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Early supplementation of starter pellets with alfalfa improves the performance of pre- and postweaning Hu lambs[1]

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ABSTRACT: This study aims to determine the effects of alfalfa supplementation on the pre- and postweaning performance, rumen development, and feed transition in starter diet–fed lambs. Six of 66 male Hu lambs were slaughtered at the age of 10 d to serve as a control. The other 60 lambs were randomly allocated to 2 dietary treatments: milk replacer and starter pellets without (STA) or with free-choice chopped alfalfa (S-ALF). The animals were offered 300 g/d of the concentrate mixture and had free access to alfalfa after weaning at the end of wk 4 (age 38 d). The alfalfa inclusion in the S-ALF group tended to increase the starter intake before weaning, significantly increased the concentrate intake soon after weaning ($P < 0.05$), and increased the BW ($P < 0.01$) and ADG ($P < 0.10$) in pre- and postweaning lambs. The S-ALF group had heavier carcasses ($P < 0.05$), rumens ($P < 0.05$), reticula ($P < 0.05$), omasums ($P < 0.10$), abomasums ($P < 0.05$), and visceral organs ($P < 0.10$) than the STA lambs after weaning. Alfalfa supplementation increased ($P < 0.05$) the rumen papillae length and the ratio of the duodenal villus height to the crypt depth; it also decreased ($P < 0.05$) the concentration and molar proportion of propionate in wk 1 and 5. The STA lambs had higher ($P < 0.01$) blood concentrations of globulin and blood urea nitrogen and lower β-hydroxybutyrate after weaning. The STA group also had a higher incidence of feed plaque. From the above results, we infer that the free-choice addition of chopped alfalfa to starter diets is beneficial to rumen development, relieves weaning stress, and improves the performance of lambs.

Key words: alfalfa, health, lamb, performance, rumen development, weaning transition

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INTRODUCTION

Weaning stress is an important challenge in ruminant feeding, which is reflected in decreased feed intake, BW loss (Budzynska and Weary, 2008; Weary et al., 2008; de Passillé et al., 2011), diarrhea (Khan et al., 2007), and other physiological responses. Proper starter intake improves rumen development (Baldwin et al., 2004), which then prevents problems related to weaning (Khan et al., 2007; Sweeney et al., 2010). However, some controversy exists regarding the chemical and physical characteristics of starter diets and the optimal rate of forage provision to preruminants (Coverdale et al., 2004; Suárez et al., 2007).

Volatile fatty acids, especially butyrate and propionate, are produced more from concentrate diets than from forage and are essential chemical stimulations for the rapid development of rumen epithelium (Flatt et al., 1958; Heinrichs, 2005). However, an increased incidence of plaque formation and poor rumen development were observed in calves fed with only concentrate diets during the preweaning period (Suárez et al., 2006a). Forage supplementation to starter diets increases the ADG and BW and improves rumen health in young calves (Castells et al., 2012; Terré et al., 2013). However, different forage sources, amounts, and particle sizes have different effects (Castells et al., 2013; Webb et al., 2013), and fewer trials have been conducted on small ruminants such as lambs, whose feeding patterns are coarser than those of calves after weaning. The change

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from a starter to a conventional diet, including restricted concentrate and ad libitum access to forages, brings many more challenges to lambs than to calves.

The Hu sheep is an extremely inbred species and is the most popular nationwide in China for its excellent prolificacy (3 to 4 lambs per parturition), rapid growth rate, and adaptation to poor quality feeds. Recently, much attention has been paid to the early weaning of Hu lambs with the development of the sheep industry in China. We hypothesized that the free-choice provision of chopped alfalfa to starter diets during the preweaning period might relieve weaning stress and promote the performance of ruminants in the postweaning period by providing physical and nutritional stimulation to the rumen, thereby increasing the adaption of the rumen to solid feed and the feed transition. Therefore, the objectives of the present study were to investigate the effects of early supplementation of chopped alfalfa to starter pellets before weaning on Hu lamb performance and rumen development before and after weaning as well as on the adaptation to a conventional feed in the postweaning period.

MATERIALS AND METHODS

Animals, Feeds, and Experimental Design

All experimental protocols were approved by the Animal Care Committee of Zhejiang University (Hangzhou, China), and the experimental procedures used in this study were in accordance with the university's guidelines for animal research. Sixty-six healthy male Hu lambs 5 d of age and with a mean BW of 3.69 kg (SD 0.67) were purchased from 1 commercial farm and then transported for 1 h to the experimental farm of the College of Animal Science (Zhejiang University). Upon arrival, the lambs were randomly divided among 11 indoor pens (1.4 by 1.4 m) with plastic slatted floors; 6 lambs were placed in each cage and subjected to a 5-d adaptation to the milk replacer (**MR**; 18.4% CP, 3.6% NDF, and 0.9% ADF) and to the new surroundings. Milk replacer was fed via baby bottles. The feeding trial began at the age of 10 d and lasted for 8 wk. Six lambs were weighed and slaughtered at the age of 10 d before feeding for use as a control. Lambs were slaughtered according to the Islamic method, which broke vessel, trachea, and esophagus at one time, in another room of the experimental farm without any transportation. The other 60 lambs were weighed and randomly assigned into 2 groups (milk replacer and starter pellets without alfalfa [**STA**] and milk replacer and starter pellets with free-choice chopped alfalfa [**S-ALF**]) with 30 lambs in each. Every 2 lambs with similar BW were considered a unit, and each unit was housed in an individual pen (1.4 by 1.4 m).

Before weaning (wk 1 to 4), the STA group was fed MR and starter pellets (24.2% CP, 16.8% NDF, and 8.7% ADF) and the S-ALF group was offered MR, starter pellets, and alfalfa chopped into 6 to 8 cm lengths (25.1% CP, 33.0% NDF, and 21.3% ADF). Starter pellets and alfalfa were offered ad libitum in individual feed bins (0.1 m wide, 0.15 m long, and 0.1 m deep) in each pen. To stimulate lambs to consume starter pellets and alfalfa, MR was given at 880 mL/d (based on consumption in the adaptation period) on the first day of wk 1 and was then reduced at a rate of 30 mL/d until reaching 180 mL/d, which was the rate given until weaning at the end of wk 4 (age 38 d). The MR was given in 3 equal portions each day.

After weaning (wk 5 to 8), all weaned lambs in both the STA and the S-ALF groups were limited to 300 g/d of the concentrate mixture (including 45% corn grain, 20% cottonseed cake, 15% soybean meal, 15% wheat bran, 2% NaCl, 1% $NaHCO_3$, and 2% $CaHPO_4$) and allowed ad libitum access to alfalfa chopped into 6-to-8-cm lengths (21.6% CP, 38.6% NDF, and 25.8% ADF). The concentrate mixture was offered in the same individual feed bins as described before, and alfalfa was offered in troughs. Each individual trough was 0.2 m wide, 0.36 m long, and 0.2 m deep. Alfalfa was chopped with a forage chopping machine (Xu Lang Machinery, Guangzhou, China) before being fed to the lambs. Orts of the starter pellets, the concentrate mixture, and the alfalfa were removed from bins and troughs at 0800 h each day, with new feed delivered 3 times daily at 0830, 1200, and 1700 h. All lambs had free access to drinking water offered in water bins (same size as the feed bins) before weaning and by duck-billed drinking fountains after weaning.

At the end of wk 1, 2, 4, 5, and 8 of the feeding trial, 3 units of 2 lambs (a total of 6 lambs) in each group were randomly selected and slaughtered according to the method described above to collect rumen samples and measure carcass and organ weights. During the feeding trial, 3 lambs died and were removed from the experiment (1 from the STA group and 2 from the S-ALF group).

Sample Collection and Performance Measurement

Fecal fluidity of lambs was evaluated daily during the feeding trial based on the 5-point-scale fecal score (Larson et al., 1977). If an animal presented a fecal score ≥3 for 3 consecutive days, it was considered diarrheic (Araujo et al., 2015).

The amount of feed offered and refused was recorded weekly during the feeding trial, and the BW was measured before the morning feeding. The feed offered and refused was recorded for 2 consecutive days each week from wk 1 to 7 and daily in wk 8 for

apparent digestibility analysis. Body weight was measured on 2 consecutive days per week throughout the feeding trial. Daily feed and orts samples were pooled by unit, and then the subsamples were used for chemical analysis to determine feed intake. Feed samples were dried in a forced-air oven at 65°C for 48 h and stored in sealed plastic containers at 4°C until analysis.

In wk 8, 3 units of 2 lambs (a total of 6 lambs) in each group were used to determine apparent digestibility of diets. All feces were collected daily with leaning feces collection plates that were hanging under the pens, and feces were separated from urine by urine discharge holes at the bottom of the plates. Fecal output was weighed, and all wet feces were dried at 65°C for 72 h and stored in sealed plastic containers at 4°C until analysis.

Blood samples (approximately 10 mL) were collected from the jugular vein into a heparinized test tube before lambs were slaughtered. Samples were then centrifuged at 3,000 × *g* at 4°C for 15 min to obtain the plasma. Plasma samples were stored at –20°C until subsequent analysis.

Immediately after slaughter, the rumen was taken and dissected along the dorsal line. The pH of the rumen content was immediately obtained using a pH meter (PB-10; Sartorius, Goettingen, Germany). A subsample of the rumen content was collected for storage at –20°C until VFA analysis. The reticulum, omasum, and abomasum were dissected. The empty weights of the forestomachs were recorded after the digesta was removed by rinsing with sterile PBS at pH 7.0. Rumen morphological characteristics were recorded using a Canon IXUS 115 camera (Canon Inc., Tokyo, Japan). The weights of the visceral organs (heart, liver, spleen, lung, and kidneys) and carcass were recorded.

Three rumen tissue samples approximately 1.5 by 1.5 cm^2 were removed from each animal: one from the ventral region and one from the region to the right and left side of the ventral line. A 1-cm duodenum sample was removed from middle part of the duodenal tract of each animal and rinsed with sterile PBS. Specimens of rumen and duodenum were fixed in solution (100 mL of formaldehyde, 50 mL of glacial acetic acid, and 850 mL of ethyl alcohol in 1 L) for morphological measurements.

Feed and Fecal Sample Analysis

All feed and fecal samples were ground to allow passage through a 1-mm sieve (HK-08A ground mill; Xu Lang Machinery) before analysis of DM (method 924.05; AOAC, 1990), CP (method 988.05; AOAC, 1990), and ADF (method 973.18; AOAC, 1990). The NDF content was determined by the method of Van Soest et al. (1991) without the addition of sodium sulfite and amylase.

Blood Analysis

Concentrations of glucose, total protein, albumin, globulin, alanine aminotransferase (**ALT**), aspartate aminotransferase, alkaline phosphatase (**ALP**), gamma glutamyl transpeptidase (**GGT**), blood urea nitrogen (**BUN**), creatinine, and β-hydroxybutyrate (**BHBA**) were determined with commercial kits (Medical System, Ningbo, China) using the Hitachi 7020 autobiochemistry instrument (Hitachi, Tokyo, Japan).

Rumen VFA Analysis

Rumen content (approximately 2 g) was vortexed in 9 mL of sterile PBS (pH 7.0), and centrifuged at 13,000 × *g* at 4°C for 15 min. The VFA sample was obtained and prepared by adding 20 μL of 85 to 90% orthophosphate acid to 1 mL of the VFA sample. The prepared sample was centrifuged again as described above to obtain the final supernatant. Volatile fatty acid measurement was performed according to Hu et al. (2005), using a gas chromatograph (GC-8A; Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector. Briefly, 2 μL of the final supernatant was injected with a syringe and the temperature of the injector/detector and the column was 260 and 220°C, respectively, with nitrogen as a carrier.

Rumen and Duodenal Morphological Analysis

Rumen epithelium containing focal or multifocal patches of papillae to which a sticky mass of feed and cell debris was adhered was marked as plaque (Suárez et al., 2007). The number of lambs with plaque was recorded. To determine the development of rumen papillae and the duodenal villus, specimens of rumen and duodenum tissue were dehydrated, embedded in paraffin, sectioned (5 μm), stained with hematoxylin and eosin, and examined under an optical microscope (Olympus, Tokyo, Japan). The width and length of the rumen papillae from the left, right, and ventral regions as well as villus height and crypt depth were measured and analyzed with Motic Image Plus 2.0 software (Motic China Group Co. Ltd., Xiamen, China).

Statistical Analyses

The results were analyzed as a completely randomized design. Each pen with 2 lambs was considered the experimental unit for the analyses of intake and digestibility. Each lamb was considered an experimental unit for other analyses. After being slaughtered at wk 2, lambs from one pen of the STA group and another pen of lambs from the S-ALF group were found with no intake of starter and alfalfa; therefore, the data of these 2 pens

were removed. All statistical analyses were performed using PROC GLM of SAS (SAS Inst. Inc., Cary, NC).

Data on the intake, BW, and ADG were pooled by week for all living lambs in that week. Data for weights of carcasses, forestomachs, and visceral organs; rumen wall and duodenal microscopic appearance; blood variables; and rumen fermentation were pooled at each slaughter time point for the 6 slaughtered lambs. These data were analyzed by a 2-way ANOVA, with a full factorial model, to examine the response to diet, age, and the interaction of diet and age. Then, the data with no significance in the interaction were removed to allow an analysis of only the main effects of diet and age. The statistical model was as follows:

$$Y_{ij} = \mu + D_i + A_j + DA_{ij} + E_{ij},$$

in which Y_{ij} is the dependent variable, μ is the overall mean, D_i is the diet effect, A_j is the age effect, DA_{ij} is the interaction of diet and age, and E_{ij} is the error term.

One-way ANOVA was used to analyze the effects of diets on digestibility in wk 8. The statistical model was as follows:

$$Y_i = \mu + D_i + E_i,$$

in which Y_i is the dependent variable, μ is the overall mean, D_i is the diet effect, and E_i is the error term.

Multiple comparisons of means among treatments were performed using Tukey's tests. Significance was defined as $P \leq 0.05$ and trends were defined as $P \leq 0.10$.

RESULTS

Diarrhea, Feed Intake, and BW Gain

At the preliminary feeding period, approximately 42.8% lambs presented a fecal score of 3 and other lambs presented a fecal score <3. During the feeding trial, diarrhea improved as lambs were adapted to the artificial feeding and no lamb was observed with diarrhea.

The feed intake of starter, concentrate mixture, and alfalfa increased with age (see Fig. 1). Lambs in the S-ALF group consumed 8 to 54 g/d of alfalfa during the preweaning period but did not increase their alfalfa intake after weaning compared with the STA group without alfalfa supplementation before weaning (Fig. 1b). The alfalfa supplementation tended to promote ($P < 0.10$) starter intake at weaning (358 g/d in the S-ALF group vs. 313 g/d in the STA group) and increased ($P < 0.05$) concentrate intake for the first 2 wk after weaning (Fig. 1a). Alfalfa supplementation increased ($P < 0.01$) total DMI, CP intake, and NDF intake from wk 3 (Fig. 1c–1e). Due to weaning, total DMI and CP intake decreased in both groups, but less decrease was observed for S-ALF than STA; NDF intake increased more quickly for S-ALF than for STA.

Initial BW of lambs in both groups were similar, but BW of lambs in the S-ALF group were greater than those in the STA group from wk 3 onward ($P < 0.01$; Fig. 2a). Before lambs were weaned, ADG increased from wk 2 to 4 for both groups, and ADG of lambs in the S-ALF group was increased faster than those in the STA group from wk 3 to 4 ($P < 0.01$; Fig. 2b). During the postweaning period, ADG slowed from 199 to 71 g/d for the STA group and from 244 to 163 g/d for the S-ALF group in wk 5 and then returned to 229 and 259 g/d for the STA and the S-ALF, respectively. No differences were observed for apparent digestibility of DM, CP, NDF, and ADF between the 2 groups at wk 8 ($P > 0.10$; Table 1).

Carcass and Organ Weights

Weights of carcasses and forestomachs showed a similar pattern of increase from wk 1 to 8 for both groups (Fig. 3). Both diet ($P < 0.05$) and age ($P < 0.01$) affected weights of carcasses and forestomachs. No differences existed between the 2 groups before weaning, although a numeric increase was observed in abomasum weight in the S-ALF group when weaning ($P < 0.10$); however, the S-ALF group had greater carcass and forestomach weights after weaning ($P < 0.05$).

Visceral organ weights changed according to a similar pattern (Table 2). Diet affected the weights of the heart ($P = 0.05$), liver ($P = 0.02$), lung ($P = 0.02$), and kidney ($P < 0.01$). The S-ALF lambs had heavier visceral organs than the STA lambs from weaning onward.

Rumen pH and VFA Concentration

Rumen pH was affected by age ($P < 0.01$). Molar proportion of propionate was affected by age and interaction effect ($P < 0.01$; Table 3) and decreased ($P < 0.05$) for the S-ALF lambs at wk 1 and 5. Rumen pH showed a similar pattern of change in the 2 groups throughout the whole feeding trial. Before weaning, rumen pH decreased from 6.83 to 4.96 in the STA lambs and from 6.83 to 5.17 in the S-ALF lambs after they

Table 1. Apparent digestibility of nutrients from wk 8 in growing lambs fed on a diet of milk replacer and starter pellets without (STA) or with free-choice chopped alfalfa (S-ALF)

Item, %	STA	S-ALF	SEM	*P*-value
DM	70.83	68.21	1.194	0.325
CP	70.96	68.54	1.226	0.380
NDF	65.00	52.22	1.487	0.241
ADF	51.12	47.37	1.642	0.300

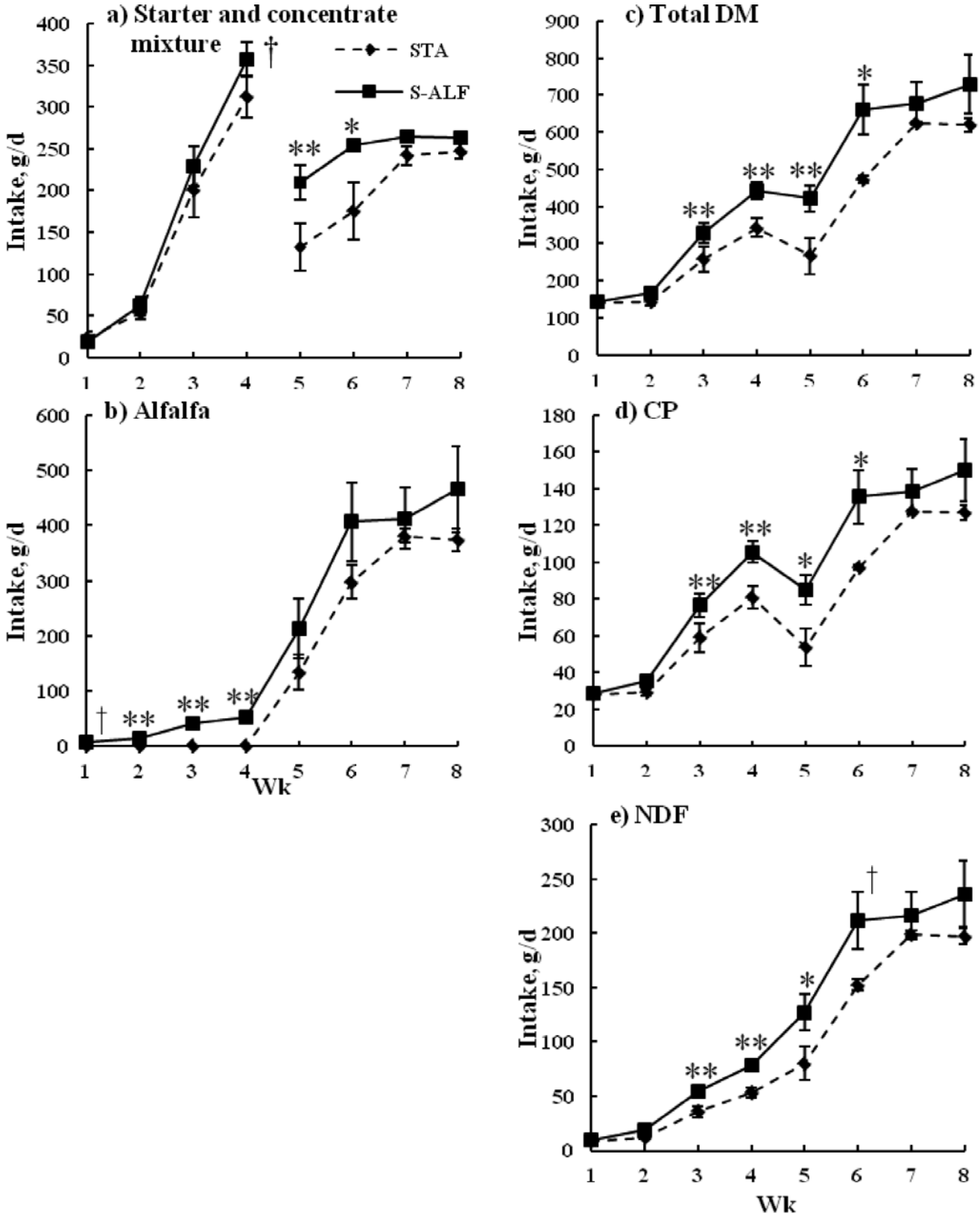

Figure 1. Change in the intake of starter and concentrate mixture (a), alfalfa (b), DM (c), CP (d), and NDF (e) with age by growing lambs fed on a diet of milk replacer and starter pellets without (STA) or with free-choice chopped alfalfa (S-ALF). Bars indicate the SEM. †$P < 0.10$; *$P < 0.05$; **$P < 0.01$.

had begun to consume the starter pellet. After weaning, rumen pH increased to 7.26 in the STA lambs and 7.25 in the S-ALF lambs within 1 wk. No difference for VFA concentrations were observed between the 2 groups.

Appearance of the Rumen Wall and Duodenum

Feed plaque was observed in both groups at wk 2 and could be observed in a few lambs of the STA group at wk 4 and 5 but was not observed in the S-ALF group after wk 4 (Fig. 4). Age affected ($P < 0.01$) length and width of rumen papillae, crypt depth, and the ratio of villus height to crypt depth (**V:C**) of the duodenum (Table 4). Diet had an effect on length ($P < 0.05$) but not width of the rumen papillae. Diet exerted the greatest effect on papillae length on the ventral side ($P = 0.01$) among the left, right, and ventral sides of the rumen. Rumen papillae length was longer on the ventral side at weaning in S-ALF lambs than in STA lambs. The duodenal villus height and crypt depth were not affected by diet, whereas the V:C increased ($P < 0.05$) in S-ALF lambs.

Plasma Variables

Concentrations of total protein, albumin, globulin, ALT, ALP, GGT, BUN, and BHBA were not affected by diet but were affected by age ($P < 0.05$), whereas concentration of glucose was increased by diet ($P < 0.01$) but was not affected by age (Table 5). The S-ALF lambs had greater concentrations of ALT and BHBA after weaning.

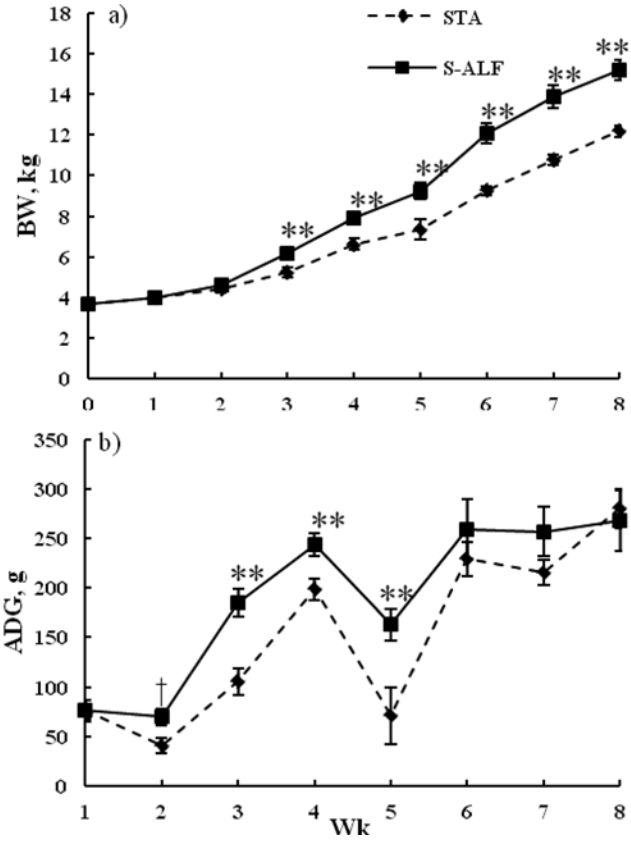

Figure 2. Change in the cumulative BW (a), and ADG (b) with age of growing lambs fed on a diet of milk replacer and starter pellets without (STA) or with free-choice chopped alfalfa (S-ALF). Bars indicate the SEM. †$P < 0.10$; *$P < 0.05$; **$P < 0.01$.

DISCUSSION

Rumen Development and Growth Performance

Consistent with forage supplementation to calves (Coverdale et al., 2004; Beiranvand et al., 2014), alfalfa supplementation to the starter diet of lambs during the preweaning period promoted feed intake and increased BW and ADG in pre- and postweaning lambs in the current study. In an earlier study, calves fed chopped grass hay (3–4 cm) had greater DM intake and reduced nonnutritive oral behaviors compared with calves fed ground hay (2 mm; Montoro et al., 2013), indicating the benefits of forage on feed intake with regard to physical stimulation. Physical stimulation from forage diets are necessary for the development of rumen muscle (Žitnan et al., 1998), cellulolytic microbiota (Beharka et al., 1998), and rumen capacity (Khan et al., 2012).

Table 2. Weight of visceral organs from wk 1 to 8 in growing lambs fed on a diet of milk replacer and starter pellets without (STA) or with free-choice chopped alfalfa (S-ALF)

	STA with feeding time, wk						S-ALF with feeding time, wk					SEM[1]			*P*-value[1]		
Item, g	0	1	2	4	5	8	1	2	4	5	8	Diet	Age	Diet × age	Diet	Age	Diet × age
Heart	25^{d}	27^{d}	32^{cd}	38^{c}	39^{c}	49^{b}	28^{d}	23^{d}	42^{bc}	46^{bc}	68^{a}	1.1	1.8	2.6	0.05	<0.01	<0.01
Liver	77^{e}	92^{e}	106^{e}	162^{d}	160^{d}	255^{b}	83^{e}	87^{e}	198^{c}	208^{c}	317^{a}	5.0	8.6	12.1	0.02	<0.01	0.03
Spleen	6^{d}	7^{d}	8^{d}	11^{cd}	11^{cd}	16^{b}	6^{d}	6^{d}	14^{bc}	12^{c}	19^{a}	0.4	0.7	1.0	0.15	<0.01	–
Lung	60^{e}	56^{e}	75^{de}	114^{cd}	105^{d}	164^{b}	57^{e}	54^{e}	133^{c}	131^{cd}	200^{a}	3.8	6.6	9.3	0.02	<0.01	–
Kidney	22^{d}	24^{d}	26^{d}	36^{c}	35^{c}	53^{b}	25^{d}	24^{d}	41^{c}	47^{bc}	69^{a}	1.0	1.8	2.5	<0.01	<0.01	0.02

[a–e]Means within a row with different superscripts differ ($P < 0.05$).

[1]Diet = the effect of diet; age = the effect of age; diet × age = the interaction between diet and age.

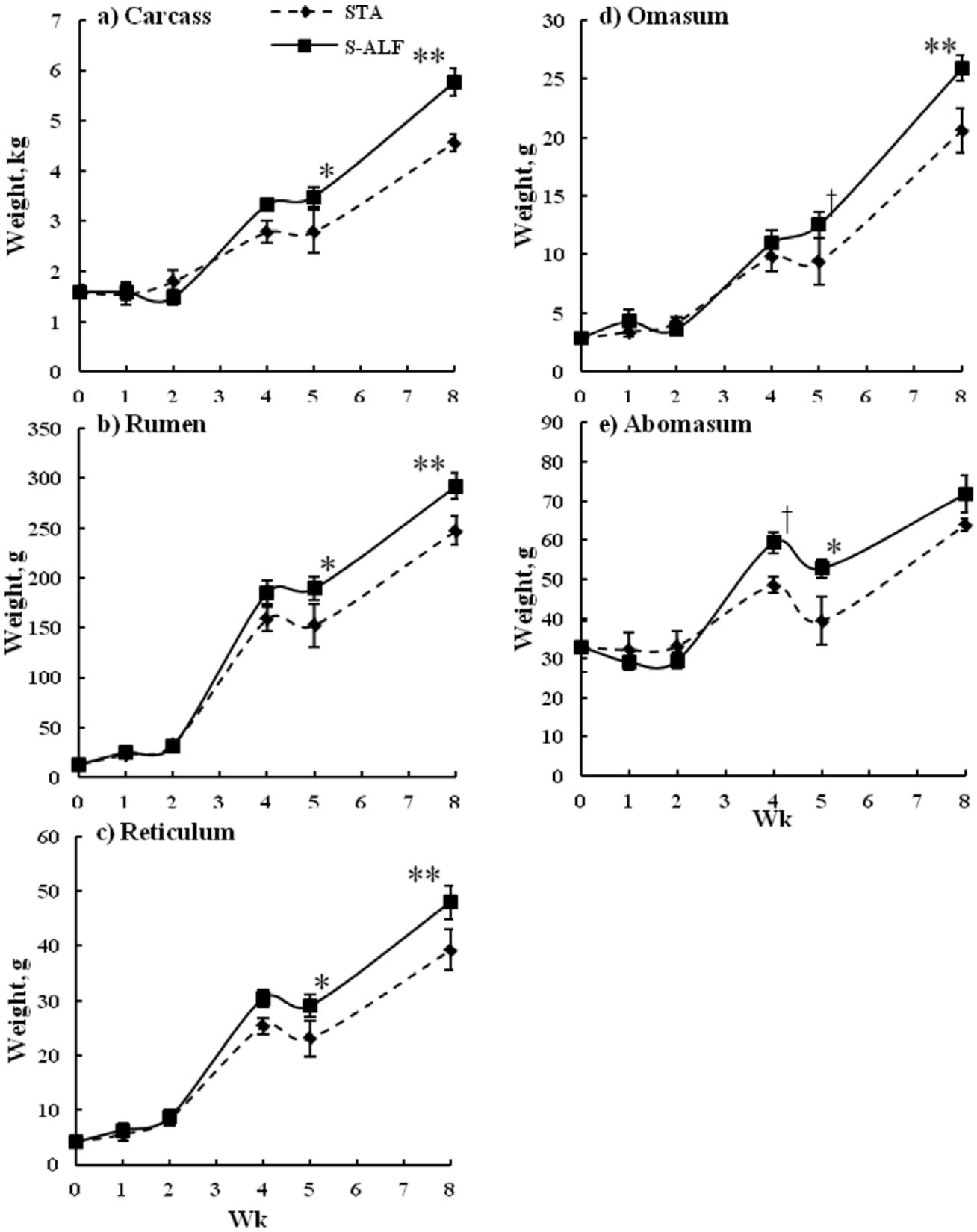

Figure 3. Change in the fresh weight of carcass (a), rumen (b), reticulum (c), omasum (d), and abomasums (e) of growing lambs fed on a diet of milk replacer and starter pellets without (STA) or with free-choice chopped alfalfa (S-ALF). Bars indicate the SEM. †$P < 0.10$; *$P < 0.05$; **$P < 0.01$.

Table 3. Rumen pH and VFA concentrations from wk 1 to 8 in growing lambs fed on a diet of milk replacer and starter pellets without (STA) or with free-choice chopped alfalfa (S-ALF)

	STA with feeding time, wk						S-ALF with feeding time, wk					SEM[1]			*P*-value[1]		
Item	0	1	2	4	5	8	1	2	4	5	8	Diet	Age	Diet × age	Diet	Age	Diet × age
pH	6.83^{ab}	5.88^{c}	5.79^{cd}	4.96^{d}	7.26^{a}	6.60^{b}	5.59^{cd}	5.40^{cd}	5.17^{d}	7.25^{a}	6.76^{ab}	0.084	0.146	0.206	0.433	<0.001	–
Concentration, mg/g																	
Total VFA	0.64^{b}	0.90^{b}	1.83^{ab}	2.41^{a}	2.67^{a}	1.50^{ab}	0.70^{b}	1.39^{ab}	1.81^{ab}	1.68^{ab}	1.88^{ab}	0.201	0.348	0.492	0.757	0.121	–
Acetate	0.54^{b}	0.61^{b}	1.37^{ab}	1.57^{ab}	1.66^{a}	1.14^{ab}	0.55^{b}	1.05^{ab}	1.13^{ab}	1.23^{ab}	1.26^{ab}	0.130	0.226	0.320	0.802	0.119	–
Propionate	0.04^{b}	0.19^{b}	0.31^{ab}	0.61^{ab}	0.85^{a}	0.24^{b}	0.08^{b}	0.20^{b}	0.52^{ab}	0.31^{b}	0.49^{ab}	0.067	0.226	0.164	0.776	0.107	–
Butyrate	0.06^{b}	0.09^{b}	0.15^{ab}	0.23^{a}	0.16^{ab}	0.13^{ab}	0.07^{b}	0.14^{ab}	0.17^{ab}	0.11^{b}	0.13^{ab}	0.016	0.027	0.037	0.485	0.178	–
Molar proportion, mM/100 mM																	
Acetate	79.3^{a}	62.1^{bc}	67.4^{b}	60.2^{bc}	58.6^{bc}	71.0^{ab}	73.9^{ab}	69.7^{ab}	56.6^{c}	65.9^{b}	62.5^{bc}	1.20	2.07	2.93	0.738	0.576	–
Propionate	7.9^{c}	25.0^{ab}	20.8^{bc}	27.2^{ab}	32.8^{a}	17.4^{bc}	12.0^{c}	15.5^{bc}	30.5^{ab}	23.0^{b}	27.0^{ab}	1.22	2.11	2.99	0.264	0.004	0.002
Butyrate	12.9^{ab}	12.9^{ab}	11.8^{ab}	12.7^{ab}	8.6^{b}	11.5^{ab}	14.1^{a}	14.8^{a}	12.9^{ab}	11.2^{ab}	9.5^{b}	0.55	0.95	1.35	0.810	0.152	–

$^{a–d}$Means within a row with different superscripts differ ($P < 0.05$).

[1]Diet = the effect of diet; age = the effect of age; diet × age = the interaction between diet and age.

For the present study, the stimulation provided by alfalfa increased rumen capacity, indicating heavier empty forestomachs, especially the rumen. Longer papillae length of S-ALF lambs reflected the effect of alfalfa supplementation for promotion of rumen morphological development. Additionally, the greater BHBA for S-ALF lambs after weaning might reflect a faster metabolic development in the rumen at the very beginning of the postweaning period, as BHBA concentration in the blood is considered to be related to the metabolic development of the rumen wall (Khan et al., 2011) and to fiber digestibility (Suárez et al., 2006b).

The VFA results in the current study showed that molar proportion of propionate in the S-ALF group decreased shortly after the start of alfalfa supplementation and weaning, during the feed transition period, compared with the STA group. This phenomenon was also observed by Suárez et al. (2007) when forage was supplemented to a concentrate diet and by van Ackeren et al. (2009) when the forage amount was increased in a total mixed ration. However, DM intake did not decrease in the S-ALF group when lambs suffered from weaning and feed transition stress. This indicated that propionate might play some role during this particular period. Among the 3 major VFA, propionate is a main gluconeogenic substrate for ruminants (Bergman, 1990) and a probable feed intake regulator for nonruminants and potentially satiety inducing for humans (Arora et al., 2011). The proportion of propionate was also related to the dietary digestible carbohydrate (van Ackeren et al., 2009) and corresponding microorganisms (Dijkstra, 1994). Whether the decreased propio-

Table 4. Change in rumen papillae and duodenum from wk 1 to 8 in growing lambs fed on a diet of milk replacer and starter pellets without (STA) or with free-choice chopped alfalfa (S-ALF)

	STA with feeding time, wk						S-ALF with feeding time, wk					SEM[1]			*P*-value[1]	
Item	0	1	2	4	5	8	1	2	4	5	8	Diet	Age	Diet × age	Diet	Age
Rumen papillae length, μm																
Left	392^{c}	482^{c}	462^{c}	1,585^{ab}	1,257^{b}	1,468^{ab}	509^{c}	533^{c}	1,730^{a}	1,612^{ab}	1,799^{a}	47.4	82.1	116.1	0.04	<0.01
Right	355^{d}	442^{d}	443^{d}	1,468^{ab}	1,023^{c}	1,339^{bc}	487^{d}	593^{d}	1,851^{a}	1,158^{bc}	1,569^{ab}	51.5	89.2	126.1	0.04	<0.01
Ventral	384^{d}	506^{d}	546^{d}	1,310^{ab}	851^{c}	1,179^{b}	439^{d}	629^{cd}	1,506^{a}	1,164^{b}	1,510^{a}	34.7	60.1	85.0	0.01	<0.01
Rumen papillae width, μm																
Left	134^{c}	199^{bc}	209^{bc}	230^{b}	171^{bc}	223^{b}	196^{bc}	210^{bc}	305^{a}	170^{bc}	207^{bc}	9.3	16.0	22.7	0.51	<0.01
Right	149^{c}	171^{bc}	227^{abc}	287^{a}	220^{abc}	254^{ab}	219^{abc}	245^{ab}	302^{a}	232^{abc}	250^{ab}	10.3	17.8	25.2	0.34	<0.01
Ventral	172^{c}	247^{b}	242^{b}	280^{b}	229^{bc}	242^{b}	260^{b}	274^{b}	348^{a}	225^{bc}	260^{b}	7.7	13.4	19.0	0.12	<0.01
Duodenum																
Villus height, μm	343^{ab}	336^{ab}	286^{b}	339^{ab}	376^{ab}	379^{ab}	339^{ab}	293^{ab}	386^{ab}	408^{a}	407^{a}	12.4	21.5	30.4	0.36	0.08
Crypt depth, μm	150^{c}	193^{abc}	214^{ab}	212^{ab}	182^{abc}	178^{bc}	166^{c}	224^{a}	191^{abc}	172^{bc}	163^{c}	4.9	8.5	12.0	0.10	<0.01
Villus height: crypt depth	2.4^{ab}	1.8^{abc}	1.4^{c}	1.6^{bc}	2.1^{ab}	2.1^{ab}	2.1^{ab}	1.3^{c}	2.0^{abc}	2.4^{ab}	2.6^{a}	0.08	0.15	0.21	<0.05	<0.01

$^{a–d}$Means within a row with different superscripts differ ($P < 0.05$).

[1]Diet = the effect of diet; age = the effect of age; diet × age = the interaction between diet and age.

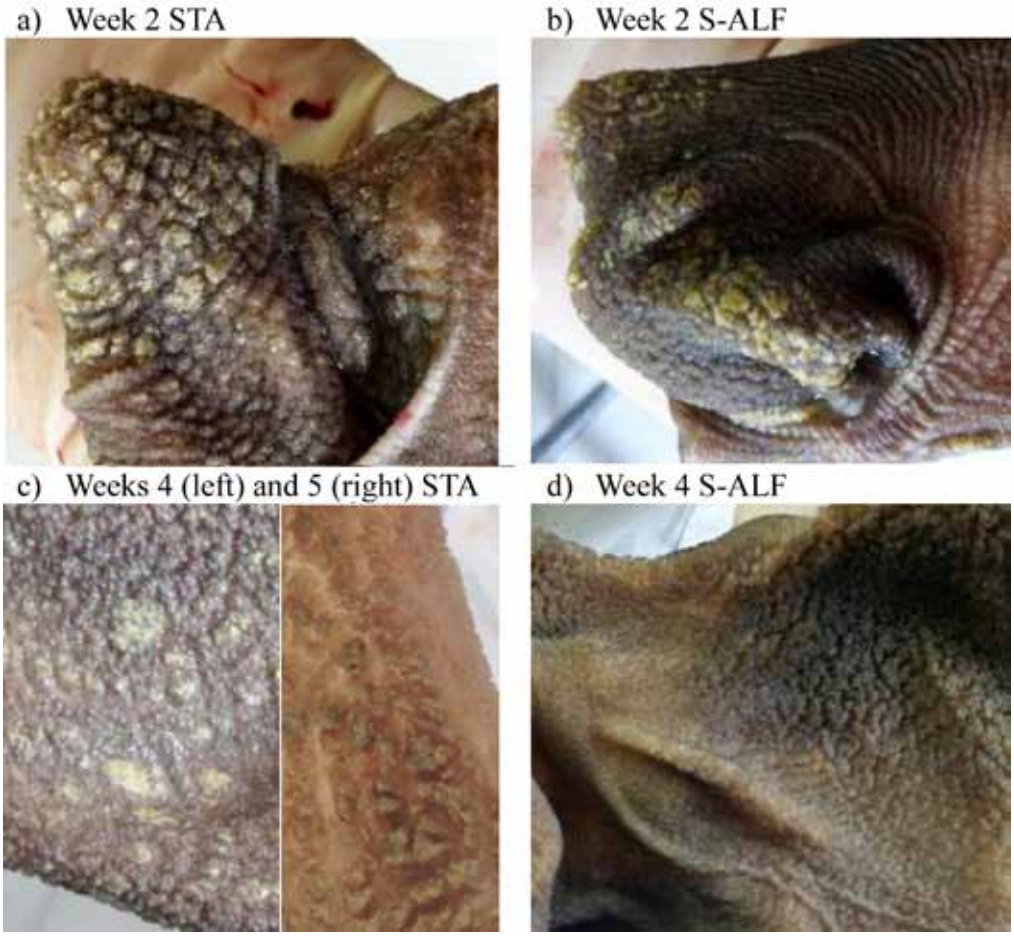

Figure 4. Feed plaque on the rumen mucosa of growing lambs fed on a diet of milk replacer and starter pellets without (STA) or with free-choice chopped alfalfa (S-ALF). Feed plaque was observed in both groups from wk 2 (a and b) and still could be observed in a few lambs of the STA group at wk 4 and 5 (c) but was not observed in the S-ALF lambs after wk 4 (d).

Table 5. Change in concentrates of plasma variables with age in growing lambs fed on a diet of milk replacer and starter pellets without (STA) or with free-choice chopped alfalfa (S-ALF)

	STA with feeding time, wk						S-ALF with feeding time, wk					SEM[2]			*P*-value[2]		
Item[1]	0	1	2	4	5	8	1	2	4	5	8	Diet	Age	Diet × age	Diet	Age	Diet × age
Glucose, mmol/L	4.51^{ab}	2.48^{b}	2.63^{b}	4.60^{ab}	4.07^{b}	4.37^{ab}	4.07^{b}	5.84^{ab}	6.08^{a}	5.43^{ab}	4.63^{ab}	0.254	0.440	0.623	<0.01	0.487	–
TP, g/L	67.9^{bc}	75.8^{b}	71.6^{bc}	67.0^{bc}	85.3^{a}	68.4^{bc}	78.1^{ab}	68.9^{bc}	63.4^{c}	76.9^{ab}	72.9^{bc}	1.31	0.26	3.20	0.96	<0.01	–
ALB, g/L	24.5^{b}	26.4^{ab}	26.3^{ab}	22.7^{b}	26.5^{ab}	26.3^{ab}	26.7^{ab}	24.4^{b}	23.5^{b}	29.1^{a}	24.6^{b}	0.40	0.69	0.98	0.23	<0.01	–
GLOB, g/L	43.4^{bc}	49.5^{b}	45.3^{bc}	44.4^{bc}	58.7^{a}	42.1^{bc}	51.4^{ab}	44.5^{bc}	40.0^{c}	47.8^{bc}	48.3^{bc}	1.25	2.16	3.06	0.70	0.03	–
ALT, units/L	10.0^{c}	10.6^{c}	12.8^{bc}	15.6^{bc}	18.9^{bc}	19.9^{b}	13.1^{bc}	11.5^{c}	21.1^{ab}	28.2^{a}	18.2^{bc}	1.06	1.83	2.59	0.12	<0.01	–
AST, units/L	69.6^{b}	124.7^{a}	90.5^{b}	111.0^{ab}	90.6^{b}	96.6^{ab}	86.6^{b}	85.8^{b}	105.6^{ab}	107.6^{ab}	82.4^{b}	4.17	7.22	10.21	0.97	0.518	–
ALP, units/L	489^{c}	358^{c}	315^{c}	1,405^{a}	642^{bc}	743^{bc}	401^{c}	377^{c}	1,465^{a}	859^{b}	461^{c}	44.5	77.1	109.0	0.3	<0.01	–
GGT, units/L	151.2^{ab}	190.2^{a}	79.3^{b}	94.0^{b}	89.6^{b}	66.1^{b}	166.0^{ab}	78.0^{b}	83.0^{b}	90.4^{b}	73.8^{b}	11.13	19.27	27.26	0.49	<0.01	–
BUN, mmol/L	7.74^{bc}	5.73^{c}	5.09^{c}	5.36^{c}	14.67^{a}	10.24^{b}	6.88^{c}	5.61^{c}	6.51^{c}	10.54^{b}	10.35^{b}	0.416	0.720	1.019	0.788	<0.01	–
CREA, µmol/L	37.6^{a}	34.0^{ab}	32.0^{ab}	31.9^{ab}	38.9^{a}	25.3^{b}	30.9^{ab}	29.2^{ab}	30.8^{ab}	35.0^{a}	31.4^{ab}	1.34	2.33	3.29	0.65	0.16	–
BHBA, µmol/L	173^{cd}	257^{cd}	236^{cd}	241^{cd}	400^{bc}	471^{bc}	120^{d}	134^{cd}	340^{c}	790^{a}	560^{b}	26.3	45.5	64.4	0.19	<0.01	<0.01

$^{a–d}$Means within a row with different superscripts differ ($P < 0.05$).

[1]TP = total protein; ALB = albumin; GLOB = globulin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGT = gamma glutamyl transpeptidase; BUN = blood urea nitrogen; CREA = creatinine; BHBA = **β**-hydroxybutyrate.

[2]Diet = the effect of diet; age = the effect of age; diet × age = the interaction between diet and age.

nate plays a role in maintaining feed intake and releasing stress during the feed transition period cannot be determined at present and needs further study.

Physical Condition and Gastrointestinal Health

The starter feed was pelleted in the present study; this supplied physical stimulation to some extent, but the lack of coarse material in the rumen caused a greater incidence of feed plaque in the STA group. Intestinal villus height, crypt depth, and V:C are indicators of digestion and absorption of nutrients (Chiang et al., 2010). Greater V:C of the duodenum of S-ALF lambs indicated that alfalfa promoted duodenal health.

Weights of visceral organs responded rapidly to nutrition (Scheaffer et al., 2001; Caton et al., 2009; Meyer et al., 2010). For the present study, greater feed intake by S-ALF lambs was consistent with heavier visceral organs. The increased size of organs (heart, liver, lung, and kidneys) reflected greater synthesis and transition of nutrients and metabolic end products (Craig et al., 1972; Burrin et al., 1990); therefore, the heavier weights of visceral organs from S-ALF lambs indicated a faster whole-body metabolic rate, consistent with the greater carcass weight and decreased BUN, especially during the feed transition. The spleen is the largest blood filter of the body, similar to a large lymph node in structure; it accommodates efficient phagocytosis of erythrocytes and recycling of iron, capture and destruction of pathogens, and induction of adaptive immune responses (Mebius and Kraal, 2005). Greater spleen weight, within normal ranges, for S-ALF lambs indicated a stronger immunity, which was confirmed by the lower plasma concentrations of globulin after weaning in the S-ALF lambs. Globulin fraction increase is associated with acute inflammatory disease (Carapeto et al., 2006).

Further work is needed to investigate whether the benefits of alfalfa supplementation are just a result of physical stimulation increasing the nutrient intake and absorption in the rumen or the entire gastrointestinal tract or a result of physical and chemical co-effects.

Conclusions

The free-choice provision of chopped alfalfa with starter pellets in preweaning lambs (from 10 d of age) increased feed intake, BW, ADG, and carcass weight in the pre- and postweaning periods, especially by stimulating rumen development during the transition from pellet starter to concentrate powder at the start of weaning. Chopped alfalfa increased rumen papillae length, duodenal V:C, and visceral organ weights and decreased globulin concentration and the occurrence of feed plaque. Therefore, free-choice provision of chopped alfalfa during the preweaning period might relieve weaning stress and improve the pre- and postweaning performance of lambs.

LITERATURE CITED

Araujo, G., C. Yunta, M. Terre, A. Mereu, I. Ipharraguerre, and A. Bach. 2015. Intestinal permeability and incidence of diarrhea in newborn calves. J. Dairy Sci. doi:10.3168/jds.2015-9666.

Arora, T., R. Sharma, and G. Frost. 2011. Propionate. Anti-obesity and satiety enhancing factor? Appetite 56:511–515. doi:10.1016/j.appet.2011.01.016.

Association of Official Analytical Chemists (AOAC). 1990. Official methods of analysis. 15th ed. AOAC, Arlington, VA.

Baldwin, R. L., VI, K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J. Dairy Sci. 87(E. Suppl.):E55–E65. doi:10.3168/jds.S0022-0302(04)70061-2.

Beharka, A. A., T. G. Nagaraja, J. L. Morrill, G. A. Kennedy, and R. D. Klemm. 1998. Effects of form of the diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. J. Dairy Sci. 81:1946–1955. doi:10.3168/jds.S0022-0302(98)75768-6.

Beiranvand, H., G. R. Ghorbani, M. Khorvash, A. Nabipour, M. Dehghan-Banadaky, A. Homayouni, and S. Kargar. 2014. Interactions of alfalfa hay and sodium propionate on dairy calf performance and rumen development. J. Dairy Sci. 97:2270–2280. doi:10.3168/jds.2012-6332.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70:567–590.

Budzynska, M., and D. M. Weary. 2008. Weaning distress in dairy calves: Effects of alternative weaning procedures. Appl. Anim. Behav. Sci. 112:33–39. doi:10.1016/j.applanim.2007.08.004.

Burrin, D. G., C. L. Ferrell, R. A. Britton, and M. Bauer. 1990. Level of nutrition and visceral organ size and metabolic activity in sheep. Br. J. Nutr. 64:439–448. doi:10.1079/BJN19900044.

Carapeto, M. V., R. Barrera, M. C. Mañe, and C. Zaragoza. 2006. Serum α-globulin fraction in horses is related to changes in the acute phase proteins. J. Equine Vet. Sci. 26:120–127. doi:10.1016/j.jevs.2006.01.007.

Castells, L., A. Bach, G. Araujo, C. Montoro, and M. Terré. 2012. Effect of different forage sources on performance and feeding behavior of Holstein calves. J. Dairy Sci. 95:286–293. doi:10.3168/jds.2011-4405.

Castells, L., A. Bach, A. Aris, and M. Terré. 2013. Effects of forage provision to young calves on rumen fermentation and development of the gastrointestinal tract. J. Dairy Sci. 96:5226–5236. doi:10.3168/jds.2012-6419.

Caton, J. S., J. J. Reed, R. Aitken, J. S. Milne, P. P. Borowicz, L. P. Reynolds, D. A. Redmer, and J. M. Wallace. 2009. Effects of maternal nutrition and stage of gestation on body weight, visceral organ mass, and indices of jejunal cellularity, proliferation, and vascularity in pregnant ewe lambs. J. Anim. Sci. 87:222–235. doi:10.2527/jas.2008-1043.

Chiang, G., W. Q. Lu, X. S. Piao, J. K. Hu, L. M. Gong, and P. A. Thacker. 2010. Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. Asian-Australas. J. Anim. Sci. 23:263–271.

Coverdale, J. A., H. D. Tyler, J. D. Quigley, and J. A. Brumm. 2004. Effect of various levels of forage and form of diet on rumen development and growth in calves. J. Dairy Sci. 87:2554–2562. doi:10.3168/jds.S0022-0302(04)73380-9.

Craig, M. C., C. M. Nepokroeff, M. R. Lakshmanan, and J. W. Porter. 1972. Effect of dietary change on the rates of synthesis and degradation of rat liver fatty acid synthetase. Arch. Biochem. Biophys. 152:619–630. doi:10.1016/0003-9861(72)90258-5.

de Passillé, A. M., T. F. Borderas, and J. Rushen. 2011. Weaning age of calves fed a high milk allowance by automated feeders: Effects on feed, water, and energy intake, behavioral signs of hunger, and weight gains. J. Dairy Sci. 94:1401–1408. doi:10.3168/jds.2010-3441.

Dijkstra, J. 1994. Production and absorption of volatile fatty acids in the rumen. Livest. Prod. Sci. 39:61–69. doi:10.1016/0301-6226(94)90154-6.

Flatt, W. P., R. G. Warner, and J. K. Loosli. 1958. Influence of purified material on the development of the ruminant stomach. J. Dairy Sci. 41:1593–1600. doi:10.3168/jds.S0022-0302(58)91138-X.

Heinrichs, J. 2005. Rumen development in the dairy calf. Adv. Dairy Technol. 17:179–187.

Hu, W. L., J. X. Liu, J. A. Ye, Y. M. Wu, and Y. Q. Guo. 2005. Effect of tea saponin on rumen fermentation in vitro. Anim. Feed Sci. Technol. 120:333–339. doi:10.1016/j.anifeedsci.2005.02.029.

Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, K. S. Ki, J. K. Ha, H. G. Lee, and Y. J. Choi. 2007. Pre- and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90:876–885. doi:10.3168/jds.S0022-0302(07)71571-0.

Khan, M. A., D. M. Weary, and M. A. von Keyserlingk. 2011. Hay intake improves performance and rumen development of calves fed higher quantities of milk. J. Dairy Sci. 94:3547–3553. doi:10.3168/jds.2010-3871.

Khan, M. A., D. M. Weary, D. M. Veira, and M. A. von Keyserlingk. 2012. Postweaning performance of heifers fed starter with and without hay during the milk-feeding period. J. Dairy Sci. 95:3970–3976. doi:10.3168/jds.2011-5027.

Larson, L. L., F. G. Owens, J. L. Albright, R. D. Appleman, R. C. Lamb, and L. D. Muller. 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. J. Dairy Sci. doi: 10.3168/jds.S0022-0302(77)83975-1.60:989–991.

Mebius, R. E., and G. Kraal. 2005. Structure and function of the spleen. Nat. Rev. Immunol. 5:606–616. doi:10.1038/nri1669.

Meyer, A. M., J. J. Reed, K. A. Vonnahme, S. A. Soto-Navarro, L. P. Reynolds, S. P. Ford, B. W. Hess, and J. S. Caton. 2010. Effects of stage of gestation and nutrient restriction during early to mid-gestation on maternal and fetal visceral organ mass and indices of jejunal growth and vascularity in beef cows. J. Anim. Sci. 88:2410–2424. doi:10.2527/jas.2009-2220.

Montoro, C., E. K. Miller-Cushon, T. J. DeVries, and A. Bach. 2013. Effect of physical form of forage on performance, feeding behavior, and digestibility of Holstein calves. J. Dairy Sci. 96:1117–1124. doi:10.3168/jds.2012-5731.

Scheaffer, A. N., J. S. Caton, M. L. Bauer, and L. P. Reynolds. 2001. Influence of pregnancy on body weight, ruminal characteristics, and visceral organ mass in beef heifers. J. Anim. Sci. 79:2481–2490.

Suárez, B. J., C. G. Van Reenen, G. Beldman, J. van Delen, J. Dijkstra, and W. J. Gerrits. 2006a. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: I. Animal performance and rumen fermentation characteristics. J. Dairy Sci. 89:4365–4375. doi:10.3168/jds.S0022-0302(06)72483-3.

Suárez, B. J., C. G. Van Reenen, W. J. Gerrits, N. Stockhofe, A. M. van Vuuren, and J. Dijkstra. 2006b. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen development. J. Dairy Sci. 89:4376–4386. doi:10.3168/jds.S0022-0302(06)72484-5.

Suárez, B. J., C. G. Van Reenen, N. Stockhofe, J. Dijkstra, and W. J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. J. Dairy Sci. 90:2390–2403. doi:10.3168/jds.2006-524.

Sweeney, B. C., J. Rushen, D. M. Weary, and A. M. de Passille. 2010. Duration of weaning, starter intake, and weight gain of dairy calves fed large amounts of milk. J. Dairy Sci. 93:148–152. doi:10.3168/jds.2009-2427.

Terré, M., E. Pedrals, A. Dalmau, and A. Bach. 2013. What do preweaned and weaned calves need in the diet: A high fiber content or a forage source? J. Dairy Sci. 96:5217–5225. doi:10.3168/jds.2012-6304.

van Ackeren, C., H. Steingaß, K. Hartung, R. Funk, and W. Drochner. 2009. Effect of roughage level in a total mixed ration on feed intake, ruminal fermentation patterns and chewing activity of early-weaned calves with ad libitum access to grass hay. Anim. Feed Sci. Technol. 153:48–59. doi:10.1016/j.anifeedsci.2009.05.009.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Weary, D. M., J. Jasper, and M. J. Hotzel. 2008. Understanding weaning distress. Appl. Anim. Behav. Sci. 110:24–41. doi:10.1016/j.applanim.2007.03.025.

Webb, L. E., E. A. M. Bokkers, L. F. M. Heutinck, B. Engel, W. G. Buist, T. B. Rodenburg, N. Stockhofe-Zurwieden, and C. G. van Reenen. 2013. Effects of roughage source, amount, and particle size on behavior and gastrointestinal health of veal calves. J. Dairy Sci. 96:7765–7776. doi:10.3168/jds.2012-6135.

Žitnan, R., J. Voigt, U. Schönhusen, J. Wegner, M. Kokardová, H. Hagemeister, M. Levkut, S. Kuhla, and A. Sommer. 1998. Influence of dietary concentrate to forage ratio on the development of rumen mucosa in calves. Arch. Anim. Nutr. 51:279–291.