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Study of Protein Characteristic of Five Feeds by CNCPS Model

T. Ghoorchi and S. Arbabi
Department of Animal Science,
Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: This study presents results on the crude protein and its components containing A, B_1, B_2, B_3, C, NPN, SP, NDIP, ADIP, degradability, effective degradability of alfalfa, soybean meal, cottonseed meal, wheat bran and beet sugar pulp. The experiments included three fistulated rams fed diets having the roughage and concentrate. The dynamics of the ruminal crude protein degradability of the above protein feedstuffs was evaluated at six incubation times: 0, 4, 8, 16, 24 and 48 h. The results of this study detected that amount of NPN were different in feedstuffs ($p<0.05$) and alfalfa had 24.85%. Soybean meal had the most of crude protein, B_1 and B_3, 40.58, 20.53, 59.93% and the less of ADIP or C and B_2, 4.11 and 4.09%, respectively. Effective degradability of dry matter with rate of passage equal 8% were in cottonseed meal 20.5%, soybean meal 30.7%, wheat bran 32.9%, beet sugar pulp 35.7% and alfalfa 31.9%.

Key words: Crude protein, degradability, true protein, unavailable nitrogen

INTRODUCTION

In ruminants, feedstuffs are fermented in the rumen before gastric and intestinal digestion and this fermentation has confounded the prediction of animal performance from dietary ingredients. Over the years, there has been considerable improvement in the feeding of ruminants, but this progress has been based on an empirical approach that has largely treated the rumen as a black box. The experience of other scientific disciplines has shown that a mechanistic understanding is usually needed for sustained development. If ruminant nutrition is to progress past the point at which diets are continually tested in virtually infinite combinations, the details of the fermentation must be considered (Van Soest and Sniffen, 1984).

Feed protein is partitioned into three fractions: Non Protein Nitrogen (NPN), true protein and unavailable nitrogen (Van Soest *et al*., 1981). These have been described as Fractions A (NPN), B (true protein) and C (bound true protein), respectively (Pichard and Van Soest, 1977). True protein is further fractionated into three subfractions (Bl, B2 and B3) based on their inherent rates of ruminal degradation (Van Soest *et al*., 1981). Roe *et al*. (1990) presented a summary of recommended procedures to determine protein fractions. Fractions A and B1 are soluble in buffer (Roe *et al*., 1990) and B1 is determined as the TCA-precipitable fraction (Van Soest *et al*., 1981).

A or NPN is rapidly converted to ammonia in the rumen. In harvested forages Fraction Bl is a small fraction of the total soluble protein (approximately 5%) and concentrates can

Corresponding Author: S. Arbabi, Department of Animal Science,
Gorgan University of Agricultural Sciences and Natural Resources,
Gorgan, Iran Tel: 098-21-20103079 Fax: 098-171-2251703

contain twice as much Fraction B1 as forages do (Krishnamoorthy *et al*., 1982). Most of the soluble protein in fresh pastures is Fraction B1 (Van Soest and Sniffen, 1984). In the CNCPS Fraction B1 is degraded in the zrumen. Unavailable or bound protein, Fraction C, is the protein that is insoluble in the acid detergent (acid detergent insoluble protein, ADIP) fraction (Pichard and Van Soest, 1977). Fraction C contains protein associated with lignin, tannin-protein complexes and Maillard products that are highly resistant to microbial and mammalian enzymes (Krishnamoorthy *et al*., 1982, 1983). The Fraction C content of feedstuffs has been measured by several researchers (Pichard and Van Soest, 1977; Krishnamoorthy *et al*., 1982; Van Soest and Sniffen, 1984).

Fraction B3 is insoluble in neutral detergent but soluble in acid detergent (neutral detergent insoluble protein [NDIPI minus ADIP] Krishnamoorthy *et al*., 1982). Fraction B3 is slowly degraded in the rumen because it is associated with the cell wall (Pichard and Van Soest, 1977; Van Soest *et al*., 1981; Krishnamoorthy *et al*., 1983). The NDIP and ADIP contents of common feedstuffs have been measured. In the CNCPS, a high percentage of Fraction B3 escapes degradation in the rumen. Buffer insoluble protein minus the protein insoluble in neutral detergent is used to estimate Fraction B2. Some Fraction B2 is fermented in the rumen and some escapes to the lower gut. The fate of Fraction B2 depends on the relative rates of digestion and passage. The Cornell Net Carbohydrate and Protein System (CNCPS) has equations that estimate the fermentation and passage of feed carbohydrate and protein fractions (Sniffen *et al*., 1992).

The Cornell Net Carbohydrate and Protein System for Cattle (CNCPS-C) is a diet evaluation and formulation system developed for use in diverse animal, feed and environmental production situations for all classes of beef, dairy and dual-purpose cattle (Fox *et al*., 2004).

The objective of this experiment was to determine the component of CNCPS model containing A, B_1, B_2, B_3, C, NPN, SP, NDIP, ADIP and also DM and protein degradability and effective degradability of alfalfa, soybean meal, cottonseed meal, wheat bran and beet sugar pulp.

MATERIALS AND METHODS

General

Five feed were chosen to provide a broad range of contents. Three samples took off each of alfalfa, soybean meal, cottonseed meal, wheat bran, beet sugar pulp. All samples were harvested from golestan state, Iran. Samples were oven-dried at 60^{0}C and ground through a 1 mm screen (Wiley mill, model 4, Arthur H. Thomas Co., Philadelphia, PA). DM, soluble CP, CP, NPN, $B_{1,}$ B_2, $B_{3,}$ neutral detergent insoluble protein (NDIP), acid detergent insoluble protein (ADIP) and ash were determined for each feed. Crude protein was measured using the macro-Kjeldahl procedure (AOAC, 1990) modified by using boric acid in the distillation process. This information can be used as a basis for predicting ME and protein absorption (Sniffen *et al*., 1992). Protein fractions were determined as described by Licitra *et al*. (1996). The following equations can be used to calculate the five protein fractions contained in the jth feedstuff from the values given in Table 1:

- Pa_j(%CP) = NPN_j (%SOLP). 0.01. $SOLP_j$(%CP)
- $PB1_j$ (%CP) = $SOLP_j$ (%CP)-A_j (%CP)
- PC_j(%CP) = $ADIP_j$ (%CP)
- $PB3_j$ (%CP) = $NDIP_j$ (%CP)-$ADIP_j$ (%CP)
- $PB2_j$ (%CP) = 100- A_j (%CP)-$B1_j$ (%CP)-$B3_j$ (%CP)-C_j (%CP)

Table 1: Chemical composition (% crude protein based) of feedstuffs examined (some components of crude protein)

Feedstuff	Dry matter (%)	Crude protein (%)	Ash (%)	NPN[1](%)	SP[2] (%)	ADIP[3] (%)	NDIP[4] (%)
Alfalfa	90.23	17.43^{c}	10.83	24.85^{a}	15.20^{b}	8.70^{d}	51.22^{f}
Soybean meal	89.05	40.58^{a}	6.79	11.34^{b}	20.53^{a}	4.11^{e}	64.04^{cd}
Cottonseed meal	92.32	35.90^{b}	8.95	6.62^{c}	15.33^{b}	12.29^{c}	65.76^{b}
Wheat bran	90.50	16.20^{d}	7.17	1.83^{d}	5.84^{d}	21.64^{b}	70.70^{a}
Beet sugar pulp	94.67	9.38^{e}	5.62	8.02^{e}	12.87^{c}	16.37^{a}	62.75^{de}

[1]Non protein nitrogen; [2]Soluble protein; [3]Acid detergent insoluble protein; [4]Neutral detergent insoluble protein; Bars with unlike letters differ ($p<0.05$)

where, CP_j(%DM) = percentage of crude protein of the jth feedstuff; NPN_j(%CP) = percentage of crude protein of the jth feedstuff that is non-protein nitrogen x 0.25; $SOLP_j$(%CP) = percentage of the crude protein of the jth feedstuff that is soluble protein; $NDIP_j$(%DM) = percentage of the jth feedstuff that is neutral detergent insoluble protein; $ADIP_j$(%DM) = percentage of the jth feedstuff that is acid detergent insoluble protein; PA_j(%CP) = percentage of crude protein in the jth feedstuff that is non-protein nitrogen; $PB1_j$(%CP) = percentage of crude protein in the jth feedstuff that is rapidly degraded protein; $PB2_j$ (%CP) = percentage of crude protein in the jth that is intermediately degraded protein; $PB3_j$(%CP) = percentage of crude protein in the jth feedstuff that is slowly degraded protein and PC_j(%CP) = percentage of crude protein in the jth feedstuff that is bound protein.

The experiments included three fistulated rams. The rams belonged to the improved zel breed. The animals were of similar weight of about 41 kg. Permanent plastic fistulas of 40-50 μm in diameter were surgically inserted into the rumen of the rams. The rams were placed in individual cages which enabled individual feeding and watering and complete control of the test animals. The animals were fed three times a day, at 8 am, 14 pm and 21 pm fistulated sheep fed a diet for supply maintenance requirements (NRC, 1985), that was alfalfa hay/grass hay, cottonseed meal; dry rolled barely, wheat bran, wheat straw. To evaluate the dynamics of the ruminal crude protein degradability of the above protein feedstuffs, six incubation times were used: 0, 4, 8, 16, 24 and 48 h. The nylon bags used in the experiment were 10×21 cm in size. They were numbered for identification. All feedstuffs were treated equally, i.e., ground to a meal through a 2.5 mm screen in a laboratory mill. Subsequently, the bags were weighed on an analytical balance first empty and then filled with the feedstuff samples.

After specific periods of incubation, the bags tied to the plastic fistulas were removed from the rumen and immediately immersed in cold water to preven further fermentation and remove feedstuff remains. Then, they were washed for 20 min. Following the washing process, the bags were separated from the plastic fistulas and dried in a dryer at a temperature of 60 to 65°C for 48 h, i.e., until a constant weight was achieved. The bags were then removed from the dryer and weighed using an analytical balance. Dried bags were weighed and DM disappearance was determined. The content of nitrogen was determined by the Kjeldahl micro method. This research project was accomplished in 2007 at the Department of Animal Science Gorgan University of Agriculture Sciences and Natural Resources.

Statistics

The chemical composition of forages was analyzed as a completely randomized design by the general linear models procedure of SAS (1999). Means were separated by Duncan test at 0.05 probability level (Duncan, 1955). The kinetics of ruminal DM disappearance *in situ* was estimated by the Naway software.

RESULTS

Chemical Composition

Soybean meal had highest crude protein (40.58%) and soluble protein (20.53%) and lowest ADIP (4.11%) comparison with others, significantly ($p<0.05$). Amount NPN of alfalfa was higher than the other and NPN of Beet sugar pulp was lower than ($p<0.05$), 24.85% and 8.02%, respectively. However, alfalfa had the lowest NDIP (51.22%) among the other examined feeds ($p<0.05$) (Table 1).

Alfalfa, soybean meal and wheat bran had highest percentage fraction A (24.85%), B_1 (20.53%) and B_2 (21.63%), respectively ($p<0.05$). Amount of fraction of B_3 in soybean meal was higher (59.93%) and wheat bran had highest of C (21.64%) ($p<0.05$) (Table 2).

In situ Ruminal DM Digestibility

Effective degradability of dry matter with rate of passage equal 8% were in cottonseed meal 20.5%, soybean meal 30.7%, wheat bran 32.9%, beet sugar pulp 35.7% and alfalfa 31.9% (Table 3). Figure 1 has shown disappearance dry matter of experiment feedstuffs.

Table 2: Percentage of different components protein of feedstuffs examined

Feedstuff	A (%)	B_1 (%)	B_2 (%)	B_3 (%)	C (%)
Alfalfa	24.85^a	15.20^b	8.73^d	42.52^e	8.70^d
Soybean meal	11.34^b	20.53^a	4.09^e	59.93^a	4.11^e
Cottonseed meal	6.62^d	15.33^b	12.29^c	53.47^b	12.29^c
Wheat bran	1.83^e	5.84^d	21.63^a	49.06^c	21.64^a
Beet sugar pulp	8.02^c	12.87^c	16.37^b	46.38^d	16.37^b

Bars with unlike letters differ ($p<0.05$)

Table 3: Degradability, effective degradability and protein components of feedstuffs examined

Feedstuff	a[1]	b[2]	c[3]	ERDP[4] (%2 h^{-1})	ERDP (%5 h^{-1})	ERDP (%8 h^{-1})	RSD
Alfalfa	18.4	26.2	0.085	39.6	34.9	31.9	2.67
Soybean meal	16.2	26.1	0.099	37.9	33.6	30.7	3.90
Cottonseed meal	11.0	9.80	2.650	20.7	20.6	20.5	4.12
Wheat bran	19.1	19.7	0.191	36.9	34.7	32.9	1.82
Beet sugar pulp	22.3	28.3	0.072	44.4	39.0	35.7	2.20

a: Water soluble protein; b: Degradable protein in the rumen by microorganisms; c: Relative rate of degradation in per hour; ERDP: Effective rumen degradable protein

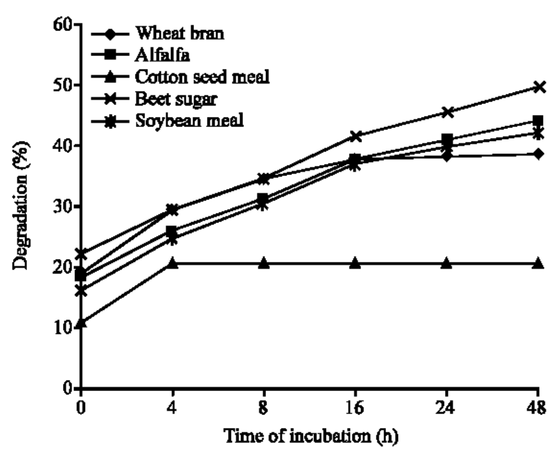

Fig. 1: Degradability dry matter of five examined feeds in the sheep rumen

DISCUSSION

Chemical Composition

Soybean meal had highest crude protein (40.58%) and soluble protein (20.53%) and lowest ADIP (4.11%) comparison with others, significantly ($p<0.05$). The CP content of Soybean meal, Cottonseed meal, Alfalfa, Wheat bran and Beet sugar pulp (40.58, 35.90, 17.43, 16.20 and 9.38%, respectively) obtained in this study were a little lower than those reported by NRC (2001).

Non protein N (ammonia, peptides and amino acids) is rapidly converted to ammonia in the rumen. Essentially all the soluble protein in silages and cut forages is in the form of NPN (Pichard and Van Soest, 1977). In this study, amount NPN of alfalfa was higher than the other and had the lowest NDIP ($p<0.05$). NPN of Beet sugar pulp was lower than other examined feedstuffs ($p<0.05$). Fraction B is subdivided to estimate rates of ruminal degradation. Fraction B1 is rapidly degraded in the rumen (Van Soest *et al*., 1981). In this experiment soybean meal had highest B_1. Type of processing effect on degradation protein supplements (NRC, 2001).

Fraction C cannot be degraded by ruminal bacteria and does not provide amino acids postruminally (Krishnamoorthy *et al*., 1982). Among the experiment feeds, wheat bran had highest of fraction C. Numerous factors affect the amount of CP in feeds that will be degraded in the rumen. The chemistry of feed CP is the single most important factor. The two most important considerations of feed CP chemistry are: (1) the proportional concentrations of NPN and the true protein and (2) the physical and chemical characteristics of the proteins that comprise the true protein fraction of the feedstuff (NRC, 2001).

Protein supplements contain a small amount of Fraction B3, but forages, fermented grains and byproduct feeds contain significant amounts of Fraction B3 (Krishnamoorthy *et al*., 1982). In the CNCPS, a high percentage of Fraction B3 escapes degradation in the rumen. In present research amount of fraction B_3 in soybean meal was highest and in alfalfa was lowest. Some Fraction B2 is fermented in the rumen and some escapes to the lower gut. Wheat bran had highest B2. Differences in 3-dimensional structure and chemical bonding (i.e., cross-links) that occur both within and between protein molecules and between proteins and carbohydrates are functions of source as well as processing. These aspect of structure affect microbial access to the proteins, which apparently is the most important factor affecting the rate and extent of degradation of proteins in the rumen (NRC, 2001). However, these information can be used as a basis for predicting ME and protein absorption (Sniffen *et al*., 1992).

In situ Ruminal DM Digestibility

A number of factors, working independently and in concert, depress fiber digestion in the rumen. Results of some studies suggest that addition of readily fermentable carbohydrates to a forage diet can, per se, cause a depression in fiber digestion. The ruminal ammonia concentration required for optimum fiber or DM digestion may be higher than that required for optimum microbial growth is supported by a summary of studies in which these values are reported (Hoover, 1986). Similarly, although it is known that most cellulolytic microbes require isoacids or other short-chain fatty acids for growth, responses to additions of these compounds to the diet have been variable regarding fiber digestion. The efficacy of isoacid additions to the diet appears to be somewhat dependent on diet composition, such as the amount and ruminal degradability of protein and on the rate of passage of the ingesta through the rumen. Other plant components, such as tannins and phenylpropanoids, may influence lag time or rate of fiber digestion (Hoover, 1986).

High levels and rates of degradability of CP and DM in soybean meal could be due to the structure and solubility characteristics of protien in soybean meal which could be easily attached by microorganisms in the rumen (Mahadevan *et al*., 1980).

It is clear from a consideration of the rumen bag technique that the absolute value of the results depends on the way in which the sample is prepared and the fineness of the material from which the bag is made. These fine particles would be rapidly fermented or else washed out of the nylon bag unfermented (Orskov, 1992).

Cottonseed meal has relatively low rumen degradability and is therefore a good source of bypass protein (Gohl, 1998). The formation of tannin-protein complexes in cottonseed meal could result in higher by-pass protein reaching the lower gut (Wanapat *et al*., 2000).

Effective DM degradability of cottonseed meal was lower than the other feeds (Table 3). This could be due to cottonseed meal containing high level of undegradable protein as it contains quite a high CP content. As reported by Wanapat *et al*. (2000) that the formation of tannin-protein complex of cottonseed meal could result in higher rumen by-pass protein in the lower gut. Effective DM degradability of beet sugar pulp (for all of rates of passage [h.sup.-1]) used in the present study was higher than the other feeds, that probably result of higher content of water soluble carbohydrate (NRC, 2001).

There are at least three fractions that nutrient groups or classes (proteins, carbohydrates) of feeds can be delineated with regard to ruminal availability (i.e., soluble, degradable and nondegradable). These are commonly referred to as the a, b and c fractions, respectively (Pichard and Van Soest, 1977). The in situ procedure can be used to quantify these different factions as well as the rate of digestion of B. However, the technique cannot be used to quantitate the rate at which A is degraded. Several researcher (Nocek, 1985, 1987) have demonstrated that a certain portion of the test feed escapes the bag prior to ruminal incubation. This varies by feed, bag porosity and sample particle size (Nocek, 1987). Reports demonstrated soluble N to be highly correlated with short-term (2 h) ruminal incubation (Stern and Satter, 1984). This fraction is generally assumed to be readily available to rumen microbes and digested at a rapid rate. Potentially degradable nutrient fractions have been described by first-order kinetic rate constants (Van soest *et al*., 1981). Primary assumptions are that the pools in question are homogeneous and that the substrate remaining will be degraded as a linear (ln transformed) function of time in the rumen. These basic assumptions are violated when the soluble and undigestible pools are not determined and subtracted from the fermentation sequence or when a heterogeneous mixture of degradable substrate (as in the case of many feeds) is simultaneously degraded at different rates. Correction for these fractions in the digestion sequence can significantly alter the rate of insoluble potentially digestible material (Nocek and English, 1986).

CONCLUSION

The CNCPS has been used as a farm management tool to optimize use of farm-specific feeds, decrease the need for purchased supplements, optimize herd size, predict the manure nutrients that will have to be managed and improve the annual return over feed cost (Tylutki *et al*., 2004; Fox *et al*., 2004). In terms of the dry matter degradability of the test feedstuffs, the highest degradability was exhibited by beet sugar pulp, at all incubation times. Lower degradability of dry matter was estimated in cotton seed meal, which showed similar degradability at shorter incubation times.

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