www.roavs.com EISSN: 2223-0343

RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Research article

In situ ruminal degradation and intestinal digestion of crude protein and amino acids of three major proteinaceous feeds for Hanwoo steers

Y. K. Oh[1], Y. J. Park[1], Y. C. Baek[1], Y. J. Do[2], D. H. Kim[3], W. S. Kwak[4] and H. Choi[1*]

[1]Animal Nutrition & Physiology Team, National Institute of Animal Science, Rural Development Administration, Wanju county 565-851, Jeon-Buk province, Republic of Korea; [2]Animal Diseases & Biosecurity Team, National Institute of Animal Science, Rural Development Administration, Wanju county 565-851, Jeon-Buk province, Republic of Korea [3]Eco-friendly Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup county 580-185, Jeon-Buk province, Republic of Korea; [4]Konkuk University, Chung-Buk province, Republic of Korea

Article history
Received:24 Oct, 2015
Revised: 16 Nov, 2015
Accepted: 18 Nov, 2015

Abstract

Three Hanwoo steers (mean body weight 520 ± 20.2 kg), each fitted with a permanent ruminal cannula and a T-shaped duodenal cannula, were used to examine digestibility of crude protein (CP), rumen undegraded protein (RUP), and individual amino acids (AA) of three proteinaceous feeds (cottonseed meal, CSM; perilla meal, PRM; rapeseed meal, RSM), by using *in situ* bag and *in situ* mobile bag techniques. The *in situ* RUP value was higher in PRM (69.1%) than in CSM (54.0%) and RSM (41.9%) ($p<0.05$). In most cases, intestinal digestibility of CP was higher than ruminal degradability of CP. The intestinal degradation of Lys was not different among CSM (41.1%), PRM (59.3%), and RSM (48.7%). The pattern of intestinal digestion of Met was similar to that of Lys. Furthermore, PRM showed the highest intestinal digestibility and total tract digestibility of AA compared with other tested feeds. Overall, these results suggest that PRM may be used as a good protein source for ruminants. These results may be used as baseline data for ruminant ration formulation.

Keywords: Crude protein; rumen undegraded protein; amino acid; Perila meal; degradation

To cite this article: Oh, YK, Park YJ, YC Baek, YJ Do, DH Kim, WS Kwak and H Choi, 2015. *In situ* ruminal degradation and intestinal digestion of crude protein and amino acids of three major proteinaceous feeds for Hanwoo steers. Res. Opin. Anim. Vet. Sci., 5(10): 395-400.

Introduction

Current recommendations for feeding proteins to cattle are based on the concept of absorbable protein (NRC, 1989). High-producing dairy cows need a significant amount of rumen un-degraded protein (RUP), which is a major source of required amino acids (AA) (NRC, 2001). Formulating diets that meet the AA requirements for ruminants is a challenging goal in animal nutrition (Polan, 1992). However, ruminant protein feeding systems are constrained by limited knowledge about the AA absorbed through the small intestine (O'Mara et al., 1997). Intestinal digestibility can vary widely depending on the feedstuff and the specific AA in question (Hvelplund, 1984, 1985; O'Mara et al., 1997).

Meat producers continuously try to decrease feed cost while improving the quality of meat. In order to

***Corresponding author:** Hyuck Choi, Ph.D., Animal Nutrition & Physiology Team, National Institute of Animal Science, Rural Development Administration, Wanju county, Jeon-Buk province, 565-851, Republic of Korea; E-mail: hchoi0504@korea.kr; Tel: +82-63-238-7451; Fax: +82-63-238-7452

improve the usefulness of a wide variety of feedstuffs, several protein sources have been developed for ruminants. Cottonseed meal (CSM), Perilla meal (PRM), and rapeseed meal (RSM) are widely used protein sources for ruminants in Korea (National Institute of Animal Science [NIAS], 2012). Although diet formulation in ruminants has been based on the digestible crude protein (CP) concept, high-performing dairy cattle have shown no response to the increase dietary CP levels (Chiou et al., 1995; Van Straalen et al., 1997). The lack of response to increased dietary CP may be due to excess protein in the diet and an imbalance in AA (Abu-Ghazealeh et al., 2001). In addition, degradability of CP is normally used as an estimate for AA degradability. Therefore, in order to address these drawbacks, improvement to an efficient ration-formulation system requires an accurate AA profile of the RUP arriving to the intestine. Scientific information on the ruminal, intestinal, and total tract digestibility of individual AA contents in CSM, PRM, and RSM is required. The effects of rumen fermentation and small intestinal digestion on the AA profile of feed protein can be studied using the rumen nylon bag and mobile nylon bag techniques (Van Straalen et al., 1993).

This study was conducted to determine the dry matter (DM), CP, and AA degradation and digestion of CSM, PRM, and RSM in the rumen, small intestine, and total gastrointestinal tract using the *in situ* nylon bag and *in situ* mobile bag techniques.

Materials and Methods

Experimental animals and feeding

All animal studies were reviewed and approved by the Animal Ethics Committee of the National Institute of Animal Science. Three healthy Hanwoo steers (mean body weight 520±20.2 kg) were selected and each was fitted with a permanent rumen cannula (Bar Diamond Inc., Parma, ID, USA) and a T-shaped duodenal cannula (Bar Diamond Inc.). The steers were housed individually in tie-stalls and fed a diet consisting of 1 kg rice straw and 3.5 kg concentrate mix (47.9% ground corn grain, 40.9% wheat bran, 5.0% soybean meal, 2.0% rapeseed meal, 2.0% beet molasses, 1.5% dicalcium phosphate, 0.4% salt, and 0.2% vitamin-mineral premix) on a DM basis. The vitamin-mineral premix contained 2,650,000 IU vitamin A, 530,000 IU vitamin D_3, 1,050 IU vitamin E, 10,000 mg/kg niacin, 4,400 mg/kg Mn, 13,200 mg/kg Fe, 440 mg/kg I, and 440 mg/kg Co.

The diet was formulated to meet NRC requirements (2001) and was fed twice daily, at 0900 and 1700. All animals had free access to water and mineral blocks to maintain a relatively stable rumen environment. Each experimental period consisted of a 14-day adaptation period and a 3-day collection period.

Feed sample collection

All tested feeds were obtained from the National Institute of Animal Science in Suwon, Korea. DM was measured after drying for 48 h in an air-forced oven at 65°C until a constant weight was obtained. All feed samples were milled to pass through a 2-mm mesh screen in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA).

In situ rumen incubation of feed samples

A previous study proposed that estimated DM and CP degradability of feed ingredients in the rumen could be measured by the nylon bag technique (Ørskov and McDonald., 1979). Nylon mobile bags (NL 130-030/330PW, NBC Inc., Tokyo, Japan), each approximately 8 × 15 cm (45-μm pore size; sample size: surface area = 16.7 mg/cm^2), were filled with 5 g of dry ground feed samples and tied with a rubber band. Bags containing feed samples were incubated in 9 replicates (3 replicates for each steer) in the rumen of steers for 0, 2, 4, 8, 16, 24, 48, and 72 h. The bags that were not incubated, representing time 0, were treated in the same manner as the incubated bags.

Values of CP degradation in the rumen were calculated according to Ørskov and McDonald (1979), with a passage rate of 4.5%/h (Van Straalen and Tamminga, 1990). All nylon bags were suspended in the rumen in a polyester mesh bag (25 × 40 cm; 3 mm pore size) and removed at the given intervals. The nylon bags were washed in a washing machine for 30 min then dried in an air-forced oven at 60°C for 48 h until a constant weight was achieved. Each bag was then weighed and feed residues analyzed for CP according to the methods of the Association of Official Analytical Chemists (AOAC, 2012). Disappearance of nutrients at each incubation time was expressed relative to the original nutrient content of the feed. Feed samples were milled to pass through a 1-mm mesh screen before AA analysis.

In situ intestinal incubation of feed samples

The small intestinal digestibility of AA was measured using a nylon mobile bag technique. Nylon bags, each approximately 3 × 4.5 cm (35 μm pore size; sample size: surface area = 37.0 mg/cm^2), were filled with 0.5 g dry ground feed samples and tied with string. Each bag containing feed samples was pre-incubated in 9 replicates (3 replicates for each steer) in the rumen of steers for 16 h. Bags were combined with a 1N HCl solution and 1 g/l pepsin (pH 2.4), and inserted into the duodenum through the T-shaped cannula at 20 min intervals. The rumen incubation time was fixed to 16 h as suggested by Varvikko and Vanhatalo (1993).

Table 1: Chemical and amino acid composition (% of DM) of tested protein feeds [1,2]

Item	Cottonseed meal	Perilla meal	Rapeseed meal
Crude protein	41.2	26.4	35.0
Ether extract	0.87	5.02	0.83
Neutral detergent fiber	28.2	58.4	25.3
Acid detergent fiber	20.2	47.3	15.0
Calcium	1.56	0.49	1.29
Phosphorus	0.42	0.73	0.36
Amino acids			
Arg	1.61	2.58	0.94
His	2.02	0.61	1.79
Ile	1.53	0.73	1.77
Leu	1.60	1.59	1.48
Lys	1.06	0.67	0.98
Met	1.11	0.65	1.10
Phe	2.28	1.20	2.42
Thr	0.61	0.90	0.64
Val	1.63	0.99	1.72
Trp	4.14	0.19	3.42
Essential amino acids[2]	17.60	10.10	16.26
Non-essential amino acids[3]	17.54	12.07	16.24
Total amino acids	35.14	22.17	32.50
Lys, % of essential amino acids	6.00	6.59	6.05
Met, % of essential amino acids	6.29	6.44	6.75

[1]On a dry matter basis; [2]Sum of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val, and Trp; [3]Sum of Ala, Asp, Cys, Glu, Gly, Pro, Ser, and Tyr

Table 2: Crude protein residues (% of DM) at each rumen incubation time of tested protein feeds[1]

Time (h)	Cottonseed meal	Perilla meal	Rapeseed Meal
0	97.0	83.0	80.9
2	89.7	75.1	58.9
4	86.3	79.0	53.3
8	79.3	76.4	51.4
16	49.0	62.9	42.9
24	33.8	60.3	32.8
48	21.7	45.6	20.3
72	10.1	33.8	11.0

[1] On a dry matter basis.

Duodenal bags were collected from the feces and washed using a washing machine until the rinse water remained clear and then dried in an air-forced oven at 60°C for 48 h until a constant weight was achieved before determination of residual DM. The tested feed samples were milled to pass through a 1 mm mesh screen before AA analysis.

Nutrient analysis and chemical composition of feeds

Feed samples were analyzed for DM, CP, and ether extract (EE) according to the AOAC method (2012). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were evaluated using a fiber analyzer (ANKOM2000, ANKOM Technology Corporation, Macedon, NY, USA). Samples were hydrolyzed in 6 M HCl at 110°C for 24 h before AA analysis. The chemical composition of feeds is presented in Table 2. The CSM was characterized as having the highest CP, RSM as having medium CP and the lowest fiber, and PRM as having the lowest CP, highest fat, and highest fiber.

The AA analysis was conducted using the ninhydrin method in a Hitachi 8900 apparatus (Hitachi, Tokyo, Japan). Calcium and phosphorus were analyzed with an ICP-AES Optima 8300 (Perkin Elmer, Inc., Waltham, Massachusetts, USA). The amino acid (AA) composition of feedstuffs is shown in Table 3. The first limiting AA was Lys for CSM (1.06%), PRM (0.67%), and RSM (0.98%). Compared with the other feedstuffs, CSM (1.06%) had a relatively high Lys content (% of DM). The second limiting AA was Met for CSM (1.11%), PRM (0.65%), and RSM (1.10%). The Met content of CSM (1.11%) was high compared with values for other feedstuffs.

Calculations and statistical analyses

The measured percentages of DM, CP, EE, and AA degradation after 16 h incubation in the rumen were calculated as the difference between the original feedstuffs and the portion remaining after incubation in the rumen, intestine, and total tract. Overall rumen-degraded protein (RDP) was estimated with an equation proposed by Ørskov and McDonald (1979):

$$RDP = A + \{B \times k_d/(k_d + k_p)\}$$

Where RDP = RDP of the feedstuff, %/ CP
A = Soluble and filterable A fraction, %/ CP
B = Degradable B fraction, %/CP
k_d = Degradation rate of degradable B fraction, %/h
k_p = Passage rate of degradable B fraction, %/h

Between-feed differences in ruminal degradation and intestinal digestion rates of DM, CP, EE, and individual AA were calculated according to Ørskov and McDonald (1979) using the PROC GLM procedure of SAS (Statistical Analysis Systems Institute Inc., 2003) with one-way ANOVA. Duncan's multiple range test was used for the comparison of means. All data presented are expressed as mean ± SE from three independent measurements. A p-value < 0.05 was considered statistically significant.

Results

In Situ rumen degradation

The CP residues at each incubation time are presented in Table 2. CSM and RSM had relatively low CP residues, and PRM had relatively high CP residues. CP fractions and RUP for tested feeds are shown in Table 3. CSM had a relatively low soluble A fraction and high degradable B fraction of CP, RSM a relatively

Table 3: Crude protein fractions and rumen undegraded protein (RUP) (% of DM) of tested protein feeds[1]

Item	Cottonseed meal	Perilla meal	Rapeseed meal	SE
Soluble and filterable A fraction	-2.0[c]	10.6[b]	29.8[a]	4.8
Degradable B fraction	94.3	64.1	61.3	6.6
Degradation rate of degradable B fraction (Kd)	0.05	0.03	0.04	0.01
RDP, % of crude protein	46.0[a]	31.0[b]	58.1[ac]	4.3
RUP, % of crude protein	54.0[b]	69.0[a]	41.9[bc]	4.3

[1] On a dry matter basis; [abc] Least square means with different superscripts within the same row differ (P<0.05).

Table 4: Ruminal degradation of crude protein and amino acids (% of DM) of the tested protein feeds[1]

Item	Cottonseed meal	Perilla meal	Rapeseed meal	SE
Crude protein	24.1[ab]	16.9[b]	45.0[a]	6.2
Arg	15.1	23.4	6.9	4.4
His	8.6[b]	17.5[b]	53.2[a]	9.0
Ile	5.4[b]	8.6[b]	49.3[a]	9.4
Leu	5.9[b]	12.6[ab]	37.9[a]	6.9
Lys	10.7	16.7	32.7	5.3
Met	72.7[a]	17.2[b]	60.7[a]	10.8
Phe	3.9[b]	12.8[b]	41.0[a]	7.7
Thr	16.9[ab]	11.8[b]	38.9[a]	6.1
Val	13.8[b]	12.2[b]	43.8[a]	7.1
Trp	21.9[b]	18.3[b]	68.4[a]	10.3

[1] On a dry matter basis; [abc] Least squares means with different superscripts within the same row differ (P<0.05).

Table 5: Intestinal digestion of crude protein and amino acid (% of DM) of the tested protein feeds[1]

Item	Cottonseed meal	Perilla meal	Rapeseed meal	SE
Crude protein	37.1	47.4	39.5	2.8
Arg	38.6[b]	39.5[b]	66.7[a]	6.1
His	40.1	47.4	35.2	3.0
Ile	42.3	49.6	37.7	3.0
Leu	42.1	47.0	43.9	2.2
Lys	40.1	59.3	48.7	7.0
Met	30.6[a]	49.8[a]	14.7[b]	9.1
Phe	41.7	42.4	45.1	2.0
Thr	40.2	45.6	47.4	2.2
Val	41.0	46.3	40.6	2.4
Trp	37.0[ab]	44.0[a]	23.9[b]	4.3

[1] On a dry matter basis; [ab]Least squares means within the same row without a common superscript differ (P<0.05).

high soluble A fraction, and PRM a medium soluble A fraction and degradable B fraction. The degradation rate of the degradable B fraction was not different among the tested feeds. The content of RDP in PRM was significantly lower than that of other feedstuffs. On the contrary, the content of RUP in PRM was higher than in CSM or RSM. These values, except for RSM, were similar to the values tabulated by NRC (2001).

In situ mobile bag digestion in the rumen

The rumen degradations of CP and AA in the tested feeds using the *in situ* mobile bag technique are shown in Table 4. The ruminal degradation of CP was highest in RSM and lowest in PRM (P<0.05). The ruminal degradation of Lys, the first limiting AA, was not significantly different among feeds. The degradation of Met, the second limiting AA, was higher in CSM and RSM than in PRM (P<0.05). Among all tested feeds, His and Met were the most degraded, Arg, Lys, Val, and Trp were intermediate, and Ile, Leu, Phe, and Thr were the least degraded in the rumen. These results were somewhat different from values tabulated by NRC (2001).

In situ mobile bag digestion in the intestine

The intestinal digestion of CP and AA contents in the tested feeds are presented in Table 5. Intestinal CP digestion was not different among tested feeds. The intestinal digestion of Lys was highest in PRM, followed by RSM and CSM. The intestinal digestion of Met was highest in PRM, then CSM and RSM (P<0.05). The AA values of the tested feeds were somewhat different from the values tabulated by NRC (2001).

In situ mobile bag digestion in the total tract

The total gastrointestinal tract digestion of CP and AA are presented in Table 6. The total tract digestion of CP was highest in RSM, followed by PRM and CSM (P<0.05). The total tract digestion of Lys and Met also was highest in RSM, followed by PRM and CSM (P<0.05).

In situ mobile bag digestion in the rumen, intestine, and total tract

The Lys and Met (g/kg of CP) absorbance of the tested feeds from the rumen, intestine, and total tract is presented in Table 7. The ruminally absorbable Lys was higher in RSM than CSM and PRM (P<0.05). The intestinally absorbable Lys was similar among tested feeds. The total tract absorbable Lys was equal in CSM and RRM and highest in RSM, and (P<0.05). The ruminally absorbable Met was similar in CSM and RSM and lowest in PRM (P<0.05). The intestinally absorbable Met was similar among feeds. The total tract absorbable Met was highest in RSM, followed by CSM and PRM (P<0.05).

Discussion

Currently, there has been a growing interest in the use of CSM, PRM and RSM as Hanwoo feed. However, we are having a limited knowledge about the

Table 6: Total tract degradation of crude protein and amino acid (% of DM) of the tested protein feeds[1]

Item	Cottonseed meal	Perilla meal	Rapeseed meal	SE
Crude protein	64.4[c]	85.3[b]	93.4[a]	5.3
Arg	60.2[c]	93.5[a]	88.8[b]	6.4
His	57.1[c]	89.5[b]	93.3[a]	7.4
Ile	55.6[c]	84.8[b]	96.3[a]	7.5
Leu	55.9[c]	86.1[b]	93.6[a]	7.1
Lys	58.1[c]	87.2[b]	93.2[a]	6.7
Met	87.2[c]	89.8[b]	98.0[a]	2.0
Phe	54.9[c]	86.0[b]	96.6[a]	7.7
Thr	61.0[c]	82.9[b]	95.5[a]	7.9
Val	59.6[c]	81.8[b]	93.9[a]	6.2
Trp	63.4[c]	88.4[b]	97.8[a]	6.3

[1] On a dry matter basis; [abc] Least squares means with different superscripts within the same row differ ($P<0.05$).

Table 7: Estimated rumen, intestinal and total tract absorbable AA (g/kg) supplied by the ruminal undegraded protein (RUP) of the tested protein feeds[1,2]

Item	Cottonseed meal	Perilla meal	Rapeseed meal	SE
Rumen				
Lys	1.13[ab]	1.11[b]	3.22[a]	0.36
Met	8.06[a]	1.12[b]	6.66[a]	1.03
Intestine				
Lys	4.24	3.94	4.79	0.31
Met	3.39	3.24	1.61	0.41
Total tract				
Lys	6.14[b]	5.80[b]	9.17[a]	0.73
Met	9.66[b]	5.84[c]	10.74[a]	0.90

[1] On a dry matter basis; [2]Absorbable AA supplied by RUP is defined as (% each organ's degradability) × (% original composition)/100; [abc] Least squares means with different superscripts within the same row differ ($P<0.05$).

rumen, intestine and total tract digestibility of individual AA contents. The *in situ* mobile bag technique is most commonly used to measure protein digestion in the small intestine of ruminants (Van der Poel et al., 2005). The objective of this study was to determine the DM, CP, and AA contents from three major proteinaceous feeds (CSM, PRM and RSM) using the *in situ* techniques. In the present study, CSM and RSM had relatively low CP residues (Table 2). These results suggest that CSM and RSM may a have higher potential for bio-utilization than PRM. Rumen-degradable protein (RDP) is needed to feed the rumen bacteria and ensure an adequate supply of microbial protein but rumen undegradable protein (RUP) is also important. Table 3 showed that variety trend of CP fraction, RDP and RUP. Particularly, content of RDP in PRM was lowest, and content of RUP in PRM was highest than other feedstuffs. It may be attributed to the high NDF contents as shown in Table 1. This variation in rumen degradation could be due to differences in physical properties (solubility, structure, etc.) of feed sources, limitation of experimental apparatus, ruminant species, and rumen exposure (Van Straalen et al., 1997; Gao et al., 2015). These results suggest that PRM, with its higher RUP, may have a high potential for digestion in the small intestine.

Our results showed varied ruminal degradation of CP and AA in the tested feeds (Table 4). In particular, compared with other feedstuffs, oil meals normally containing high amounts of branched-chain AA showed a relatively low degradation of AA (Von Keyserlingk et al., 1998). Moreover, previous reports showed that Met and Lys were more degradable than other AA in most feedstuffs (Cozzi et al., 1995; Taghizadeh et al., 2005; Gao et al., 2015). Differences in the degradation rates of individual AA in the rumen may be related to the different protein classes (albumin, globulin, etc.), physical properties, and AA composition of proteins (Clark et al., 1987; van Straalen et al., 1997; Taghizadeh et al., 2005).

Data on Lys and Met are especially important in diet formulation as suggested by Mjoun et al. (2010). The intestinal digestion of Lys and Met was highest in PRM compared the other feedstuffs (Table 5). The main reason for such variation may be different CP and AA contents in RUP as indicated by Gao et al. (2015). Moreover, intestinal digestion can vary widely depending on the feed species and individual AA composition (Hvelplund, 1985; Erasmus et al., 1994; O'Mara et al., 1997).

These *in situ* results showed total gastrointestinal tract digestion rates (Table 6) that were slightly higher than the *in vivo* digestibility results reported by Von Keyserlingk and Mathison (1989). Most notably, the estimated intestinal digestibility of PRM was higher than the estimated ruminal degradability. It suggests that the intestine has a greater capacity to digest protein than the rumen (Taghizadeh et al., 2005). Overall, these results indicate that PRM is a good source of RUP, which is mostly absorbed in the intestine. According to NRC (2001), formulation of diets for suitable RUP requires high total digestibility of AA, specifically low digestibility of AA in the rumen and high digestibility of AA in the intestine. PRM meets these criteria.

Finally, the rumen and total tract absorbability of Lys in PRM was lowest than other feedstuffs and total tract absorbability of Met in RSM was highest and RPM was lowest, with CSM in the middle (Table 7). Overall, these results indicated that PRM is good Lys and Met source for feedstuffs.

Conclusion

Ruminal degradation and intestinal digestion of CP, RUP, and AA varied substantially among CSM, PRM, and RSM for Hanwoo steers. In particular, PRM showed low CP degradability in the rumen and high intestinal digestibility of Lys and Met. Furthermore, absorbable Lys and Met results showed that PRM was a suitable RUP source for Hanwoo steers. These results can be used for beef cattle diet formulation.

Acknowledgements

This work was supported by the "Establishment of energy and protein feeding system for reducing feed cost in Hanwoo" project (PJ00938202) of the National Institute of Animal Science, RDA, Korea.

References

Abu-Ghazealeh AA, Schingoethe DJ, Hippen AR (2001) Blood amino acids and milk composition from cows fed soybean meal, fish meal, or both. J Dairy Sci 84: 1174–1181.

Association of Official Analytical Chemists (2012) Official Methods of Analysis. 17th edition. Association of Official Analytical Chemists, Arlington, VA, USA.

Chiou PWS, Chen KJ, Kuo KS, Hsu JC, Yu B (1995) Studies on the protein degradabilities of feedstuffs in Taiwan. Anim Feed SciTechnol 55: 215–226.

Clark JH, Murphy MR, Crooker BA (1987) Supplying the protein needs of dairy cattle from by-product feeds. J Dairy Sci 70: 1092–1109.

Cozzi G, Andrighetto I, Berzaghi P, Polan CE (1995) *In situ* ruminal disappearance of essential amino acids in protein feedstuffs. J Dairy Sci 78: 161–171.

Erasmus LJ, Botha PM, Cruywagen CW, Meissner HH (1994) Amino acid profile and intestinal digestibility in dairy cows of rumen-undegradable protein from various feedstuffs. J Dairy Sci 77: 541–551.

Gao W, Chen A, Zhang B, Kong P, Liu C, Zhao J (2015) Rumen degradability and post-ruminal digestion of dry matter, nitrogen and amino acids of three protein supplements. Asian-Aust J Anim Sci 28: 485-493.

Hvelplund T (1984) Intestinal digestion of protein in dairy cows. Can J Anim Sci 64(Suppl.): 193-194 (Abstr).

Hvelplund T (1985) Digestibility of rumen microbial protein and undegraded dietary protein estimated in the small intestine of sheep and by *in sacco* procedure. Acta Agric Scand Suppl 25: 132.

Mjoun K (2010) Ruminal degradability and intestinal digestability of protein and amino acids in soybean and corn distillers grains products. J Dairy Sci 93: 4144–4154.

National Institute of Animal Science (2012) Standard Tables of Feed Composition in Korea. Second revision. RDA, Suwon. Korea.

National Research Council (1989) Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington DC, USA.

National Research Council (2001) Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington DC, USA.

O'Mara FP, Murphy JJ, Rath M (1997) The amino acid composition of protein feedstuffs before and after ruminal incubation and after subsequent passage through the intestines of dairy cows. J Anim Sci 75: 1941–1949.

Ørskov ER, McDonald I (1979) The estimation of protein degradability in the rumen from incubation measurement weighted according to rate of passage. J Agric Sci (Camb.) 92: 499–503.

Polan CE (1992) Protein and amino acids for lactating cows. In: Van Horn HH and Wilcox CJ (editors), Large Dairy Herd Management. Am. Dairy Sci. Assoc., Champaign, IL USA, p: 236.

SAS Institute Inc (2003) SAS/STAT User's Guide: Version 9. 7th edition. SAS Instute INC., Cary, NC, USA.

Taghizadeh A, Danesh Mesgaran M, Valizadeh R, Eftekhar Shahroodi F, Stanford K (2005) Digestion of feed amino acids in the rumen and intestine of steers measured using a mobile nylon bag technique. J Dairy Sci 88: 1807–1814.

Van der Poel AFB, Prestlokken E, Goelema JO (2005) Feed processing: effects on nutrient degradation and digestibility. In: Dijkstra J, Forbes JM, and France J (editors), Quantitative Aspects of Ruminant Digestion and Metabolism. 2nd ed. J CABI Publishing, New York, NY, pp: 627–661.

Van Straalen WM, Odiga JJ, Mostert W (1997) Digestion of feed amino acids in the rumen and small intestine of dairy cows measured with nylon-bag techniques. Br J Nutr 77: 83–97.

Van Straalen WM, Dooper FMH, Antoniewicz AM, Kosmala I, van Vuuren AM (1993) Intestinal digestibility of protein from grass and clover in dairy cows measured with the mobile nylon bag and other methods. J Dairy Sci 76: 2970–2979.

Van Straalen WM, Tamminga S (1990) Protein degradation of ruminant diets. In: Wiseman J and Cole DJA (editors), Feedstuff Evaluation. Butterworths, London, UK, p: 55.

Varvikko T, Vanhatalo A (1993) Use of a combined synthetic fibre bag method to estimate the true total tract digestion of organic matter and nitrogen of hay and grass silage in cows. Arch Anim Nutr 43: 53–61.

Von Keyserlingk MA, Shelford JA, Puchala R, Swift ML, Fisher LJ (1998) *In situ* disappearance of amino acids from grass silages in the rumen and intestine of cattle. J Dairy Sci 81: 140-149.

Von Keyserlingk MA, Mathison GW (1989) Use of the *in situ* technique and passage rate constants in predicting voluntary intake and apparent digestibility of forages by steers. Can J Anim Sci 69: 973-987.