Effects of amount of feed intake on protein degradability in the rumen

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Abstract

Most feed proteins are extensively degraded by microorganisms in the rumen. However, bypass protein, which can escape digestion in the rumen, can be made available intact to the animal. There are many factors affecting protein degradability in the rumen. This experiment was conducted to investigate the effects of feed intake on protein degradability in the rumen and rumen charact eristics during the feeding period.

Samples of untreated soybean meal (USBM) and soybean meal treated with 0.3% formaldehyde (FSBM) were incubated for 1, 2, 8, 12 and 24 hours in nylon bags in the rumen of sheep receiving diet consisting of 60% rice straw treated with 4% ammonia and 40% commercial formula feed. Two feeding levels, high intake *ad libitum* (HI) and low intake of approximately 60% *ad libitum* (LI), were set.

USBM incubation at HI level caused reduction of rate of dry matter disappearance from nylon bags and significant reduction of protein degradability ($p<0.05$). FSBM incubation at both levels of intake, however, produced no changes in both rate of dry matter disappearance and protein degradability. According to the equation to calculate the effective degradability ($P=a+bc/(c+k)$), the calculated values for P (For HI-USBM), LI-USBM, HI-FSBM and LI-FSBM were 76.6, 84.2, 45.7 and 47.3, respectively. Rumen pH, NH_3-N concentration and total VFA concentration decreased, while acetate-propionate ratio increased at HI.

Key Words: Degradability, Feed Intake, Formaldehyde, Nylon Bags.

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Introduction

Protein ingested by ruminants is extensively degraded by microorganisms inhabiting the rumen. Ruminants utilize two types of protein, microbial protein and bypass protein. However, even in maximum feeding levels, synthesized microbial protein can not cover the requirements of high yielding dairy cows, so, the latter must be offered additional feed protein to satisfy their requirements.[11, 12]

Factors influencing protein degradability in the rumen include composition and solubility of protein in feed, physical or chemical treatment of feed, rate of passage of digesta through the forestomachs, feed intake level, energy intake level, growth rate of rumen microbes, particle size of feed, and rumen pH.[17, 22]

Protein degradability has been evaluated by measuring protein solubility in various solvents (*in vitro*), by the use of rumen and postrumen cannula method (*in vitro*) or by the nylon bag technique (*in situ*). Protein in the feed treated with heat,[2, 16] alcohol,[12] acetic acid,[24] formaldehyde (HCHO),[15, 20, 24] zine,[2] tannin,[16] monensin[25] and masonex[8] is protected from rumen degradation. HCHO treatment is a popular practice in the world especially in Europe, but it has been prohibited in Japan. HCHO protection of protein is achieved through the binding of aldehyde and free amino groups to produce a methylene bond, rendering the protein insoluble under neutral rumen condition. The gastric jiuce in the abonosum, then, breaks up this bond releasing the protein for digestion. The level of feed intake of ruminants also affect protein degradation in the rumen[7].

There are few studies on the effect of the level of feed intake on protein degradation in the rumen.

This study was conducted to examine the effect of the amount of feed intake on protein degradability in soybean meal treated with 0.3 % HCHO in the rumen using the nylon bag technique.

Materials and Methods

This experiment was conducted in Kon Kuk University, KOREA and Obihiro University of Agriculture and Veterinary Medicine, JAPAN in 1987.

Four castrated and cannulated *Corriedale* sheep, 28.9kg (19.0-33.5kg) average body weight, were used.

Experimental plots consisted of two feed intake levels, i. e. high-intake group (HI) was offered *ad libitum* and low intake group (LI) was restricted to the equivalent of 60% intake of HI. Basal diet, consisted of 60% chopped rice straw treated with 4% ammonia mixed with 40 % commercial formula feed on a dry basis. This ration contained approximately 12.5% crude protein. Two sheep each were assigned to HI and LI in the first period and were replaced in the second period of the experiment.

To determine the degradability of feed supplement, nylon bag technique[13] was applied. Incubation with cannula method was started immediately before feeding in the morning and bags were withdrawn at 1, 2, 8, 12 and 24 hours after feeding. Feed supplement consisted of untreated soybean meal and soybean meal treated with HCHO. The concentraon of HCHO was 0.3w % of crude protein contained in soybean meal.

Outflow rate of solid from the rumen was measured by the amount of soybean meal marked with sodium dichromate that passed through the nylon bag. Protein treated with sodium dichromate is insoluble and nondegr adable in the rumen. Immediately before feeding in the morning, 50g soybean meal

Table 1. Chemical composition of basal diet fed to sheep

		Ammoniated rice straw	Formula feed
Dry matter	%	80.6	84.5
Organic matter	% DM	88.3	93.2
Crude protein		11.5	14.1
Crude fat		2.3	5.1
NFE		39.6	68.7
Crude fiber		34.9	5.3
NDF		75.3	40.6
ADF		52.8	8.9
Hemi cellulose		22.5	31.7
Cellulose		42.5	5.8
Calcium		0.3	1.1
Phosphorus		0.1	0.6

Table 2. Chemical composition of soybean meal.

Level of HCHO (%)	Dry matter (%)	Organic matter (%DM)	Crude protein (%DM)
0	88.1	93.2	52.8
0.3	84.2	93.0	52.6

Table 3. Dry matter intake

Level of feed intake	Ad libitum	Restricted
% of LW kg*	2.7	1.6
g / LW $kg^{0.75}$**	60.5	37.2
DM intake (g/day)	717.6	431.1
CP intake (g/day)	111.2	66.0

* LW; Live weight.

** Metabolic body size.

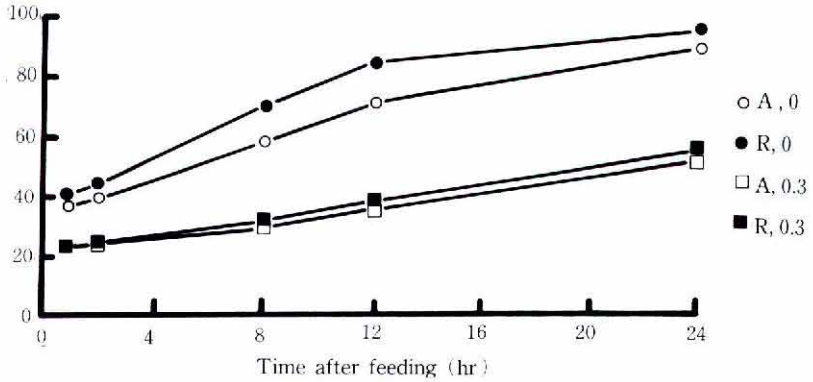

Fig. 1. The disappearance of dry matter from nylon bags incubated in the rumen of sheep.

treated with sodium dichromate was added to the feed in each nylon bag via a rumen cannula. Outflow rate of protein from the rumen was measured from the 18th and 19th day of each period. Samples of rumen solid were taken out by forceps at 1, 2, 4, 6, 9, 12, 24, 36 and 48 hours after the start of incubation.

Rumen fluid was collected on the 12th day of each period at 0, 1, 2, 3, 4, 6, 8, 10 and 12 hours after feeding in the morning.

Results and discusson

The chemical composition of the basal diet is shown in Table 1, while that of soybean meal is shown in Table 2. Untreated and ammoniated rice straw contained 11.5% and 19.4% crude protein, respectively. Dry matter (DM) intake was 2.7% at HI and 1.6 % at LI which correspond to about 60% of that at HI (Table 3). The amount of crude protein consumed was 111.2g at HI and 66.0g at LI per day.

The disappearance of DM from nylon bags incubated in the rumen significantly increased with HCHO treatment, regardless of intake levels ($p<0.01$), (Fig. 1). The differences in DM disappearance between soybean meals untreated (USBM) and treated with HCHO (FSBM) increased gradually as the time of incubation increased. The difference in the disapearance of DM in the rumen has been reported similarly by many researchers[24]. Rate of DM disappearance in USBM at HI was considerably higher than that at LI. No difference was detected between intake levels for the rate of DM disappearance in FSBM at any time of incubation. This may be due to protection of protein from degradation in the rumen. The pattern of crude protein disapprearance was similar to that of DM (Fig. 2) and conforms with the findings in investigations carried out in vitro[20] and in situ.[20]

It has been repored that protein degradation of SBM in the rumen could be reduced substantially by HCHO treatment at concentrations at 0.8% and 0.4% crude protein. In this experiment, the comparatively low HCHO concentration (0.3% of crude protein) was also effective. The protein disappearance was reduced by HCHO treatment regardless of intake levels ($p<0.01$). When USBM was incubated in the rumen, intake levels did not affect the protein disappearance at 1 and 2 hours after incubation, but the disappearance at HI was significantly lower than that at LI after 8 hours ($p<0.05$). When FSBM

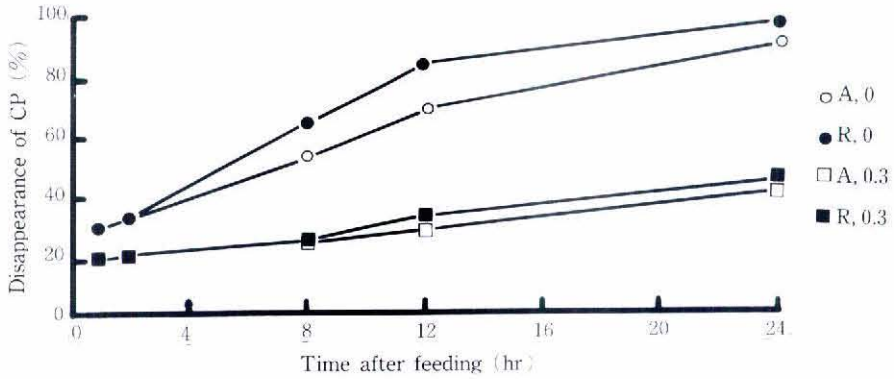

Fig. 2. The disappearance of crude protein from nylon bags incubated in the rumen of sheep.

was incubated, intake levels did affect the protein disappearance at any incubation. Firkins et al.[5] reported that the increased DM intake resulted in the increased quantity of feed protein escaping from degradation in the rumen, probably because of faster passage of digesta in vivo, but Ganev et al[7]. reported that rate of degradation was only slightly lower at high intake level than at low intake level.

ørskov et al.[15] showed that the percentage of protein disappearance from samples incubated for time t can be described by the equation, $p(t) = a + b(1-e^{-ct})$. where, a is a protein fraction which disappears rapidly in the rumen and b is another fraction that is degraded at a constant fractional rate c per unit time. Table 4 represents the values of parameters a, b and c which were assumed in this experiment. HCHO treatment raised the value of a but it reduced that of b and c regardless of intake levels. Particularly, the value of c was greatly reduced by the treatment indicating that FSBM required longer period for breakdown by rumen microorganisms than USBM. Therefore, the rate of degradability of SBM was extremely reduced with HCHO treatment. When FSBM was incubated in the rumen, values of each parameter were constant at both levels of intake, but when USBM was exposed to the rumen enviroment, the value of a was higher at HI than at LI (14.8 vs. 8.4) suggesting that protein disappearance from nylon bags at 1 hour after incubation was slightly greater at HI. Conversely, values b and c were lower at HI than at LI reflecting the decreased degradability at HI as incubation time increased.

Several reports published previously suggested that the rate of passage of particulate matter was increased by an increase in feed intake.[3, 7, 19, 21] In this study, it was shown that the rate of passage was faster at HI than at LI but the difference was not significant. It is postulated that time of exposure of feed particles to the digestive environment was reduced with the reduction of degradability.

Turnover rate of dietary components in the rumen is an important determinant for the efficiency of microbial growth and for the partitioning of digesta to the rumen and postrumen tracts. It was demonstrated that an increase in the flow of both bacterial and nonbacterial, and non-ammonia nitrogen in the duodenum resulted in higher flow of non-

Table 4. Values of parameters a, b, and c outflow rate (K) of solid-phase

Feed intake	Ad libitum		Restricted	
Level of HCHO (%)	0	0.3	0	0.3
a (%)	14.8	17.1	8.4	17.5
b (%)	85.2	82.9	91.6	82.5
c	0.095	0.019	0.014	0.017
k	0.036	0.036	0.030	0.030

* Parameter a, b and c were estimated from the exponential equation $P(t) = a + b(1-e^{-ct})$, where the constant a represents a very rapidly degradable component of the protein and b represents another fraction which is decomposed at a constant fractional rate c per unit time.

* K; the fractional rate of passage from the rumen.

ammonia nitrogen to the small intestine at high intake level.[15] This was probably due to increased nitrogen intake which occurred in association with high intake level. In this experiment, it is also postulated that the degradability was reduced at high intake level due in part to higher flow of non-ammonia nitrogen to the postrumen tracts caused by faster dilution rate and greater efficiency of microbial protein synthesis, in addition to greater nitrogen intake.

ϕrskov et al.[18] proposed that the effective degradability which is expressed as the cumulative percentage of protein degradation to time t can be obtained by the equation,

$$P(t) = a + bc\,[1-e^{-(c+k)t}]\,(c+k),$$

In the above equation, the fractional rate of passage from the rumen (k) was applied to make it more accurate. The final estimate of the percentage of protein degradation approached closer towards P = a + bc / (c + k), because the fraction of protein remaining in the rumen declines to zero as the time of incubation increases.

The effective degradability of each incubation time and the final estimated protein degradability are shown in Table 5. All the values of effective degradability at each incubation time were smaller than direct estimate using the nylon bag technique. When USBM was incubated, the final estimated calculated values were 75.5 and 82.9 at HI and LI, respectively. Both values were lower than actual values at 24 hours after incubation in nylon bags, because the effective degradability took the time of retention into account. But when FSBM was incubated, final estimates were 45.7 and 47.3 at HI and LI, respectively. Both of these values were higher than actual values at 24 hours incubation.

Changes in ruminal characteristics after feeding were observed. Lower pH was constantly observed at HI than at LI. AT HI level pH was below 6.0 at any time except for 0 hour. On the contrary, at LI, pH was kept above 6.0. These results agree with earlier observations.[10, 15, 19] Isaacs et al.[9] in his experiment with pH from 5.0 to 7.0, demonstrated that protein of SBM was relatively more soluble at higher pH in vitro.

NH_3-N concentration increased immediately after feeding and reached the highest levol after 1 hour and then declined to the initial

Table 5. Estimates of P (t), the effective degradability (%) at time t after incubation, and P, the final estimated effective degradability.

Feed intake		Ad libitum		Restricted	
Level of HCHO	(%)	0	0.3	0	0.3
Incubation time (hr)	1	22.5	18.6	20.8	18.8
	2	29.2	20.1	31.0	20.1
	8	54.4	27.2	65.6	26.6
	12	62.8	30.8	74.8	30.0
	24	72.5	37.8	82.9	37.2
P		76.6	45.7	84.2	47.3

$P(t) = a + (bc/(c+k))(1-e^{-(c+k)t})$. $P = a+bc/(c+k)$

^{a,b} : Values with different superscripts on the same line differ ($p<0.05$).

^{l,m,n}: Values with different superscripts on the same line differ ($p<0.01$).

Disappearance of DM (%)

level. NH_3-N concentration was lower at HI than at LI. Higher NH_3-N concentration at LI reflected faster degradability. These results agree with some investigations,[6, 15] except for some reports that the levels of intake had no effect on NH_3-N concentration[19, 23] and increased intake resulted in greater NH_3-N concentration.[10] Mehrez et al.[14] described that the minimal NH_3-N concentration for the maximal rate of fermentation was 23.5mg/100ml, while Owens et al.[18] suggested that NH_3-N concentration required for the maximal microbial protein synthesis was from 0.35 to 29mg/100ml. The concentration observed in this experiment was quite low. Erfle et al.[4] reported that the cause of decreased NH_3-N concentration at low pH was the loss of proteolytic bacteria *in vitro* and Abe et al.[1] reported that NH_3-N accumulated in low concentration with high dilution rate *in vitro*. There fore, it is possible to consider that low pH at HI disturbes the activity of proteolytic bacteria leading to reduced protein degradation, additionally, high dilution rate at HI reduces the rate of feed protein degradation by faster rate of flow of digesta from the rumen.

The total VFA concentration reached the highest level at 1 or 2 hours after feeding and then gradually declined. VFA concentration was higher at LI than at HI. Nevertheless, many researchers demonstrated that VFA concentration increased linearly with an increase in intake levels.[19, 32] This was probably due to increased quantity of substrates available for fermentation in the rumen. Abe et al.[1] reported that VFA accumulated in low concentration with high dilution rate *in vitro* fermentation corresponding to the observation of this experiment. Therefore, there is a possibility that high dilution rate at HI reduced VFA concentration. Molar proportion of acetate tended to be higher and that of propionate and butyrate tended to be lower at HI. The ratio of acetate to propionate was higher when fed at HI than at LI. Many investigators showed the relationship between the levels of feed intake and concentrations of VFA, but these data were quite inconsistent.[5, 10, 23] Low pH, particularly below 6.0, is unfavorable for the existence of rumen microorganisms, particularly protozoa. As a rule, decreased population of protozoa increases acetate-propionate ratio because protozoa hardly produces propionate. But in this experiment, it was likely that the harmful influence by low pH was greater for bacteria rather than for protozoa. Therefore acetate-propionate ratio was higher at HI. Moreover, protozoa hasa significant effect on nitrogen utilization. The absence of rumen protozoa reduced the utilization of dietary protein by animals. In this experiment, it could be postulated that the faster rate of passage at HI reduced the availability of substrates for fermentation and then decreased total VFA concentration. Lower pH at HI reduced the population of rumen microorganisms, particularly proteolytic bacteria, thus roducing protein degradation in the rumen.

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飼料摂取量がルーメン内における蛋白質の分解性に及ぼす影響

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摘　　要

飼料摂取量がルーメン内での蛋白質分解性およびルーメン内性状に及ぼす影響を検討した。

無処理大豆粕（ＵＳＢＭ）または0.3％ホルムアルデヒド処理大豆粕（ＦＳＢＭ）が入ったナイロンバッグを，４％アンモニア処理稲藁と市販の配合飼料を60：40で混合した飼料を給与した羊のルーメン内に１，２，８，12，24時間投入し培養した。飼料摂取水準は自由給与（ＨＩ）と，制限給与（ＬＩ－自由給与の約60％）の２水準とした。ナイロンバッグからの乾物消失率は，ＵＳＢＭの場合ＬＩ時に比べてＨＩ時では減少したが，ＦＳＢＭの場合は減少しなかった。また，蛋白質分解性はＵＳＢＭの場合飼料摂取水準による差が認められ，ＬＩ時に比べＨＩ時では有意（$P<0.05$）に低下した。しかしＦＳＢＭでは両者に差は無かった。ナイロンバック試験による実測値をもとにＰ－ａ＋ｂｃ／（ｃ＋ｋ）から算出した有効分解率Ｐは，ＨＩ－ＵＳＢＭ，ＬＩ－ＵＳＢＭ，ＨＩ－ＦＳＢＭ，ＬＩ－ＦＳＢＭにおいてそれぞれ76.6，84.2，45.7，47.3であった。

ルーメン内ＰＨ，NH_3－Ｎ濃度，総ＶＦＡ濃度はＨＩ時に低下し，酢酸のプロピオン酸に対する割合も上昇した。