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Short communication: Performance, intestinal permeability, and metabolic profile of calves fed a milk replacer supplemented with glutamic acid

M. A. Ahangarani,[1] A. Bach,[2,3] A. Bassols,[4] M. Vidal,[2] D. Valent,[4] S. Ruiz-Herrera,[4] and M. Terré[2]*
[1]Department of Animal Science, College of Agriculture, 98619-15599 Zabol, Iran
[2]Department of Ruminant Production, IRTA (Institut de Recerca i Tecnologia Agroalimentàries), 08140 Caldes de Montbui, Spain
[3]ICREA (Institució Catalana de Recerca i Estudis Avançats), 08010 Barcelona, Spain
[4]Departament de Bioquímica i Biologia Molecular, Facultat de Veterinària, Universitat Autònoma de Barcelona Cerdanyola del Vallès, 08193 Barcelona, Spain

ABSTRACT

The objective of this study was to evaluate the potential benefits of supplementing glutamic acid in milk replacers (MR) with respect to calf performance, intestinal permeability, and metabolism. Sixty Holstein male calves (3 ± 1.3 d old and 45 ± 5.9 kg body weight) were individually housed and fed a control MR without AA supplementation (24.8% crude protein and 19.1% fat, dry matter basis), or MR supplemented with 0.3% glutamic acid (25.1% crude protein and 20.3% fat, dry matter basis). Animals followed the same MR feeding program and were weaned at 56 d of the study. The amount of starter concentrate offered was restricted to limit the effect of concentrate intake on calf metabolism. Individual daily consumption and weekly body weight were measured, and 4 h after the morning feeding, blood samples were obtained at 14 and 35 d to determine general biochemical parameters and plasma AA concentrations. On d 10 of the study, we conducted an intestinal permeability test by including 21 g of lactulose and 4.2 g of D-mannitol as markers in the MR. We found no differences in calf performance or in intestinal permeability (measured as lactulose:mannitol ratio). Serum glucose concentration was greater in unsupplemented calves than in Glu-supplemented calves. At 14 d, the proportion of plasma Leu was greater in Glu-supplemented calves; the proportion of Ile tended to be greater in Glu-supplemented calves; and the proportion of Met tended to be greater in unsupplemented calves. We observed no other differences. Small changes occurred in AA metabolism when supplementing calf MR with 0.3% glutamic acid, without leading to improvements in calf performance or changes in intestinal permeability.

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*Corresponding author: marta.terre@irta.cat

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Short Communication

In recent decades, research has focused on improving newborn calf health (Cho and Yoon, 2014; Harris et al., 2017), especially diarrhea during the first 3 wk of life (Gulliksen et al., 2009; Mahendran et al., 2017). Colostrum is an excellent source of nutrients, but also a pivotal source of antimicrobials, immune-stimulating factors, and growth factors protecting against infections (Menchetti et al., 2016) that promote epithelial cell proliferation (Altomare et al., 2016) and maturation of the gastrointestinal tract (Blum, 2006; Blum and Baumrucker, 2008; Hammon et al., 2013). To support gastrointestinal tract development beyond colostrum feeding, glutamic acid might represent a good supplement, because it is a major oxidative fuel for the intestine (Burrin and Stoll, 2009) and immune cells (Huang et al., 2003). Furthermore, glutamic acid has been shown to act as a protector for intestinal mucosa, maintaining intestinal barrier function (Wang et al., 2014), and as a mediator of immune response (Ruth and Field, 2013). In addition, glutamic acid is a precursor of glutamine, an AA considered to be essential for neonates (Wu et al., 2014). The objective of this study was to evaluate the potential effects of glutamic acid supplementation on calf performance, metabolism, and intestinal permeability.

Sixty male Holstein newborn calves from a single commercial farm were enrolled in this study between April and September 2017. Calves were blocked by arrival at the farm, so that the same number of calves was enrolled per treatment group in each batch. Within the first 2 h after birth, calves were fed 3.6 ± 0.09 L of frozen colostrum. Then, they were offered 2 additional colostrum feedings of 3 L. After 3 colostrum feedings, calves were fed 4 L/d of a commercial milk replacer

(23.8% CP, 18% fat, DM basis; Nantamilk Platino; Tres Cantos, Madrid, Spain) at 12.5% DM concentration distributed in 2 feedings until they were transported to IRTA facilities at Torre Marimon (Caldes de Montbui, Spain). Calves were managed according to common animal management conditions under the supervision of IRTA technicians and with the approval of the animal care committee of the Catalonia government (authorization code 9733). On arrival, calves were 3 ± 1.3 d old and weighed 45 ± 5.9 kg. They were individually housed in pens (1 × 1.6 m) equipped with 1 bucket for water, 1 for concentrate, and 1 for chopped forage. Pens were bedded with sawdust on a daily basis. For the duration of the study, calves had ad libitum access to water. Chopped barley straw (2.37% CP, 87.7% NDF, 59.62% ADF; DM basis) was restricted for calves from d 1 to 35 d to 7.5% of the daily offer of solid feed, and then provided ad libitum from 36 d until the end of the study. Straw was chopped using a forage chopper (Seco, Curtalo, Italy) to reach the following particle size distribution: 66.0% > 20 mm, 10.0% between 8 and 20 mm, and 24.0% < 8 mm, determined using a Penn State particle separator. The amount of pelleted starter feed (19.3% CP, 16.2% NDF, 5.8% ADF, 6.3 ash, 3.5% fat; DM basis) available to calves was restricted to minimize potential confounding of effects on calf metabolism because of differences in solid feed intake. Solid feed was restricted according to the following schedule: from d 1 to 7, calves were offered a maximum of 100 g of starter feed; from 8 to 14 d, 300 g/d; from 15 to 21 d, 500 g/d; from 22 to 28 d, 600 g/d; from 29 to 35 d, 800 g/d; and starter feed was provided ad libitum thereafter. Calves were bottle-fed milk replacer (**MR**) according to the following feeding program: 2 L of MR twice a day at 12.5% concentration of solids for the first 4 d; then, MR was increased to 5 L/d at 12.5% concentration for the next 3 d; then increased to 6 L/d at 12.5% concentration for the next 7 d; and finally increased to 2 meals per day of 3 L at 15% concentration until 49 d of age. After that, MR was limited to a single meal of 3 L (also at 15% concentration) until 56 d, when calves were fully weaned. Calves were fed MR (47.2% lactose, 25.1% CP, 20.3% fat, 7.5 ash; DM basis; Nukamel, Weert, the Netherlands) supplemented with 0.3% glutamic acid (Fuxing Pharmaceutical, Ningxia, China) replacing skim milk powder (**GLU**) or MR (48.2% lactose, 24.8% CP, 19.1% fat, 7.5 ash; DM basis; Nukamel) with no AA supplementation (control). Milk replacers were formulated to have the same nutrient composition but a different AA profile (Table 1).

Calves were weighed using an electronic scale (Mobba SC-01, Badalona, Spain) when they arrived at the farm and weekly basis. Individual consumption of MR and solid feed were determined daily by measuring leftovers. A daily score for digestive health status was recorded [1 = no digestive disorder; 2 = moderate signs (soft/watery diarrhea but calf reacting to caretaker stimulus); and 3 = severe signs (watery or bloody diarrhea, and calf listless)]. At 14 and 35 d of the study, blood samples were obtained 4 h after the morning feeding of MR. Samples were collected in 10-mL Vacutainer tubes (BD Diagnostics, Wokingham, UK) without anticoagulant to obtain serum and determine biochemistry profiles, and with lithium heparin to determine plasma AA concentrations. Serum and plasma were obtained by centrifugation at 1,500 × *g* for 10 min and stored in aliquots at −20°C until further analysis. At 10 d of study, a permeability test was conducted by adding 21 g of lactulose and 4.2 g of D-mannitol to the MR as markers (Araujo et al., 2015). Blood samples were collected in Vacutainer (BD Diagnostics) tubes without additives 1 h after the morning feeding of MR to assess serum concentration of lactulose and D-mannitol. Feeds were analyzed for DM (4 h at 103°C), ash (550°C calcination), and CP using a Kjeldahl automatic distiller (Kjeltec Auto 1030 Analyzer, Tecator, Valley City, OH) with copper sulfate/selenium as a catalyst instead of copper sulfate/titanium dioxide (method 988.05; AOAC, 1990); NDF was analyzed with sodium sulfite and heat-stable α-amylase (Van Soest et al., 1991); ADF was analyzed following the method of Robertson and Van Soest (1981); ether extract was analyzed using petroleum ether for distillation instead of diethyl ether with a previous acid hydrolysis (method 920.39; AOAC, 1990); and total AA and total tryptophan concentrations were determined using fluorescence HPLC and UV HPLC, respectively, after strong acid hydrolysis with orthophthaldialdehyde and 9-fluorenyl-methoxycarbonyl chloride (UNE-EN-ISO 13903:2005 and UNE-EN-ISO 13904:2005, respectively; UNE, 2005a,b). We determined serum concentrations of lactulose and D-mannitol using ultra-HPLC mass spectrometry (Xevo G2 Tof; Waters, Milford, MA) with an electrospray ionization source operating in negative mode following the method of Araujo et al. (2015). We measured plasma AA by HPLC as described in Yu et al. (2018) using an Elite LaCHrom (Hitachi, Tokyo, Japan) equipped with a UV detector (L-24200; Hitachi) with a Novapak C18 column (300 mm × 3.9 mm; Waters). We used EZChrom Elite system V3.1.7 software (Agilent, Santa Clara, CA) for system control and data acquisition. Serum biochemistry analyses were performed using an Olympus AU400 analyzer (Beckman Coulter, Hamburg, Germany) using the Olympus System Reagent (Beckman Coulter).

Performance and blood data were analyzed using a mixed-effects model accounting for the fixed effects of treatment, time of measurement, their 2-way interac-

Table 1. Ingredients and AA composition of milk replacers, unsupplemented (control) or supplemented with 0.3% glutamic acid (GLU)

Item	Control	GLU	Starter feed
Ingredient composition, %			
Skim milk powder	39	38.7	—
Whey protein concentrate 35	15	15	—
Whey protein concentrate 60	4	4	—
Fatted whey 50	39	39	—
Premix[1]	3	3	—
Glutamic acid	0	0.3	—
AA composition, g/100 g (DM basis)			
Asp	2.15	2.17	1.55
Glu	4.94	5.14	3.33
Ser	1.30	1.29	0.85
His	0.54	0.57	0.41
Gly	0.60	0.54	0.76
Thr	1.28	1.30	0.70
Arg	0.73	0.76	1.17
Ala	0.95	0.93	0.79
Tyr	0.86	0.89	0.58
Val	1.38	1.47	0.77
Met	0.57	0.58	0.21
Phe	0.98	0.96	0.82
Ile	1.33	1.32	0.66
Leu	2.46	2.39	1.35
Lys	2.14	2.03	1.06
Hyp	<0.030	<0.030	0.043
Pro	2.13	2.05	1.05
Trp	0.30	0.36	0.20

[1]Vitamin A 25,000 IU, vitamin D_3 4,500 IU, vitamin C 300 mg/kg, vitamin B_1 16 mg/kg, vitamin B_2 10 mg/kg, vitamin B_6 10 mg/kg, vitamin B_{12} 80 μg/kg, vitamin K_3 5.5 mg/kg, biotin 160 μg/kg, niacin 50 mg/kg, pantothenic acid 23 mg/kg, folic acid 1 mg/kg, Fe 150 mg/kg, Cu 10 mg/kg, Zn 170 mg/kg, Mn 40 mg/kg, I 1.3 mg/kg, Se 0.4 mg/kg.

tion, and the random effect of calf, with the arrival batch of calves at experimental farm as a block. Time entered the model as a repeated measure using an autoregressive covariance matrix. Digestive health status scores were analyzed with a generalized linear model, considering glutamic acid supplementation as a fixed effect and the number of days with a digestive score >1 per calf within a defined period of time as the dependent variable.

No benefits in calf performance were observed when MR was supplemented with 0.3% glutamic acid (Table 2). Despite the fact that we limited the solid feed offered, some calves did not consume all of their feed allowances on some days, which added some variation to the results (although this variation would have been much greater had we not limited the solid feed offered). We found an increase in straw intake in the last 2 wk of the study in GLU calves compared with control calves, but this difference did not lead to improvements in growth performance. The prevalence of digestive health scores greater than 1 was similar for both treatment groups in the 3 periods studied (d 1 to 14, d 15 to 35, and d 36 to 56, Table 2). Intestinal permeability was also similar in both treatment groups at 10 d (Table 2). Most of the analyzed biochemical parameters were also similar between treatments (insulin, urea, creatinine, cholesterol, triglycerides, fatty acids, total proteins, hepatic enzymes, and glutathione peroxidase), with the exception of serum glucose concentration, which was lower ($P < 0.05$) in GLU calves compared with control calves (Table 2). Most plasma concentration AA profiles were similar for both treatments (Table 3), including glutamic acid. However, Leu and Ile plasma concentration profiles were lower ($P < 0.05$) or tended ($P = 0.054$) to be lower in control than in GLU calves at 14 d, and similar at 35 d. Although not significant, the Val plasma concentration profile showed the same numerical trend ($P = 0.11$) as plasma Leu and Ile concentration profiles at 14 d. The proportion of plasma Met tended to be greater ($P = 0.09$) in control calves compared with GLU calves.

This study was inspired by the reported benefits of glutamic acid in intestinal cells as a main energy source. However, we observed changes in only the plasma branched-chain AA profile at 14 d of that study, and a trend for a higher Met plasma profile. We did not expect to observe changes in plasma Glu concentrations, because enterocytes use most of the glutamic acid intake,

Table 2. Performance, feed intake, and serum biochemical parameters of calves fed an unsupplemented milk replacer (control) or a milk replacer supplemented with 0.3% glutamic acid (GLU)

	Treatment			*P*-value[1]		
Variable	Control	GLU	SEM	T	W	T × W
Performance and feed intake						
Initial BW, kg	44.4	44.9	1.14	0.789	—	—
Final BW, kg	89.0	89.4	0.74	0.448	<0.001	0.621
Initial hip height, m	0.82	0.82	0.007	0.498	—	—
Final hip height, m	0.93	0.93	0.004	0.846	<0.001	0.634
ADG, g/d	800	809	18.5	0.731	<0.001	0.609
DMI, g/d						
Milk replacer	750	749	1.8	0.663	<0.001	0.564
Starter feed	475	442	36.7	0.520	<0.001	0.808
Straw	32	37	3.4	0.251	<0.001	0.008
Total	1,258	1,227	37.5	0.559	<0.001	0.896
Gain:feed ratio	0.66	0.68	0.011	0.108	<0.001	0.591
Fecal score (period 1)[2]	0.10	0.11	0.015	0.65	—	—
Fecal score (period 2)[3]	0.10	0.08	0.011	0.24	—	—
Fecal score (period 3)[4]	0.05	0.04	0.008	0.29	—	—
Serum						
Lactulose:mannitol ratio	2.5	2.2	0.19	0.12	—	—
Glucose, mmol/L	6.38	6.00	0.133	0.049	0.920	0.158
Urea, mmol/L	2.49	2.62	0.122	0.586	0.099	0.872

[1]T = effect of glutamic acid supplementation; T × W = effect of the interaction of glutamic acid supplementation with week of study; W = effect of week of study.

[2]Prevalence of fecal score >1 of calves in each treatment from 1 to 14 d of the study.

[3]Prevalence of fecal score >1 of calves in each treatment from 15 to 35 d of the study.

[4]Prevalence of fecal score >1 of calves in each treatment from 36 to 56 d of the study.

and small fractions of luminally administered Glu are absorbed into the mesenteric venous blood (Janeczko et al., 2007), even if Glu intake is excessive (Burrin and Stoll, 2009). Moreover, we observed no differences in serum urea concentrations, suggesting that glutamic acid supplementation did not increase AA oxidation.

Table 3. Plasma AA profile at 14 and 35 d of the study in calves fed an unsupplemented milk replacer (control) or a milk replacer supplemented with 0.3% glutamic acid (GLU)

	Treatment			*P*-value[1]		
AA, μmol/100 L	Control	GLU	SEM	T	D	T × D
Asn/Ser	5.86	5.86	0.121	0.995	0.179	0.748
Glu	2.64	2.72	0.106	0.592	<0.001	0.797
Gly	11.82	11.51	0.303	0.469	0.03	0.162
Gln	11.40	11.36	0.224	0.885	0.209	0.156
His	2.40	2.32	0.069	0.432	<0.001	0.751
Arg	7.06	6.97	0.288	0.834	0.195	0.771
Cit	2.44	2.68	0.104	0.119	<0.001	0.418
Thr	6.62	6.49	0.237	0.708	<0.001	0.957
Ala	8.44	8.43	0.210	0.981	0.062	0.506
Pro	4.45	4.47	0.090	0.878	0.020	0.948
Tyr	2.22	2.25	0.068	0.709	<0.001	0.779
Val	6.68	6.88	0.160	0.384	0.003	0.112
Met	1.45	1.28	0.074	0.093	<0.001	0.364
Orn	2.40	2.46	0.094	0.656	<0.001	0.888
Lys	7.76	7.44	0.224	0.315	0.072	0.898
Cys	4.46	4.67	0.141	0.283	<0.001	0.743
Ile	3.73	3.82	0.074	0.405	0.059	0.054
Leu	4.76	4.90	0.119	0.388	0.024	0.022
Phe	1.99	2.00	0.060	0.865	0.003	0.598
Trp	1.44	1.52	0.042	0.199	<0.001	0.991

[1]D = effect of the sampling day; T = effect of feeding milk replacer supplemented or not with 0.3% glutamic acid; T × D = effect of the interaction of glutamic acid supplementation and sampling day.

In contrast, when greater amounts (1%) of glutamine supplementation were used in a soy protein concentrate MR, an increase in plasma urea N concentration was observed (Drackley et al., 2006). Similar to our results, Burrin and Stoll (2009) and Rezaei et al. (2013) also reported that an increase of key gut oxidative substrates reduced the gut metabolism of branched-chain AA, resulting in increased plasma levels. Branched-chain AA are known to upregulate glucose transporters and activate glucose synthesis (Holeček, 2018), and this might explain the lower glucose plasma concentrations in the GLU calves compared with control calves. Although plasma Met concentrations also increased when glutamate was fed to postweaned pigs in a previous study (Rezaei et al., 2013), the observed trend in the present study was in the opposite direction, suggesting that Met might be a limiting AA in calves.

The lack of benefits associated with glutamic acid supplementation in the present study might be explained because of an insufficient supply of glutamic acid in the MR, as reported in Rezaei et al. (2013) which supplemented glutamate in pigs at 0.5%, rather than 1%, 2%, or 4% supplemented doses. Another explanation might be that calves in the present study were not in a stressful situation (e. g., illness, injury, heat stress), in which Glu or Gln requirements increase, as observed in broilers under heat stress conditions (Porto et al., 2015).

In conclusion, under commercial rearing conditions, supplementation with 0.3% glutamic acid in an all-milk protein MR with 25% CP and 19.5% fat did not improve calf performance, but did alter the metabolism of some branched-chain AA.

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