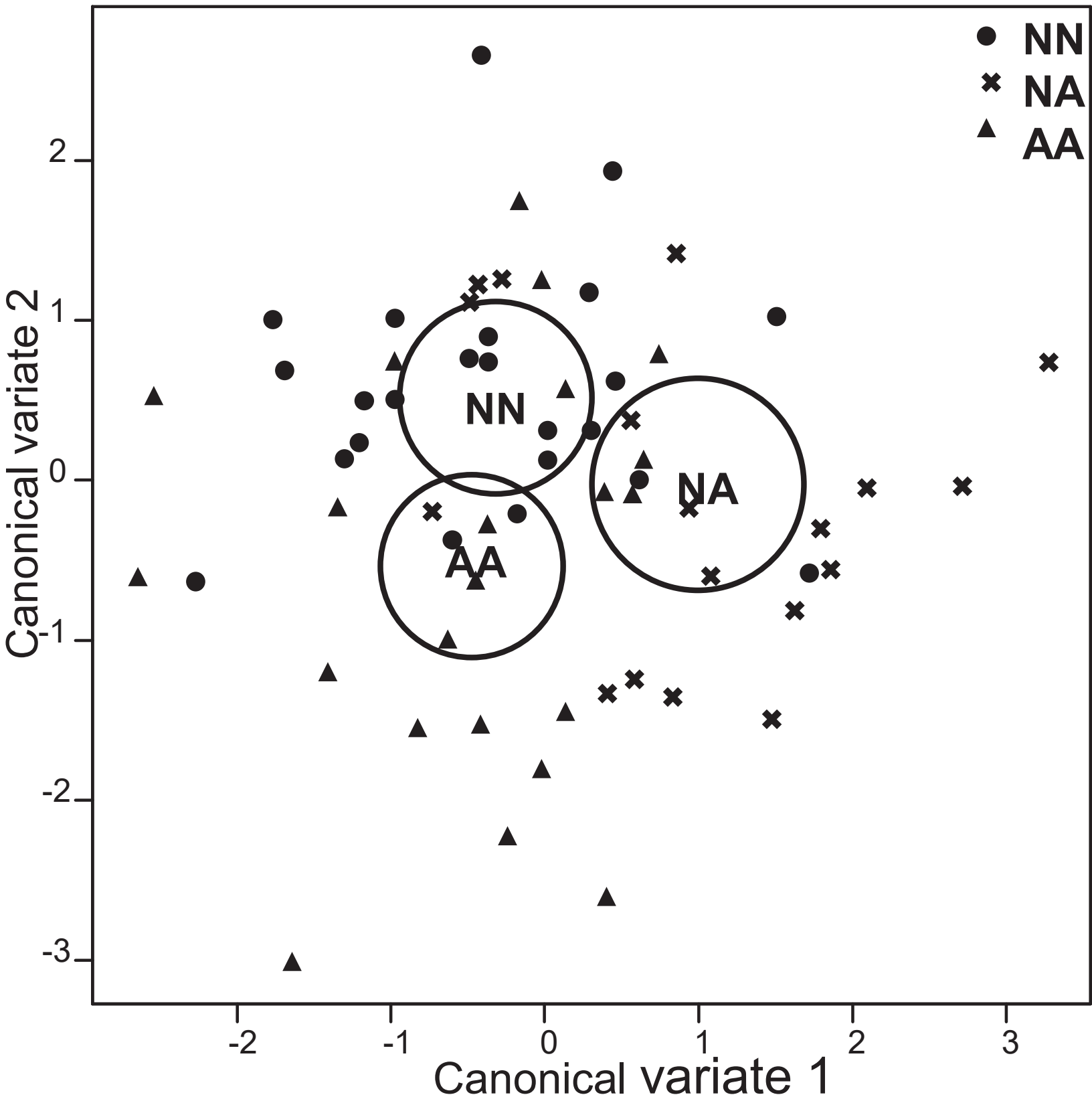


Figure 2 hi-resolution



ANIMAL Journal, SUPLEMENTARY MATERIALS

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

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

Table S1. Chemical composition (g/kg DM) of the feeds consumed by the experimental lambs

Items	Creep-feed	Hay	Pasture
Organic matter	926	936	904
Crude protein	183	61	114
Neutral detergent fibre	528	644	510
Acid detergent fibre	139	346	221
Carbon / Nitrogen ratio	15.0	45.4	24.0

Table S2. Permutational analysis of variance illustrating the effect of colostrum alternative and artificial lactation on the faecal Fourier-Transform Infrared spectra (FTIR) in grazing lambs (23 weeks old).

Type of lactation	**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

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