Original Research Article

Protein Fractions of Some Plant and Animal Protein Sources Using CNCPS Method

ABSTRACT

Aims: This experiment was conducted with the aim of investigating fractions of protein of some plant and animal protein sources using the CNCPS method.
Study design: After preparing the desired feed items, the protein fractions of feed samples were determined.
Methodology: This experiment was conducted on sources of plant protein (soybean meal, rapeseed meal, and cottonseed meal) and sources of animal protein (poultry offal meal, fish meal and blood meal).The protein fractions of feed samples were determined by Licitra et al, 1996. These fractions were included non-protein nitrogen, true protein, soluble true protein, insoluble protein, soluble protein in neutral detergent, insoluble protein in neutral detergent but soluble in acid detergent and insoluble in acid detergent and attached.

Results: After testing, the amount of non-protein nitrogen (fraction A) in soybean meal, cottonseed meal and rapeseed meal was 8.52, 6.33 and 4.55 %, and in poultry offal meal, fish meal and blood meal in slaughterhouses were 10.38, 13.63 and 16.08% of crude protein, the amount of B1 in soybean meal, cottonseed meal and rapeseed meal was 2.30, 3.32 and 13.68 % respectively, and in poultry offal meal, fish meal and blood meal, in slaughterhouses were 3.45, 7.44 and 7.16 % of crude protein respectively , the amount of B2 in soybean meal, cottonseed meal and rapeseed meal was 80.49, 77.50 and 68.40%, in poultry offal meal, fish meal and blood meal, respectively, 66.36, 55.03 and 61.66 % respectively, the amount of B3 for soybean meal, cottonseed meal and rapeseed meal was 6.24, 2.63 and 9.11 %, respectively, and poultry offal meal, fish meal and blood meal in slaughterhouses were 7.50, 6.74 and 11.91 % of crude protein respectively , the protein C portion assumed in the rumen's indissoluble CNCPS system in soybean meal, cottonseed meal, and rapeseed meal was 2.45, 9.92, and 4.77 %, respectively, in poultry offal meal, fish meal and blood meal, respectively, 12.21 , 17.16 and 3.18 % of crude protein
Conclusion: The results indicate that the use of CNCPS and NRC data for portion fractions of different feeds cannot be considered absolutely, and domestic research and results should be used to extract samples from different regions and different growth conditions, so that the dietary regimens With these foods, it's real and more balanced and with less waste of nutrients.

Keywords: CNCPS, Fractions protein, Protein sources.

1. INTRODUCTION

The CNCPS (Cornell Net Carbohydrate and Protein System) system is a semi-mechanical approach that evaluates the rate of degradation of feed in the rumen, passes through the rumen's undigested material, as well as the amounts of energy and protein metabolism used

for ruminant tract [2]. The protein fractions in the CNCPS system is A, B1, B2, B3, and C. Non-protein nitrogen (fraction A) is a part of the crude protein that is dissolved quickly (zero time) in the rumen fluid, and its decomposition rate is assumed to be unlimited. Fraction A is chemically part of the crude protein that is dissolved in borate-phosphate buffer [1]. The degradation rate in the rumen is rapid and directly into the rumen ammonia tank [3]. B1 is the percentage of total crude protein dissolved in borate phosphate buffer and precipitated with Trichloroacetic acid (TCA; TCAA; also known as trichloroethanoic acid), and its rate of degradation in the rumen is rapid, the rate of degradation of this part in the rumen is about 200-300 percent, and its degradation in the intestine 100 percent. Fraction B2 is a protein that partly breaks down into the rumen and is calculated as the difference in the total amount of protein A, B1, B3 and C from the crude protein. The fate of this sector is rapidly degradation and the speed of passing through the rumen depends. The digestion of the amino acids of B1 and B2 is 100% and 80% of the B3 sectors. The protein part B3 contains prolamins and fermented proteins, which are found to be extremely low in most of the feed, especially protein. Part C is a non-soluble protein in acidic detergent that is assumed to be in the Cornell net carbohydrate and protein system in the rumen and has a direct correlation with the thermal damage of protein and indigestible protein [4]. In CNCPS, it is assumed that protein C does not decompose in the rumen, this part has a direct relation to heat damage [5]. Therefore, the proper and controlled temperature is very important during thermal processes. Increasing cell biodegradability results in the release of proteins bound to the cell wall and decreases the protein C fraction [6]. In CNCPS, the reduction of A and B1 and the increase of protein B2 and B3 are associated with a decrease in protein degradation in the rumen and an increase in RUP [3], which, in the absence of a negative effect on RUP degradation the intestine can have a positive effect on the production and reproductive performance of lactating cows [7].

This experiment was conducted with the aim of investigating fractions of protein of some plant and animal protein sources using the CNCPS method.

2. MATERIALS AND METHODS

This experiment was conducted at the animal science laboratory of Mohaghegh Ardebili University in Iran. This experiment was conducted on sources of plant protein (Soybean meal, rapeseed meal, and cottonseed meal) and sources of animal protein (Poultry offal meal, fish meal and blood meal). The studied samples were obtained from feed compound manufacturers, the agricultural sector and the local slaughter house of North West Iran Ardebil province (Meshgin, Germi and Ardabil), over the years 2014 and 2016. The prepared samples from local factories, for preventing degradation and degreasing, were used carrier materials or moisture adsorbent such as wheat bran. Therefore, some of its analyzes did not match to world feed standard analysis and their cell wall values were higher. the samples were randomly selected for the survey. Then, two local associations were randomly selected from each of the famous regions. A systematic sampling was done in each of the selected associations until total fifteen farmers or agricultural sector were selected for the study, which brings the number of farmers selected to thirty in every region. the chemical composition of the feed by conventional methods of AOAC.

2.1 Determine different fractions of nitrogen

The protein fractions of feed samples by [1] as follows were determined as presented in Table 1.

Table 1: Protein fraction of feed content

Fraction	Grouping*	Style abbreviation	Enzymatic decomposition	Method of estimation and definition

Non-protein nitrogen	A	NPN	impractical	Insoluble and dissolved
True protein	-	TP	-	It is precipitated with trichloroacetic acid
Soluble true protein	B1	BSP	rapid	Buffer solution, but insoluble
Insoluble protein	-	IP	-	Insoluble in buffer
Soluble protein in neutral detergent	B2	IP-NDIP	Variable	The difference between the insoluble in the buffer and the insoluble in the neutral detergent
Insoluble Protein in neutral detergent but soluble in acid detergent	B3	NDIP-ADIP	Variable and Slow	Insoluble in neutral detergent but soluble in acid detergent
Insoluble in acid detergent and attached	C	ADIP or ADIN	Indigestible	Protein has a thermal damage seen and attached to lignin

** Based on Van Soest [8]*

2.2 NPN or A (Non-Protein Nitrogen):

Initially, 0.5 g of the desired dry feed sample was weighed and spilled into the 125 ml Erlenmeyer. Then, 50 ml of distilled water, and then, 8 ml of sodium tungstate 10% solution was added and placed until the Erlenmeyer remained at 20-25 ℃ for 30 minutes. After this period of pH checked, add 10 ml of sulfuric acid (0.5 M) until to reach pH=2 and was restarted until the Erlenmeyer remains at room temperature overnight. Pull the filter paper and place it in a cone funnel. First, the paper was wetted, then the filter was done and then left, washed twice with distilled water, and the paper was transferred to the Kjeldahl flask and the remaining nitrogen was estimated to deduct the remaining nitrogen from the total nitrogen feed and NPN fraction calculated.

2.3 BSN (Nitrogen Soluble in Buffer):

Initially, 0.5 g of the desired dry feed sample was weighed and poured into an Erlenmeyer 125 ml and then 50 ml of borate-phosphate buffer and then, 1 ml of sodium Azide solution was added to it and laid to Erlenmeyer stays at room temperature for 3 hours. After this time, the filter paper was passed and the filter was done and the remainder was washed with 250 ml of distilled water and Nitrogen was estimated in the remainder of the Kjeldahl, which is the same protein as the insoluble, and the soluble protein can also be distinguished by difference The total crude protein was calculated and the real protein with NPN fraction (A) was obtained from BSN.

B1 or TP = BSN – A

2.3 ADIN or C (Insoluble Nitrogen in Acid Detergent):

An acid insoluble fiber method was used to dissolve the acidic solution in the ANKOM method [9] using filter paper 541. The nitrogen present in the sediment is characterized by ADIN, using the Kjeldahl method.

2.4 NDIN (Insoluble Nitrogen in Neutral Detergent):

Using the method of determining the insoluble fiber in neutral detergent in the incubator [9], the amount of nitrogen present in the precipitate was determined on the filter paper as insoluble nitrogen in neutral detergent by the Kjeldahl method.

A = NPN

B1 = BSN - NPN
B2 = CP - (A+B1+B3+C)
B3 = NDIN – ADIN
C = ADIN

2.5 Methods of Data Analysis and Statistical Model

Comparing the average of the least significant difference (LS MEAN) was performed. Other data in a completely randomized design with 3 repeats and 3 treatments were evaluated and comparison of means using Duncan test when P≤0.05. Statistical model research design is as:

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where: Y_{ij} is the observation, μ is the population mean, A_i is the effects of experimental treatments and e_{ij} is the residual error.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition

The chemical compositions of test feed are presented in Table 2. Blood meal content had higher percentage of protein than any of the other plant and animal protein. The maximum amount of crude fat 31.3% for poultry offal meal (POM) and the highest ash content of 20% were observed for fish meal (FM). Highest of NDF and ADF (70.6% and 58.4%) for cottonseed meal (CM) and the lowest NDF and ADF were obtained 45.7 and 33.3% for soybean meal (SM), respectively.

Table 2. Chemical composition of some plant and animal protein sources

Protein sources	DM	CP	EE	Ash	NDF	ADF
Plant						
Soybean meal	92.4	50	1.6	6.1	45.7	33.3
Rapeseed meal	91.4	37	1.2	8	51.5	46.1
Cottonseed meal	93	24	1.4	4.7	70.6	58.4
Animal						
Poultry offal meal	94.4	55	31.3	7.3	48.9	34.8
Fish meal	93.6	50	18.1	20	61.2	40.6
Blood meal	70.6	59	1.6	5	55.3	33.4

**DM = dry matter (percent), CP = crude protein (%DM), EE= crude fat (%DM), Ash = ash (%DM) NDF = Neutral detergent fiber (%), ADF= Acid detergent fiber (%)*

3.2 Protein Fractions

The results of various nitrogen fractions based on the CNCPS method in various protein sources are shown in Table 3. The amount of non-protein nitrogen (part A) in soybean meal, cottonseed meal and rapeseed meal was 8.52, 6.33 and 4.05 %, and in poultry offal meal, fish meal and blood meal in slaughterhouses were 10.38, 13.63 and 16.08 % of crude protein (Table 3). The highest amount of the A part was related to blood meal and the lowest was related to rapeseed meal ($p < 0.05$), which was different from the reported amounts by [10], probably due to the method used for measuring non-protein nitrogen, location Crop cultivation, harvesting method, drying and storage of feed, as well as the type of protein precipitators in different experiments. Therefore, when using blood meal in order to regulate the diet due to its high non-protein nitrogen, sufficient amount of energy should be provided to facilitate the synthesis of microbial protein and to use the NPN fraction well. The measured values of the true soluble protein (Fraction B1) in soybean meal, cottonseed meal and rapeseed meal were 2.30, 3.32 and 13.68 % respectively, and in poultry offal meal, fish meal and blood meal in slaughterhouses were 3.45, 7.44 and 7.16 %of crude protein. There was not a significant difference between the mean of fish meal and blood meal ($p > 0.05$). This part was the least in soybean meal and the highest in rapeseed meal ($p < 0.05$). The results of this study were not consistent with the values reported by other researchers [11] and [12], which probably are part of the difference between various reports related to the use of different buffers [13]. The protein with a medium degradation function in the rumen (Fraction B2) is, in fact, a nitrogen-free solution in neutral detergent, part of which is broken down into the rumen and part of the intestine, passing through this part of the rumen to the relative rate of digestion and the passage of dependence has it. The amount of B2 in soybean meal, cottonseed meal, and rapeseed meal was 80.49, 77.50 and 68.40%, respectively, and in poultry offal meal, fish meal and blood meal samples were 66.36, 55.03 and 61.66 %, respectively, in crude protein. In fact, the highest proportion of B2 was related to soybean meal and the lowest was fish meal ($p < 0.05$) [11]. The amount of fast digestible protein in the rumen was 40 % for cottonseed meal and 72.7 % for soybean meal, but this parameter in another study [12] reported for cottonseed meal 12.29 and soybean meal 4.09 % of crude protein. Because this fraction is calculated from the discrepancy, so all the measurement errors in this fraction are gathered, which is probably one of the reasons for the difference between the amounts reported by various researchers. The heating of feedstuffs destroys B2 proteins and makes them insoluble, in which case fractions B3 and C increase [14]. Low protein digestibility in the rumen (fraction B3) for soybean meal, cottonseed meal and rapeseed meal was 6.24, 2.63 and 9.11 % respectively, and for poultry offal meal, fish meal and blood meal in slaughterhouses were 7.50, 6.74 and 11.91 % of the crude protein. In the present study, the highest level of B3 was estimated for blood meal, which was higher than other protein sources in the table 3 ($p < 0.05$). In [11], the amount of protein with ruminal degradation was 10% for cottonseed meal and 0.8% for soybean meal, and [15], for soybean meal, 1% of crude protein as fraction B3 reported. The fraction B3 Protein is very low in most feedstuffs, especially plant proteins. These proteins are bound to the cell wall and are insoluble in neutral detergent. Fraction of C protein in the rumen is assumed that in the system CNCPS is non-biodegradable and these were in soybean meal , cottonseed meal and rapeseed meal 2.45, 9.92 and 4.77%, respectively, and in poultry offal meal, fish meal and blood meal, respectively, 12.21, 17.16 and 3.18 % of crude protein. The highest part of C was related to crude protein of fish meal and the lowest was related to raw protein content of soybean meal ($p < 0.05$). MirzaiiAlamoti *et al* [11] reported the amount of crude protein C for cottonseed meal 12.7 % and for soybean meal 5 %, while Ghoorchi *et al* [12] is presented for cottonseed meal 12.29 % and for soybean meal, 11.4 % of crude protein. Fraction C has a very strong relationship with indigestible nitrogen in rumen of feed, and therefore the proper and controlled temperature during thermal processes is very important.

Table 3. protein fractions of some protein sources based on the CNCPS (based on dry matter percent)

Chemical composition	Meal type			Powder type			SEM	p-value
	Plant protein source			Animal protein source				
	Soybean	Rapeseed	Cottonseed	Poultry offal	Fish	Blood		
Crude protein	50.09^{c}	23.84^{e}	36.98^{d}	55.00^{b}	50.09^{c}	59.06^{a}	0.22	<0.001
A	8.52^{cd}	6.63^{d}	4.05^{e}	10.38^{c}	13.63^{b}	16.08^{a}	0.76	<0.001
B1	2.30^{c}	3.32^{c}	13.67^{a}	3.54^{c}	7.44^{b}	7.16^{b}	0.91	<0.001
B2	80.49^{a}	77.50^{a}	68.40^{b}	66.36^{bc}	55.03^{d}	61.66^{c}	1.83	<0.001
B3	6.24^{bc}	2.63^{c}	9.11^{ab}	7.50^{b}	6.74^{b}	11.91^{a}	1.32	0.008
C	2.45^{c}	9.92^{b}	4.77^{c}	12.22^{b}	17.16^{a}	3.19^{c}	0.16	<0.001

The different letters in each row represent the difference between the averages. A: Non-Protein Nitrogen, B1: The true protein fast parsing in the rumen, B2: The true protein is parsing, B3: The true protein is decomposed, C: Inaccessible protein

4. CONCLUSION

The results show that there is a difference between the average concentration of insoluble fiber in neutral detergent, crude protein, soluble protein, non-protein nitrogen, insoluble protein in neutral detergent and insoluble protein in the acidic detergent of feed samples. Therefore, the use of CNCPS and NRC data for portion fractions of various feeds cannot be considered absolutely, and domestic research and results should be used to extract samples from different regions and different growth conditions, so that diets adjusted with these feeds, Realistic and more balanced and with less waste of nutrients.

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