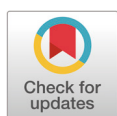


Effects of supplementing limiting amino acids on milk production in dairy cows consuming a corn grain and soybean meal-based diet

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Competing interests

No potential conflict of interest relevant
to this article was reported.

Abstract

Limiting amino acids (AAs) for milk production in dairy cows fed on a concentrate diet of corn grain and soybean meal was evaluated in this study. Four lactating and multiparous Holstein cows (in third or fourth parities, with an average body weight of 633 ± 49.2 kg), 8 to 9 weeks into their lactation period, were used in a 4×4 Latin square design. The experiment comprised four dietary treatments: (1) no intravenous infusion (control); (2) control plus intravenous infusion of an AA mixture of 6 g/d methionine, 19.1 g/d lysine, 13.8 g/d isoleucine, and 15.4 g/d valine (4AA); (3) control plus intravenous infusion of the AA mixture without methionine (no-Met); and (4) control plus intravenous infusion of the AA mixture without lysine (no-Lys). All animals were fed on a controlled diet (1 kg/d alfalfa hay, 10 kg/d silage, 14 kg/d concentrate mixture, *ad libitum* timothy hay). The AA composition of the diet and blood were determined using an automatic AA analyzer. Milk composition (protein, fat, lactose, urea nitrogen, and somatic cell counts) was determined using a MilkoScan. The results showed that feed intake for milk production did not differ from that of intravenous infusion using a limiting AA mixture. The 4AA treatment numerically had the highest milk yield (32.4 kg/d), although there was no difference when compared with the control (31.2 kg/d), no-Met (31.3 kg/d), and no-Lys (31.7 kg/d) treatments. The concentration of AAs in blood plasma of cows in all treatments, mainly isoleucine and valine, increased significantly compared with that of control. The no-Met treatment increased ($p < 0.05$) the concentration of lysine in the blood relative to the control and no-Lys treatments, whereas the no-Lys treatment increased ($p < 0.05$) the concentration of methionine relative to the control and no-Met treatments. In conclusion, milk production increased when feeding 10 g/d methionine to the cows, together with their concentrate diet of corn grain and soybean meal.

Keywords: Limiting amino acid, Methionine, Lysine, Dairy cow

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Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Park JK, Kim CH.

Data curation: Park JK, Kim CH.

Formal analysis: Park JK.

Methodology: Park JK, Kim CH.

Software: Park JK, Bae GS.

Validation: Yeo JM, Kim CH.

Investigation: Park JK, Yeo JM, Bae GS, Kim EJ, Kim CH.

Writing - original draft: Park JK, Kim EJ, Kim CH.

Writing - review & editing: Park JK, Yeo JM, Bae GS, Kim EJ, Kim CH.

Ethics approval and consent to participate

The protocol and the standard operating procedures of this study were reviewed and approved by the National Institute of Animal Science's Institutional Animal Care and Use Committee (No. 2010-066, C-grade).

INTRODUCTION

The efficiency of nutrient utilization of the dairy cow is relatively low compared with monogastric animals, and this is particularly so with absorbed nitrogen (N) in the form of amino acids (AAs). Therefore, efforts have been made to balance AAs in the diet of dairy cows to produce more milk and milk protein. These efforts have been increased due to the demand from environmental sectors, highlighting the environmental impact of the ruminant industry on the planet [1]. Further efforts have been focused on reducing this environmental impact by reducing N excretion. In developed countries, several studies have been conducted on limiting AAs and their effects on milk production in dairy cows. The French National Institute for Agricultural Research (INRA) has conducted research to establish the requirements of methionine and lysine [2,3]. In the United States, the requirements of essential AAs were established through modeling in the Cornell Net Carbohydrate and Protein System (CNCPS) [4,5], at Cornell University, and by the National Research Council (NRC) [6].

Methionine [3,7] and lysine [8,9] are widely known as first-limiting AAs, which affect the milk production and milk protein of dairy cows under many dietary regimes. In addition, lysine and methionine have long been regarded as potentially rate-limiting factors for milk production, either alone or in combination [6,10–12]. The production responses to dairy cows can be greater when methionine, used in combination with lysine, is directed into the small intestine and bloodstream [13,14]. In various experimental diets, differences in the response to supplemented AAs are likely to be caused by variations in the quantity and proportion of AAs in the microbial and dietary protein that are digested and absorbed by the small intestine. The inconsistency in the response to supplemental methionine and lysine may indicate that these are not always clearly first-limiting. The first-limiting AA for milk production was reported to be histidine from a grass silage diet [15,16]. Therefore, although methionine and lysine are known as the first-limiting AA in the milk production of dairy cows under many dietary regimes, these AAs may not be the first-limiting AAs in all feeding conditions [17,18]. In Korea, dairy cows are typically fed a high level of concentrate diets, containing a large quantity of corn grain and soybean meal [19]. With methionine and lysine, often isoleucine and valine may be limiting AAs, and these AAs may also be insufficient for milk production in a diet of dairy cows that contains corn grain and soybean meal. However, there have been limited studies on the requirements and efficiency of AA use in the metabolic protein of dairy cows. This study aimed to investigate the effects of first-limiting AAs of dairy cows fed concentrate diet containing corn grain and soybean meal.

MATERIALS AND METHODS

The protocol and the standard operating procedures of this study were reviewed and approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science, Korea (No. 2010-066, C-grade).

Animals, experimental treatments, and design

This study employed four lactating multiparous Holstein dairy cows in third or fourth parities with a similar dairy performance and was conducted at an experimental farm at the National Institute of Animal Science located in Cheonan-si, Korea. The cows, with an average body weight of 633 ± 49.2 kg, were 8 to 9 weeks into their lactation period at the beginning of the experiment. The animals were individually housed in metabolism stalls that were installed with floor mattresses to minimize foot problems and stress and were milked twice a day at 06:00 and 18:00. The cows received a 14

kg/d concentrate mixture (63.0% ground corn, 20.0% soybean meal, 9.4% cottonseed meal, 6.0% sugar beet pulp and 1.6% mineral and vitamin mixture on a dry matter basis), 10 kg/d of corn silage, and 1 kg/d of alfalfa hay on a fresh weight basis. They were also given access, *ad libitum*, to timothy hay. The diet was formulated to supply the cows with metabolizable energy and metabolizable protein in excess of their normal requirements [6]. Feed was given equally in the two daily feedings after milking. Dry matter intakes were measured daily by weighing the amount of feed offered. The refusal was removed from the feed bin at 07:00 in the morning prior to the next feeding. The dry matter content of the feed was determined once every other week for concentrates and timothy. Then, dry matter intake was calculated as the difference between the amounts offered and refused in terms of dry matter. The amount of timothy hay offered was adjusted to ensure that there was refusal of up to 15%. The chemical composition and AA content of the basal diets are presented in Tables 1 and 2.

Table 1. Chemical composition (% of dry matter unless otherwise stated) of concentrate, corn silage, alfalfa hay and timothy hay used in the experiments

Item	Concentrate ¹⁾	Corn silage	Alfalfa hay	Timothy hay
Dry matter	86.7	24.7	89.9	89.5
Crude protein	22.4	8.0	15.2	6.4
Ether extract	2.2	2.5	1.2	1.2
Crude ash	4.4	3.9	9.4	4.9
Neutral detergent fiber	24.1	53.9	55.5	71.5
Acid detergent fiber	8.4	34.4	42.5	41.1

¹⁾Contained corn grain 63.0%, soybean meal 20.0%, cottonseed meal 9.4%, sugar beet pulp 6.0%, mineral 0.7%, and vitamin mixture 0.9%.

Table 2. Amino acid (AA) profiles (% of crude protein) of concentrate, corn silage, alfalfa hay and timothy hay used in the experiment

Item	Concentrate	Corn silage	Alfalfa hay	Timothy hay
Essential AA				
Arginine	5.83	3.32	3.42	3.70
Histidine	3.16	1.74	2.84	2.69
Isoleucine	3.23	3.06	3.34	3.02
Leucine	8.85	8.24	6.69	6.16
Lysine	5.01	2.60	5.69	6.18
Methionine	0.88	2.54	1.23	1.41
Phenylalanine	5.05	4.09	4.57	4.59
Threonine	3.67	3.48	4.37	3.98
Valine	3.82	4.16	4.33	4.15
Non-essential AA				
Alanine	5.18	10.72	5.02	5.43
Aspartic acid	9.13	4.77	12.53	10.31
Cysteine	0.80	2.97	1.31	1.59
Glycine	3.90	4.29	4.43	4.26
Glutamic acid	19.76	9.37	9.70	9.48
Proline	6.36	6.09	9.37	10.02
Serine	5.01	2.85	4.90	4.31
Tyrosine	3.57	1.58	2.73	2.51

All animals received the basal diet for at least 21 days before the start of the experiment. The four experimental treatments were (1) the basal diet as described above (control); (2) control with a continuous intravenous infusion of the AA mixture of 6 g/d methionine, 19.1 g/d lysine, 13.8 g/d isoleucine, and 15.4 g/d valine (4AA); (3) control with an infusion of the AA mixture, without methionine (no-Met); and (4) control with the infusion of the AA mixture, without lysine (no-Lys). The total experimental period was 60 days (including 21 days of basal diet feeding). The amounts of four amino acids infused were calculated approximate difference between the amounts absorbed from the basal diet and the amounts contained in milk protein according to NRC [6]. The study employed a 4 × 4 Latin square design, and each period lasted 10 days. A solution of AA (pharmaceutical grade; Forum Chemicals, Redhill, UK) for intravenous infusion was prepared by gradually adding a daily dose of the AA to distilled water. The pH was adjusted to 7.4, and the volume made up to four liters. All solutions were filtered through a Whatman cellulose nitrate membrane filter (pore size 0.45 µm; Whatman International, Maidstone, UK) and autoclaved at 121 °C for 15 min. The solution of AA in a volume of 4 L/d was infused into the jugular vein. The infusion rate was set to deliver the required volume within a 23 h period, and the solution was changed at 09:00 each day. An indwelling polyethylene catheter (Arrow International, Reading, MA, USA) was inserted into the jugular vein approximately 24 h before the start of infusion. The area around the entry point of the catheter was protected with an adhesive bandage, which was changed every 2 days. The catheter was changed at the end of each experimental period and was flushed with sterile citrate saline before the start of each day's infusion to allow the infusates to flow freely. No infusion was given to the control in the experiment. The dosage sets of the AA solution were replaced every period.

The body weight of the cows was measured, using an electric scale, for 2 days prior to the experiments. Feed intake and milk yield were recorded daily. The milk composition was determined using a representative, composite sample from the previous four consecutive milkings. Samples of blood were taken from the vein of the tail muscle area of the cows using a heparin-treated 10 mL vacutainer® tubes (BD, Texarkana, TX, USA) on the last day of each experimental period. The blood samples were centrifuged (VS-6000CFi, Vision Scientific, Seoul, Korea) immediately to separate the plasma, which was then stored at -20 °C, pending further analysis. No evidence of hemolysis was found.

Chemical analysis

Feed samples were taken, dried in a forced-air oven at 60 °C, ground to pass through a 1 mm screen, and analyzed for chemical composition using the appropriate AOAC [20] and Van Soest methods [21]. Milk samples were analyzed for milk protein, milk fat, lactose, milk urea-N (MUN), and somatic cell numbers with a Milkoscan 4000 series (Foss Electric, Hillerød, Denmark). For the AA in the feed samples collected on the first day of each experimental period, 6 N HCl was added to the feed for hydrolysis, the HCl was then immediately removed through drying, using a concentrator (V805, BÜCHI, Flawil, Switzerland), and the dried materials were dissolved with a buffer solution and then analyzed with an AA analyzer (Beckman 6300, Beckman Coulter, Brea, CA, USA). For plasma AA, the plasma samples were mixed with 8% 5-sulfosalicylic acid (No. 2130, Sigma-Aldrich, St. Louis, MO, USA) at a 1:1 ratio and centrifuged. The retrieved supernatants were filtered using a membrane filter with a 0.2 µm pore size and then analyzed with an automatic AA analyzer (Biochrom 20, Biochrom, Cambridge, UK).

Statistical analyses

The mean values of feed intake, milk yield, and milk compositions were recorded for the last 7 days of each experimental period, and AA in the blood and body weight were measured for the last 2

days of each experimental period. The results were subjected to the analysis of variance using GenStat 5 [22], and the least significant difference values were calculated when the F values were significant ($p < 0.05$).

RESULTS

Feed intake, milk yield and milk composition

To determine the first-limiting AAs in the milk production of Holstein dairy cows, the diets were formulated to exceed total AA requirements by 15% according to NRC [6]. The dry matter intake of the cows did not differ ($p > 0.05$), and the values were 20.4, 21.4, 20.6, and 20.7 kg/d for control, 4AA, no-Met and no-Lys, respectively. Timothy hay was offered *ad libitum*, and the intake was numerically ($p = 0.185$) greater in cows infused with the 4AA compared with the others (Table 3).

The milk production of the lactating dairy cows that were infused with the mixture of lysine, methionine, isoleucine and valine by aqueous infusion into the jugular vein was numerically higher (32.4 kg/d) than in the rest of the treatments, although there was no significant difference when compared with the control (31.2 kg/d), no-Met (31.3 kg/d), and no-Lys (31.7 kg/d) treatment. No-Met had lower milk protein content ($p < 0.05$) and yield ($p < 0.05$), when compared with the 4AA and the no-Lys treatment ($p < 0.05$), and was similar to the control. As for lactose content, no-Met had a higher content (4.84%) when compared with the other treatments ($p < 0.05$); however, there was no difference in lactose yield.

Blood amino acids

In terms of blood plasma AA concentrations, isoleucine and valine were lower in the control ($p < 0.05$) than in the other treatments (Table 4). No-Met showed a lower ($p < 0.05$) methionine concentration in blood plasma, when compared with those that had methionine supplemented to their diets. Likewise, the diets without lysine supplements (i.e., control and no-Lys) showed a relatively

Table 3. Effects of intravenous infusions of amino acids (AA) on feed intake, and milk composition and production of Holstein dairy cows

Item	Treatment ¹⁾				SED	p-value
	Control	4AA	no-Met	no-Lys		
Dry matter intake (kg/d)						
Concentrates	12.1	12.1	12.1	12.1	-	
Corn silage	2.5	2.5	2.5	2.5	-	
Alfalfa hay	0.9	0.9	0.9	0.9	-	
Timothy hay	4.9	5.9	5.1	5.2	0.42	0.185
Total	20.4	21.4	20.6	20.7	-	
Milk yield (kg/d)	31.2	32.4	31.3	31.7	0.64	0.284
Fat (g/kg)	31.6	32.9	34.0	33.0	1.74	0.617
Fat (g/d)	987	1,067	1,068	1,049	45.0	0.318
Protein (g/kg)	29.3 ^a	30.8 ^b	29.1 ^a	30.7 ^b	0.44	0.015
Protein (g/d)	913 ^a	997 ^b	910 ^a	970 ^b	18.3	0.008
Lactose (g/kg)	48.1 ^b	48.0 ^b	48.4 ^c	47.6 ^a	0.11	0.003
Lactose (g/d)	1,503	1,561	1,518	1,509	30.3	0.305

¹⁾Control, no infusion; 4AA, mixture of 6 g/d methionine, 19.1 g/d lysine, 13.8 g/d isoleucine and 15.4 g/d valine; no-Met, mixture without methionine; no-Lys, mixture without lysine.

^{a-c}Means with different letters within a row differ at $p < 0.05$.

SED, standard error of the differences.

Table 4. Effects of intravenous infusions of amino acid on the concentrations ($\mu\text{mol/L}$) of amino acids (AA) in blood plasma of Holstein dairy cows

Item	Treatment ¹⁾				SED	p-value
	Control	4AA	no-Met	no-Lys		
Essential AA						
Arginine	92	92	89	80	9.8	0.601
Histidine	65	58	65	63	6.0	0.591
Isoleucine	111 ^a	162 ^{ab}	208 ^b	197 ^b	26.4	0.038
Leucine	151	140	158	143	17.4	0.737
Lysine	81 ^a	121 ^{ab}	162 ^b	82 ^a	21.5	0.026
Methionine	30 ^a	52 ^{ab}	29 ^a	68 ^b	14.0	0.083
Phenylalanine	49	44	51	46	5.7	0.681
Threonine	215 ^b	197 ^a	186 ^a	194 ^a	6.4	0.017
Valine	209 ^a	302 ^b	367 ^b	346 ^b	37.3	0.021
Non-essential AA						
Alanine	170	165	178	154	23.0	0.754
Aspartic acid	12	10	14	13	3.2	0.626
Cysteine	24	26	27	32	3.5	0.199
Glycine	246	211	244	230	28.8	0.627
Glutamic acid	126	130	144	144	17.1	0.649
Proline	76	65	68	68	11.2	0.804
Serine	74	64	69	69	9.4	0.556
Tyrosine	49	39	42	42	5.5	0.335

¹⁾Control, no infusion; 4AA, mixture of 6 g/d methionine, 19.1 g/d lysine, 13.8 g/d isoleucine and 15.4 g/d valine; no-Met, mixture without methionine; no-Lys, mixture without lysine.

^{a-b}Means with different letters within a row differ at $p < 0.05$.

SED, standard error of the differences.

lower ($p < 0.05$) concentration in plasma, compared with 4AA and no-Met, although there was substantial variation between treatments (Table 4). In addition to the four AAs associated with supplemental treatments, there were no differences ($p > 0.05$) between the treatments, in terms of the individual AAs; however, threonine was significantly higher ($p < 0.05$) in cows that were offered the control diet, relative to the other treatments.

DISCUSSION

Dry matter intake in animals varies depending on the N level in the diets. The levels of essential AA and crude protein in the diets significantly affect dry matter intake. These are also affected when there is a large difference between the AA level in feed and the amount of AA required by the animal [12,23–26]. It is notable that a significant proportion of corn grain was used as a source of feed ingredient in this study. However, the composition of essential AAs in the crude protein of corn grain is known to be unbalanced, since it is low in lysine [27,28]. Therefore, dairy diets formulated with high a level of corn grain (a typical feeding regime in Korea) do not supply sufficient lysine to high-producing dairy cows. It is also understood that corn- and soybean-rich diets are not satisfactory for supplying methionine to high-producing dairy cows [29,30]. Even in a monogastric animal, such as rats, when such cereal grains were fed to them, there was a remarkable decrease in feed intake and weight gain, as well as a lowering of the efficiency of AA utilization in the body [31]. When using feeds that have a low crude protein content, or that have an unbalanced AA compo-

sition, it is possible to improve feed intake, weight gain, and the efficiency of AA utilization in the body of the animal by supplementing the deficient essential AAs [32].

In the present study, with an increased supply of lysine and/or methionine, the dairy cow's productivity seemed to improve. The response in the milk production of dairy cows to the supply of lysine and methionine is usually observed in the early lactation period rather than in the mid-lactation or late-lactation period [33]. Literature has shown that the increased supply of lysine and methionine to dairy cows, following the highest lactation period, induced a more sensitive response in milk yield than in milk protein content [34–36]. Nimrick et al. [37] reported that, when urea was supplied as the only source of N, the experimental animals exhibited a deficiency in lysine, methionine, and threonine and that the infusion of lysine and methionine into the abomasum caused an improvement in the usability of protein in dairy cow, sheep, and deer [38–41].

Accordingly, the results of the present study showed that milk protein content and milk yield varied depending on the supply of either lysine or methionine and were independent of the supply of either isoleucine or valine. However, the response seemed more pronounced when methionine was supplemented in the diet (Table 3). This may indicate that methionine is more limiting than that of lysine in this study, where the diets were mainly formulated with corn grain and soybean meal. Our result is in agreement with Socha et al. [42], who reported that cows were responded favorably to additional rumen-protected methionine compared with rumen-protected Lys. Further study by Patton [11] proved the effect of supplemental rumen-protected methionine in terms of milk protein yield. We did not expect methionine to be the first-limiting because corn was the primary energy sourced in the experimental diet. However, since NRC [6] concluded that lysine and methionine are the two most limiting AAs in dairy diets, a diet formulation for dairy cows may be an essential factor to decide which AA is more limiting than the other. Overton et al. [43] reported that, following supplementation of rumen-protected methionine in the diet of dairy cows, the plasma methionine concentration increased; however, there was a decrease in the plasma histidine concentration. In addition, the plasma concentrations of arginine and lysine, following rumen-protected methionine supplementation, were lower than those of the plasma from the cows offered with the non-treated diet. This indicates that methionine may have an impact on utilization of other AAs. Overton et al. [43] further reported that there was a diet \times methionine interaction, where the concentrations of phenylalanine, threonine, and cysteine in plasma differed depending on their basal diets (i.e., ground shelled corn vs. corn gluten feed). In this study, the concentration of threonine in plasma from the control diet was higher than those of 4AA, no-Met and no-Lys, and this may indicate that methionine (and/or lysine) affects the metabolism of other AAs for example, the absorption and utilization of other AAs, resulting in a change in the concentration of other plasma AA.

CONCLUSION

With a typical feeding regime where the level of concentrate was relatively high, capable of producing well over 9,000 kg of milk per annum, Holstein dairy cows received intravenously a mixture of AAs, such as methionine, lysine, isoleucine, and valine, or the same mixture without either methionine or lysine. The cows that were fed with the control diet did not receive an infusion. The composition of AA in blood plasma was altered with the mixed AAs, with or without either methionine or lysine. These changes in blood plasma were reflected in milk protein content, although the changes were marginal. Nevertheless, the results of this study may provide some insight into the AA requirements of dairy cows in Korea, which are offered diets with corn grain and soybean meal mostly imported from other countries. Further studies are needed to identify AA requirements in

various dietary regimes, and this is very important for animals fed on total mixed rations where the feed ingredients change more rapidly.

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