Sheep & Goat Research Journal
Volume 36, 2021 - December

Case Study: Effects of Supplemental Leucine in Milk Replacer on Pre- and Post-weaning Growth, Carcass Characteristics, Serum Amino Acids, and Visceral Tissue Mass in Lambs[1]

J. N. Reiners[*], *T. W. Hoffman*[*], *S. E. Anderson*[*], *and K. C. Swanson*[*,2]

[*] *Department of Animal Science, North Dakota State University, Fargo, ND 58102, USA*

[1] *This research was partially funded by the North Dakota State Board of Agricultural Research and Education. Authors would like to thank the employees of the NDSU Sheep Unit, Animal Nutrition and Physiology Center, Meat Laboratory, Nutrition Laboratory, and students who assisted with this project.*

[2] *Corresponding author: kendall.swanson@ndsu.edu*

Summary

The objective was to determine the effects of leucine supplementation during the pre-weaning period on growth performance until slaughter. Nineteen commercial Dorset ram lambs (5.07 ± 0.15 kg) were used. Leucine was added to milk replacer at 0 (control) or 2.9% of DM and provided to lambs for ad libitum intake for 42 d using LAC-TEK automated milk feeders. Lambs were then fed a corn-based finishing diet and slaughtered in two blocks based on final body weight (BW). Data were analyzed using the MIXED and GLM procedure of SAS. Leucine supplementation increased ADG (P = 0.007) during the pre-weaning period. Because milk replacer intake was not measured during the pre-weaning period, it is difficult to conclude if effects of Leu on pre-weaning growth were influenced by differences in milk replacer intake. Final BW and ADG during the finishing period were not affected by pre-weaning Leu supplementation. Mass of the reticulorumen tended (P = 0.09) to be greater in lambs supplemented with Leu pre-weaning but no other tissue masses were affected ($P \geq 0.39$) by pre-weaning Leu supplementation. Hot carcass weight and 12th rib fat thickness were unaffected by treatment. Thickness at the body wall was greater (P = 0.05) in lambs supplemented with Leu pre-weaning. Longissimus area, yield grade, quality grade, and percent boneless, closely trimmed retail cuts were not different between treatments. Results suggest that supplemental Leu to lambs fed milk replacer via automated feeders during the pre-weaning period increases growth in the pre-weaning period, especially in low birth weight lambs, without negatively affecting lamb performance in the finishing period. Additionally, Leu supplementation to lambs fed milk replacer may be useful to increase ADG of lighter weight lambs in the pre-weaning period.

Key Words: Developmental Programming, Leucine, Milk Replacer, Neonatal, Sheep

Introduction

Leucine is an essential amino acid (AA) and is required in diets to meet physiological needs, although in typical diets it is not believed to be limiting for production (Wu, 2009). Leucine has numerous effects on metabolism (Wu, 2009; Dodd and Tee, 2012; Millward, 2012) and is the primary AA signal for increasing muscle protein synthesis (Pedroso et al., 2015). Leucine supplementation to pre-weaned pigs has been shown to increase skeletal muscle protein synthesis (Escobar et al., 2010), mass of the longissimus dorsi, and BW (Columbus et al., 2015). Data are limited on the effects of supplemental Leu in pre-weaned lambs.

Lambs from twin or triplet births and twin lambs with a greater range in birth weights have been reported to have lower survivability during the pre-weaning period (Borg et al., 2007; Miller et al., 2010; Juengel et al., 2018; Notter et al., 2018). Removing lambs with lower birth weights and rearing with milk replacer may increase lamb survivability and production later in life. Chai et al. (2018) reported that removing a twin lamb from its dam after 10, 20, or 30 days and reared on milk replacer until 60 days of age resulted in greater ADG than their siblings that remained with their dam. Additionally, Soberon et al. (2012) reported that increasing ADG during the pre-weaning period in Holstein heifers increased milk production during the first lactation suggesting pre-weaning programming of productivity later in life.

The objectives of this study were to determine the effects of supplemental Leu to lambs fed milk replacer using an automated feeding system during the pre-weaning period on ADG and serum AA during the pre-weaning period, DMI and ADG during the finishing period, visceral organ masses, and carcass characteristics of lambs. We hypothesized that supplemental Leu to lambs fed milk replacer via automatic feeders would increase ADG during the pre-weaning period, ADG and gain:feed during the finishing period, and the cutability of carcasses.

Table 1. Dietary composition and nutrient concentrations of milk replacer and milk replacer supplemented with leucine (DM basis)[1]

Ingredient	Milk replacer	Milk replacer + leucine
Milk replacer, %	100	97.1
Supplemental Leu	0	2.9
Nutrient Composition		
Ash	6.15	5.98
Fat	16.0	15.5
CP	24.4	26.6
Ca	0.990	0.962
P	0.728	0.707
AA		
Glu	3.95	3.84
Leu	2.41	5.25
Asp	2.36	2.29
Lys	2.21	2.15
Thr	1.68	1.63
Pro	1.67	1.62
Val	1.58	1.54
Ile	1.53	1.49
Ser	1.25	1.21
Ala	1.14	1.11
Phe	0.977	0.949
Tyr	0.850	0.826
Arg	0.850	0.826
Met	0.648	0.630
Gly	0.573	0.557
His	0.563	0.547
Cys	0.467	0.454
Trp	0.446	0.433

[1] AA = amino acid; CP = crude protein; DM = dry matter.

Materials and Methods

Animals, Facilities, and Experimental Design

All procedures involving the use of animals were approved by the North Dakota State University (NDSU) Institutional Animal Care and Use Committee. Nineteen (n= 10 control, n = 9 Leu) neonatal fall-born ram lambs (5.07 ± 0.15 kg; twin-born n = 16, triplet-born n = 3) predominately of Dorset breeding were used. Lambs remained with ewes (2.8 ± 0.4 years old) for 12 h post-birth, so that lambs received colostrum, and then were randomly allotted to either a control milk replacer (Shepherd's Choice, Premier1 Supplies, Washington, IA, USA; n = 10; 8 twin lambs and 2 triplet lambs or the control milk replacer with 2.9% (DM basis) added Leu (n = 9; 8 twin lambs and 1 triplet lamb). Lambs were housed at the NDSU Sheep Unit and were fed using automated LAC-TEK Stainless 61450 milk dispensers (Biotic Industries, Inc., Bell Buckle, TN, USA; 1 per treatment;) which allowed for continuous ad libitum consumption of milk replacer. LAC-TEK machines were calibrated to deliver one part milk replacer (Table 1) and four parts heated water before the initiation of the experiment.

Lambs were allowed access to milk replacer for 42 d to provide a sufficient length of time to observe effects on growth performance. Individual intake of milk replacer was not measured making it difficult to conclude if treatment effects were because of the effects of Leu on milk replacer intake or other physiological effect(s). Water and starter feed, consisting of a creep feed and chopped alfalfa hay (Table 2), were provided for ad libitum intake when individual lambs reached 14 d of age. A partition within each pen was used to divide lambs that were less than 14 d of age from older lambs to reduce competition for the nipple feeders (nipple feeders separated along partition allowing a single LAC-TEC feeder to be used per treatment). On d 42, lambs were weaned and

removed from pre-weaning pens, comingled with weaned lambs from both treatments, and provided ad libitum access to water, creep feed, and chopped alfalfa hay.

After all lambs were weaned, lambs were moved to the NDSU Animal Nutrition and Physiology Center and penned in groups of four or five (0.91 × 2.4 m pens) in a temperature-controlled room (14ºC) on Tenderfoot flooring (Tandem Product, Inc., Minneapolis, MN) for 39 ± 3.6 d. Lambs were penned in groups until individual lambs reached approximately 30 kg BW (backgrounding period; from weaning until beginning of finishing period) or 12 weeks of age, and then were penned individually (0.91 × 1.2 m pens) for the remainder of the experiment (finishing period) to monitor daily feed intake. Creep feed and chopped alfalfa hay were provided for ad libitum intake and lambs were transitioned to a finishing diet (Table 2) over 14 d by feeding (DM basis) 75% of the backgrounding diet and 25% of the finishing diet for 5 d, 50% of the backgrounding diet and 50% of the finishing diet for 4 d, and 25% of the backgrounding diet and 75% of the finishing diet for 5 d. The finishing diet consisted of 90% pellets and 10% chopped alfalfa hay (DM basis) and was formulated to meet or exceed nutritional requirements for growing lambs gaining 300 g/d (NRC, 2007). Lambs were provided the finishing diet at 5% of BW to ensure ad libitum intake, and feeding amounts were adjusted every 14 d based on lamb weight.

The five heaviest lambs from each treatment were selected for slaughter after 68 (± 3.4) days of feeding the finishing diet and all remaining lambs were slaughtered after 96 (± 3.6) days of feeding the finishing diet. Lambs were slaughtered in two groups to increase uniformity in carcasses between lambs and because of constraints in slaughter capacity. The five heaviest lambs from each treatment were selected for the first day of slaughter to assure similar days on feed between treatment groups and because BW is a more objective measure than visual assessment of fatness or degree of finish. Hot carcass weight (HCW) and dressing percent was determined after slaughter. After a 24-h chill, carcasses were knife-ribbed between the 12th and 13th rib. Carcasses were evaluated for longissimus muscle area (LMA), fat thickness at the 12th rib (BF), body wall thickness (BWT), yield grade, leg score, flank streaking, and quality grade by trained personnel. Standard USDA grading procedures were used to derive a calculated yield grade. Percent of boneless, closely trimmed retail cuts (%BCTRC) was calculated, as described by Savell and Smith (2000).

Table 2. Nutrient composition of feeds provided to lambs (DM basis)[1]

	Creep		Finishing	
Nutrient composition	**Pellet[2]**	**Hay**	**Pellet[3]**	**Hay**
DM	89.1	89.7	88.0	87.7
Ash	6.87	8.80	4.31	9.33
CP	21.8	14.0	18.2	16.7
EE	3.38	0.77	3.18	0.815
NDF	13.5	57.7	11.1	52.4
ADF	7.70	40.3	3.27	37.9
Ca	0.767	1.04	0.686	1.08
P	0.434	0.295	0.330	0.284

[1] CP = crude protein; DM = dry matter; EE = ether extract.
[2] Creep pellet consisted of (DM basis) corn (46.6%), soybean meal (30%), beet pulp (19%), limestone (1.5%), urea (0.10%), and trace mineral salt supplement (2.8%).
[3] Finishing pellet consisted of (DM basis) corn (86.1%), soybean meal (9.6%), urea (1.65%), limestone (1.1%), and trace mineral salt supplement (1.58%).

Sample Collection

Lambs were weighed after birth and every 7 d until weaning. After weaning, lambs were weighed every 14 d through the backgrounding and finishing periods and 2 consecutive days before slaughter. Blood samples were collected via jugular venipuncture on d 1, 21, and 42 of the pre-weaning period at 1200 h for serum metabolite and AA concentration analyses. Blood samples were allowed to clot for 30 min at room temperature before being placed on ice and transferred to the laboratory. Serum was harvested by centrifugation (3,000 × g at 4ºC) for 20 min, transferred to microcentrifuge tubes, and stored at -20ºC until subsequent analysis. Samples of milk replacer (mixed with water), creep feed, chopped alfalfa hay, and finishing diet were sampled weekly. At slaughter, contents of the digestive tract were emptied and trimmed from the mesentery, and the mass and length of the small intestine, and mass of the reticulorumen, abomasum, omasum, colon, cecum, liver, pancreas, spleen, visceral fat, and kidneys were recorded.

Sample analysis

Feed samples were thawed at room temperature and subsequently dried (60ºC) in a forced-air oven for 48 h before being ground to pass a 1-mm screen. Feed samples were analyzed (AOAC, 1990) for DM, OM, N, and ether extract (EE). The techniques of Van Soest et al. (1991) were used to quantify neutral detergent fiber (NDF) and acid detergent fiber (ADF) nonsequentially. Crude protein concentration was calculated as 6.25 × N. Amino acid concentration of milk replacer was analyzed by high performance liquid chromatography after acid hydrolysis (AOAC, 1990). To assure accurate and precise analyses in our laboratory, samples are run in duplicate to ensure acceptable coefficients of variation and forage and concentrate control samples are run periodically to ensure consistency over time.

Serum glucose concentration was measured using the hexokinase/glucose-6-phosphate dehydrogenase method (Farrance, 1987) using the Infinity Glucose hexokinase kit (Thermo Trace, Louisville, KY, USA). Serum urea concentration was measured (Jung et al., 1975) using the QuantiChrom Urea Assay Kit (BioAssay Systems, Hayward, CA, USA). Serum free AA concentrations were analyzed by reversed phase ultra-performance liquid chromatogra-

phy after pre-column derivatization of AA with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Salazar et al., 2012; Lemley et al., 2013) and using an ethylene bridged hybrid C18 column (2.1 × 150 mm; 1.7 µm; Waters Corp., Milford, MA, USA).

Statistical Analysis

Pre-weaning and backgrounding data were analyzed as a completely randomized design. Lamb pre-weaning weight, serum AA, and serum metabolites were analyzed using the MIXED procedure in SAS (SAS 9.4, SAS Institute Inc., Cary, NC, USA) with repeated measures. The experimental unit was lamb. However, because lambs were offered milk replacer from a single feeder, it may be considered pseudoreplication rather than true replication. Because individual milk replacer intake was not measured, it is difficult to conclude if treatment effects were because of the effects of Leu on intake of milk replacer or some other physiological effect(s). The repeated effect was day, and the model included effect of day and treatment and their interaction. The REG procedure of SAS was used to examine how Leu supplementation influenced the relationship between birth weight and pre-weaning ADG. Tissue mass, initial and final finishing weight, and ADG during the finishing period were analyzed using the GLM procedure in SAS. The experimental unit was lamb, and the model included effects of treatment; days on feed during the backgrounding period was used as a covariate for finishing period data. Carcass characteristics data were analyzed as a randomized complete block design (slaughter date; n = 2 blocks). The experimental unit was lamb, and the model included effects of block and treatment. Significance was declared at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$.

Figure 1. Body weight (kg; A; pooled SEM = 0.563) and average daily gain (ADG, kg/d; B) of lambs during the pre-weaning period. Week 0 corresponds to lamb weight at birth. Effects for body weight were treatment × week ($P < 0.01$), treatment ($P < 0.01$), and week ($P < 0.01$), and effect for ADG was treatment ($P < 0.01$). * indicates significance between treatments within day.

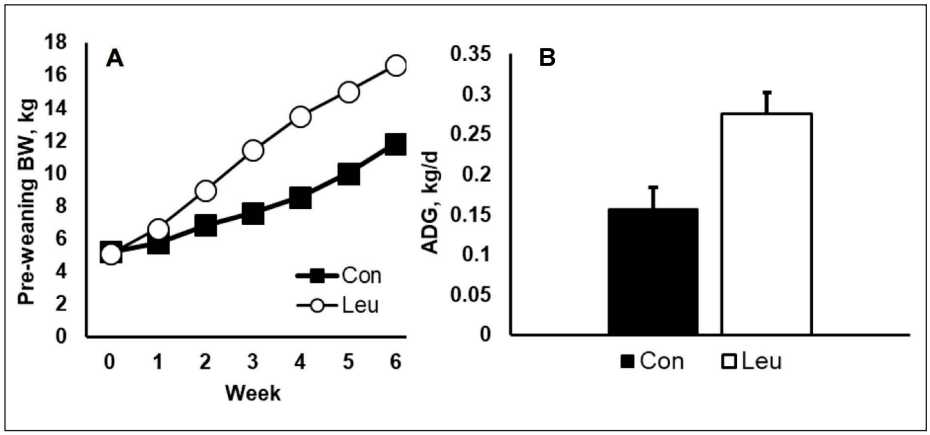
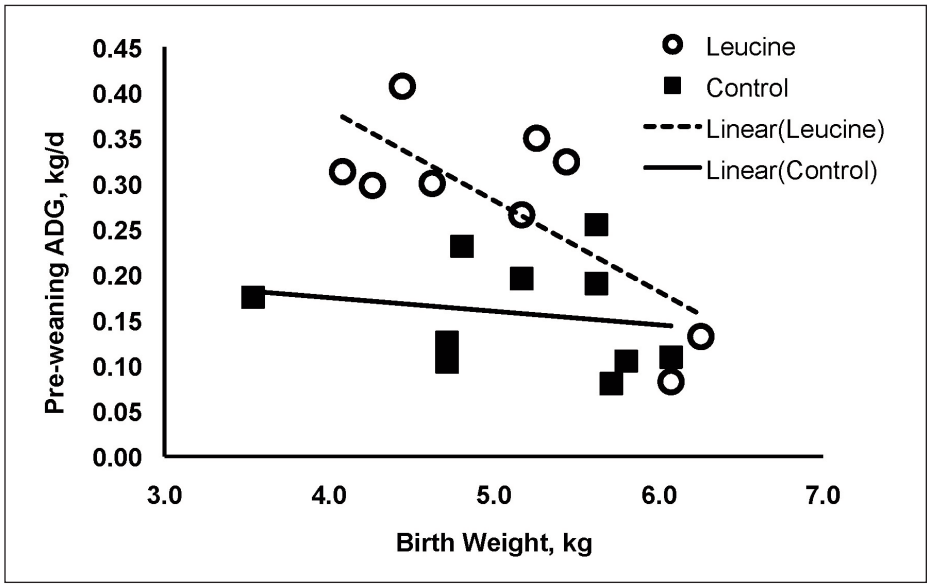


Figure 2. Linear regression of birth weight and pre-weaning average daily gain (ADG) for lambs during the pre-weaning period in Leu-supplemented and control lambs. Solid square and solid line are control, open circle and dashed line are leucine supplemented lambs. Linear regression of birth weight and pre-weaning ADG in control lambs: y = -0.0149x + 0.2343, r^2 = 0.04 (P = 0.60). Linear regression of birth weight and pre-weaning ADG in lambs supplemented with Leu pre-weaning: y = -0.1001x + 0.7818, r^2 = 0.56 (P = 0.02).



Results

Birth weight did not differ between treatments (Figure 1A). A treatment × week interaction was observed ($P < 0.01$) for pre-weaning BW with BW greater ($P \leq 0.04$) in lambs supplemented with Leu pre-weaning from day 7 until weaning. Average daily gain of lambs during the pre-weaning period (Figure 1B) was greater in lambs supplemented with Leu pre-weaning than control lambs ($P < 0.01$). In lambs supplemented with Leu pre-weaning, ADG decreased as lamb birth weight increased (r^2 = 0.56; P = 0.02; Figure 2) but in control lambs birth weight was not associated with ADG (r^2 = 0.04; P = 0.60).

No day × treatment interactions were observed for serum AA concentrations (Supplementary Table 1). Serum Leu concentrations were greater ($P < 0.01$) in lambs supplemented with Leu pre-weaning (Table 3). Lambs supplemented with Leu pre-weaning had greater (P = 0.03) serum Asp concentrations than control lambs. Serum concentrations of Arg, His, Ile, Lys, Met, Phe, Thr, Val, total EAA (essential AA), Ala, Asn, Gln, Glu, Gly, Pro, Ser, Tyr, total NEAA (non-essential AA) and total AA were not affected by treatment. A day effect was observed ($P < 0.01$) for

Table 3. Serum AA profile of lambs fed milk replacer with or without supplemental leucine during the pre-weaning period[1]

	Treatment			
Item, µM	**Control**	**Leucine**	**SEM**	***P*-value**
Arg	255	255	22.2	0.98
His	144	143	9.97	0.91
Ile	84.7	94.7	10.23	0.50
Leu	167	308	23.6	<0.01
Lys	160	180	10.8	0.22
Met	35.5	44.9	4.60	0.17
Phe	87.1	89.1	5.80	0.81
Thr	369	438	33.0	0.16
Val	294	281	22.4	0.67
Total EAA	1679	1911	101.8	0.13
Ala	233	240	9.7	0.61
Asn	88.6	95.5	5.73	0.40
Asp	13.0	15.2	0.69	0.03
Gln	161	151	12.8	0.60
Glu	240	270	18.4	0.27
Gly	517	476	28.7	0.32
Pro	224	234	15.9	0.67
Ser	135	150	8.1	0.20
Tyr	99.4	104.0	7.5	0.69
Total NEAA	1737	1732	46.7	0.94
Total AA	3416	3643	137.9	0.26
Metabolites, mg/dL				
Urea	19.0	21.4	1.09	0.13
Glucose	94.1	107.0	4.46	0.06

[1] AA = amino acids; EAA = essential amino acids; NEAA = non-essential amino acids

serum concentrations of His, Lys, Met, Phe, Val, total EAA, Asp, Gln, Glu, Pro, and Tyr where serum AA concentrations increased over the experimental period (Supplementary Table 1). Serum concentrations of His, Ile, Thr, Ala, Asn, Gly, Ser, total NEAA, and total AA decreased ($P < 0.01$) from d 1 to 21, then increased on d 42. Serum concentrations of Arg increased ($P < 0.01$) from d 1 to 21, then decreased on d 42.

There was no effect of treatment on serum urea N but a day effect was observed ($P < 0.01$) where serum urea N decreased from d 1 to 21, then increased on d 42 (Supplementary Table 1). Serum glucose concentrations tended to be greater ($P = 0.06$) in lambs supplemented with Leu pre-weaning than control lambs (Table 3). A day effect was observed ($P = 0.02$) for serum glucose, where concentrations increased from d 1 to 21, then decreased on d 42 (Supplementary Table 1).

Days on feed for the backgrounding period was not affected by treatment (Table 4) although Control lambs had numerically 24% greater (43 vs 36 days, respectively, $P = 0.11$) days on feed than Leu lambs. Control lambs had greater weight gain ($P = 0.01$) and ADG ($P = 0.05$) during the backgrounding period compared to lambs supplemented with Leu during the pre-weaning period. Lambs supplemented with Leu pre-weaning were transitioned to the finishing diet at heavier weights than control lambs ($P = 0.05$). For the finishing period, final BW, ADG, days on feed, average DMI, gain:feed, and age at slaughter were not affected by Leu supplementation.

Supplemental Leu pre-weaning did not affect small intestinal length or mass at slaughter (Table 5). Mass of the reticulorumen tended ($P = 0.09$) to be greater in lambs supplemented with Leu pre-weaning. Supplemental Leu pre-weaning did not affect mass of the abomasum, omasum, colon, cecum, liver, pancreas, spleen, visceral fat, or kidneys on an absolute or on a g tissue/kg BW basis.

Hot carcass weight, dressing percent, and back fat at the 12th rib was not affected by treatment (Table 6). Body wall thickness was greater ($P = 0.05$) in lambs supplemented with Leu pre-weaning. Longissimus dorsi area, yield grade, percent of boneless closely trimmed retail cuts, leg score, flank streakings, and quality grade were not affected by treatment.

Discussion

Supplemental Leu has been shown to increase muscle mass and growth in piglets (Escobar et al., 2010; Columbus et al., 2015). Additionally, increasing ADG of Holstein heifers during the pre-weaning period increased milk produc-

Table 4. Backgrounding and finishing performance of lambs fed milk replacer with or without supplemental leucine during the pre-weaning period[1]

	Treatment			
Backgrounding	**Control**	**Leucine**	**SEM**	***P*-value**
Days on feed	43.3	34.8	3.59	0.11
Weight gain, kg	13.7	9.8	0.98	0.01
ADG, g	320	284	12.2	0.05
Finishing				
Initial finishing BW, kg	27.7	30.1	0.78	0.05
Final finishing BW, kg	56.3	59.3	1.50	0.18
ADG, g	374	374	14.1	0.99
Days on feed	81.1	82.5	1.47	0.53
Daily feed intake, kg	1.46	1.40	0.078	0.65
Gain:feed	0.284	0.282	0.0272	0.66
Age at slaughter, d	179	181	1.69	0.39

[1] ADG = average daily gain; BW = body weight.

Table 5. Tissue mass of lambs fed milk replacer with or without supplemental leucine during the pre-weaning period[1]

	Treatment			
Item	**Control**	**Leucine**	**SEM**	***P*-value**
Small intestine				
Length, m	23.6	24.4	0.63	0.40
g	576	595	34.1	0.70
g/kg BW	10.4	9.98	0.628	0.66
Reticulorumen				
g	1052	1169	45.5	0.09
g/kg BW	19.0	19.5	0.67	0.61
Abomasum				
g	151	162	8.9	0.40
g/kg BW	2.71	2.73	0.164	0.92
Omasum				
g	116	127	17.4	0.67
g/kg BW	2.13	2.13	0.309	0.99
Colon				
g	425	449	38.2	0.66
g/kg BW	7.68	7.48	0.621	0.82
Cecum				
g	48.9	57.6	6.90	0.39
g/kg BW	0.880	0.989	0.1310	0.57
Liver				
g	965	1006	49.8	0.57
g/kg BW	17.4	16.9	0.88	0.68
Pancreas				
g	65.0	59.8	5.97	0.55
g/kg BW	1.16	1.01	0.092	0.27
Spleen				
g	90.5	98.0	4.07	0.21
g/kg BW	1.62	1.61	0.072	0.96
Visceral fat				
g	2928	3014	250.0	0.81
g/kg BW	52.2	50.2	3.62	0.71
Kidneys				
g	131	129	7.5	0.84
g/kg BW	2.34	2.18	0.129	0.39

[1] BW= body weight.

tion up to the third lactation (Soberon et al., 2012). However, data are limited on effects of supplemental Leu to lambs fed milk replacer via automated feeders during the pre-weaning period on pre- and post-weaning growth. Therefore, our objectives were to evaluate the effects of Leu supplemented pre-weaning on pre- and post-weaning growth of lambs, serum AA, visceral organ mass, and carcass characteristics.

In the current study, pre-weaning supplemental Leu to lambs fed for ad libitum intake using an automated feeders increased BW and ADG in the pre-weaning period, suggesting that Leu supplementation in pre-weaning lambs increases growth up until weaning at 42 days of age. Because lambs were group-fed and individual milk replacer intake was not measured, it is difficult to conclude if effects of Leu supplementation were mediated through changes in Leu intake or other physiological effect(s). However, it is unlikely that the magnitude of the observed positive effects on ADG could be mediated solely by differences in DMI. Future research is needed to monitor milk replacer intake in lambs supplemented with Leu. Our results differ from results of Mao et al. (2019) who reported no effects of Leu on BW or ADG in Hu lambs up to 30 d of age. Reasons for the discrepancy in results could be because lambs from the Mao et al. (2019) experiment nursed their dams until 5 d of age, Leu supplementation started at 11 d of age which resulted in the supplementation period of only 19 d, and lambs were limit-fed. This may suggest that Leu needs to be supplemented earlier in life and/or for a longer time period to elicit effects on growth during the pre-weaning period.

Interestingly, the stronger relationship between birth weight and ADG in light-weight lambs supplemented with Leu in the current study suggests that increasing Leu concentration in milk replacers may be beneficial for lambs with low birth weights. Although it is commonly assumed that lambs with lower birth weights have decreased ADG during the pre-weaning period, Greenwood et al. (1998) reported that lambs with low birthweight have the capacity to grow at similar rates than heavier lambs when fed milk replacer. Also, Wardrop (1968) reported a positive relationship between birth weight and pre-weaning ADG in female but not in male lambs. However, previous research has shown that low birth weight twin or triplet lambs and twin lambs with a greater range of birth weights had lower incidences of survivability in the pre-weaning period (Miller et al., 2010; Juengel et al., 2018). Removing lambs with low birth weights or lambs from triplet births from ewes and feeding with milk replacer supplemented with Leu could result in increased ADG and survivability. Further research is needed to confirm or refute the role that Leu supplementation may have on growth of low birth weight lambs.

As expected, serum Leu concentration was greater in lambs supplemented with Leu pre-weaning. Our results are similar to those of Nair et al. (1992) and Cao et al. (2018) who reported an increase in plasma Leu in humans and calves when supplemented with Leu. Supplemental Leu during the pre-weaning period had minimal effects on serum concentrations of other AA, suggesting that Leu was not inhibiting uptake or utilization of other AA, or that other AA were not limiting for growth in lambs supplemented with Leu pre-weaning. Supplemental Leu did increase serum Asp concentrations in lambs supplemented with Leu pre-weaning. These results contradict those of Zheng et al. (2019) and Mao et al. (2019) who reported increases in serum Met, Thr,

Table 6. Carcass characteristics of lambs fed milk replacer with or without supplemental leucine during the pre-weaning period[1]

	Treatment			
Item	**Control**	**Leucine**	**SEM**	***P*-value**
Hot carcass weight, kg	30.10	31.90	0.89	0.31
Dressing percent	54.0	53.3	0.51	0.33
12th rib back fat, mm	9.70	11.1	0.922	0.34
Body wall thickness, cm	2.84	3.16	0.087	0.05
Longissimus area, cm^2	15.8	15.6	0.75	0.20
Yield grade	4.10	4.79	0.315	0.22
% BCTRC	44.7	43.6	0.47	0.12
Leg score[2]	2.20	2.04	0.278	0.27
Flank streaking[3]	2.50	2.07	0.360	0.37
Quality grade[4]	1.90	1.76	0.198	0.45

[1] BCTRC = Boneless closely trimmed retail cuts
[2] Leg scores on scale 1-4; 1 = Low Choice, 2 = Average Choice, 3 = High Choice, 4 = Low Prime
[3] Flank scores on scale 1-4; 1 = Slight, 2 = Small, 3 = Modest, 4 = Moderate
[4] Quality grade on scale 1-3; 1 = High Choice, 2 = Low Prime, 3 = Average Prime

His, EAA, Gly, and Ser and increases in plasma concentrations of Ile and decreases in plasma concentrations of Ala and Met from calves and lambs, respectively, receiving supplemental Leu. These differences between studies could be because of differences in feeding management. Lambs in the current study were allowed ad libitum access to milk replacer, whereas calves from Zheng et al. (2019) and lambs from Mao et al. (2019) were meal-fed two or three times each day, respectively. El-Kadi et al. (2012) reported that continuous enteral delivery of milk replacer to piglets has limited effects on plasma AA, whereas bolus enteral delivery of milk replacer increased plasma AA concentrations. Boutry et al. (2013) also reported that bolus feedings increase EAA and NEAA concentrations in piglets compared to piglets that had a continuous orogastric infusion of milk replacer. It is possible that the lack of response in most of the serum AA is because of the continuous access to feed for lambs in the current study.

In the current study, lambs supplemented with Leu pre-weaning had a tendency for greater serum glucose concentrations. Leucine is unlikely to directly affect glucose production as it is a ketogenic AA (Voet and Voet, 2008). These results were not expected as Leu is known to increase insulin sensitivity in humans, which helps regulate circulating glucose levels (Zanchi et al., 2012). Previous research has reported no effects of supplemental Leu on glucose concentrations in pre-weaned piglets, calves, and lambs (Manjarín et al., 2016; Cao et al., 2018; Mao et al., 2019). Jarrett et al. (1964) reported that as lambs develop a functional rumen, glucose concentrations in the blood decreases. Although unlikely because control lambs had lower ADG, lambs on the control milk replacer could have had greater ruminal development, leading to lower serum glucose concentration than lambs supplemented with Leu pre-weaning.

In this study, an increase in total weight gain and ADG during the backgrounding period was observed in lambs fed the control milk replacer pre-weaning. The lambs likely experienced compensatory growth during this period, as at weaning they were 29% lighter than lambs supplemented with Leu pre-weaning. Similarly, Greenwood and Café (2007) reported that calves with lower weaning weights had greater ADG during the backgrounding period, when fed until a common age or weight, but ADG during the finishing period was unaffected. However, further research is needed to better understand how pre-weaning Leu-supplementation influences post-weaning growth in different growing and finishing systems.

During the finishing period, initial BW of lambs supplemented with Leu pre-weaning was greater than control lambs; this was likely because Leu lambs reached the target weight more rapidly than control lambs. However, final weight of lambs supplemented with Leu pre-weaning was not different than control lambs. Lambs supplemented with Leu pre-weaning in this experiment attained a heavier weight at weaning, and numerically started the finishing period after a shorter backgrounding period (36 vs. 43 d) than control lambs. This resulted in a numerical increase in days on feed for the finishing period for lambs supplemented with Leu pre-weaning as lambs within both treatments were slaughtered after a common time on feed on one of two dates. Gain:feed often decreases as animals mature and deposit more fat than protein (Ferrell, 1988). The greater body wall fat thickness observed in lambs supplemented with Leu pre-weaning could suggest greater fat deposition later in the finishing period. More efficient feed conversion during the finishing period may have been observed in lambs supplemented with Leu pre-weaning if slaughtered at a common body fatness rather than common days on feed. This could suggest that the resulting increase in ADG during the pre-weaning period in lambs supplemented with Leu pre-weaning may be beneficial in reducing the number of days lambs spend on feed and attain market weight, without negatively affecting carcass yield and quality.

In conclusion, supplemental Leu to lambs fed milk replacer with automated feeders during the pre-weaning period increased lamb ADG by 75% at weaning and increased weaning weight by 5 kg, but the increased ADG did not persist through the finishing period. Because individual milk replacer intake was not measured, it is difficult to conclude if effects of Leu on ADG were influenced by differences in milk replacer intake. Additionally, Leu supplementation to lambs fed milk replacer may be useful to increase ADG of lighter weight lambs in the pre-weaning period, and produce lambs for harvest at a similar compositional endpoint in fewer days for finishing. Future research is needed to monitor milk replacer intake in lambs supplemented with Leu and to further study the potential role that Leu supplementation may have on growth of low birth weight lambs.

References

AOAC. 1990. Official Methods of Analysis, Arlington, VA.

Borg, R. C., D. R. Notter, L. A. Kuehn, and R. W. Kott. 2007. Breeding objectives for Targhee sheep. J. Anim. Sci. 85:2815-2829. doi: 10.2527/jas.2006-064

Boutry, C., S. W. El-Kadi, A. Suryawan, S. M. Wheatley, R. A. Orellana, S. R. Kimball, H. V. Nguyen, and T. A. Davis. 2013. Leucine pulses enhance skeletal muscle protein synthesis during continuous feeding in neonatal pigs. Am. J. Physiol. Endocrinol. Metab. 305:E620-E631. doi: 10.1152/ajpendo.00135.2013

Cao, Y. C., X. J. Yang, L. Guo, C. Zheng, D. D. Wang, C. J. Cai, S. M. Liu, and J. H. Yao. 2018. Effects of dietary leucine and phenylalanine on pancreas development, enzyme activity, and relative gene expression in milk-fed Holstein dairy calves. J. Dairy Sci. 101:4235-4244. doi: 10.3168/jds.2017-13987

Chai, J., Q. Diao, J. Zhao, H. Wang, K. Deng, M. Qi, M. Nie, and N. Zhang. 2018. Effects of rearing system on meat quality, fatty acid and amino acid profiles of Hu lambs. Anim. Sci. J. 89:1178-1186. doi: 10.1111/asj.13013

Columbus, D. A., M. L. Fiorotto, and T. A. Davis. 2015. Leucine is a major regulator of muscle protein synthesis in neonates. Amino Acids 47:259-270. doi: 10.1007/s00726-014-1866-0

Dodd, K. M., and A. R. Tee. 2012. Leucine, and mTORC1: a complex relationship. Am. J. Physiol. Endocrinol. Metab. 302:E1329-E1342. doi: 10.1152/ajpendo.00525.2011

El-Kadi, S. W., A. Suryawan, M. C. Gazzaneo, N. Srivastava, R. A. Orellana, H. V. Nguyen, G. E. Lobley, and T. A. Davis. 2012. Anabolic signaling and protein deposition are enhanced by intermittent compared with continuous feeding in skeletal muscle of neonates. Am. J. Physiol. Endocrinol. Metab. 302:E674-E686. doi: 10.1152/ajpendo.00516.2011

Escobar, J., J. W. Frank, A. Suryawan, H. V. Nguyen, C. G. Van Horn, S. M. Hutson, and T. A. Davis. 2010. Leucine and alpha-ketoisocaproic acid, but not norleucine, stimulate skeletal muscle protein synthesis in neonatal pigs. J. Nutr. 140:1418-1424. doi: 10.3945/jn.110.123042

Farrance, I. 1987. Plasma glucose methods, a review. Clin. Biochem. Rev. 8:55-68.

Ferrell, C. L. 1988. Energy metabolism. In: D. C. Church, editor, The Ruminant Animal - Digestive Physiology and Nutrition. Prentice-Hall, Englewood Cliffs, NJ. p. 250-268.

Greenwood, P. L., and L. M. Cafe. 2007. Prenatal and pre-weaning growth and nutrition of cattle: Longterm consequences for beef production. Animal 1:1283-1296. doi: 10.1017/S175173110700050X

Greenwood, P. L., A. S. Hunt, J. W. Hermanson, and A. W. Bell. 1998. Effects of birth weight and postnatal nutrition on neonatal sheep: I. Body growth and composition, and some aspects of energetic efficiency. J.Anim.Sci. 76:2354. doi: 10.2527/1998.7692354x

Jarrett, I., G. Jones, and B. Potter. 1964. Changes in glucose utilization during development of the lamb. Biochem. J. 90:189-194. doi: 10.1042/bj0900189

Juengel, J. L., G. H. Davis, R. Wheeler, K. G. Dodds, and P. D. Johnstone. 2018. Factors affecting differences between birth weight of littermates (BWTD) and the effects of BWTD on lamb performance. Anim. Reprod. Sci. 191:34-43. doi: 10.1016/j.anireprosci.2018.02.002

Jung, D., H. Biggs, J. Erikson, and P. U. Ledyard. 1975. New colorimetric reaction for end-point, continuous-flow, and kinetic measurement of urea. Clin. Chem. 21:1136-1140. doi: 10.1093/clinchem/21.8.1136

Lemley, C. O., L. E. Camacho, A. M. Meyer, M. Kapphahn, J. S. Caton, and K. A. Vonnahme. 2013. Dietary melatonin supplementation alters uteroplacental amino acid flux during intrauterine growth restriction in ewes. Animal 7:1500-1507. doi: 10.1017/S1751731113001006

Manjarín, R., D. A. Columbus, A. Suryawan, H. V. Nguyen, A. D. Hernandez-García, N.-M. Hoang, M. L. Fiorotto, and T. Davis. 2016. Leucine supplementation of a chronically restricted protein and energy diet enhances mTOR pathway activation but not muscle protein synthesis in neonatal pigs. Amino Acids 48:257-267. doi: 10.1007/s00726-015-2078-y

Mao, H., C. Wang, and Z. Yu. 2019. Dietary leucine supplementation enhances the health of early weaned Hu lambs. Anim. Feed Sci. Technol. 247:248-254. doi: 10.1016/j.anifeedsci.2018.11.020

Miller, D. R., D. Blache, R. B. Jackson, E. F. Downie, and J. R. Roche. 2010. Metabolic maturity at birth and neonate lamb survival: association among maternal factors, litter size, lamb birth weight, and plasma metabolic and endocrine factors on survival and behavior. J. Anim. Sci. 88:581-593. doi: 10.2527/jas.2009-2170

Millward, D. J. 2012. Knowledge gained from studies of leucine consumption in animals and humans. J. Nutr. 142:2212S-2219S. doi: jn.111.157370

Nair, K. S., D. E. Matthews, S. L. Welle, and T. Braiman. 1992. Effect of leucine on amino acid and glucose metabolism in humans. Metabolism 41:643-648. doi: 10.1016/0026-0495(92)90057-h

Notter, D. R., M. R. Mousel, T. D. Leeds, G. S. Lewis, and J. B. Taylor. 2018. Effects of rearing triplet lambs on ewe productivity, lamb survival and performance, and future ewe performance. J. Anim. Sci. 96:4944-4958. doi: 10.1093/jas/sky364

NRC. 2007. Nutrient Requirements of Small Ruminants. National Academies Press, Washington, DC.

Pedroso, J. A., T. T. Zampieri, and J. Donato, Jr. 2015. Reviewing the effects of L-leucine supplementation in the regulation of food intake, energy balance, and glucose homeostasis. Nutrients 7:3914-3937. doi: 10.3390/nu7053914

Salazar, C., J. M. Armenta, and V. Shulaev. 2012. An UPLC-ESI-MS/MS assay using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivatization for targeted amino acid analysis: application to screening of arabidopsis thaliana mutants. Metabolites 2:398-428. doi: 10.3390/metabo2030398

Savell, J. W., and G. C. Smith. 2000. Laboratory Manual for Meat Science. 7th ed. American press, Boston, MA.

Soberon, F., E. Raffrenato, R. W. Everett, and M. E. Van Amburgh. 2012. Preweaning milk replacer intake and effects on long-term productivity of dairy calves. J. Dairy Sci. 95:783-793. doi: 10.3168/jds.2011-4391

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

Voet, D., and J. G. Voet. 2008. Amino acid metabolism, Fundamentals of Biochemistry. John Wiley & Sons, Hoboken, NJ, USA. p. 732-790.

Wardrop, I. D. 1968. Birth weight, liveweight gain in early life, and subsequent sain in sheep and cattle. Aus. J. Agr. Res. 19:837-844. doi: 10.1071/Ar9680837

Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. Amino Acids 37:1-17. doi: 10.1007/s00726-009-0269-0

Zanchi, N. E., L. Guimarães-Ferreira, M. A. Siqueira-Filho, J. P. Gabriel Camporez, H. Nicastro, D. F. Seixas Chaves, P. Campos-Ferraz, A. H. Lancha, and C. R. De Oliveira Carvalho. 2012. The possible role of leucine in modulating glucose homeostasis under distinct catabolic conditions. Med. Hypotheses 79:883-888. doi: 10.1016/j.mehy.2012.09.018

Zheng, C., J. Yao, L. Guo, Y. Cao, Z. Liang, X. Yang, and C. Cai. 2019. Leucine induced promotion of post absorptive EAA utilization and hepatic gluconeogenesis contributes to protein synthesis in skeletal muscle of dairy calves. J. Anim. Phys. Anim. Nutr. 103:705-712. doi: 10.1111/jpn.13072

Supplementary Material

Supplementary Table S1. Serum AA and metabolites of lambs fed milk replacer with or without supplemental leucine during the pre-weaning period[1]

	Treatments							*P*-value		
	Control			Leucine						
AA, µM	**1**	**21**	**42**	**1**	**21**	**42**	**SEM[1]**	**Day**	**Trt**	**Day × Trt**
Arg	153	371	240	176	338	252	33.5	<0.01	0.98	0.80
His	295	67.1	71.2	273	73.2	82.1	17.3	<0.01	0.91	0.61
Ile	96.8	77.1	80.2	115	67.5	102	14.8	0.01	0.50	0.26
Leu	226	147	127	334	337	253	37.4	0.13	<0.01	0.31
Lys	142	140	198	158	168	213	20.9	0.08	0.22	0.93
Met	49.5	28.7	28.4	54.4	35.7	44.7	7.21	0.01	0.17	0.70
Phe	136	71.1	54.1	139	70.3	57.6	8.62	<0.01	0.81	0.91
Thr	604	197	306	570	472	272	67.3	<0.01	0.16	0.11
Val	459	222	202	498	175	169	32.4	<0.01	0.67	0.54
Total EAA	2309	1368	1359	2379	1807	1547	176	<0.01	0.13	0.57
Ala	394	141	162	425	133	161	15.8	<0.01	0.61	0.57
Asn	145	49.9	71.2	151	66.5	69.1	10.3	<0.01	0.40	0.68
Asp	17.7	10.0	11.2	24.2	11.0	10.5	1.31	<0.01	0.03	0.06
Gln	62.3	20.9	17.6	22.3	22.0	18.5	20.6	<0.01	0.60	0.28
Glu	471	132	118	542	116	152	25.5	<0.01	0.27	0.10
Gly	537	319	697	471	363	592	52.2	<0.01	0.32	0.36
Pro	439	108	124	433	127	141	20.8	<0.01	0.67	0.94
Ser	241	68.1	95.0	251	85.2	113	11.5	<0.01	0.20	0.96
Tyr	193	54.9	50.4	213	53.0	45.7	10.61	<0.01	0.69	0.72
Total NEAA	2580	1082	1550	2524	1164	1508	96.0	<0.01	0.94	0.80
Total AA	4888	2450	2909	4902	2971	3055	231.4	<0.01	0.26	0.50
Metabolites, mg/dL										
Urea	24.6	17.9	14.5	27.2	15.9	21.1	1.87	<0.01	0.13	0.08
Glucose	82.6	101	98.5	93.7	126	101	7.43	0.02	0.06	0.14

[1] AA, amino acids; EAA, essential amino acids; NEAA, non-essential amino acids.