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In situ Ruminal Dry Matter and Crude Protein Degradability of Plant and Animal Derived Protein Sources in Northwest of Iran

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Abstract: Three plant and animal derived protein sources including Fish Meal (FM), Poultry By-products (PBM) and Whole Cottonseed (WCS) were evaluated with *in situ* technique. The using incubation times were 0, 4, 8, 12, 16, 24 and 48 h. There were significant differences between protein sources in terms of DM and CP disappearances. Effective degradabilities of Dry Matter (DM) and Crude Protein (CP) of WCS at 3 outflow rates were significantly higher than those of the other protein sources. Animal-derived protein sources such as PBM and FM had significantly lower effective DM and CP degradability values than those of plant-derived protein sources such as WCS. Therefore, animal-derived protein sources with a low degradability can be used to increase the bypass protein. The CP degradation parameters obtained in this experiment using sheep would be very useful in improving the accuracy of formulation of sheep diets.

Key words: *In situ*, protein by products, rumen, crude protein, degradability, plant and animal, dry matter

INTRODUCTION

Feed protein for ruminant animals has been evaluated traditionally in terms of DM or CP. The new protein systems (ARC, 1980; AFRC, 1992; NRC, 2001) were introduced to substitute the digestible crude protein system in most parts of the world. All of the new systems require accurate information on the kinetics of ruminal protein degradation to successfully apply the new systems of ruminant ration formulation. The *in situ* nylon bag technique (Ørskov and McDonald, 1979) has become a popular method to estimate the ruminal degradation kinetics, which is used in the new protein systems.

Although, it is easy to determine the CP of protein sources in most laboratories in Iran, the determination of the ruminal protein degradation kinetics is very difficult and time consuming. Despite of these problems, since it offer precise information about ruminal degradation, this technique is developed. The information related to degradability of CP in rumen is limited. Therefore, many feed factories, sheep and goat nutritionists use the tabulated values obtained from experiments carried out in different countries. Many researchers showed that the ruminal degradation kinetics of plant and animal-derived proteins are affected by many factors such as source of protein, processing method, bag pore size and substrate particle size (Weakley *et al*., 1977; Nocek *et al*., 1979; Wadwa *et al*., 1998). Differences found in protein degradabilities between laboratories are too large to be acceptable. It is not advisable for researchers and advisers to use absolute figures for protein degradability determined in another laboratory (Madsen and Hvelpund, 1994). Therefore, the tabulated values related to protein degradation kinetics in the rumen may not be accurate when used under different conditions. As a result of this, the accuracy of ration formulation in terms of protein may be lower than expected.

The aim of this experiment was to determine *in situ* ruminal degradability characteristics, fractional rates of digestion and effective degradability of DM and CP of FM, PBM and WCS which used in northwest of Iran.

MATERIALS AND METHODS

Protein sources: The animal and plant by-products containing FM, PBM and WCS, which widely used in ruminant feeds in northwest of Iran were used in this experiment.

Chemical analysis: Dry matter was determined by drying the samples at 105°C overnight and ashed by igniting the samples in a muffle furnace at 525°C for 8 h. N content was measured by the Kjeldhal method (AOAC, 1990). CP was calculated as N×6.25. Ether Extracts (EE) and Ash were determined by the method of AOAC (1990).

***In situ* DM degradation:** The nylon bag technique (Ørskov and McDonald, 1979) was used to measure the kinetics of DM and CP degradation of 3 plant and animal

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derived protein sources. Samples were milled in a hammer mill through a 1mm sieve and subjected to standard rumen degradability procedures using 3 fistulated Gizil male sheeps with (37±0.9 kg) live weight. The sheep were fed a diet containing 60% concentrate and 40% alfalfa hay at the maintenance level. The concentrate contain barley grain and mineral/vitamin supplement. The sheep were given free access to fresh water and mineral salt licks.

Throughout the experimental period, dacron bags with 45-50 μm poor size containing approximately 5 g samples in duplicate were incubated in each sheep for each of the testing time periods: 4, 8, 12, 16, 24 and 48 h. The bags were removed after incubation in the rumen of sheep and washed in cold running water until the washing ran clear and colorless. Time 0 h samples were not incubated in the rumen, but were washed in cold water as above to determine solubility at time 0 h. The bags were oven dried at 60°C for 48 h. The DM and CP degradation data were fitted to the exponential equation (Ørskov and McDonald, 1979).

$$y = a+b\,(1-e^{-ct})$$

Where, p is the disappearance of nutrient during time t; a the soluble nutrient fraction which is rapidly washed out of the bags and is assumed to be completely degradable; b the proportion of insoluble nutrient, which is potentially degradable by micrograms; c the degradation rate of fraction b per hour and t is the time of incubation.

The effective degradability of samples was calculated using the equation shown below, using rumen fractional outflow rates (r) of 0.02 h^{-1}.

$$ED = a + \frac{b \times c}{c + r}$$

ED is the effective degradability of DM. Data of DM and CP disappearance, degradation kinetics were analyzed by Analysis of Variance (ANOVA) using ANOVA of SAS (version 9.00). Significance between individual means was identified using the LSD's multiple range test Mean differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Chemical composition: Chemical compositions of the protein sources used are shown in Table 1. There was a considerable variation between protein sources in chemical composition. The animal-derived protein sources had higher CP contents than WCS as a protein and energy source, but EE content of WCS was higher than the other test feeds. The ash contents of animal-derived protein sources were considerable higher than WCS. The chemical compositions of WCS and animal derived protein sources aren't consistent with those reported by NRC (2001). The chemical composition of FM and PBM is consistent with those reported by Kamalak *et al.* (2005). Differences in chemical composition may result from differences in variety, whether condition or industrial processing methods employed in different countries.

Table 1: Chemical composition (%) of animal- and plant-derived protein sources

Ingredients	DM(%)	CP(%DM)	EE(%DM)	Ash(%DM)
WCS	90	22.16	17.8	3.8
FM	92.14	60.94	6.5	15.7
PBM	89.15	59.82	14.8	15.9

Table 2: The degradation kinetics of DM and effective DM degradability at three outflow rates

	Degradation kinetics			Effective degradability (%)	
Ingredient	a(%)	b(%)	c(h^{-1})	k = 0.02	RSD
WCS	34.87	35.38	0.0374	57.9	0.95
FM	26.75	40.15	0.0202	46.9	1.19
Pbm	26.67	44.77	0.0399	56.5	0.12

Table 3: The degradation kinetics of protein and effective protein degradability at 3 outflow rates

	Degradation kinetics			Effective degradability (%)	
Ingredient	RSD	WCS	c(h^{-1})	k = 0.02	RSD
WCS	2.17	FM	0.1015	84.3	2.17
FM	0.89	Pbm	0.0191	64.7	0.89
Pbm	4.01	Ingredient	0.0709	54.5	4.01

***In situ* DM disappearance and estimated parameters:** Dry matter disappearance from nylon bags incubated in the rumen increased with increasing incubation time. At 4 h incubation time DM disappearance of WCS was significantly higher than that of the other feedstuffs. The DM disappearance of WCS and PBM was significantly higher than FM. The estimated parameters and effective degradabilities Of DM are given in Table 2.

Dry matter degradation rate (c) of PBM was significantly higher than those of FM and WCS. The quickly degradable DM fraction (a) of WCS was significantly higher than those of FM and PBM. The potentially degradable DM fraction (b) of PBM was significantly higher than the other feedstuffs.

The effective degradability of a feed is a measure of its digestion in the rumen over time, while considering the rate at which it flows from the rumen to the small intestine.

The estimated parameters of degradability of DM in plant-derived protein sources were significantly higher than those of animal origin. The values obtained here for DM disappearance and estimated parameters of degradability; c, a and b of FM, PBM and WCS were consistent with findings by Filya *et al.* (2002), Kamalak *et al.* (2005) and Arieli *et al.* (1989), respectively.

***In situ* CP disappearance and estimated parameters:** Disappearance of crude protein from the bags incubated in the rumen increased with increasing time.

At 4 h incubation time, protein disappearance of WCS protein was significantly higher than the others. Also at 72 h incubation time, crude protein disappearance of WCS was significantly higher than the other protein sources (Table 3). The estimated parameters of WCS, in except of a and c, were significantly higher than the others due to high protein disappearance from nylon bags.

Crude protein degradation rate (c) of PBM was significantly higher than that of WCS and FM but the quickly soluble protein fraction (a) of PBM was significantly less than the other protein sources. Slowly degradable protein fraction (b) of FM was significantly less than that of PBM and WCS.

The effective CP degradabilities were calculated using rumen outflow rate of 2%. The effective CP degradability values decreased with increased outflow rates. the effective degradability of WCS was numerically higher than the other protein sources. The crude protein disappearance and estimated parameters; c, a and b of FM and PBM were consistent with findings of Kamalak *et al.* (2005) and the crude protein disappearance and estimated parameters; c, a and b of WCS were consistent with findings of Arieli *et al.* (1989) and NRC (2001).

The *in situ* crude protein degradation kinetics (a, b and c) of FM obtained in this experiment were different with those shown in NRC (2001). These differences may be due to differences in protein sources, pore size of nylon bag and milling screen size or used animals. The variation between laboratories in the determination of protein degradability was mainly associated with differences between laboratories in the methods used for sample preparation and processing. It was likely also influenced by the poor repeatabilities between laboratories in the determination of crude protein content and by the type of filter and nylon bag material used (Madsen and Hvelpund, 1994).

Large difference in disappearance and digestion kinetics of substrate with *in situ* conditions have been observed (Weakley *et al.*, 1977, 1983; Nocek *et al.*, 1979; Wadwa *et al.*, 1998). It has been shown that nylon bags with small pores decreased the influx of digesting agents into bags and limited efflux of digested residues from bags (Weakley *et al.*, 1983). Furthermore, few differences were observed between cattle and sheep, when comparing *in situ* degradation of DM after 9 and 24 h incubation (Ørskov *et al.*, 1983). The CP degradability value of FM were similar to those obtained by Gorgulu *et al.* (1999). They used fistulated sheep fed alfalfa hay as in this experiment. The effective CP degradability of FM at was comparable to that obtained by Kristensen *et al.* (1985) and Sampath *et al.* (1989). They used cows and nylon bags with a similar pore size (36 μm). On the other hand, the effective CP degradability of FM found in this experiment was considerably higher than that obtained by Martillotti *et al.* (1995) and Ha and Kennelly (1985) possibly due to differences in raw materials and manufacturing process such as the length of time the raw fish are stored before processing, whether or not formaldehyde is added as a preservative, type of dryer used, duration of heating, etc. (Harris and Staples, 1992).

The CP degradabilities of PBM calculated at outflow rate 2 were consistent with findings of Moreira *et al.* (2003) but lower than that observed by Souza *et al.* (2000). All these differences in the results obtained by different authors may be due to manufacturing processes used. Heating is an inherent part of the processing of animal- and plant-derived protein sources. Excessive heat treatment reduced the protein degradability in the rumen of cows (Ljokjel *et al.*, 2000).

Effective CP degradability of animal derived protein was significantly lower than the WCS. Therefore, FM and PBM can be used to increase the bypass protein or to replace the readily degradable protein sources in diet to reduce the losses due to excessive degradation of protein in the rumen.

CONCLUSION

In this experiment, a comparative data set of *in situ* DM and CP degradation parameters for three commercially available animal- and plant-derived protein sources used in sheep nutrition in Northwest of Iran were determined. This experiment clearly showed that there were significant differences between protein sources in terms of DM and CP disappearance and degradability parameters. There was a trend towards a low protein degradability for animal-derived protein sources. Therefore, animal-derived protein sources with a low degradability can be used to increase the bypass protein. The CP degradation parameters obtained in this experiment using sheep would be useful in improving the accuracy of formulation of sheep diet.

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