Effects of leucine supplementation on growth and plasma metabolites in preweaned Holstein dairy calves

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Leucine supplementation during the preweaning phase altered plasma amino acid and glucose profiles, suggesting changes in nutrient utilization. These metabolic shifts may have implications for long-term productivity and warrant further investigation.

Summary

This study evaluated the effects of leucine and alanine supplementation in milk replacer on performance and blood metabolites of Holstein calves during the preweaning phase. No overall effects on body weight or weekly gain were observed. However, leucine altered plasma amino acid profiles and glucose dynamics, suggesting shifts in nutrient utilization. These findings indicate that while growth performance remained unchanged, leucine may influence metabolic pathways with potential implications for long-term development.

Introduction

Rapid growth during the preweaning phase programs lifetime productivity in dairy heifers. A meta-analysis showed that every additional 0.2 lb/d of average daily gain (ADG) before weaning yields ~190-240 lb more milk in the first lactation (Soberon & Van Amburgh, 2013). Because rumen function is minimal at this age, the nutrient profile of milk or milk replacer largely dictates anabolic potential.

Beyond supplying amino acids (AA) for protein accretion, certain AA act as metabolic signals. Leucine, in particular, activates the mTOR pathway, promoting translation initiation and muscle protein synthesis (Kimball & Jefferson, 2006). While leucine enhances lean tissue deposition in nonruminant neonates, data in young ruminants remain limited and inconclusive.

Reiners et al. (2022) supplemented Holstein bull calves with up to 0.8 g leucine/kg BW/day in milk replacer. Serum leucine concentration increased linearly, concentrations of several other amino acids linearly decreased, but ADG and nitrogen retention were unaffected by leucine supplementation. However, because added leucine increased nitrogen (and thus crude protein [CP]) intake, the study could not isolate leucine-specific effects from higher nitrogen supply. To our knowledge, no published research has evaluated isonitrogenous leucine supplementation that separates signaling effects from CP-related responses.

Therefore, the objective of this study was to evaluate the effects of supplementing milk replacer with leucine on growth performance and blood concentrations of AA, glucose and blood urea nitrogen in preweaned Holstein heifer calves.

Experimental Procedures

All animal procedures followed North Dakota State University animal care guidelines and were approved by the institutional committee. The study was conducted at the NDSU Dairy Unit in Fargo, North Dakota, between June 2023 and May 2024. Thirty-five newborn Holstein heifer calves were enrolled as they became available. At birth, each calf was separated from its dam, weighed and fed maternal colostrum within two hours to ensure good transfer of immunity; colostrum feeding continued for the first three days of life. Starting on day 4, all calves received a commercial milk replacer (Amplifier Max BOV MOS, Land O'Lakes).

Calves were randomly assigned, one out of every three births, to one of three treatments. Control (CON) calves received the milk replacer (20.4% CP) mixed as directed on the product label. Leucine (LEU) calves received the milk replacer with an inclusion of 5 % crystalline leucine (99.7 % purity) on a dry matter basis. Calves on the alanine (ALA) treatment received the milk replacer plus 3.47 % of crystalline L-alanine (99.7 % purity) to provide equal amounts of total nitrogen intake per day as the LEU calves. Milk replacer was offered twice daily at 5 a.m. and 2 p.m. During the first week, each feeding consisted of 0.69 lb of milk

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replacer powder blended into 2 qt of warm water; from the second week onward, 1.09 lb of powder was mixed into 3 qt of water per feeding. Calves also had ad libitum access to starter pellets and water from day 4 onward, with refusals weighed weekly to estimate starter intake.

Following NDSU Dairy Unit management procedures, calves were housed in a climate-controlled group pen during the first week. On day 8, they were moved to individual outdoor hutches bedded with straw, where they remained for the rest of the 56-day milk replacer-feeding phase, which ran from study day 4 through day 59. Body weight was recorded at the start (days 3-4), every seven days and at the end (day 59) of milk replacer-feeding. Blood samples were taken from the jugular vein three hours after feeding on days 28 and 56 of the milk replacer-feeding phase. Samples were allowed to clot for 30 minutes, kept on ice and spun in a refrigerated centrifuge, and the serum was stored at minus 20 degrees Celsius. Serum AA concentrations were measured by high-performance liquid chromatography, and glucose and urea nitrogen by ultraviolet/visible spectrophotometry.

All analyses were carried out with SAS 9.4 (SAS Institute Inc., Cary, NC). Growth variables (body weight and weekly gain) were fitted with a mixed-effects repeated-measures model that included fixed effects of dietary treatment (CTL, ALA, LEU), week (1 to 8) and their interaction. For the body weight response, the model was adjusted for initial weight at week 0, whereas this covariate was omitted when weekly gain (lb week^{-1}) was the dependent variable. Two a priori contrasts were tested: control versus the average of the two AA treatments (CTL vs. [ALA + LEU]) and ALA versus LEU. Statistical significance was declared at $P \leq 0.05$, and $0.05 < P \leq 0.10$ was interpreted as a tendency. Serum variables used the same model framework but replaced week with sampling day.

Results and Discussion

Dietary treatment did not affect BW or weekly gain during the eight-week milk replacer-feeding phase ($P > 0.10$; Figure 1). However, a treatment × week interaction was detected for weekly gain ($P = 0.042$; Figure 2) as LEU calves showed greater gain than CTL and ALA in week 3 ($P < 0.05$) with tendencies for reduced gain compared to CTL in week 6 and to ALA in week 7 ($P < 0.10$). These transient effects likely explain the lack of differences in final BW. Overall, leucine supplementation caused week-specific shifts in gain that were not consistent or cumulative, suggesting no performance advantage of ALA or LEU under these conditions.

Plasma urea nitrogen (BUN) concentration was not affected by treatment, day or their interaction ($P > 0.10$). For plasma glucose concentration, there was a treatment × day interaction ($P = 0.021$). Among calves fed the LEU diet, glucose concentration increased from day 28 to day 56 ($P = 0.042$), whereas no change over time occurred for CTL or ALA. Despite this, glucose concentrations were not different among treatments on both days, indicating a transient LEU effect on glucose metabolism.

For plasma essential amino acids (EAA), leucine ($P < 0.001$), threonine ($P = 0.010$) and valine ($P = 0.001$) were higher in supplemented groups (ALA + LEU) vs. CTL, with a tendency for lower phenylalanine ($P = 0.061$). Comparing the two supplemented groups, calves fed ALA had greater plasma concentrations of isoleucine ($P < 0.001$), lysine ($P = 0.013$) and methionine ($P = 0.003$), whereas calves fed LEU had markedly greater leucine concentrations ($P < 0.001$). Regarding nonessential amino acids (NEAA), supplementation had an

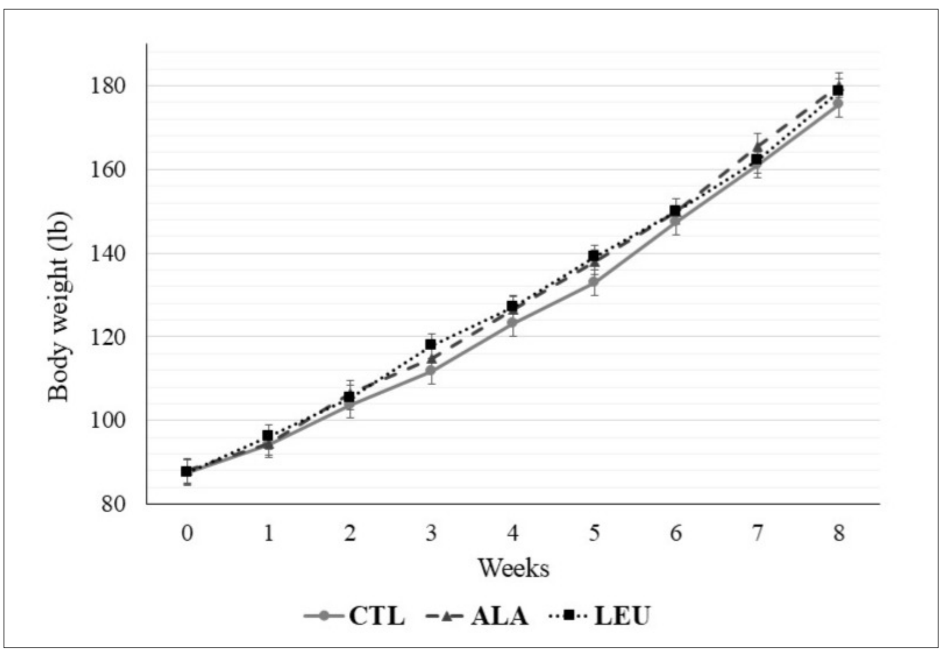

Figure 1. Average body weight (BW; mean ± SE) of Holstein heifer calves during the 8-week milk-replacer phase. Calves received CTL = unsupplemented milk replacer, ALA = milk replacer + alanine or LEU = milk replacer + leucine. *P*-values: Treatment = 0.664; Week < 0.001; Treatment × Week = 0.137. Planned contrasts: CTL vs (ALA + LEU) = 0.647; ALA vs LEU = 0.427.

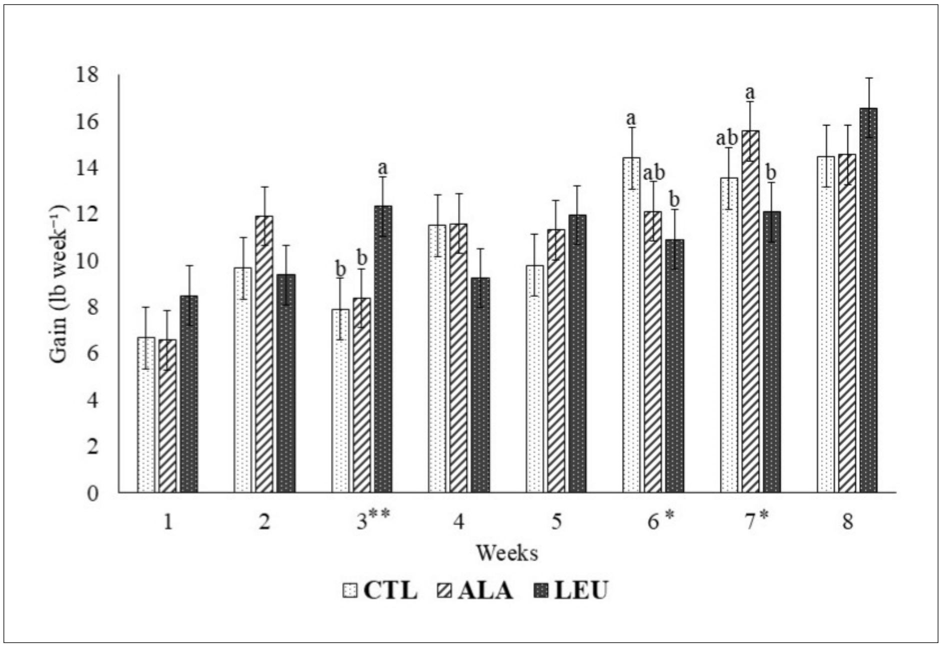

Figure 2. Weekly body-weight gain (lb week^{-1}) of Holstein heifer calves during the 8-week milk-replacer phase. Calves received CTL = unsupplemented milk replacer, ALA = milk replacer + alanine, or LEU = milk replacer + leucine. *P*-values: Treatment = 0.757; Week < 0.001; Treatment × Week = 0.043. Planned contrasts: CTL vs (ALA + LEU) P = 0.597; ALA vs LEU *P* = 0.593. Symbols below the x-axis mark overall treatment effects within each week: ** = significant ($P \leq 0.05$); * = tendency ($0.05 < P \leq 0.10$). Within a week, bars that do not share a common superscript letter differ ($P<0.10$).

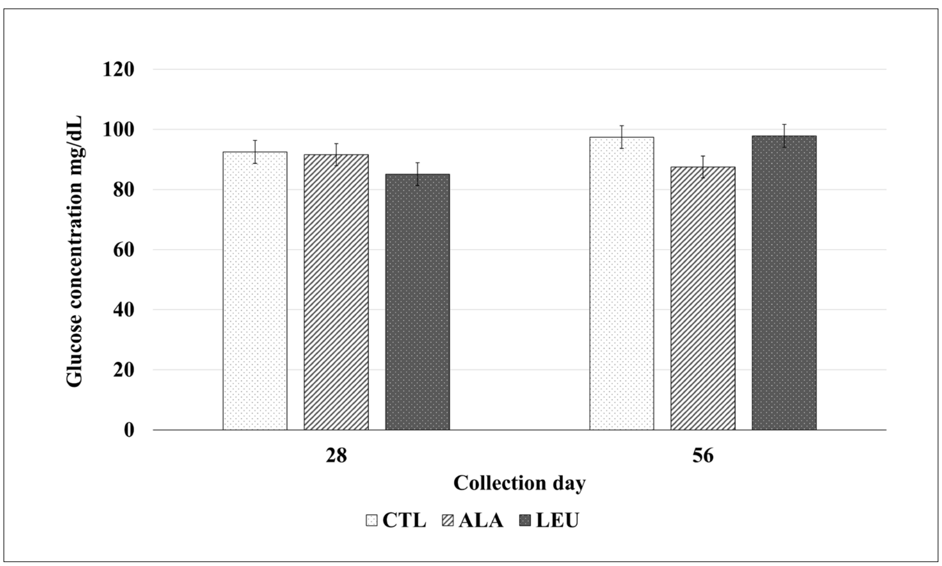

Figure 3. Plasma glucose concentrations (mean ± SE) of Holstein heifer calves on days 28 and 56 of the milk-replacer phase. Calves received CTL = unsupplemented milk replacer, ALA = milk replacer + alanine, or LEU = milk replacer + leucine. *P*-values: Treatment = 0.494; Day = 0.062; Treatment × Day = 0.022. The Treatment × Day interaction was driven by an increase in glucose from day 28 to 56 in the LEU group ($P = 0.042$).

effect on plasma alanine ($P = 0.013$), proline ($P < 0.001$) and tyrosine ($P = 0.007$) and a tendency for increased glutamic acid in the supplemented groups ($P = 0.072$). When comparing ALA and LEU groups, ALA calves had higher concentrations of asparagine ($P = 0.040$), cystine ($P = 0.038$) and proline ($P < 0.001$). No treatment × day interactions were observed for any NEAA.

Although no consistent improvements in performance were observed, the inclusion of leucine in the milk replacer led to distinct shifts in the plasma amino acid profile that cannot be attributed solely to increased nitrogen intake. Compared with alanine, which served as an isonitrogenous control, leucine supplementation altered concentrations of several essential and nonessential amino acids, suggesting changes in metabolic utilization and possibly enhanced protein synthesis. The transient increase in glucose concentration and the marked rise in circulating leucine itself further support the idea that leucine modulates nutrient partitioning during early development. From a practical standpoint, such metabolic adaptations could contribute to differences in muscle growth potential or efficiency in later productive stages, even if not immediately reflected in preweaning body weight.

In conclusion, supplementation of leucine in milk replacer during the preweaning phase did not improve short-term growth but elicited measurable metabolic responses that suggest altered amino acid utilization. These findings emphasize the need for further research to determine whether such early-life metabolic shifts influence long-term productivity in dairy heifers.

Table 1. Plasma amino-acid concentrations (μmol L^{-1}) of Holstein heifer calves fed a commercial milk replacer alone (CTL), or the same milk replacer supplemented with alanine (ALA) or leucine (LEU).

	Treatments				*P*-values		
Items	Control	Alanine	Leucine	SEM	CP level[1]	LEUvsALA[2]	Treatment x Day[3]
Essential amino-acids (EAA)							
Histidine	29.5	34.2	28.2	2.72	0.623	0.131	0.235
Isoleucine	108.8	116.9	87.1	4.89	0.272	<0.001	0.288
Leucine	152	164	526	27.1	<0.001	<0.001	0.078
Lysine	101.2	108.6	89.0	5.21	0.714	0.013	0.407
Methionine	177	183	141	9.0	0.192	0.003	0.244
Phenylalanine	41.4	35.0	37.8	2.06	0.061	0.345	0.306
Threonine	214	174	167	12.6	0.010	0.663	0.319
Tryptophan	49.4	52.8	51.7	2.40	0.350	0.749	0.411
Valine	80.1	65.2	61.2	3.81	0.001	0.472	0.125
Non-essential amino-acids (NEAA)							
Alanine	219	182	172	12.6	0.013	0.600	0.469
Arginine	154	165	157	9.3	0.518	0.535	0.578
Asparagine	34.8	39.8	29.3	3.48	0.954	0.040	0.215
Aspartic acid	214	237	213	13.5	0.538	0.217	0.709
Cystine	126	128	105	7.5	0.325	0.038	0.362
Glutamic acid	84.4	100.0	91.8	4.94	0.072	0.247	0.782
Glutamine	8.94	9.97	10.66	1.633	0.504	0.768	0.851
Glycine	293	306	318	16.7	0.359	0.593	0.541
Proline	352	642	286	23.8	<0.001	<0.001	0.493
Serine	155	146	130	8.0	0.108	0.170	0.910
Tyrosine	39.0	30.5	32.6	2.04	0.007	0.490	0.722

[1]Planned contrast comparing the CTL group with the mean of the two amino-acid–supplemented groups (ALA + LEU).
[2]Planned contrast comparing the Alanine group with the Leucine group. [3]P-value for the overall Treatment × Day interaction.
Differences were declared significant at $P \leq 0.05$ and considered a tendency when $0.05 < P \leq 0.10$.

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