Effects of Heat Treatment of Three Animal by-products on Ruminal Degradation Characteristics and Intestinal Availability of Crude Protein

Y. H. Moon*, S. C. Lee** and B. K. Kim***

Department of Animal Science and Biotechnology, Chinju National University*,

National Livestock Research Institute, RDA**,

Kyungbuk Livestock Research Institute***

동물성 부산물 사료 세 종류에 대한 열처리가 조단백질의 반추위내 분해특성 및 하부장기내 이용성에 미치는 영향

문여황* · 이상철** · 김병기***

진주산업대학교 동물생명과학과*, 축산기술연구소**,

경북축산기술연구소***

ABSTRACT

In order to investigate the effects of heat treatment of three animal by-products(feather meal, tallow meal, viscera meal) on *in situ* ruminal degradation characteristics and gastrointestinal availability of dietary crude protein(CP), three ruminally and duodenally cannulated dry Holstein cows were employed. Cows were fed a diet containing 60% concentrate and 40% orchard grass hay, and had free access to water and mineral block. Experimental feeds were processed for 4 hr at 149℃ in a forced-air oven, and were passed through a 1-mm screen. Degradation kinetics of feed protein in the rumen were fitted to an exponential type model, and intestinal availability was estimated by the mobile nylon bag technique.

Effective CP degradabilities in the rumen for feather meal, tallow meal and viscera meal were 30.2%, 75.0% and 56.4% at 5% passage rate per hour(k=0.05), respectively. In addition, heat treatment increased effective ruminal CP degradability on feather meal and viscera meal treatments, whereas decreased in tallow meal treatment($P<0.05$).

Gastrointestinal CP disappearances of feather meal, tallow meal and viscera meal were 56.2%, 18.6%, and 37.9%, respectively. In addition, heat treatment decreased the gastrointestinal CP disappearance on feather meal and viscera meal treatment, but increased in tallow meal treatment($P<0.05$). Intestinal availability of rumen undegradable protein(A-UDP) was 80.4% for feather meal, 83.8% for tallow meal and 86.9% for viscera meal. In addition, heat treatment increased A-UDP on feather meal and tallow meal treatment, 94.0% and 91.3%, respectively, but decreased on viscera meal treatment, 76.5%($P<0.05$).

(**Key words** : Animal by-products, Heat treatment, Mobile nylon bag, Ruminal degradability, Intestinal availability)

Corresponding author : S. C. Lee, National Livestock Research Institute, R.D.A., Omockchun-dong Suwon 441-350, Korea. (Tel: 031-290-1647, e-mail: lee647@rda.go.kr)

Ⅰ. INTRODUCTION

Application of dry heat on feedstuffs having highly soluble proteins has been suggested as a means of improving utilization. Heat treatment can be applied to decrease nitrogen solubility by coagulating or denaturing proteins(Chalupa, 1975), and can decrease proteolysis by blocking reactive site for microbial proteolytic enzymes (Broderick and Craig, 1980). However, severe thermal treatment of proteins may result in structural changes such as hydrolysis of peptide bonds, modification of amino side chains, and the formation of new covalent isopeptide cross-links(Cheftel, et al., 1985). Since the objectives of heat treatment on protein feeds are originally to increase the undegradable crude protein in rumen(UDP) and the intestinal availability of dietary protein(Broderick and Craig, 1980), heat treatment on the *in situ* degradability of animal-origin feedstuffs having a relatively high UDP were rarely investigated.

In order to know the availability of dietary protein in ruminants, the estimation of intestinal digestion of UDP is required. Although the quantity of protein reaching the small intestine of ruminants was largely increased by various treatments or supplementation of protein sources with low rumen degradation, it is a loss if not being digested in the gastrointestinal tract(de Boer et al., 1987). Therefore, appropriate treatments of protein feeds for ruminant should be rather emphasized on the intestinal digestibility than the ruminal degradability. However, because of difficulties associated with using animals surgically prepared with intestinal cannula(Stern et al., 1985), there is limited research concerning the effect of treatments on intestinal protein supply in ruminants. The mobile nylon bag technique for ruminant was developed to permit rapid and reliable estimation of the intestinal availability of rumen undegradable protein by de Boer et al(1987).

In this study the effect of heat treatment on ruminal degradation characteristics and intestinal UDP availability of proteins of feather meal, tallow meal and viscera meal were investigated using the mobile nylon bag technique.

Ⅱ. MATERIALS AND METHODS

1. Animals and Diets

Three ruminally and duodenally(T-type) cannulated dry Holstein cows(average body wt. 550kg) were used to estimate the effects of heat treatment for three animal by-products(feather meal, tallow meal, viscera meal). Cows were fed a diet containing 60% concentrate and 40% orchard grass hay on the dry matter basis twice daily(07:30 and 19:30) by 110% of NRC(1988) requirements for maintenance, and had free access to water and mineral block. Three experimental feeds were processed for 4 hr at 149℃ in a forced-air oven. Then untreated- and heat treated feeds were passed through a 1-mm screen, which was used for the estimation of *in situ* degradabilities in the rumen and intestine of cows. Chemical composition of experimental feeds is presented in Table 1.

2. *In situ* measurements

The nylon texture(NYTAL, Swiss Screen Co.) with 45μm pore size was used to estimate the *in situ* rumen and intestinal degradabilities for six experimental feeds(feather meal, heated-feather meal, tallow meal, heated-tallow meal, viscera meal, heated-viscera meal) by mobile nylon bag technique(de Boer et al., 1987). Large bag(LB) for ruminal degradability were cut to an internal size 9.0×15 cm(LB) and were double stitched with curved corners and closed off at the top with a nylon drawstring.

Table 1. Chemical compositions of experimental feedstuffs

Items	Feather meal		Tallow meal		Viscera meal		SEM[1)]
	Untreated	Heated	Untreated	Heated	Untreated	Heated	
	% DM						
Dry matter	86.98	90.29	90.38	90.50	90.15	94.05	0.91
Crude protein	75.42	73.06	67.44	63.19	64.94	60.21	2.39
Ether extract	16.28	12.78	12.47	6.20	20.49	18.18	2.07
Crude ash	7.76	7.53	16.56	8.70	13.11	13.01	1.50

[1)] Standard error of the mean.

Five grams sample of experimental feedstuff was inserted into the LB. Small bag(SB) for intestinal degradability was cut into 7.0×5.5 cm pieces and folded in half. SBs were formed 3 mm from each of two free edges by heat sealing machine. After inserting the sample of 1g the SBs(3.5×2.5 cm, final dimensions) were thoroughly sealed by heat. Three LB were removed from the rumens of each three cows after incubation of 0, 1, 2, 4, 6, 8, 12 and 16 hrs for each feed, respectively. Nylon bags(LB, SB) containing the test feeds were inserted into a polyester mesh bag, and for ventral sac suspension a nylon string(75cm long) of mesh bag with glass beads was connected to the outside of the cannula. Just before placing in the rumen, nylon bags with feed samples were presoaked for 5 min in 39℃ water, and were inserted into the rumens of cows just after 07:30 hr feeding. Nine SBs per experimental feedstuff were alloted to three cows for intestinal diappearance. SBs experienced for 16 hr incubation in the rumen were passed to the lower digestive tract through duodenal cannulas by intervals of 30 min. SBs not inserted immediately the duodenum after rumen withdrawal were kept at 4℃ without washing. SBs inserted into the duodenum were recovered from feces after 12~14 hrs. Respective LB and SB retrieved from rumen and feces were mechanically(particularly devised machine washer) rinsed in cold water until the rinse water was clear. After the rinsed bags were dried in a forced air oven set at 60℃ for 48 hr, bags with contents were weighed, and contents were placed in vinyl bag individually for each animal and each incubation time for nitrogen analysis. Nitrogen was determined by Kjeldahl analysis using Kjeltec Auto 1030(Tecator, Sweden).

3. Data analyses

Degradation kinetics of feed protein in the rumen were fitted to an exponential type model as follows :

$$P = a + b(1 - e^{-ct})$$

as reported by Ørskov and McDonald(1979) ; P is percentage disappearance at time 't' suspended in the rumen; a, the intercept at time zero, is an expression of the soluble material which is immediately fermented or disappeared from the bag; b is the non-soluble fraction which can be degraded; c is the rate of degradation of the "b" fraction. Estimated values for a, b, and c were derived from a iterative least squares procedure (Marquardt, 1963). Effective degradability(ED) where fractional outflow rate(k) is accounted for its effect on degradability was also estimated by the equation of Ørskov and McDonald(1979). Three

functional outflow rates of 0.02, 0.05 and 0.08/hr were assumed.

The estimated values of quickly degradable crude protein(QDP), slowly degradable crude protein(SDP) and undegradable crude protein (UDP) in the rumen(AFRC, 1993) were applied by outflow rate(k) of 0.05. From parameters(a, b, c) of above non-linear equation ED, QDP, SDP and UDP were calculated as follows (AFRC, 1993).

$ED(\%) = a + (b \times c) / (c+k)$

$QDP(\%) = a \times [CP]$

$SDP(\%) = \{(b \times c)/(c+0.05)\} \times [CP]$

$UDP(\%) = [CP] - \{[QDP] + [SDP]\}$

CP and DM disappearances were calculated from the proportion remaining in the bag. Whole digestive tract(rumen plus intestine) disappearance of crude protein was obtained from the trial using SB, and intestinal disappearance was calculated by subtracting the rumen disappearance(%) from whole digestive tract disappearance(%). In this case, rumen disappearance was used the ED value after 16 hr incubation in the trial using LB. Intestinal availability of UDP(A-UDP) was calculated as follows.

A-UDP=(UDP - CP residue after rumen plus intestinal incubation)/UDP

4. Statistical analysis

All data were analysed by two-way ANOVA analysis of variance according to a 3×2 factorial design(SAS, 1988). Significant differences between control and heat treatment in each dietary group were tested using student's t-test when a significant interaction between dietary treatments was detected for a parameter.

Ⅲ. RESULTS AND DISCUSSION

1. CP degradation kinetics in the rumen

Ruminal CP degradation characteristics of experimental feedstuffs are presented in Table 2.

Since "c" value means the degradation parameter of "b" fraction per hour, "b" fraction of dietary CP was rapidly degraded(36.7%/hr) for tallow meal, intermediated for viscera meal (7.9%/hr), and slowest for feather meal(2.9%/hr). And the effect of heat treatment on "c" value appeared differently among feedstuffs; feather meal was increased, but tallow meal was decreased, and viscera meal was not affected.

In order to estimate the effective degradability(ED), three functional outflow rates of 0.02, 0.05 and 0.08/hr were assumed. ARC (1984) concluded that this rates were suitable to animals fed at a low level of feeding(about $1\times$ maintenance) for 0.02/hr, calves, low yielding dairy cows(<15kg milk/d), beef cattle and sheep on higher levels of feeding, but less than $2\times$ maintenance for 0.05/hr, and high yielding dairy cows(>15kg milk/d) and greater feeding than $2\times$maintenance for 0.08/hr. Fractional outflow rate of 0.05/hr in rumen has been reported, and are commonly used(Janick and Stallings, 1988).

ED of dietary CP of untreated feedstuffs was high in the order of tallow meal, viscera meal and feather meal.

Heat treatment of tallow meal caused a decrease in the effective CP degradability by 31% compare to the untreated one, whereas those of feather meal and viscera meal increased by 58% and 13%, respectively. There are few reports on the increment of CP degradability by heat treatment of protein in feeds. However heat treatment on feather meal and viscera meal increased the dietary CP degradability in the rumen.

Rumen degradable crude protein(RDP) consisted of quickly degradable protein(QDP), NPN and water soluble small protein molecules, and slowly degradable protein in the rumen(SDP) which is a function of level of feeding and outflow rate. The amount of [SDP] is [RDP]-

Table 2. Effect of heat treatment for three animal by-products on *in situ* crude protein degradation kinetics in the rumen of dairy cow.

Items	Feather meal		Tallow meal		Viscera meal		SEM[6]	Significance[7]		
	Untreated	Heated	Untreated	Heated	Untreated	Heated		F	T	F×T
Parameters[1]										
a	14.39^{b}	33.54^{a}	59.62^{a}	34.02^{b}	43.87	42.77	6.10	*	ns	**
b	42.99^{a}	18.69^{b}	17.47^{b}	23.69^{a}	20.39^{b}	33.63^{a}	4.12	ns	ns	*
c	0.029^{b}	0.149^{a}	0.367^{a}	0.158^{b}	0.079	0.084	0.05	**	ns	**
ED[2]										
k=.02	39.83^{b}	50.02^{a}	76.19^{a}	54.04^{b}	60.14^{b}	69.95^{a}	5.45	ns	ns	*
k=.05	30.17^{b}	47.54^{a}	75.00^{a}	52.00^{b}	56.36^{b}	63.89^{a}	6.22	ns	ns	*
k=.08	25.83^{b}	49.71^{a}	73.97^{a}	49.73^{b}	54.00	60.03	6.45	ns	ns	**
QDP[3]	14.39^{b}	33.54^{a}	59.62^{a}	34.02^{b}	43.87	42.77	6.10	ns	ns	**
SDP[4]	15.78^{a}	12.63^{b}	15.38	17.98	12.49^{b}	21.11^{a}	1.34	*	*	**
UDP[5]	69.84^{a}	53.83^{b}	25.00^{b}	48.00^{a}	43.64	36.12	6.27	ns	ns	**

[1] a,b,c = Fitted exponential constants for crude protein.
[2] Effective degradability of crude protein at rates of passage(k), % DM basis.
[3] Quickly degraded crude protein in the rumen at k=0.05, % DM basis.
[4] Slowly degraded crude protein in the rumen at k=0.05, % DM basis.
[5] Undegraded crude protein in the rumen at k=0.05, % DM basis.
[6] Pooled standard error of the mean.
[7] F=main effect of feed source, T=main effect of heat treatment, F×T=interaction of feed source and heat treatment.
^{a,b} Significant difference between control and heat treatment in each animal by-products(P<0.05).
**, P<0.01; *, P<0.05; ns, no significance.

[QDP], both of which are now to hand for a fractional outflow rate of 0.05. In the effects of heat treatment on experimental feedstuffs, QDP content was increased(P<0.05) on feather meal, but decreased(P<0.05) on tallow meal, and did not affected on viscera meal. SDP content increased in viscera meal by heat treatment, but was not effect in feather meal and tallow meal. UDP content increased in tallow meal by heat treatment, but decreased in feather meal and viscera meal. In general, heat application to feedstuffs is aim to reduce the ruminal degradability by coagulating or denaturing the protein of feeds having high solubility(Chalupa, 1975) and can decrease proteolysis by blocking reactive site for microbial proteolytic enzymes (Broderick and Craig, 1980). Consequently, heat treatment has been used to increase the supply of dietary protein to the duodenum by decreasing ruminal N degradation. In this experiment, however, heat treatment on feather meal and viscera meal resulted in higher ruminal CP degradability rather than the untreated ones. This indicates that appropriate temperature and heating time according to the structure and the composition of feed protein should be applied, and the composition and the structure of feed protein have been suggested to be the main reasons for the differences of N degradation between feedstuffs. Ruminal hydrolysis rate of peptides depends on the structure of the N-terminus of a low degradability of protein in the rumen(Stock et al., 1981). Protein degradation can be decreased also by the high content of

hair in the meal(Stock et al., 1981), because of abundance of disulfide bond in hair. Animal by-products, particularly feather meal, largely comprised by disulfide bonds in protein structure, had a low ruminal N degradability inherently(Mahadevan et al., 1980), which may induce a breakdown rather than protection of protein matrix by heat treatment. UDP content of feather meal obtained from the study was similar to the value(71%) appeared in NRC (1988).

2. CP degradability in digestive tracts

Intestinal and whole digestive tract CP degradation coefficients of experimental feedstuffs were presented in Table 3.

Intestinal CP degradability of untreated feedstuffs was high in the order of feather meal, viscera meal and tallow meal. Heat treatment caused a decrease in intestinal CP degradability which is contradictory to the ruminal degradability; Intestinal CP degradability of tallow meal was increased by heat treatment as much as near 138%, but those of feather meal and viscera meal were decreased by 20% and 33%, respectively.

Almost all the CP of experimental feedstuffs was degraded(near or above 95%) through the whole digestive tract except for the untreated feather meal which showed 86.3% disappearance. However feather meal was also significantly($P<0.05$) increased by heat treatment in the CP degradability through whole digestive tract. de Boer et al.(1987) mentioned that whole digestive tract degradability measured, using the mobile nylon bag technique described in this study, can be considered as an estimate of true rather than apparent digestibility. It can be easily believed that protein sources of low ruminal degradability or the heat treatment enhances ruminant animal performance thanks to the increase in the amount of protein available in the small intestine. However abound in the rumen escaped proteins may be, it will be rather disadvantageous if being not digested in intestinal tract. Therefore it is necessary to estimate the intestinal availability of rumen undegraded protein(A-UDP).

In this study, A-UDP was high in the order of viscera meal, tallow meal and feather meal when they were not treated. Heat treatment of

Table 3. Effects of heat treatment for three animal by-products on digestive tract degradation and intestinal availability of crude protein in dairy cow.

Items	Feather meal		Tallow meal		Viscera meal		SEM[3]	Significance[4]		
	Untreated	Heated	Untreated	Heated	Untreated	Heated		F	T	F×T
Digestive tract	..... % DM									
Rumen[1]	30.17[b]	47.59[a]	77.81[a]	51.57[b]	56.36[b]	66.39[a]	7.34	**	ns	*
Intestine	56.17[a]	48.93[b]	18.60[b]	44.23[a]	37.92[a]	25.32[b]	6.39	**	ns	**
Whole tract	86.34[b]	96.52[a]	96.41	95.78	94.28	97.59	1.69	ns	*	*
A-UDP[2]	80.44[b]	93.96[a]	83.82[b]	91.33[a]	86.89[a]	76.50[b]	2.61	ns	ns	*

[1] Percent of whole crude protein disappearing during 16 hours suspension in the rumen.
[2] Percent of intestinal disappearance of protein fraction remaining after 16 hs rumen suspension.
[3] Pooed standard error of the mean.
[4] F=Main effect of feed source, T=main effect of heat treatment, F×T=interaction of feed source and heat treatment.
[a,b] Significant difference between control and heat treatment in each animal by-products($P<0.05$).
**, $P<0.01$; *, $P<0.05$; ns, no significance.

Table 4. Effects of heat treatment for three animal by-products on the amount of crude protein degraded in digestive tracts of dairy cow

Items	Feather meal		Tallow meal		Viscera meal		SEM[1]
	Untreated	Heated	Untreated	Heated	Untreated	Heated	
	..... g/kg DM						
CP	754	731	674	632	649	602	24.0
Rumen data							
QDP	109	255	402	215	285	258	38.9
SDP	119	92	104	114	81	127	7.1
UDP	526	383	169	303	283	217	52.2
Small bag data							
Intestinal disappearance	423	357	125	279	246	152	51.7
Whole tract disappearance	651	705	650	605	612	588	17.3
Unavailable CP	103	25	24	27	37	15	13.2

[1] Standard error of the mean.

feather meal and tallow meal increased their A-UDP by 9~17%, but viscera meal decreased by 12%. Although heat application to feather meal reduced the intestinal CP degradability due to an increase of the ruminal disappearance, but remarkably($P<0.05$) increased in A-UDP.

The calculated amounts of degradable protein in digestive tracts of cow are shown in Table 4. The amounts of UDP and intestinal degradable protein were high in the order of feather meal, heated-feather meal, tallow meal, heated-tallow meal, viscera meal, and heated-viscera meal. Although heat application for three animal by-products reduced the content of dietary CP, feather meal and tallow meal increased the amount of CP degraded in whole digestive tract or intestine, respectively. However, heat application for viscera meal produced negative effects on the CP degradations in intestine and whole digestive tract.

Ⅳ. 요 약

동물성 부산물 사료(우모분, 우지박, 내장분) 단백질의 반추위내 분해특성과 하부장기내 이용성에 대한 열처리 효과를 구명하기 위하여 반추위와 십이지장에 누관이 장착된 Holstein 건유우 3두를 공시하였다. 시험사료에 대한 열처리는 149℃가 유지되는 oven에서 4시간동안 처리한 후, 1 mm체를 통과시켰다. 시험사료의 반추위내 분해특성은 발효시간별 분해율에서 비선형회귀식을 유도하여 구하였고, 사료단백질의 하부장기내 이용성은 mobile nylon bag기법으로 추정되었다. 농후사료와 orchard grass를 60:40의 비율로 급여하였으며, 물과 mineral block은 자유섭취토록 하였다.

조단백질의 반추위내 유효분해도(k=0.05) 및 하부장기내 소실율에 있어서 우모분은 각각 30.2% 및 56.2%, 우지박은 75.0% 및 18.6% 그리고 내장분은 56.4% 및 37.9%였다. 시험사료에 대한 열처리효과에 있어서 조단백질의 반추위내 유효분해도는 우모분과 내장분은 증가하였으나 우지박은 감소되었고($P<0.05$), 하부장기내 조단백질 소실율에서는 우지박은 증가된 반면, 우모분과 내장분은 감소되어($P<0.05$) 상반되는 결과를 나타내었다. 반추위 미분해 사료단백질의 하부장기내 이용율은 우모분, 우지박 및 내장분에 대해서 각각 80.4%, 83.8% 및 86.9%였으며, 열처리를 함으로써 우모분과 우지박은 각각 94.0% 및 91.3%로 향상되었으나,

내장분은 76.5%로 낮아졌다($P<0.05$).

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