

## Evaluation of Protein Quality in Hydrolyzed Protein Meals by Biological Assay Methods in Broiler Chickens

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**Abstract:** This study was conducted to determine chemical composition, mineral contents, protein quality and the effects of hydrolyzed protein meals (HPM) on broiler chicken performance during two experiments. Six samples of Hydrolyzed protein meal were provided from commercial processing plants. Average values of Dry Matter (DM), Ether Extract (EE), Crude Protein (CP), Crude Fiber (CF), Total Volatile Nitrogen (TVN) and Ash content were 97, 0.12, 70, 0, 50 and 19.68%, respectively. The average Apparent Metabolizable Energy (AME) value for the Hydrolyzed protein meal samples was 3056 kcal/ kg. The mean values of major elements including Ca, P and Na were 3.89, 0.76 and 3.9%, respectively. In experiment 1, Protein efficiency ratios (PER) and net protein ratio (NPR) were determined in chicks fed a semipurified diets containing 10% CP from HPM as the only source of dietary protein. The PER and NPR values for the HPM averaged 1.73 and 2.05, respectively. In experiment 2, three corn-soybean meal based diets containing 0, 7.5 or 15 percent of HPM was fed to four groups of ten Ross 308 male chicks from 11 to 32 d of age. The data were analyzed using completely randomized designs. When HPM substituted at 7.5%, growth performance was significantly better than the other levels ( $P<0.05$ ). Growth performance was decreased by using of HPM when substituted at 15% of the diet. The results of this study indicated that using of 7.5% HPM in the practical diets has any adverse effects on broiler chicken performance and HPM has substantial nutritional value for poultry.

**Key words:** Hydrolyzed protein • PER • NPR • Performance

### INTRODUCTION

Nutrition has the main role in the successful of poultry industry. The new perspective for economical formulation of poultry diet, using of unconventional feedstuff in a part of diet. Animal protein sources are useful components in poultry diets. They provide a high level of protein or amino acids, highly available phosphorus, a number of other minerals and moderate amounts of energy. One of the feed resources for this means is the waste from leather processing plants. The nutrient composition and amino acid profile of HPM have suitable and applicable in poultry diet. In addition, has led to produce economical savings and reduce bioenvironmental pollution. Hydrolyzed protein meal is the waste from leather processing plants that consist of skin soft scrap, leather shavings and collagen.

HPM contains abundant of protein, nitrogen and organic materials that can be used as an alternative protein source for poultry nutrition. This product is obtained from leather trimmings that are cooked with the addition of steam, sodium hydroxide and calcium hydroxide. This product can be used as a liquid or as a powder [1]. The composition matrix of this meal was similar to fishmeal but methionine, lysine and histidine were less than this. Using of leather waste until 4% levels in broiler chicken diet have any adverse effects on broiler chicken performance in grower and finisher periods [2]. Research evaluating protein quality of rendered by-product meals has been done by Parsons *et al.* [3]; Wang and Parsons [4]. Unfortunately little research has been published on the protein quality of HPM or the effect of this meal on broiler chicken performance. Indeed this meal is the novel high protein content source

for poultry nutrition. Due to shortage of protein source in the developing country such as *iran*, the HPM can be used as an alternative protein supplementation. Therefore, the objective of the current series of experiments were to determination chemical composition, minerals content and evaluation the quality of hydrolyzed protein meal in broiler chicken diets.

## MATERIALS AND METHODS

Hydrolyzed Protein meal samples were collected from commercial leather processing plant in Iran so that samples were stored at -20°C until further analysis. The Dry Matter (DM), Ether Extract (EE), Crude Protein (CP), Crude Fiber (CF), Total Volatile Nitrogen (TVN) and ash contents of the Hydrolyzed Protein meal samples were determined. Also Calcium was determined by atomic absorption, phosphorus by spectrophotometry [5].

Experiment 1 was conducted to determine the protein quality of HPM using a protein efficiency ratio (PER) and net protein ratio (NPR) assay. Chicks were fed a N-free cornstarch basal diet, or the basal diet supplemented with a HPM as a sole source of protein to provide 10% CP (Table 1). Each of the two diets was fed to three groups of six male chicks from 13 to 22 d post hatching. The feed intake and body weight of each experimental unit was determined following an overnight fasting after 10 days of test. This research project was conducted from 2 April 2011 to 2 May 2011 in agriculture research station of Tabriz University. The PER value was calculated as BW gain (grams) divided by CP intake (grams). The NPR value was calculated as [BW gain (grams) minus BW gain (grams) of chicks fed N-free basal diet] divided by CP intake (grams). The chicks were housed in batteries place in an environmentally regulated room. Feed and water were supplied in *ad libitum*. Experiment 2 was conducted to determine the growth performance of chicks fed diets containing different levels of HPM when substituted for corn and soybean meal. Chicks were fed a corn-soybean meal diet or diets containing 7.5 or 15% of a HPM (Table 2). All diets were formulated to provide 21.00% crude protein and 3050 kcal AME/kg and to meet all other NRC [6] nutrient requirements. The three diets were fed to four groups of ten Ross 308 male chicks from 11 to 32 d post hatching and raised in litter pens. During this experiment feed intake, weight gain, feed conversion ratio (FCR) and body weight were recorded weekly basis. Protein efficiency ratio and European efficiency factor (EEF) in end of the experiment was calculated were follow:

Table 1: Feed ingredients and nutrients composition of nitrogen free basal diet and hydrolyzed protein diet

| Item                            | Nitrogen free basal diet (%) | Hydrolyzed protein diet (%) |
|---------------------------------|------------------------------|-----------------------------|
| Corn Starch                     | 91.2                         | 75.8                        |
| Soybean Oil                     | 4                            | 5.3                         |
| Hydrolyzed protein              | 0                            | 14.3                        |
| Oyster shell                    | 1.7                          | 1.7                         |
| Dicalcium Phosphate             | 2.2                          | 2.2                         |
| Common Salt                     | 0.4                          | 0.20                        |
| Vitamin Premix <sup>1</sup>     | 0.25                         | 0.25                        |
| Mineral Premix <sup>2</sup>     | 0.25                         | 0.25                        |
| Calculated Analysis             |                              |                             |
| Metabolizable Energy (kcal/ kg) | 3680                         | 3680                        |
| Crude Protein                   | 0                            | 10                          |

1- Vitamin supplementation: B1: 3.3 g; B2: 0.72 g; K3: 1.6 g; Vitamin E: 14.4 g; Vitamin D: 7 g; Vitamin A: 7.7 g; Pantothenic acid: 12 g; Pyridoxine: 6.2 mg, B12: 14.4 g, Choline chloride: 440 mg.

2-Mineral supplementation: Mn: 64 g; Zn: 44 g; Fe: 100 g; Cu: 16 g; I: 0.64 g.

Table 2: Composition of experimental diets and nutrient profile

| Item                    | Control | 7.5%  | 15%   |
|-------------------------|---------|-------|-------|
| Ingredient (%)          |         |       |       |
| Corn                    | 55.00   | 64.37 | 70.13 |
| Soybean meal            | 36.60   | 23    | 10    |
| Soybean oil             | 3.60    | 2     | 1.6   |
| Hydrolyzed protein meal | -       | 7.5   | 15    |
| CaCO <sub>3</sub>       | 1.40    | 0.5   | -     |
| DCP                     | 1.50    | 1.3   | 1     |
| Salt                    | 0.25    | 0.25  | 0.25  |
| Lys                     | 1.00    | -     | -     |
| Met                     | 0.15    | 0.013 | .013  |
| Mineral premix          | 0.25    | 0.25  | 0.25  |
| Vitamin premix          | 0.25    | 0.25  | 0.25  |
| sand                    | -       | -     | 1.5   |
| Nutrient composition    |         |       |       |
| Metabolizable energy    | 3050    | 3050  | 3050  |
| Crud protein            | 21      | 21    | 21    |

1- Vitamin supplementation: B1: 3.3 g; B2: 0.72 g; K3: 1.6 g; Vitamin E: 14.4 g; Vitamin D: 7 g; Vitamin A: 7.7 g; Pantothenic acid: 12 g; Pyridoxine: 6.2 mg, B12: 14.4 g, Choline chloride: 440 mg.

2-Mineral supplementation: Mn: 64 g; Zn: 44 g; Fe: 100 g; Cu: 16 g; I: 0.64 g.

$$\text{European Efficiency Factor (EEF)} = \frac{\text{average live body weight (kg)} * \text{viaability}}{\text{feed efficiency ratio} * \text{day old}}$$

**Statistical Analyses:** Data from all assays were subjected to GLM for completely randomized designs. Comparison of means was conducted by Duncan's multiple range tests [7].

## RESULTS AND DISCUSSION

The chemical composition and mineral contents of the hydrolyzed protein meal from leather processing plants samples are shown in Table 1. The average DM, EE, CP, CF, TVN and ash were 97, 0.12, 70, 0, 50 and 0, 19.68 percent, respectively. The average values of macro elements including Ca and P were 3.89 and 0.76 percent. The average apparent metabolizable energy was 3056 kcal/kg. The chemical composition of hydrolyzed protein meal was similar to hydrolyzed leather meal [8]. except for its lower protein and ether extract values. The high ash content of hydrolyzed protein meal from leather was due partially to the calcium and sodium in this meal.

Evaluation of protein quality of animal protein sources by integrative methods such as PER and NPR, present some information on probably heat damage in during processing and availability of amino acids in tissue level for birds. The results of the PER and NPR assays are shown in Table 3. Chicks fed the N-free diet lost weight during the assay period, whereas chicks fed hydrolyzed protein meal had a positive weight gain response. Calculated PER values averaged 1.53 and NPR values averaged 1.15. The mean PER value of the hydrolyzed protein meals was lower than that of the poultry by-product meal evaluated here in and those evaluated by Escalona *et al.* [9] and Parsons *et al.* [3]. The lower PER of hydrolyzed protein meal compared to poultry by product meal is probably due to the high ash and collagen content in hydrolyzed protein meal. In the other protein source for poultry feeding, whereas the result of Shirley and Parsons [10] showed that ash content no effect on AA digestibility; however, they did observe a significant decrease in PER due to decreasing concentrations of essential AA when ash increased. Its in agreement in present study. Interestingly, PER was negatively correlated with ash. As discussed by Partanen [11] ash has been suggested to be a proper indicator for protein quality because of it reflects the bone and collagen (poor quality protein) content of the meals. However, little or no data have been published to support the latter hypothesis in HPM. When 7.5% of a HPM was substituted into a corn soybean meal basal diet, chick growth was significantly affected by HPM (Table 6); however, growth was depressed by HPM at level of 15%. The 7.5% level of HPM improved ( $P < 0.05$ ) feed conversion ratio, PER and EEF compared to the 15% HPM diet. However the lowest growth performance was observed in 15% HPM diet than the other experimental diets.

Table 3: Chemical composition of hydrolyzed protein meal

| Component               | (%)   |
|-------------------------|-------|
| Moisture                | 3     |
| Crude protein           | 70    |
| Ether extract           | 0.12  |
| Ash                     | 19.68 |
| Calcium                 | 3.89  |
| Phosphorus              | 0.70  |
| AME kcal/kg             | 3056  |
| Total volatile nitrogen | 50    |

Table 4: Determination of protein quality of hydrolyzed protein meal<sup>1</sup>

| Dietary treatments | Weigh gain (g) | PER <sup>2</sup> (g:g) | NPR <sup>3</sup> (g:g) |
|--------------------|----------------|------------------------|------------------------|
| Basal (N-free)     | -18            | -                      | -                      |
| Basal + HPM        | 97             | 1.73                   | 2.05                   |

1. Means of three groups of five male chicks from 13 to 22 d posthatching.

2. PER = protein efficiency ratio = weight gain (grams) divided by protein intake (grams).

3. NPR = net protein ratio = [weight gain (grams) - weight gain (grams) chicks fed N-free basal diet] divided by protein intake (grams).

Table 5: Growth performance of chicks fed a corn soy bean meal diet or diets containing hydrolyzed protein meal

| Diets Parameter | Control   | 7.5(%)   | 15(%)    | SEM*  |
|-----------------|-----------|----------|----------|-------|
| BW 1            | 423.33a   | 428.67a  | 362.00b  | 12.43 |
| FI 1            | 403.33a   | 402.33a  | 334.33b  | 12.92 |
| WG1             | 242.22a   | 253.11a  | 175.94b  | 14.69 |
| FCR1            | 1.95      | 1.66     | 1.59     | .082  |
| BW 2            | 660.67b   | 725.00a  | 583.33c  | 21.11 |
| FI 2            | 458.33ab  | 510.00a  | 438.67b  | 13.50 |
| WG2             | 237.33    | 296.33   | 221.33   | 15.78 |
| FCR2            | 2.00      | 1.72     | 2.01     | 0.11  |
| BW 3            | 1081.67b  | 1167.33a | 990.00   | 26.33 |
| FI 3            | 849.67b   | 880.33ab | 914.33a  | 12.32 |
| WG3             | 421.00    | 442.33   | 406.67   | 10.42 |
| FCR3            | 2.02      | 2.00     | 2.24     | 0.056 |
| BW total        | 1081.67   | 1167.33  | 990.00   | 26.33 |
| FI total        | 1711.63ab | 1792.67a | 1687.33b | 21.07 |
| WG total        | 900.56b   | 991.78a  | 803.94c  | 28.69 |
| FCR total       | 1.58b     | 1.53b    | 1.70a    | 0.028 |
| PER             | 2.50a     | 2.63a    | 2.26b    | 0.061 |
| EEF             | 220.72b   | 245.35a  | 187.42c  | 8.82  |

a,b,c: In the same row differently superscripted are significantly ( $P < 0.05$ ) different.

\* Standard Error Mean

The reduced chick performance obtained with the 15% HPM diets was probably largely due to differences in ash content and lower PER value. The poor quality of protein this protein source in broiler chicken diets, when used in high inclusion level, as result of high ash and collagen contents in these diets. Wisman and Engell [12] find out when hydrolyzed leather fed at dietary levels up to 8% was not decreased growth performance in chicks provided that amino acid supplementation was furnished. This result may be to imbalance of amino acid profile in

these meals. Therefore in our study the decline of growth performance was observed when used of 15% HPM in diet. But the result of this study was conflict with Waldroup *et al.* [13]. This investigator indicated that Leather meal had not greater body-weight at 4 or 8 weeks than soya bean meal. Also reported that diet with 2.5% fish meal without or with 2, 4, 6 or 8% leather meal was fed to chickens from day-old to 28 days no adverse effects on weight, mortality and efficiency of feed conversion. Also replacing soybean meal protein by hydrolyzed cattle hide scrap meal protein up to 50% in rabbit diet did not produce any effect on weight gain, feed intake and carcass weight [14]. The reason of this paradox in this study with previous research, probably duo to raw material source, duration of the raw material storage prior to rendering, methods and condition processing which used in producing the product. As well no health problems were observed during of hydrolyzed protein meal feeding trials. That according with the result of other study Knowlton *et al.* [8].

### CONCLUSION

The results showed that HPM from leather processing plants as the novel protein source for using in broiler chicken diet. Thus as much as 7.5% HPM could be included in chick practical diets with no detrimental effect on performance in grower phase and HPM has substantial nutritional value for poultry.

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