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SHORT COMMUNICATION

Effects of feeding rumen–protected amino acids on the performance of feedlot calves

Mitra Mazinani[1], Abbas Ali Naserian[1], Brian J. Rude[2], Abdol Mansour Tahmasbi[1], Reza Valizadeh[1]
[1]Department of Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
[2]Department of Animal and Dairy Sciences, Faculty of Agriculture, Mississippi State University, Starkville, USA

ABSTRACT

Objective: This study was conducted to produce and evaluate protected amino acids (AAs) against degradation in the rumen with greater bioavalibility and without the problems associated with polymer coating and the effect this has on calf performance.
Materials and Methods: In the first step, essential AAs methionine and lysine were reacted with two chemical compounds (Benzaldehyde and Glutaraldehyde) in an attempt to make ligands for producing protected AAs. The physico-chemical characterization, melting point, and mass spectrometric of products were estimated. These products were fed to 36 Holstein dairy calves with 110 ± 0.50 kg of average body weight and an age of 110 ± 10 days. Calves were randomly assigned to six treatments. This study was done with six treatments as a completely randomized one-way design.
Results: Feed consumption and average daily gain were less for control animals and those fed methionine and lysine glutaraldehyde compared to other treatments. The largest chewing time was observed for methionine and lysine glutaraldehyde, respectively, and the least was control. There was no difference for energy consumption, dry matter intake, or blood metabolites among the six treatments. The greatest total protein content was related to methionine and lysine glutaraldehyde treatment and the least total protein was observed in control treatment.
Conclusion: It can be concluded that the use of chemical methods to protect AAs can be applied and may have some beneficial effects.

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Introduction

Protein is considered as one of the most important and high-cost components of the animal diet and is considered as a limiting nutrient, especially for high-producing cows [1]. Excess protein in the rumen is broken into non-nutrient like ammonia. This ammonia can be absorbed which increases blood urea concentration and eventually is excreted as urea and ammonia. This leads to increased adverse effects to health, decrease of reproductive and productive performance, and increased environmental pollution. Only a small fraction of dietary protein consumed by the animals passes through the rumen. However, most dietary protein is either broken down into microbial protein or after hydrolysis, deamination occurs and its amino acid breakdown to ammonia and carbon skeleton. Therefore, supplying free essential amino acids (EAAs) to ruminants in their diet is not successful. Because the research has shown that the presence of microorganisms in the rumen will degrade amino acid sources, such as lysine and methionine [2].

Thus, the presence of microorganisms in the rumen, despite being useful in the synthesis of many AAs and vitamins as well as assisting in the digestion of fiber, can have a negative effect on utilization of EAAs, especially by high-yielding animals that may require more EAAs. One way to improve amino acid utilization animal diet is to add these AAs in form which protects them from rumen degradation but are able to be degraded post-rumenly. Previous research has shown that it is possible to protect AAs against rumen microbial digestion via physical protecting methods. Schwab [3] discussed that several methods which have been evaluated to protect AA from rumen degradation. The

Correspondence Abbas Ali Naserian ✉ naserian@um.ac.ir Department of Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

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AA protection is usually done by mechanical and physical methods [4]. One method is known as pH sensitive encapsulation and has been used to encapsulate lysine and methionine [5]. These polymers are resistant to degradation in rumen pH but breakdown when exposed to abomasal pH. Some of these products may be ineffective when used with mixed diet or corn silage. Depending on the ruminal pH, the efficacy of these products has limited use when feeding diets which decrease ruminal pH (such as concentrate-based diets). Because the rumen pH is normally between 5.5 and 7, there is a significant difference between pH of the rumen and post rumen parts of the intestinal tract which is between 2 and 3. The pH-sensitive polymer coats are used based on this pH difference. However, despite the high cost, making such capsules has its disadvantages. For example, any type of rumen-resistant coating may be damaged during chewing, feed processing, and rumination. If this damage happens during mixing and processing of the diet, the amino acid will be broken down in the rumen [3].

Another way of protecting AAs is encapsulation with neutral lipids. Researchers in South Dakota conducted several studies using fatty acid capsules (58%) and methionine (30%) and observed variable results for improved milk production. They concluded that the encapsulation of methionine improved its availability. New products have now been developed which include calcium salts containing fatty acids. The potential problem with this method is that the amino acid can overprotect; therefore, these complexes that are greatly neutral in the rumen may not digested in the post ruminal parts and small intestine, so there is a constant competition between good rumen protection and bioavailability. This method is a simple procedure, but the results are unsatisfactory, primarily due to the low biodegradability of protected AA in the small intestine. The production of amino acid analogs and their derivatives is another solution for protecting AAs. Methionine hydroxy analog (MHA) is the most protected form of methionine studied with this method. St-Pierre et al. [6] reported that more than 70% of the initial concentration of MHA and only 15% of diethyl methionine remained after 12 h of incubation with rumen bacteria. Alimith, a liquid form of rumen MHA, was similar to methionine-resistant solid hydroxy analogs [6]. In the early 1970s, it was hypothesized that amino acid derivatives (a free amino acid with a chemical group added to the alpha-amine group or carboxyl group) or analogs, such as the replacement of the alpha-amine moiety with non-nitrogen group may alter resistance to rumen degradation but still able to be absorbed in the small intestine. Many evidences point to increased bacterial protein synthesis, increased number of protozoa, increased fiber digestion, increased rumen fat synthesis, milk yield, percentage of milk fat, in lactating cow using MHA. Increased milk production and percentage of milk fat occurred mostly in fresh cows and in herds using high percentage of concentrates [7]. Results from using this product in other studies were variable. Therefore, manufacturing protected commercial AAs with good quality is difficult. Scientists who have been researching this have been aware of this problem for years. This commonly accepted that even recent products are still far from ideal. Achieving quality and sustainable products will require a lot of refinement of current procedures. The aim of this study was to use a new technical method to produce protected AAs from degradation in the rumen without the problems associated with polymer.

Materials and Methods

Step 1: Synthesis of rumen-protected amino acid

Essential AA, including lysine and methionine reacted by two chemical compounds (benzaldehyde and glutaraldehyde), were used to produce protected AA. This part of the study was done in the chemistry lab of Ferdowsi University of Mashhad. The protected AAs were produced with chemical reactions. Different solvents and temperatures were used to produce pH-sensitive protected AA. When the reaction finished, produced ligands were dried and weighed. At the end, physico-chemical characterization, mass spectrometric, and melting point, were estimated [8].

Step 2: in vivo test

Thirty six Holstein dairy calves with 110 ± 0.50 kg average weight and age of 110 ± 10 days. Calves were handled following regulations established by the Animal Experiment Committee of Ferdowsi University. This protocol study and experimental procedures were approved by the Ethics Committee of the Faculty of Agriculture—Ferdowsi University of Mashhad- Iran, for care and use of experimental animals (approval number: 47699). Calves were randomly assigned to six treatments. The experiment was conducted for 28 days (with a 15-day adaptability period). Daily feed consumption was measured daily and blood samples were taken on day 28. Treatments were four protected amino acid treatments that were made in step 1 of the trial unprotected amino acid, and the control. To equalize the amount of amino acid obtained from different treatments (considering the ratio of amino acid to aldehyde in each product), the equivalent of 2 gm methionine and 6 gm lysine per product were calculated. Therefore, the treatments used were as follows: 1) control, 2) free amino acid (2 gm methionine and 6 gm lysine), 3) benzaldehyde methionine and benzaldehyde lysine (3.95 gm methionine and 10.79 gm protected lysine), 4) glutaraldehyde methionine and glutaraldehyde lysine (2.67 gm methionine and 7.64 gm protected lysine), 5) methionine sucrose and lysine sucrose (7.12 gm of methionine and 18.55 gm of protected lysine), and 6) vanillin methionine and vanillin lysine (4.03 gm methionine

and 12.30 gm protected lysine). Diets were balanced according to calves' nutritional requirements using NRC 2001 [7] software. Five percent wheat straw and %15 alfalfa hay plus the amino acid treatment were added to each group diet.

Observation and rumination behavior

Eating, rumination, and chewing activity (total eating and rumination) of calves were evaluated on day 25 of the experiment by visual observation. Different behaviors included eating, ruminating, etc. (any behavior, including lying down, eating, and moving). Calves were evaluated every 5 min and their behavior was recorded. The total time the animal spent ruminating or eating was considered chewing time. By subtracting this time from a 24 h day, resting time (not chewing) was obtained [8].

Data analysis

Data were analyzed as a one-way analysis of variance using General Linear Models (GLM) procedure of SAS 9.1. AA treatments were considered the only sources of variation. The significance of differences between control and treatments was estimated with Duncan's post-hoc test, and alpha level of $p < 0.05$ was used to assess the significance among means.

Results and Discussion

Feed intake and growth performance

Feed intake and daily body weight gain are reported in Table 1. These factors were decreased for the control treatment and methionine and lysine glutaraldehyde treatments compared to others. Therefore, adding protected AAs may have some benefits on these parameters by enhancing rumen undegradable protein (RUP). Improvements in feed efficiency were reported for beef calves fed methionine and lysine. Results for gaining on the data from the 21-day trial periods are not very reliable [9]. Mantano et al. [10] added protected methionine and lysine to the diet of feedlot calves and prepared that this did not alter daily feed intake but improved daily weight gains and feed efficiency. The consumption of these protected AAs increased the availability of methionine and lysine in the intestine, post-ruminal segments, and the entire gastrointestinal tract. The results are in agree with Zhou et al. [11] who found feed efficiency and daily weight gain were improved as a result of increased metabolizable amino acid. Torrentra et al. [12] using feedlot calves, fed a methionine-supplemented diet, found no improvement for weight gain. According to NRC (2001), methionine and lysine required by feedlot calves (190 kg weight and 1.24 kg average daily gain) was 9.6 kg and 30.6 gm/day, respectively.

Eating and ruminating activity

The results of eating activity, rumination, and chewing time are presented in Table 2. Different treatments had an effect on the time duration of eating and rumination in calves. The increased chewing time was observed for methionine and lysine glutaraldehyde, respectively, and the least for control.

In one study, the addition of tryptophan to calves diet did not significantly increase spent time for feeding and

Table 1. Effect of adding protected amino acid ligands on feed intake and weight gain of calves.

Parameter	Treatments						SEM	*p* value
	1	2	3	4	5	6		
Daily weight gain (kg)	1.15	1.22	1.24	1.28	1.25	1.12	0.032	0.681
Daily feed consumption (kg)	5.75 ^{b}	6.10 ^{ab}	6.38 ^{a}	6.44 ^{a}	6.30 ^{a}	5.99 ^{b}	0.089	0.194
Conversion factor	5.01	5.01	5.13	5.04	5.05	5.35	0.185	0.868

1 – in each row the numbers with different letters have significant difference (p <0.05).
2 – Treatment 1) Control 2) free amino acid 3) Methionine and lysine benzaldehyde 4) Methionine and glutaraldehyde lysine 5) Methionine and lysine sucrose 6) Methionine and lysine vanillin.

Table 2. Effect of adding protected amino acid ligands on eating, rumination and total chewing time.

Parameter	Treatments						SEM	*p* value
	1	2	3	4	5	6		
Eating time (minutes per day)	116^{bc}	126 ^{ac}	136 ^{a}	132 ^{a}	127 ^{ac}	130 ^{ab}	1.71	0.014
Duration of the rumination time (minutes per day)	268 ^{b}	285 ^{ab}	282 ^{a}	280 ^{ab}	275 ^{ab}	290 ^{ab}	3.13	0.014
Chewing time (minutes per day)	384	411	418	412	402	412	4.62	0.19
Rest (not doing chewing activity)	1,056	1,029	1,022	1,022	1,038	1,020	4.62	0.19

1 – In each row the numbers with different letters have significant difference (p < 0.05).
2 – Treatment 1) Control, 2) free amino acid, 3) Methionine and lysine benzaldehyde, 4) Methionine and glutaraldehyde lysine, 5) Methionine and lysine sucrose, and 6) Methionine and lysine vanillin.

their motility decreased compared to control. In the tryptophan treatment, the social behaviors of calves were also reduced. These results may be related to the effect of tryptophan on serotonin secretion, which causes drowsiness in domestic animals. In another study, the injection of antibiotics to calves increased feed intake [13]. In another study with female calves, two energy sources (maize and barley) and two protein sources (soybean meal and sunflower meal) were used and the effects of these sources on feed consuming behavior were evaluated. The results showed that the experimental treatments had no effect on animal behavior. Calves spent 9.97% of their day for eating, 2.11% for drinking water and 25.13% ruminating and 16.97% on the other activities, e.g., licking and social behavior. 45.82% of the time was spent resting or other acts. Eating and drinking water and social behaviors were performed in a standing position, while resting and ruminating were often in a lying position. Eating was often in the first 4 h after a meal, and more rumination occurred during night hour. Calves fed more balanced and fermented diets, feed intake decreased, and chewing time increased. In diets including high concentrate, chewing activity was reciprocally depends on feed texture [14]. Abdullahzadeh and Abdulkarimi [15] stated that dietary fiber takes part a fundamental role in dry matter intake and stimulation of rumen chewing and fermentation activity. In an experiment on male calves that were fed two treatments of 8% and 16% tomato pomace, the rumination and chewing time of the calves were increased. In a statistical analysis, Zebeli et al. [16] found that in early lactation cows total feeding time varied from 425 to 969 min per day (mean 691 min) and rumination time was from 151 to 632 min per day (mean 434 min). Cows that eat 22 kg dry matter per day should spend at least 16 min on rumination each kg of dry matter. Animal response to chewing activity is more strongly associated with insoluble fiber, and this type of insoluble fiber is primarily supplied from forage sources. All rations and insoluble fiber provided from the forage were similar. Changes in eating and ruminating time in the present experiment may be linked to increased palatability of the diet, which may be influenced by glutaraldehyde and vanillin odor. Amanlou et al. [17] reported that to prevent gastrointestinal diseases, chewing activity for cattle should be from 35 to 38.75 min for each kg of dry matter. The time spent for chewing per kg of dry matter consumed and the total rumination time indicated animals were within this normal time range.

Yuangklang et al. [18] reported that chewing activity was related to physical context of the feed ($p < 0.05$), but in this study, by feeding four different sizes of rolled barley, there was no difference for chewing activity ($p < 0.05$).

Blood metabolites

Since most of the factors affecting blood metabolites are related to energy metabolism and dry matter intake, therefore, there was no difference between energy consumption and dry matter intake, so blood metabolites were not affected. The greatest total protein content was related to methionine and lysine glutaraldehyde treatment and the least total protein was observed in control treatment which was 7.94 and 7.01 gm / dl, respectively (Table 3).

The reduction in blood urea nitrogen in protected amino acid treatments can be due to increased ruminal uptake of what and increased utilization efficiency for tissue growth as well as reduced amino acid deamination. Movaliya et al. [2] observed similar results in heifers fed a protected methionine-lysine supplement. The results of the present experiment were in agreement with the studies of Socha et al. [5], who observed similar results in cows using lysine and methionine. Torrentera et al. [12] observed increased methionine and nitrogen uptake by adding methionine to the diet, as well as no difference for plasma lysine, possibly reflecting a low rumen-crossing rate.

Table 3. Effect of adding protected amino acid ligands on eating, rumination and total chewing time.

Parameter	Treatments						SEM	*p* value
	1	2	3	4	5	6		
Glucose (mg/dl)	95.10	96.60	100.71	98.35	99.71	97.71	0.60	0.10
Cholesterol (mg/dl)	136.00[a]	134.51[ab]	130.44[b]	132.86[a]	131.44[a]	130.44[b]	0.64	0.02
Urea Nitrogen (mg/dl)	16.99	17.89	17.23	17.10	17.43	17.30	0.40	0.99
Total protein (gm/dl)	7.01	7.21	7.68	7.94	7.53	7.48	0.40	0.99

1 – In each row the numbers with different letters have significant difference ($p < 0.05$).
2 – Treatment: 1) Control, 2) free amino acid, 3) Methionine and lysine benzaldehyde, 4) Methionine and glutaraldehyde lysine, 5) Methionine and lysine sucrose, and 6) Methionine and lysine vanillin.

Conclusion

According to the results, the chemical techniques for protecting AA could be useful and decrease AA breakdown in the rumen. In the animal experiments part, the protected amino acid treatments showed a beneficial effect on feed intake, weight gain, and some blood factors. Therefore, using protected AAs and balancing diet according to bypass protein could have some beneficial effects on animal performance.

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Conflict of interests

The authors have declared that they have no conflict of interest.

Authors' contribution

AAN and BR designed the study. MM did the farm and laboratory part of this experiment. Laboratory works was monitored by all the authors, also data analyzing, and manuscript writing. BR critically reviewed the manuscript. All the authors read and approved for publication.

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