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Impact of early weaning on rumen fermentation profiles of artificially reared lambs

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Abstract

This study investigated the impact of weaning age on rumen fermentation profiles in lambs. Mixed-sex lambs (n=32) were randomly allocated to one of two weaning groups: early-weaning (4wk) and control (6wk). Milk replacer (24% CP and 25% fat, DM basis) was offered at 20% of initial body weight. Lambs were weaned at either four or six weeks after commencement of the trial using a three-week step-down procedure, initiated at the start of week 2 (early weaned group) or week 4 (control group). Lambs were individually penned indoors and concentrate and chopped meadow hay were offered *ad libitum* and individual feed intakes recorded until week six. At the end of week six, lambs were moved onto a ryegrass-based pasture and transitioned to an all pasture diet by week ten. Rumen contents were collected at slaughter, half of the lambs from each group at week four and sixteen, to determine rumen pH, and short chain fatty acid (SCFA) and ammonia concentrations. Ammonia concentrations were 132% greater ($P<0.05$) in early weaned than control lambs at week four, but did not differ at week sixteen. Weaning age did not affect ruminal pH and SCFA total concentrations and proportions at week four or sixteen. The results suggest that ruminal fermentation can be established in lambs weaned off milk by four weeks using a step-down weaning method.

Keywords: Early weaning; artificial rearing; solid feed intake; rumen fermentation

Introduction

Early weaning of artificially reared lambs offers the potential to decrease rearing costs of lambs in commercial dairy sheep production systems. Dietary transition from liquid milk to solid feed is an important component of a successful rearing program in ruminants (Davis & Drackley 1998). Studies of rumen development have indicated that the beginning of solid feed intake and production of fermentation by-products stimulates rumen differentiation and facilitates weaning transition in young ruminants (Khan et al. 2011). However, how weaning age influences solid feed intake and, thereby, rumen fermentation profiles during transition from milk to pasture is not yet fully understood in artificially reared lambs.

Weaning off milk triggers behavioural, metabolic and physiologic changes in young ruminants to support a transition from a reliance on glucose (lactose) supplied from milk to the short chain fatty acids (SCFA) derived from fermentation of solid feed in the rumen (Baldwin et al. 2004). The ingestion of solid diets stimulates the production of fermentation byproducts (SCFA and ammonia [NH_3]) and changes in ruminal pH (Van Soest 1994). Increases in the production of ruminal SCFA and NH_3, as well as changes in pH, stimulate papillae development, facilitating the metabolic adaptations required for weaning off milk (Liu et al. 2016). In artificially reared lambs, solid-food intake is affected by management practices including access to milk and starter diets (Walker & Hunt 1981; Wang et al. 2016), and weaning age and method (Lane et al. 1986; Bimczok et al. 2005).

A better understanding of the effects of pre-weaning management practices on ruminal fermentation of young lambs is important to optimise weaning transitions. No studies have yet investigated the effect of age at weaning on rumen fermentation profiles of artificially reared lambs during transition from milk to pasture. The aim of this study was to investigate the effect of weaning age on rumen fermentation profiles in artificially reared lambs.

Materials and methods

Animals and trial design

All animal manipulations in this study including animal welfare and husbandry, experimental procedures and sampling were reviewed and approved (AE 13233) by the Animal Ethics Committee of AgResearch Grasslands, Palmerston North, New Zealand.

Thirty-two mixed-sex twin-born lambs from composite ewes, one twin per ewe (16 males and 16 females), were sourced from a commercial farm in the Hawke's Bay region of New Zealand on the same day. Lambs were separated from their dams at 5 ± 2 days of life and randomly allocated to one of two weaning groups (n=16/group): early weaning (EW; 4 weeks) and control (Ctrl; 6 weeks) balanced by sex and starting body weight. Each lamb was reared at individual pen (~1.1 m^2) with kiln-dried wood shavings as bedding. Animals remained indoors with *ad libitum* concentrate and hay until week six, followed by a ten-week outdoor period where both groups were managed as one mob on pasture.

Diets and feeding management

Reconstituted (200 g/L) milk replacer (Anlamb, Fonterra Ltd., Auckland, New Zealand) was individually fed to lambs at 20% of their initial body weight (BW). Gradual weaning off milk was achieved over a three-week period by reducing 25% of milk allowance per week prior to completely removing milk by week four or six of the experiment. Concentrate (mash form; barley, maize, oats, soya, brollard, molasses and general premix) and chopped meadow hay (Wenham Grain & Seed, Palmerston North, New Zealand) were offered *ad libitum* in individual feeders from day one to week six during the indoor period. At the end of week six, lambs were moved as one mob onto a ryegrass/white clover pasture and transitioned to an all pasture diet by gradually removing concentrate and meadow hay by week ten. The lambs grazed on the pasture in a mob until week sixteen. Individual dry matter intakes (DMI) of concentrate and hay were recorded daily until week six. Samples from each batch of milk replacer, concentrate and hay were analysed for chemical composition (Table 1) by wet chemistry methods (Hill Laboratories Ltd, Hamilton, New Zealand). The chemical composition of the pasture was analysed by Near Infrared Reflectance Spectroscopy (NIRS; AgResearch, Palmerston North, New Zealand; Table 1) in accordance with the methods of Corson et al (1999).

Rumen pH, short chain fatty acids (SCFA) and ammonia (NH_3)

Eight animals per weaning group were randomly selected and euthanised by captive bolt stunning and exsanguination at weeks four (last day of milk for the EW lambs) and sixteen of the trial. Ruminal contents were collected to evaluate pH, SCFA and NH_3. At week sixteen, pH was measured using a portable pH meter (EZDO-7011 waterproof tester; GOnDO Electronic CO. Ltd, Taipei, Taiwan) immediately after sample collection, however, pH at week four was not measured due to limited amount of rumen contents available. For SCFA and NH_3 analyses, a subsample was snap frozen and stored at -20°C. Samples were prepared as described by Hammond et al. (2013). Short chain fatty acids were determined as described by Attwood et al. (1998) in a Hewlett-Packard 6890 series gas chromatograph equipped with an auto-sampler fitted with a Zebron ZB-FFAP 30.0m x 0.53mm I.D. x 1um film column and a flame ionisation detector (Tavendale et al., 2005). Ammonia was analysed using a modified and downscaled version of the colorimetric method used by Weatherburn (1967). A standard curve was prepared using 0, 1, 2, 4, 6, 9, 12, 15 mM NH_4^+ using $(NH_4)_2SO_4$; triplicate reactions per sample were prepared adding 100 µl of phenol nitroprusside solution, 10 µl of sample or standard and 100 µl of alkaline hypochlorite into a round bottom plate; the mix was homogenised and then incubated for 30 min at 37°C. Two 75 µl aliquots from each reaction were transferred on to a microtitre plate (three plates in total, two columns on each plate for each reaction); and their absorbance read at 625 nm.

Table 1 Chemical composition (% DM) of the milk replacer, concentrate and hay offered to pre-weaned lambs, and forage grazed by post-weaned lambs.

	Milk	Concentrate	Hay	Forage
Dry matter[1]	94.9	88.9	88.2	12.7
Crude protein[2]	24.0	20.1	11.1	31.7
ADF[3]	-	6.4	35.9	18.1
NDF[4]	-	15.7	55.2	40.5
Organic matter[5]	94.5	88.9	91.0	87.0
Soluble sugars[6]	40.5	5.8	5.4	7.3
Starch[7]	-	35.6	-	-
Ether extract[8]	25.0	2.7	1.6	5.7
Ash[9]	5.5	11.1	9.1	10.9

[1] AOAC 945.15
[2] AOAC (OMA) 992.15, 18th Edition.
[3] Acid detergent fibre; AOAC (1990) 7.074
[4] Neutral detergent fibre; AOAC (1990) 7.074
[5] Clarke, T., Flinn, P.C. and McGowan, A.A. 1982. Low cost pepsin-cellulase assays for prediction of digestibility of herbage. Grass and Forage Sciences 37:147-150.
[6] Paul, A.A and Southgate, D.A. The Composition of Foods. 4th Edition, 1978.
[7] AOAC Method 996.11
[8] Total Fat* Subcontracted test, Cawthron Institute, Nelson
[9] AOAC 942.05, 19th Edition

Statistical analysis

Data were analysed using the residual maximum likelihood algorithm in the linear mixed effect models, employing the NLME package of R (R Core Team, 2013; Pinheiro et al., 2015). Data from average feed DMI during weeks four and six, and ruminal fermentation profiles at each slaughter sampling time point were analysed fitting a linear mixed model with weaning group and sex as fixed effects and animal as a random effect. Additionally, fermentation profiles between sampling times were compared fitting a linear mixed model with age at sampling as a fixed effect, and animal as a random effect. Weaning group, sex, age at sampling, and the interaction between weaning group and sex were assessed, and predicted means from the model together with estimated standard errors, were obtained and compared using the PREDICTMEANS package of R (Luo et al., 2014).

Results

Results are presented as the mean ± standard error of the difference. Early weaned lambs consumed 52% (0.1 vs 0.2±0.07 kg/d; P=0.08) and 74% (0.1 vs 0.5±0.09 kg/d; P<0.01) less hay dry matter than did Ctrl lambs at week four and week six, respectively. Concentrate DMI did not vary between EW and Ctrl lambs at week four (1.5 vs 1.1±0.30 kg, P=0.27) and week six (4.6 vs 4.5±0.63 kg, P=0.98). Total solid food DMI (concentrate and hay) was similar between EW and Ctrl lambs at week four and six. No sex effect or weaning group by sex interactions were observed for DMI.

Ruminal SCFA concentrations and proportions, NH_3 concentrations and pH of EW and Ctrl lambs at week four and sixteen are given in Table 2. Ammonia concentrations were 2.3 times greater (P=0.05) in EW compared to Ctrl lambs, while total concentration, and individual proportions and concentrations of SCFA did not vary between groups at week four. Proportions of caproate were 1.7 times greater (P=0.02) in EW compared to Ctrl lambs,

Table 2 Effect of weaning lambs at week 4 (early weaning; EW) and 6 (control; Ctrl) on total short chain fatty acids (SCFA) concentration and the proportion and concentrations of individual SCFA , ammonia (NH_3) concentrations , and pH in the rumen at week four and sixteen, when lambs were consuming starter diets[1] and forages, respectively. Data presented are predicted means, standard error of the difference (SED) and P-values (P).

	Week 4				Week 16			
Parameter	Ctrl	EW	SED	P	Ctrl	EW	SED	P
SCFA (mM)	87.6	114.5	23.43	0.27	85.2	101.6	13.21	0.24
Acetate (%)	57.2	56.5	4.13	0.75	60.4	60.9	2.02	0.81
Propionate (%)	28.0	29.7	5.72	0.70	23.0	20.8	1.82	0.25
Butyrate (%)	11.1	9.7	3.15	0.71	11.7	12.6	1.03	0.42
Valerate (%)	1.4	1.3	0.61	0.92	1.3	1.4	0.09	0.09
Caproate (%)	0.4	0.4	0.32	0.99	0.2	0.4	0.06	0.02
Isobutyrate (%)	1.1	1.1	0.55	0.88	1.5	1.8	0.23	0.29
Isovalerate (%)	1.0	1.3	0.58	0.57	1.8	2.1	0.28	0.33
Ace:Pro ratio	2.5	2.1	0.58	0.47	2.7	3.0	0.35	0.36
Acetate (mM)	47.9	63.6	11.60	0.22	50.9	60.8	6.00	0.13
Propionate (mM)	27.7	36.2	11.21	0.43	20.1	22.0	4.58	0.68
Butyrate (mM)	8.9	10.3	2.46	0.57	10.2	13.2	2.47	0.26
Valerate (mM)	1.3	1.7	0.86	0.65	1.1	1.5	0.21	0.08
Caproate (mM)	0.4	0.5	0.42	0.83	0.2	0.4	0.05	>0.01
Isobutyrate (mM)	0.8	1.0	0.24	0.41	1.3	1.7	0.12	>0.01
Isovalerate (mM)	0.8	1.3	0.90	0.19	1.5	2.0	0.18	0.01
NH_3 (mmol/L)	9.2	21.4	5.39	0.05	10.3	17.8	4.27	0.11
pH	--	--	--	--	6.5	6.4	0.21	0.34

[1] EW lambs were consuming 25% of their milk allowance and ad lib concentrate, while the controls were consuming 75% of their milk allowance and ad lib concentrate.

while the total concentration and individual proportions of SCFA did not differ between treatments at week sixteen. Individual concentrations of minor SCFA at week sixteen were 1.4 times greater (5.6 vs 4.0±0.5 mM; P<0.01) in EW lambs as a result of increased isobutyrate (1.3 times; P<0.01), isovalerate (1.3 times; P=0.01), caproate (2 times; P<0.01) and tendency for increased valerate (1.4 times; P=0.08) compared to Ctrl lambs; concentrations of acetate, propionate and butyrate did not vary between treatment groups. Rumen pH and NH_3 did not differ between the weaning groups at week sixteen. Similar rumen fermentation profiles were observed for sex and the interaction treatment by sex at week four and sixteen (P>0.05).

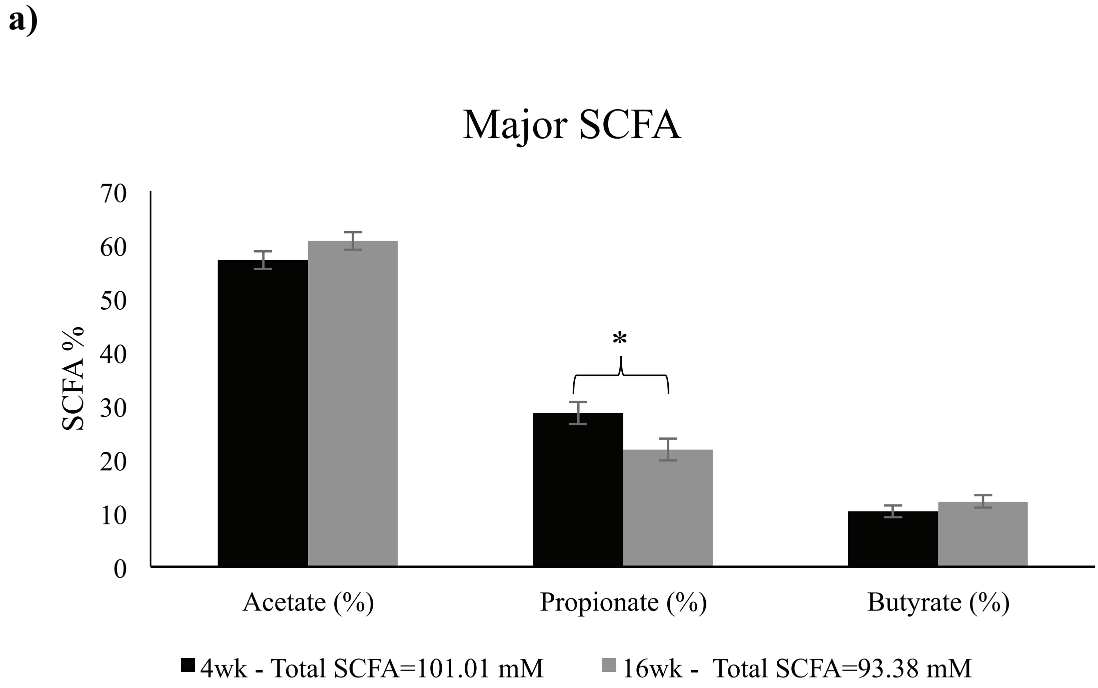
From week four to sixteen, the rumen fermentation profiles showed that the proportion of propionate decreased from 28.6% to 21.9% (P=0.024; Figure 1a), while the proportions of isovalerate and isobutyrate increased from 1.1% to 1.9% (P=0.02) and from 1.1% to 1.7% (P=0.06) (Figure 1b), respectively.

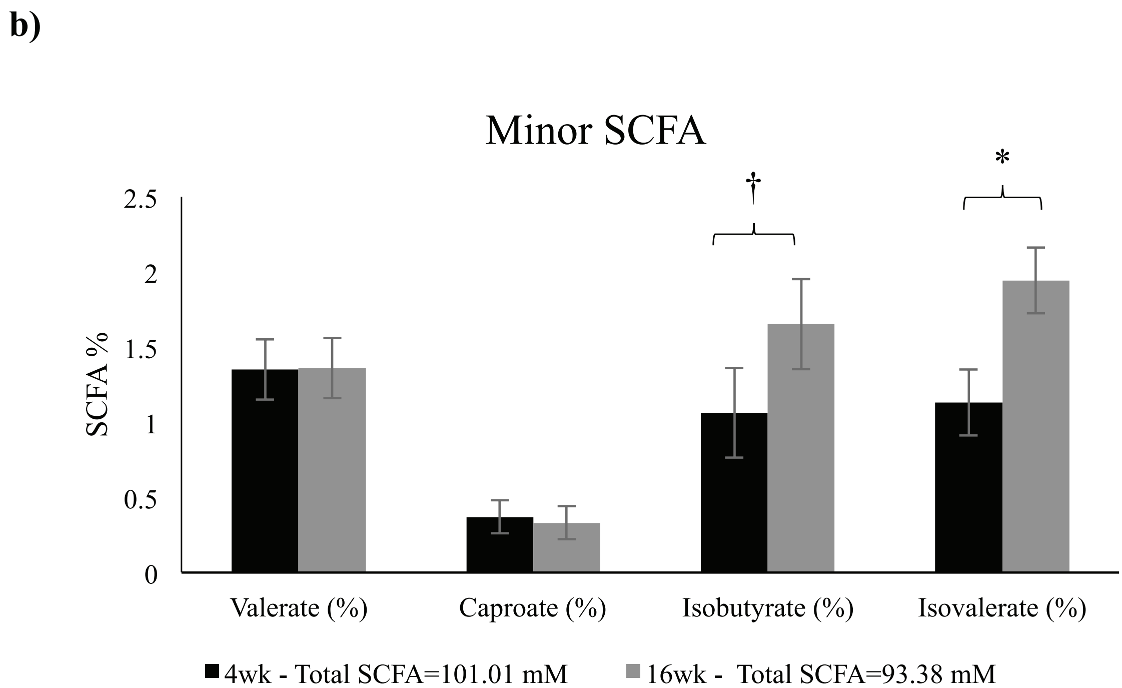
Discussion

This study investigated the effect of age at weaning on rumen fermentation profiles of artificially reared lambs. The key findings of this study were that ammonia concentrations differed at week four, but total concentrations and individual proportion of SCFA and fermentation profiles were similar between groups at week sixteen, indicating the establishment of considerable ruminal fermentation activity at an early age in lambs.

In young ruminants, while the microbial colonisation of the rumen occurs soon after birth (Yáñez-Ruiz et al. 2015), the production of ruminal SCFA is related to the ingestion of fermentable substrates (Lane et al. 2000) and the total amount of organic matter digested by the ruminal microorganisms (Weston & Hogan 1968). In the current study, the total solid feed intake recorded in EW and Ctrl lambs at week four agrees with the similarities found in ruminal SCFA concentrations and proportion between weaning groups. The molar proportions of acetate, propionate and butyrate observed at week four were similar to those reported by Liu et al. (2016) in lambs of similar age consuming concentrates, while the total concentrations of SCFA at week four and sixteen were in the range of adult sheep grazed on ryegrass (Leng et al. 1968). The time at which lambs start to increase the production of ruminal SCFA is determined by the increased ingestion of solid feed (Žitnan et al. 1993), which is stimulated by restricting milk intake prior to weaning (Owen et al. 1969; Heaney et al. 1984; Bimczok et al. 2005). In this study, the use of the step-down weaning method in EW and Ctrl lambs stimulated and increased the early consumption of starter diets, reaching adult-like SCFA concentration levels by week 4 of the trial. These results differ from those observed in lambs naturally reared. Wardrop and Coombe (1961) reported that lambs reared by their dams and with access to pasture achieved adult-like SCFA concentration levels

Figure 1 Comparisons of (a) major (acetate, propionate and butyrate) and (b) selected minor (valerate, caproate, isobutyrate and isovalerate) short chain fatty acids (SCFA) at week four and sixteen. Data represent the means ± SEM (standard error of the mean) of values from lambs sampled at week four and sixteen. † P<0.10, * P<0.05.





by eight weeks of age, while Liu et al. (2016) observed that lambs reared indoors with restricted access to their dams, but with *ad libitum* concentrate supplementation at early (day 7) or delayed (day 42) age reached adult-like SCFA concentrations by six and eight weeks, respectively. Therefore, providing lambs with early access to, and allowing increased consumption of, solid feed favours the establishment of the ruminal fermentation independent of weaning age.

Ammonia concentrations arise in the rumen through the diet and urea in plasma (absorbed NH_3 converted by the liver) recycled via ruminal wall and salivary secretion (Lapierre & Lobley 2001). Ruminal NH_3 concentrations in lambs at week four agree with those reported by Wardrop and Coombe (1961), who indicated that lambs reach adult-like NH_3 concentrations when they start eating solid feed. In ruminants, approximately 60-90% of the nitrogen that enters the rumen is converted to NH_3 (Membrive 2016).

Greater crude protein (CP) intake could potentially increase NH_3 concentration in the rumen. The nature and amount of both CP and carbohydrates available at the rumen dictate the availability and utilisation of carbon and nitrogen for microbial growth and fermentation (Sinclair et al. 1993; Bach et al. 2005). In the current study, EW lambs consumed numerically more concentrate and less hay than Ctrl lambs, which indicates that more CP was available for microbial action and NH_3 production in the rumen. Baldwin and Denham (1979) showed that forages may supply N as highly degradable protein or non-protein N, while concentrates may slowly supply N mainly as peptides and/or amino acids needed for microbial protein synthesis. Therefore, in EW lambs the release of NH_3 from dietary protein may not have matched the release of usable energy from starch to improve microbial N utilisation. Czerkawski (1976) showed that sheep fed with concentrate-hay diets had greater ruminal microbial growth than those fed concentrate and hay separately. Lack of difference by sixteen weeks between groups, when the lambs were eating only pasture, indicate that differences observed in NH_3 concentration at week four were likely related to the differences in feed and protein intake.

Rumen pH is closely related to the production of SCFA from fermentation in the rumen and the absorption, passage, neutralisation, and buffering of those acids (Owens et al. 1998). The ruminal pH at sixteen weeks did not differ between weaning groups, showing an inverse relationship to the total SCFA concentrations. Sun et al. (2012) observed that the ruminal pH of sheep fed with ryegrass varied between the feeding time and measuring time, which explain the variations in ruminal pH measured in this study that ranged between 5.53 and 6.94. Additionally, further individual variations in ruminal pH may be associated with the SCFA clearance capacity of the ruminal epithelium, neutralisation with salivary buffers, and passage to the lower digestive tract (Schlau et al. 2012).

In conclusion, the present study demonstrated that the early access to starter diets stimulated the early establishment of ruminal fermentation in lambs by week four independently of weaning age. These findings will contribute to the development of artificial-rearing management options for commercial farming practices (e.g., rearing of orphan lambs and dairy-sheep and -goat systems).

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