Original Research Article

Effect of ZnO Nanoparticles on Protein Fractions of Some Plant and Animal Protein Sources Using CNCPS Method

ABSTRACT

Aims: This experiment was conducted with the aim of investigating effect of zno nanoparticles on fractions protein of some plant and animal protein sources using cncps method.

Study design: After preparing the desired food items, the protein fractions of feed samples were determined by Licitra et al., 1996.

Methodology: 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 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

Results: 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.

12 13

Keywords: CNCPS, Fractions protein, nZnO, Protein sources.

14 15

1. INTRODUCTION

16 17

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 **Comment [Aa1]:** These are not results, results are your observations. These sound like conclusions

11

1

25 degradation rate in the rumen is rapid and directly into the rumen ammonia tank [3]. B1 is 26 the percentage of total crude protein dissolved in borate phosphate buffer and precipitated 27 with Trichloroacetic acid (TCA; TCAA; also known as trichloroethanoic acid), and its rate of 28 degradation in the rumen is rapid, the rate of degradation of this part in the rumen is about 29 200-300 percent, and its degradation in the intestine 100 percent. Fraction B2 is a protein 30 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 31 32 degradation and the speed of passing through the rumen depends. The digestion of the 33 amino acids of B1 and B2 is 100% and 80% of the B3 sectors. The protein part B3 contains 34 prolamins and fermented proteins, which are found to be extremely low in most of the feed, 35 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 36 37 with the thermal damage of protein and indigestible protein [4]. In CNCPS, it is assumed that 38 protein C does not decompose in the rumen, this part has a direct relation to heat damage [5]. Therefore, proper and controlled temperature is very important during thermal 39 processes. Increasing cell biodegradability results in the release of proteins bound to the cell 40 41 wall and decreases the protein C fraction [6]. In CNCPS, the reduction of A and B1 and the 42 increase of protein B2 and B3 are associated with a decrease in protein degradation in the 43 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 44 45 performance of lactating cows [7].

| Fraction | Grouping* | Style abbreviation | Enzymatic decomposition | Method of estimation and definition |
|--|------------|--------------------|-------------------------|--|
| Non-protein nitrogen | А | 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 | \bigcirc | IP | - | Insoluble in buffer |
| Soluble protein in neutral detergent | B2 | IP-NDIP | Variable | the insoluble in the buffer and the insoluble in the neutral detergent |
| Insoluble Protein in neutral detergent but soluble in acid | B3 | NDIP-ADIP | Variable and Slow | Insoluble in neutral detergent but soluble in |
| detergent | | | 0.011 | acid detergent |
| Insoluble in acid detergent and attached | С | ADIP or ADIN | Indigestible | Protein has a thermal damage seen and attached to lignin |

Table 1: Protein fraction of feed content

46

* Based on Van Soest [8]

| 47 48 | This experiment was conducted with the aim of investigating effect of ZnO nanoparticles on fractions protein of some plant and animal protein sources using CNCPS method. | |
|----------------------|--|--|
| 49 50 51 | 2. MATERIALS AND METHODS | |
| 51 52 53 54 | 2.1 Determine Different Parts of Nitrogen The protein fractions of feed samples by [1] as follows were determined. | |
| 55 56 57 58 | 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 °C for 30 minutes. After this | |
| 59 | period of pH, add 10 ml of sulfuric acid to a pH of 0.5 to 2 M and was restarted until the | Comment [Aa2]: ?? |
| 60 61 | Erienmeyer remains at room temperature overnight. Puil 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 | Comment [Aa3]: ?? |
| 62 | with distilled water, and the paper was transferred to the Kjeldahl flask and the remaining | Comment [Aa4]: ? |
| 63 64 65 | nitrogen was estimated to deduct the remaining nitrogen from the total nitrogen feed and NPN fraction calculated. | |
| 66 | 2.3 BSN (Nitrogen Soluble in Buffer): | Comment [Aa5]: improve |
| 67 | Initially, 0.5 g of the desired dry feed sample was weighed and poured into an Erlenmeyer | |
| 68 60 | 125 ml and then 50 ml of borate phosphate buffer and then, 1 ml of sodium azide solution | |
| 69 70 | the filter paper was passed and the filter was done and the remainder was washed with 250 | |
| 71 | ml of distilled water and Nitrogen was estimated in the remainder of the Kjeldahl, which is | |
| 72 | the same protein as the insoluble, and the soluble protein can also be distinguished by | |
| 73 | difference The total crude protein was calculated and the real protein with NPN fraction (A) | |
| 74 75 | Was obtained from BSN. B1 or TP - BSN - A | |
| 76 | | |
| 77 | 2.3 ADIN or C (Insoluble Nitrogen in Acid Detergent): | Comment [Aa6]: improve |
| 78 | An acid insoluble fiber method was used to dissolve acidic solution in ANKOM method [9] | |
| 79 80 | using filter paper 541. The nitrogen present in the sediment is characterized by ADIN, using the Kjeldahl method. | |
| 81 | 2.4 NDIN (Insoluble Nitrogon in Neutral Detergent): | Commont [Ap7] |
| o∠ 83 | Using the method of determining the insoluble fiber in neutral detergent in the incubator [9] | Comment [Aa7]: improve |
| 84 85 | the amount of nitrogen present in the precipitate was determined on the filter paper as insoluble nitrogen in neutral detergent by the Kjeldahl method. | |
| 86 | | |
| 87 | A = NPN | |
| 00 89 | $B_{2} = CP \cdot (A + B_{1} + B_{3} + C)$ | |
| 90 | B3 = NDIN – ADIN | |
| 91 | | Comment [Aa8]: what happened with the ZnO? |
| 92 | | Comment [Aa9]: DM, EE, ASH, FDN & FDA? |
| 93 | 3. RESULTS AND DISCUSSION | How many times you repeated the procediment for sample |
| 94 95 | 3.1 Chemical Composition | Statistical analysis |
| 96 | F | |
| 97 98 | 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 | |

| 99 | amount of crude fat 31.3% for poultry offal meal (POM) and highest ash content of 20% was |
|-----|---|
| 100 | observed for fish meal (EM). Highest of NDE and ADE (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.

103

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

| Protein sources | DM | СР | EE | Ash | NDF | ADF | |
|--------------------|------|----|------|-----|------|------|--|
| Plant | | | | | 4 | | |
| 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 | | | 4 | | | | |
| 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 (%)

104 3.2 Protein Fractions

105

106 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, 107 cottonseed meal and rapeseed meal was 8.52, 6.33 and 4.05 %, and in poultry offal meal, 108 109 fish meal and blood meal in slaughterhouses were 10.38, 13.63 and 16.08 % of crude 110 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 111 [10], probably due to the method used for measuring non-protein nitrogen, location Crop 112 113 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 114 the diet due to its high non-protein nitrogen, sufficient amount of energy should be provided 115 to facilitate the synthesis of microbial protein and to use the NPN fraction well. The 116 117 measured values of the true soluble protein (Fraction B1) in soybean meal, cottonseed meal 118 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 119 120 was a significant difference between the mean of fish meal and blood meal. This part was 121 the least in soybean meal and the highest in rapeseed meal (p < 0.05). The results of this 122 study were not consistent with the values reported by other researchers [11] and [12], which 123 probably are part of the difference between various reports related to the use of different 124 buffers [13]. The protein with a medium degradation function in the rumen (Fraction B2) is in 125 fact a nitrogen-free solution in neutral detergent, part of which is broken down into the rumen 126 and part of the intestine, passing through this part of the rumen to the relative rate of

Comment [Aa10]: You should review, these results are different to the show them in the table

127 digestion and the passage of dependence has it. The amount of B2 in soybean meal, 128 cottonseed meal, and rapeseed meal was 80.49, 77.50 and 68.40%, respectively, and in 129 poultry offal meal, fish meal and blood meal samples were 66.36, 55.03 and 61.66 %, 130 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 131 132 rumen was 40 % for cottonseed meal and 72.7 % for soybean meal and 72.7 %, [12] for cottonseed meal 12.29 and soybean meal 4.09 % of crude protein reported. Because this 133 fraction is calculated from the discrepancy, so all the measurement errors in this fraction are 134 135 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 136 them insoluble, in which case fractions B3 and C increase [14]. Low protein digestibility in 137 138 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 139 140 slaughterhouses were 7.50, 6.74 and 11.91 % of crude protein. In the present study, the 141 highest level of B3 was estimated for blood meal, which was higher than other protein 142 sources in the table (p <0.05). In [11], the amount of protein with ruminal degradation was 143 10% for cottonseed meal and 0.8% for soybean meal, and [15], for soybean meal, 1% of 144 crude protein as Fraction B3 reported. Part B3 Protein is very low in most feedstuffs, especially plant proteins. These proteins are bound to the cell wall and are insoluble in 145 146 neutral detergent. The protein C portion assumed in the rumen's indeterminable CNCPS 147 system in soybean meal, cottonseed meal and rapeseed meal was 2.45, 9.92 and 4.77%, 148 respectively, 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 149 150 the lowest was related to raw protein content of soybean meal (p <0.05). [11] reported the 151 amount of crude protein C for cottonseed meal 12.7 % and for soybean meal 5 %, while in 152 [12] for cottonseed meal 12.29 % and for soybean meal, 11.4 % of crude protein is 153 presented. Fraction C has a very strong relationship with indigestible nitrogen in rumen of 154 feed, and therefore the proper and controlled temperature during thermal processes is very 155 important.



Comment [Aa12]: improve Comment [Aa13]: review

Comment [Aa14]: ???

156

157 158

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

| percenty | | | | | | | | | |
|---------------|------------------|-----------------------|--------------------|--------------------|-----------------------|--------------------|--------------------|------|---------|
| | | Meal type Powder type | | | | | | | |
| | | Plant pr | otein source | e A | Animal protein source | | | | |
| Cher compo | nical osition | Soybean | Rapeseed | Cottonseed | Poultry offal | Fish | Blood | SEM | p-value |
| Crude | protein | 50.09 [°] | 23.84 ^e | 36.98 ^d | 55.00 ^b | 50.09 ^c | 59.06 ^a | 0.22 | <0.001 |
| ļ | 4 | 8.52 ^{cd} | 6.63 ^d | 4.05 ^e | 10.38 ^c | 13.63 ^b | 16.08 ^a | 0.76 | <0.001 |
| В | 1 | 2.30 ^c | 3.32 ^c | 13.67 ^a | 3.54 ^c | 7.44 ^b | 7.16 ^b | 0.91 | <0.001 |
| В | 2 | 80.49 ^a | 77.50 ^a | 68.40 ^b | 66.36 ^{bc} | 55.03 ^d | 61.66 ^c | 1.83 | <0.001 |
| В | 3 | 6.24 ^{bc} | 2.63 ^c | 9.11 ^{ab} | 7.50 ^b | 6.74 ^b | 11.91 ^a | 1.32 | 0.008 |
| (|) | 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

Comment [Aa15]: What happens with your aim? With the ZnO effect?

159 **4.** CONCLUSION 160

161 The results show that there is a difference between the average concentration of insoluble 162 fiber in neutral detergent, crude protein, soluble protein, non-protein nitrogen, insoluble 163 protein in neutral detergent and insoluble protein in acidic detergent of feed samples. 164 Therefore, the use of CNCPS and NRC data for portion fractions of various feeds cannot be 165 considered absolutely, and domestic research and results should be used to extract samples 166 from different regions and different growth conditions, so that diets adjusted with these 167 feeds, Realistic and more balanced and with less waste of nutrients.

170 **REFERENCES**

169

171 172

173

174

175 176

177 178 179

180 181

182 183

184

185

186 187

192

193

194 195

196 197

198

199 200 201

202

203 204 205

206

207

208

- 1. Licitra C, Hernandez TN, Van Soest PJ. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim Feed Sci Technol. 1996; 57: 347-358.
- Van Soest PJ, Sniffen CJ, Oconnor JD, Fox DG, Russel JB. A net carbohydrate and protein system for evaluating cattle diets: 2. Carbohydrate and protein availability. J Anim Sci. 1992; 70: 3562-3577.
- 3. Lanzas CL, Tedeschi O, Seo S, Fox DG. Evaluation of protein fractionation systems used in formulating rations for dairy cattle. J Dairy Sci. 2007; 90: 507-521.
- Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG, Russell J B. A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. J Anim Sci. 1992; 70: 3562–3577.
- 5. Mjoun K, Kalscheur KF, Hippen AR, Schingoethe DJ. Ruminal degradability and intestinal digestibility of protein and amino acids in soybean and corn distillers grains products. J Dairy Sci. 2010; 93: 4144-4154.
- Shahbazi HR, Sadeghi AA, Fazaeli H, Raisali G, Chamani M, Shawrang P. Effects of electron beam irradiation on dry matter degradation of wheat straw in the rumen. Pakistan J Biological Sci. 2008a; 11(1): 676-679.
- NRC. Nutrients requirements of dairy cattle. National academy press. Washington, D. C. 2001.
- 8. Van Soest PJ. Nutritional Ecology of the Ruminant (2nd Ed.). Comstock Publishing Associates, Ithaca, NY; 1994.
- Riasi A, Allaheresani A, Naimipour H, Fathi MH. Comparison of methods for measuring insoluble fibers in neutral detergents and insoluble fiber in acid detergents in fourage and feed products. J Anim Sci. 2009; 19(1); 91-103.
- Khezri A, Yousefi Ansari M, Mohammad Abadi MR. Investigating subunits of protein fractions in comparison with soybean meal and cottonseed using SDS-PAGE and CNCPS electrophoresis method, J Agri Sci. 2003; 5 (1):12-18.

- MirzaiiAlamoti HR, Amanloo H, Nikkhah A. Protein and Carbohydrate Fractions of Common Feedstuffs in the Cornell Net Carbohydrate and Protein System. Iranian J Agri Sci. 2005; 36 (2): 409-414.
 - 12. Ghoorchi T, Arbabi S. Study of protein Characteristic of five feeds by CNCPS model. Asian J Anim and Veterinary Advances. 2010; 5: 584-591.
 - 13. Krishnamoorthy U, Muscato TV, Sniffen C J, Van Soest PJ. Nitrogen fractions in selected feedstuffs. J Dairy Sci. 1982; 65: 217–255.
- 14. Arieli A. Whole cottonseed in dairy cattle feeding: a review. Anim Feed Sci Technol. 1998; 72: 97-110.
- Shannak S, Suedekum KH, Susenbeth A. Estimating ruminal crude protein degradation with in situ and chemical fractionation procedures. Anim Feed Sci Technol. 2000; 85: 195-214.