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Research Article

The Effect of Soybean Meal Treated with Formaldehyde on Amount of Protected Protein in the Rumen and Absorption of Amino Acid from Small Intestines*

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Abstract: This study was conducted to determine the effects of soybean meal (SBM) treated with formaldehyde on rumen fermentation pattern, microbial protein synthesis, amount of by-pass protein, and amino acid absorption from small intestines. Four 1-year-old rams with ruminal, duodenal, and ileal cannulas were fed 800 g of dry clover plus a concentrate mixture containing 100 g of SBM treated with 0%, 0.3%, 0.6%, and 0.9% formaldehyde. The amount of crude protein passed through the duodenum was not affected by the formaldehyde treatment; however, the amount of microbial protein decreased in rams fed with SBM treated with both 0.3% and 0.6% formaldehyde ($P < 0.05$). Digestibility of acid detergent fibre (ADF) and neutral detergent fibre (NDF) in the duodenum and ileum did not differ by treatments. In the rams fed SBM treated with both 0.3% and 0.6% formaldehyde, absorbability of arginine, histidine, leucine, methionine, phenylalanine, and valine (essential amino acids), as well as alanine and aspartic acid (non-essential amino acids) significantly increased, whereas tyrosine absorbability notably decreased ($P < 0.05$). Formaldehyde treatment increased the absorbability of other essential amino acids (leucine and valine) and non-essential amino acids (serine and tyrosine) in the ileum ($P < 0.05$). Increasing the percentage of formaldehyde linearly increased total essential amino acid absorption in the duodenum ($P < 0.05$). In conclusion, 0.3% and 0.6% formaldehyde treatment enhanced the bioavailability of protein in SBM.

Key Words: Soybean meal, formaldehyde, by-pass protein, microbial protein synthesis, amino acid absorption

Formaldehit ile İşlem Görmüş Soya Küspesinin Rumende Korunmuş Protein Miktarı ve İnce Bağırsaklarda Aminoasit Emilimi Üzerine Etkisi

Özet: Bu araştırma formaldehit ile işleme tabi tutulmuş soya küspesinin rumen fermantasyonu, mikrobiyal protein sentezi korunmuş protein (by-pass protein) miktarı ve ince bağırsaklarda amino asit emilimi üzerine etkilerini incelemek amacıyla yapılmıştır. Araştırmada hayvan materyali olarak, rumen duodenal ve ileal kanül takılmış 4 adet 1 yaşlı morkaraman koç kullanılmıştır. Yem materyali olarak günlük 800 g kuru yonca ve ağırlıklarının %8'i oranında su ve ham protein miktarlarının % 0, 0,3, 0,6 ve 0,9 düzeyinde saf formaldehit içeren solüsyonlarla işleme tabi tutulmuş 100 g soya küspesi verilmiştir. Duodenuma geçen günlük ham protein miktarı formaldehit işleminden etkilenmezken, günlük mikrobiyal ham protein miktarı özellikle % 0,3 ve 0,6 formaldehitli gruplarda azalmıştır ($P < 0,05$). Duodenum ve ileum ADF ve NDF sindirimleri bakımından gruplar arasında farklılık olmamıştır. Özellikle % 0,3 ve 0,6 düzeyinde uygulanan formaldehit işlemi duodenumdan esansiyel amino asitlerin (arjinin, histidin, löysin, metionin, fenilalanin ve valin) ve nonesansiyel amino asitlerin (alanin, aspartic asit) emilimini önemli derecede artırırken, tirozin emilimini azaltmıştır ($P < 0,05$). Esansiyel (löysin, valin) ve nonesansiyel amino asitlerin (serin, tirozin) ileumdan emilimi formaldehit uygulaması ile önemli derecede artmıştır ($P < 0,05$). Duodenumdan günlük toplam esansiyel amino asit emilimi de formaldehit uygulaması ile önemli derecede artırmıştır ($P < 0,05$). Sonuç olarak, % 0,3 ve % 0,6 formaldehit muamelesi soya küspesinin biyoyararlanilabilirliğini artırmıştır

Anahtar Sözcükler: Soya küspesi, formaldehit, by-pass protein, mikrobiyal protein sentezi, amino asit emilimi

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Introduction

For optimal performance in high-producing ruminants, there should be enough soluble readily-fermentable protein to support microbial growth and fermentation in the rumen, plus a source of less fermentable protein, which can pass directly to the abomasum and small intestine for a normal proteolytic digestion and absorption process. The latter is often called "rumen undegradable", "by pass", or "escape" protein. The increased undegradable protein in the rumen and its availability for absorption in the lower gastrointestinal tract may benefit rapidly growing calves and high-production dairy cows (1).

Soybean meal (SBM) is the most commonly used protein supplement in beef and dairy rations. It is quite palatable and has a good amino acid balance with high availability. Relative to other commonly used proteins, SBM is rich in lysine, but not methionine, valine, or isoleucine, which are the 1st, 2nd, and 3rd limiting amino acids, respectively (2).

Lactating dairy cows and rapidly growing ruminants cannot meet their protein requirements with microbial protein. Thus, by-pass proteins are important for these animals. Therefore, protecting high-quality protein sources, such as legumes and seed meals from ruminal fermentation positively affects animal performance. Various methods for treating proteins have been used to reduce their degradation in the rumen. These can be broadly categorised as chemical and physical treatments (3). Chemical treatments can be further divided into methods in which the chemicals actually combine with the proteins, e.g., formaldehyde treatment, and those in which the chemicals denature the proteins, e.g., treatment with alcohol, sodium hydroxide, and propionic acid (4,5).

Total amino acid that is synthesised in the rumen and then absorbed in the small intestine is microbial in origin. Several methods are used to determine the amount of microbial protein syntheses in the rumen. The main logic on which most of them are based is chemical compounds that are located in microbial cells, such as ribonucleic acid (RNA), deoxyribonucleic acid (DNA), diaminopimelic acid (DAPA), D-alanine, total purine and pyrimidine, aminoethylphosphoric acid (AEP), adenosinetriphosphate (ATP), and amino acid profile. These compounds are used to distinguish microbial proteins from feed proteins. Several researchers (6,7) reported that degradation of dry matter (DM), crude protein (CP), and effective protein degradation of vegetable protein sources decreased in relation to formaldehyde treatment level. It was reported that formaldehyde treatment decreased both rumen pH and NH_3-N (8,9). The objective of the present study was to determine the effects of SBM treated with formaldehyde on rumen fermentation pattern, microbial protein synthesis, amount of by-pass protein, and amino acid absorption from the small intestine.

Materials and Methods

Four approximately 1-year-old Morkaraman rams (average weight: 35 kg) were fed rations containing dried alfalfa and SBM treated with various amounts of formaldehyde.

The rams were kept in individual cages for a month to acclimate to their environment. Then duodenal and ileal cannulas made of silicon were placed in the animals according to Atasoy and Taş (10). Rumen cannulas were also placed in the animals 15 days after placement of the duodenal and ileal cannulas as described by Atasoy and Taş (10).

Formaldehyde solutions were prepared in 8% water based on the weight of SBM to contain 0%, 0.3%, 0.6%, and 0.9% formaldehyde, proportional to the CP content of SBM. The formaldehyde solutions were sprayed on SBM samples in polyethylene bags and mixed homogeneously. Bags were tightly sealed and incubated for 72 h, after which the bags were opened and the samples were dried under laboratory conditions.

The experiment was carried out using a 4 × 4 Latin Square design with 10-day adaptation and 6-day sampling periods. The animals were offered 800 g of dried alfalfa and 100 g of SBM treated with 0.5%, 0.3%, 0.6%, and 0.9% formaldehyde. Drinking water and vitamin-mineral blocks were available ad libitum.

Starting from the third day of the adaptation period, 1 g of markers composed of 2% chromium (Cr_2O_3) fixed to neutral detergent fibre (NDF) (11) was given to each animal through the rumen cannula, at 08:00 and 20:00 h. After determining feed intake by animal by period, faecal samples from the rectum and 100 ml of duodenal samples through the duodenal cannula were collected at 6-h intervals from day 11 to 15.

On the 15th day of the experiment, to determine NH_3-N and volatile fatty acid (VFA) levels of rumen fluid, 10 ml samples were taken at the 0, 2nd, 4th, 6th, 8th, and 10th h relative to feeding, and were put into tubes containing 1:1 diluted HCl. These samples were kept at -20 °C until analysed. At the end of each period, 2 l of rumen fluid was taken at the 2nd, 4th, 6th, 8th, and 10th h from each animal to determine total number of micro-organisms. DM, ash, and organic matter (OM), and CP of alfalfa, soybean meal, duodenal samples, and faecal samples were determined according to methods described by AOAC (12). NDF content was determined using the method described by Van Soest et al. (13), and acid detergent fibre content was determined with the technique of Karslı (14). Purine from isolated rumen micro-organisms and duodenal content was measured using the method described by Zinn and Owens (15). In order to determine NH_3-N content of ruminal and duodenal samples, a Kjeldahl distillation unit was used. Chromium concentrations of the marker in duodenal and faecal samples were measured according to the method of Williams et al. (16). Daily dry matter out of the duodenum and daily faecal output were calculated as follows:

Daily faeces amount (dry matter, g) = daily consumed indicator (g/day)/indicator concentration in the faeces (g/kg, DM).

The amino acid profiles of feeds and samples were determined using an Eppendorf LC 3000 Amino Acid Analyser at the TÜBİTAK Marmara Research Centre, Turkey.

Statistical Analysis

All data obtained in the present study were analysed using SAS software in a 4 × 4 Latin Square experiment pattern (17). Differences between mean values were also determined by Duncan's test (18).

Results

The nutrient composition of alfalfa and SBM are presented Table 1, and pH and NH_3-N level of the rumen are presented in Table 2. While rumen pH was not affected by formaldehyde treatment at 0.6% and 0.9% levels, rumen NH_3-N decreased significantly ($P < 0.05$). CP passed to the duodenum and its fraction is presented in Table 3. There were no differences in the amounts of

Table 1. Chemical composition of alfalfa and soybean meal, %.

	DM	Ash	CP	Ether Extract	NDF	ADF	OM
Alfalfa	94.42	8.39	13.44	0.98	44.80	34.70	86.03
SBM	91.18	6.07	42.66	1.06	18.39	10.80	85.11

Table 2. The effects of treating SBM with formaldehyde on ruminal pH and NH_3-N. (n = 4).

Formaldehyde, %	Hours				
	0	3	6	9	12
pH					
0	6.28	6.13	5.99	6.13	6.17
0.3	6.37	6.16	6.04	6.15	6.16
0.6	6.22	6.10	6.08	6.20	6.23
0.9	6.26	6.19	6.11	6.14	6.11
S.E.M	0.046	0.042	0.052	0.160	0.161
NH_3-N (mg/100ml)					
0	64.71	66.36a	50.62^a	47.62^a	40.53
0.3	57.60	56.62ab	47.11^{ab}	46.41^a	38.17
0.6	60.64	45.71b	41.93^{ab}	40.24^b	35.91
0.9	56.94	46.01b	40.22^b	37.74^b	37.22
S.E.M	2.50	3.61	3.02	1.85	4.73

Different letters (a, b) in the same columns indicate significant differences ($P < 0.05$)

Table 3. Amount of daily crude protein (CP) passed to the duodenum and its fractions. (n = :4).

	Formaldehyde, %				
	0	0.3	0.6	0.9	S.E.M
Daily CP passed to the duodenum, g	66.30	64.38	63.31	66.68	2.21
Daily microbial protein passed to the duodenum, g	57.23[a]	51.09[b]	50.23[b]	55.70[ab]	1.80
Daily by-pass protein passed to the duodenum, g	7,37[b]	11.67[a]	11.54[a]	9.08[ab]	1.06
Microbial CP/daily CP passed to the duodenum, %	86.26[a]	79.47[b]	79.39[b]	83.58[ab]	13.66
By-pass protein/daily CP passed to the duodenum, %	11.15[b]	18.02[a]	18.17[a]	13.58[b]	1.38

Different letters (a, b) in the same rows indicate significant differences (P < 0.05).

daily CP passed to the duodenum; however, the amount of daily microbial protein passed to the duodenum was highest in the control. The ratio of by-pass protein to daily CP passed to the duodenum was significantly higher in the 0.3% and 0.6% formaldehyde treated SBM-fed rams than in the control ram. Digestibility of ADF and NDF in the duodenum and ileum are given in Table 4. Formaldehyde treatment did not affect digestibility of ADF and NDF in the duodenum and ileum. Formaldehyde treatment of SBM, in general, increased the amount of amino acid absorbed from the duodenum (Table 5) and this effect was notable in the rams fed 0.3% and 0.6% formaldehyde-treated SBM ($P < 0.05$). The effect of formaldehyde-treated SBM in the ileum was not as great as in the duodenum (Table 6). The absorption of leucine and valine (essential amino acids), and tyrosine and serine (non-essential amino acids) were higher than the absorption of other amino acids in the ileum.

Discussion

The effectiveness of microbial protein synthesis changes in relation to ration composition and/or different applied treatments to feed (19). In the present experiment, the formaldehyde levels did not affect rumen pH, but 0.6% and 0.9% formaldehyde levels decreased the rumen NH_3-N concentration. This result may be associated with a decrease in protein degradation, which may depend on increasing formaldehyde level (Table 2). Formaldehyde treatments did not alter amounts of CP

Table 4. Digestibility of ADF and NDF in the duodenum and ileum, (n = 4).

	Formaldehyde, %					
	0	0.3	0.6	0.9	S.E.M.	
Digestibility of ADF in the duodenum, g/d	149.76	151.39	165.16	166.30	8.77	N.S
Digestibility of ADF in the duodenum, %	51.93	52.49	57.26	57.66	3.04	N.S
Digestibility of ADF in the ileum, g/day	247.54	249.03	234.91	237.78	5.78	N.S
Digestibility of ADF in the ileum, %	85.83	86.35	81.45	82.45	2.00	N.S
Digestibility of NDF in the duodenum, g/d	189.43	179.37	186.17	205.90	15.04	N.S
Digestibility of NDF in the duodenum, %	50.28	47.60	49.41	54.65	3.99	N.S
Digestibility of NDF in the ileum, g/day	320.51	320.11	308.17	304.92	8.27	N.S
Digestibility of NDF in the ileum, %	85.06	84.95	81.79	80.61	2.64	N.S

N.S: Not significant (P > 0.05)

Table 5. Amount of amino acid absorbed in the duodenum and total amount of essential amino acid and nonessential amino acid, g/day (n = 4).

Amino acid	Formaldehyde,%				
	0	0.3	0.6	0.9	S.E.M.
Arginine	4.86^{b}	5.82^{ab}	5.56^{a}	5.15^{ab}	0.14
Histidine	0.39^{b}	0.48^{ab}	0.78^{a}	0.30^{b}	0.10
Isoleucine	2.72	2.70	3.05	2.56	0.47
Leucine	5.28^{b}	6.14^{a}	6.25^{a}	5.35^{b}	0.24
Lysine	3.81	4.03	4.06	3.94	0.31
Methionine	0.96^{b}	1.09^{a}	1.08^{a}	0.94^{b}	0.03
Phenylalanine	3.61^{b}	3.71^{ab}	3.92^{a}	3.77^{ab}	0.08
Threonine	2.87	3.16	3.04	2.98	0.11
Valine	2.53^{b}	3.26^{ab}	3.61^{a}	3.05^{ab}	0.25
Alanine	3.42^{b}	3.39^{b}	3.80^{a}	3.58^{ab}	0.11
Aspartic acid	11.47^{b}	11.95^{ab}	12.41^{a}	11.51^{b}	0.26
Glutamic acid	15.31	15.73	15.72	14.88	0.40
Glycine	3.55	3.58	3.44	3.17	0.28
Serine	4.90	5.24	5.22	5.07	0.41
Tyrosine	1.75^{a}	1.49^{ab}	1.41^{b}	1.75^{a}	0.08
EAA	27.01^{b}	29.83^{ab}	31.35^{a}	28.00^{ab}	1.26
NEAA	41.50	43.00	43.79	41.07	1.17

Different letters (a, b) in the same rows indicate significant differences ($P < 0.05$).
Essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine)
Non-essential amino acids (alanine, aspartic acid, glutamic acid, glycine, serine, and tyrosine)

Table 6. Amount of amino acid absorbed in the ileum and total amount of essential amino acid and nonessential amino acid, g/day.

Amino acid	Formaldehyde, Level,%				
	0	0.3	0.6	0.9	S.E.M.
Arginine	5.49	5.82	5.86	5.35	0.18
Histidine	0.83	1.01	1.08	0.57	0.18
Isoleucine	3.29	3.56	3.81	3.09	0.26
Leucine	6.51^{ab}	6.82^{b}	7.21^{a}	5.93^{b}	0.36
Lysine	4.46	4.60	4.88	4.34	0.28
Methionine	0.99	1.08	1.19	0.94	0.09
Phenylalanine	4.18	4.45	4.99	4.68	0.44
Threonine	3.23	3.55	3.66	3.08	0.31
Valine	3.68^{ab}	4.09^{ab}	4.36^{a}	3.46^{b}	0.24
Alanine	3.58	3.83	4.00	3.84	0.38
Aspartic acid	12.18	12.78	13.22	12.95	0.45
Glutamic acid	15.44	15.68	15.88	14.76	0.49
Glycine	3.60	3.71	4.06	3.51	0.29
Serine	5.14^{b}	5.95^{a}	5.99^{a}	4.97^{b}	0.11
Tyrosine	2.92^{b}	3.16^{a}	3.33^{a}	2.87^{b}	0.01
EAA	32.67	34.97	37.01	31.44	1.74
NEAA	42.88	45.11	46.48	42.30	1.58

Different letters (a, b) in the same rows indicate significant differences ($P < 0.05$).
Essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine)
Non-essential amino acids (alanine, aspartic acid, glutamic acid, glycine, serine, and tyrosine)

passed to the duodenum, but decreased the amount of microbial protein passed to the duodenum ($P < 0.05$) (Table 3). This was not a surprise, because formaldehyde treatment decreased rumen NH_3-N concentration, which is related to protein degradation; thus, microbial protein synthesis was decreased (5,20). The ratio of microbial protein to daily CP passed to the duodenum was the highest (86.26%) in the untreated ram and the lowest (79.39%) in the ram fed 0.6% formaldehyde-treated SBM ($P < 0.05$).

Similarly, the ratio of daily by-pass protein to daily CP passed to the duodenum was higher in the rams fed 0.3% and 0.6% formaldehyde-treated SBM than in the control ram (18.02% and 18.17%, respectively, $P < 0.05$). Although research on the effect of formaldehyde-treated SBM on microbial protein synthesis and amino acid profile in the small intestine of sheep is limited, these findings are in agreement with Varviko et al. (5), who reported that formaldehyde treatment increased the amount of by-pass protein passed to the duodenum. Unexpected results were obtained for the amount of microbial protein passed to the duodenum in the ram fed 0.9% formaldehyde-treated SBM. Treating SBM with formaldehyde did not significantly affect the level of digestibility of ADF and NDF in the duodenum or ileum (Table 4). These findings are in agreement with Lynch et al. (20) and McKinnon et al. (21), who reported that using certain chemicals, such as lignosulfate and organic acids, did not affect the digestibility of ADF and NDF in the small intestine.

There were differences between the profiles of absorbed amino acids in the duodenum and ileum (Tables 5 and 6). Treating SBM with formaldehyde, in general, increased the amount of essential amino acid absorbed in the duodenum. The absorption of essential amino acid in the duodenum, except tyrosine, increased significantly in the rams fed 0.3% and 0.6% formaldehyde-treated SBM ($P < 0.05$). This effect was more obvious in the essential amino acid profile than in the non-essential amino acid profile. Only absorption of the non-essential amino acids tyrosine and aspartic acid differed significantly from all other non-essential amino acids ($P < 0.05$). The amount of daily total essential amino acid absorbed in the duodenum was affected significantly by formaldehyde treatment of SBM ($P < 0.05$). The highest amounts were 29.83 and 31.35 g in the rams fed 0.3% and 0.6% formaldehyde-treated SBM, respectively. The differences in daily totally non-essential amino acid absorbed in the duodenum were not significant.

The effect of formaldehyde treatment on the amount of amino acid absorbed in the ileum was not as significant as in the duodenum. The absorption of the essential amino acids leucine and valine and the non-essential amino acids tyrosine and serine were affected significantly ($P < 0.05$). No differences were observed among the rams in the amount of daily total non-essential amino acid absorbed in the ileum. Disappearance of the amino acid profile of the absorbed fraction of by-pass protein can be different from that of the original feed (22), making prediction of absorbed amino acid difficult. More information is needed on the content of by-pass amino acid. These findings are in agreement with the results of Erasmus et al. (22).

O'Mara et al. (23) reported that the disappearance of lysine, methionine, phenylalanine, and histidine was, in general, quite similar to the disappearance of total amino acids in formaldehyde-treated SBM.

On the other hand several researchers (20,24) reported that treating canola with heat and organic acids did not change the amino acid profile, but the absorption of leucine, isoleucine, and aspartic acid increased in the small intestine. Erasmus et al. (22) reported that lysine seems to be one of the more degradable of the important amino acids. Methionine is an amino acid that is resistant to ruminal degradation, as is phenylalanine (25).

In conclusion, treating SBM with formaldehyde reduced its ruminal degradation without adversely affecting its intestinal protein digestion and absorption. Moreover, this treatment significantly increased the amount of by-pass protein and essential amino acid absorbed in the duodenum. Further research is also needed to increase the understanding of animal responses to the inclusion of SBM treated with formaldehyde in ruminant rations.

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