

Full Length Research Paper

Determination of protein quality and true metabolizable energy of high oil poultry by-product meal

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The three high oil poultry by-product meal (PBPM) samples were obtained from three poultry slaughter-houses in East Azerbaijan Province, Iran. For evaluating of protein quality, 126 male broiler chicks in a completely randomized design as factorial arrangement was used and fed experimental diets from 8 to 17 days of age. The experimental diets consisted of a nitrogen-free basal diet based on corn starch and glucose and six semipurified diets. Semipurified diets were obtained by replacing each of the three PBPM samples with portion of cornstarch and glucose in the nitrogen-free basal diet to provide 6 and 10% crude protein (CP). Sixteen cecectomized roosters (4 roosters per PBPM sample and 4 roosters for determining endogenous energy loss) were used for determination of true metabolizable energy (TME_n) content of PBPM samples. TME_n content of PBPM samples was different significantly ($p < 0.05$) and was 3696 kcal/Kg as average. PER and NPR values varied from 1.29 to 1.727 and 2.463 to 3.001 and showed significant differences among PBPM samples ($p < 0.05$). The results showed that with increasing CP level, protein efficiency ratio (PER) values was increased ($p < 0.05$). Net protein ratio (NPR) value was not affected by CP level. As the assay length increased from 6 to 10 days, PER and NPR values decreased ($p < 0.05$). Protein quality ranking, with PER, among three PBPM samples is the same in two period assays.

Key words: High oil poultry by-product meal, protein quality, metabolizable energy, cecectomized rooster.

INTRODUCTION

Poultry by-product meal (PBPM) is produced from the inedible parts of the poultry carcasses including the head, feet and the viscera organs, except the feathers (Buhnert et al., 1999; Senkoğlu et al., 2005). In recent years, poultry industry has been increasingly developed in Iran and one of its main by-product is PBPM. PBPM is produced in large amount in Iran by simple low cost technology. The product is rich in oil (Gheshlagh et al., 2010a) and has different nutrient composition in comparison to that reported in NRC (1994). Many studies have been conducted on protein quality changes and other nutritional characteristics of PBPM in several

countries (Aimiwu and Lilburn, 2006; Bhargava and O'Neil, 1975; Dale et al., 1993; Dozier et al., 2003; Escalona et al., 1986, 1987; Johnson and Parsons, 1997; Johnson et al., 1998; McNaughton et al., 1977). Little research was conducted by the PBPM produced in Iran. The product was used in poultry diets; broilers, layers and rainbow trout diets as protein source in three production studies (Janmohammadi et al., 2009; Gheshlagh et al., 2010 a, b; Maleki et al., 2010). In those conducted studies, protein quality and metabolizable energy of PBPM was not determined. Protein quality determination of animal protein sources is necessary for evaluation of heat damage in during processing and producing. On other hand, evaluation of metabolizable energy also is necessary for balance formulation diets. There are some reports that showed metabolizable energy of PBPM (Nadeem et al., 2005; Johnson and Parsons., 1997) is high than that in the NRC (1994). Thus, the objective of this study was to evaluate the protein quality and metabolizable energy of three samples of PBPM in poultry slaughter-houses in east

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Abbreviations: PBPM, Poultry by-product meal; TME, true metabolizable energy; TME_n, tme corrected to zero nitrogen balance; PER, protein efficiency ratio; NPR, net protein ratio; DM, dry matter; CP, crude protein; EE, ether extract; GE, gross energy.

Table 1. Feed ingredient and composition of nitrogen- free basal diet.

Ingredient	Percent
Corn starch	61.94
Glucose	30.97
Sunflower oil	2.37
Oyster sell	1.13
Dicalcium phosphate	2.67
Iodized salt	0.4
Vitamin permix ¹	0.25
Mineral permix ²	0.25
Antioxidant	0.01
Calculated analysis	
ME (Kcal/Kg)	3600
Crude protein (%)	0
Calcium (%)	1
Phosphorus (%)	0.5

¹Vitamin premix provided the following per kilogram: vitamin A, 7.2 g, vitamin B₁, 0.72 g, vitamin B₂, 3.3 g, vitamin B₃, 4 g, vitamin B₆, 1.2 g, vitamin B₁₂, 0.6 g, vitamin D₃ 1.6 g, vitamin E, 14.4 g, choline Cl, 400 mg, menadione, 1.6 g, ²Mineral premix provided the following per kilogram: manganese, 64 g; zinc, 100 g, iron, 44 g, copper, 16 g, Calcium iodated, 0.64 g.

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MATERIALS AND METHODS

Providing poultry by - product meal samples

The PBPM samples were randomly collected from rendering units of three industrial poultry slaughter-houses in east Azerbaijan Province, Iran. Samples were maintained in -20°C in the freezer until their chemical composition analysis and biological assays.

Chemical analysis

The dry matters (DM), crude protein (CP), ether extract (EE) and the crude ash contents of the three PBPM (A, B and C) were analyzed according to the procedure of the Association of Official Analytical Chemists (AOAC, 2005). The Labsco adiabatic oxygen calorimeter bomb was used for measuring the gross energy (GE).

True metabolizable energy assay

Cepectomized Ross-308 roosters were used in 48 h assays (Sibbald, 1986). Cepectomy was performed according to the procedure of Parsons (1985), except that lidokain used instead of pentobarbital for surgery operation, when the birds were 20 weeks of age. The roosters were 25 weeks old at the time of the balance assay. The birds were housed in an environmentally controlled room and kept in individual cages with raised wire floors and subjected to 16 h light and 8 h dark daily. Before the experiment, the water was supplied *ad libitum* to the roosters and the feed was supplied to the extent for maintenance requirement. Following a 24

h period without feed, roosters received 25 g of PBPM through crop intubation. Each product was replicated four times as well as four replications for endogenous collection. A plastic tray was placed under each cage and excreta were collected quantitatively for 48 h after crop intubation. Endogenous energy losses and N excretion were measured from roosters that were deprived of feed for 48 h. The excreta were oven-dried, ground, and N and GE concentrations were determined according to the procedures described previously. TME and nitrogen-corrected TME (TME_n) was calculated according to the method of Parsons et al. (1982).

Biological assay

Three hundred one-day-old Ross-308 chicks were purchased from a local hatchery. Chicks were sexed with feather sexing method and 126 male chicks were separated. The chicks were fed a 23% CP corn-soybean meal pretest diet during the first week post-hatching. Following an overnight period without feed, the chicks were weighed and allotted to dietary treatments as described by Sasse and Baker (1973). Triplicate groups of six chicks were assigned to each dietary treatment. The experimental diets were fed from 8 to 17 days post-hatching and weight gain and feed consumption were measured for each group of chicks in 6 and 10 day of assay (13 and 17 days of age). The chicks were housed in an environmentally controlled room and kept in cages with raised wire floors and subjected to 23 h light and 1 h dark daily. Feed and water were consumed as *ad libitum*. An N-free basal diet was used in the chick assays for determining protein quality of the PBPM samples. Dietary treatments consisted of the N-free basal diet (Table 1) or the basal diet plus 6 or 10% CP provided solely by one of the PBPM samples which replaced in portion of the cornstarch and glucose. The PER and NPR was calculated as follows with all measurements in grams:

PER = body weight gain /CP intake

NPR=[(body weight gain of chicks fed semipurified diets - body weight gain of chicks fed nitrogen free basal diet)/CP intake.

At the end of the experiment, blood samples was taken of two chicks per replicate from the neck vein and concentration of uric acid, calcium and phosphorus in the blood serum was determined by using the standard kits. After the blood taking, two chicks from the each replicate were slaughtered and the right tibias of the chicks were separated. Tibia ash content of chicks was determined according to Cramer et al. (2007). Right tibias were autoclaved under 6.82 kg of pressure for 12 min. Bones were allowed to cool and were cleaned of all tissue, including cartilage caps. Bones were dried at 105°C for 24 h. The samples were then weighed, ashed overnight at 600°C, and weighed again. The percentage of tibia ash was calculated as the proportion of the dry, pre-ashed tibia multiplied by 100 (Hall et al., 2003).

Biological data assay were analyzed in completely randomized design as factorial arrangement (two level CP x two period assay x three PBPM samples, A, B and C), metabolizable energy and chemical composition data were analyzed in completely randomized design with GLM procedure of SAS (SAS institute, 2003). Comparison of the means was performed by using Duncan's Multiple Range test (p<0.05). Univariate procedure of SAS was used for obtaining the descriptive statistical parameters.

RESULTS

Chemical composition content

The chemical composition of PBPM samples is shown in

Table 2. Chemical composition (% as fed) of the PBPM samples¹.

Item	Sample A	Sample B	Sample C	MSE	NRC
CP	50.907 ^d	54.300 ^a	54.127 ^a	1.2499	60
EE	19.762 ^a	17.231 ^d	20.493 ^a	0.9202	13
DM	93.70 ^a	89.56 ^d	93.78 ^a	0.042	93
Ash	11.33 ^a	9.49 ^a	10.81 ^a	2.366	-

¹Means within each row with different superscripts are significantly different (p<0.05).

Table 3. GE, TME, TME_n (Kcal/Kg) and True metabolizable dry matter (DM met), (%) in PBPM samples¹.

Item ²	PBPM A	PBPM B	PBPM C	Mean	MSE	NRC
GE	5760±163 ^a	5419±185 ^d	5481±219 ^{ab}	5553.5±231	201	-
TME	3376±117 ^{ab}	3234±77 ^d	3465±110 ^a	3359±136	101	-
TME _n	3764±116 ^a	3476±73 ^d	3846±108 ^a	3695.5±189	103	3120
TME _n /GE (%)	65.42±3.75 ^{ad}	64.21±3.20 ^d	70.21±2.35 ^a	66.61±3.93	3.161	-
DM met	60.6±2.64 ^u	64.19±4.68 ^{du}	70.17±4.22 ^a	64.98±5.45	3.949	-

¹Values are on based of as fed, ²Means within each row with different superscripts are significantly different (p<0.05).

Table 2. Chemical composition, except ash case, showed significant differences among PBPM samples. The mean value of EE, CP, DM and ash were 19.16, 53.11, 92.34 and 10.54%, respectively.

Metabolizable energy

GE, TME, TME_n, GE/TME_n and true metabolizable dry matter values are shown in Table 3. True metabolizable dry matter and energetic values of PBPM samples were found to be different significantly (p< 0.05). True metabolizable dry matter was the lowest for sample A and TME, TME_n, and GE/TME_n was the lowest in sample B. Average values of GE, TME, TME_n and GE/TME_n were 5553.5, 3359, 3696 Kcal/Kg and 66.61%, respectively for PBPM samples.

Biological assay

The effects of assay period, CP level and PBPM sample are shown in Table 4. Effect of CP level on PER values of PBPM samples was significant (p<0.0003). With increasing CP level, PER value was increased. NPR value was not affected by CP level. Effect of period assay was significant on protein quality values. As the assay length increased from 6 to 10 days, PER and NPR values decreased. The results of this study indicated that PER and NPR values of PBPM samples were influenced by level of CP and length of period assay. PER, NPR and weight gain values were found to be different significantly (p<0.05). These values were the lowest in sample B.

Tibia ash percent, breast weight as percent of live body weight and values of uric acid, calcium and phosphor in blood serum of chicks are shown in Table 6. Tibia ash

and breast weight percent were not different among PBPM samples. Among blood serum metabolites, only uric acid values were significantly (p<0.05) different in PBPM samples.

DISCUSSION

Chemical composition content

Basically, because of using different raw materials and processing conditions in animal protein sources rendering plants, determination of chemical composition in these feeds should be done continuously. These data is used in diets formulation or in estimation of amino acid and metabolizable energy contents. The chemical composition data reported here on the PBPM may be as considered first ones. A 19.16% average for EE were found for the PBPM and in agreement with data reported on EE of 22 PBPM samples by Dale et al. (1993), was higher than in NRC (1994) and report of Cramer et al. (2007). The high amount of EE in the PBPM samples used in this study was due to fat that is not removed from final product in rendering units because of processing and producing of the PBPM by simple low cost technology, in Iran. Although more amount of the fat could enhanced available energy content for poultry but it may cause deterioration because of oxidation, and will decrease nutritional value of the product finally.

Average CP of PBPM samples was lower than that reported by NRC (1994) and Dozier and Dale (2005). Lower amount of CP in our PBPM samples than that reported by NRC (1994) may be due to the high level of EE in our samples. Ash content varied from 9.49 to 11.33% and was as average of 10.54%. Yilmaz et al. (2003) reported average value of 18.34% for the crude

Table 4. Protein quality values for the PBPM samples during 6¹ and 10² days assay period.

Main effects	PER ³	NPR ⁴	Weight gain ⁵
Protein level			
6 %	1.316 ^b	2.812 ^a	62.294 ^b
10 %	1.776 ^a	2.797 ^a	109.722 ^a
P-Value	0.0003	0.9011	< 0.0001
SEM	0.07076	0.08694	4.809
Assay period			
6 day	1.675 ^a	3.062 ^a	76.471 ^b
10 day	1.417 ^b	2.547 ^b	96.333 ^a
P-Value	0.0268	0.0002	0.0034
SEM	0.07076	0.08694	4.809
Sample			
A	1.621 ^a	2.949 ^a	100.000 ^a
B	1.29 ^b	2.463 ^b	68.250 ^b
C	1.727 ^a	3.001 ^a	92.917 ^a
P-Value	0.0087	0.0018	0.0029
SEM	0.08666	0.1065	5.896

1: Assay length of 6 days = 8 to 13 days post hatching, 2: Assay length of 10 days = 8 to 17 days posthatching, 3: Protein efficiency ratio calculated as PER = body weight gain (g)/CP intake (g), 4: Net protein ratio calculated as NPR = [(body weight gain of chicks fed experimental diets (g)-body weight gain of chicks fed nitrogen free basal diet (g)]/CP intake (g), 5: Average initial body weight at the start of assay was 131.67 g.

ash of PBPM samples. Johnson et al. (1998) reported crud ash of PBPM in the range of 7.5 to 17%, which was in agreement with the present study. Considering that the bird legs are used in human consumption and not as raw material in producing the PBPM, lower mean ash value is not unexpected. Janhanin et al. (2007) also is reported low ash values for PBPM samples from other part of Iran.

Metabolizable energy

Average values of GE, TME, TME_n and GE/TME_n were 5553.5, 3359, 3696 Kcal/Kg and 66.61%, respectively for PBPM samples. GE value of sample B were the lowest, probably due to the low amount of its DM and EE. GE values of the three PBPM samples was in range values (4830 and 5652 kcal/kg) of reported by Johnson and Parsons (1997).

TME_n values of studied PBPM were higher than that reported by NRC (1994). The higher ME in the PBPM samples can be the result of their higher EE. The mean EE content of the PBPM is partially seen in NRC (1994). The biological methods of ME determination may be another reason for this difference. Basically, the methods vary depending on age of the birds, type of feed presentation, level of test feed used in diets based on practical or purified ones. There are some reports that support our findings here on ME content of PBPM (Dozier and Dale, 2005; Lesson and Summers, 2001; McDonald

et al., 2002 and Pesti et al., 1986). For example, Dale et al. (1993) found a TME_n range between 3626 and 5247 kcal/kg for 22 sample poultry offal meals and also, Han and Parsons (1990) found TME_n values between 2863 and 3390 kcal/kg. These findings on ME content of PBPM recommend that using of recent data instead of NRC (1994) in formulating broiler diet with PBPM, could be decreased fat content of broilers carcass.

Biological assay

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. At the present study, PER values of PBPM samples was lower than that reported by Escalona et al. (1986), Douglas et al. (1997), Johnson (1988) and Jahanian et al. (2007). Average NPR value for the PBPM samples in this study was also lower than that reported by Cramer et al. (2007) and was higher than that reported by Jahanian et al. (2007). Basically, protein quality values reported here is depends on raw material source, duration of the raw material storage prior to rendering, methods and condition processing which used in producing the product (Johnson and Parsons., 1997; Johnson et al., 1998; McNaughton et al., 1977; Robbins and Firman, 2006; Tamim and Doerr, 2003). Since, it is

Table 5. protein quality ranking of the PBPM samples by level of CP¹ and assay length².

CP Level (%)	Sample A ⁵		Sample B		Sample C	
	PER ³	NPR ⁴	PER	NPR	PER	NPR
6	1.4908 (#1)	3.177 (#1)	0.9688 (#3)	2.456 (#3)	1.4900 (#2)	2.802 (#2)
10	1.715 (#2)	2.721(#2)	1.612 (#3)	2.410 (#3)	1.964 (#1)	3.200 (#1)
Assay (length/ day)						
6	1.756 (#2)	3.172 (#2)	1.431 (#3)	2.711 (#3)	1.837 (#1)	3.302 (#1)
10	1.486 (#2)	2.726 (#1)	1.150 (#3)	2.215 (#3)	1.616(#1)	2.701 (#2)

1: Assay length of 10 days = 8 to 17 days posthatching, 2: Assay length of 6 days = 8 to 13 days post hatching, 3: Protein efficiency ratio calculated as PER = body weight gain (g)/CP intake (g), 4: Net protein ratio calculated as NPR = [(body weight gain of chicks fed experimental diets (g)-body weight gain of chicks fed nitrogen free basal diet (g)]/CP intake (g), 5: Average initial body weight at the start of assay was 131.67 g.

Table 6. Values of tibia bone ash, uric acid¹, calcium and phosphor in blood serum and breast as % of body weight.

Item	Level of CP (%)	PBPM A	PBPM B	PBPM C	MSE ²
Tibia ash %	10	38.9 ^a	37.1 ^a	38.48 ^a	1.318
Uric acid(mg/dl) ¹	10	0.695 (5.13) ^d	0.834 (7.36) ^a	0.663 (4.68) ^d	0.101
Calcium(mg/dl)	10	11.18 ^a	11.75 ^a	11.16 ^a	0.021
Phosphor(mg/dl)	10	7.66 ^a	6.98 ^b	7.71 ^a	0.558
Breast %	10	13.94 ^a	14.28 ^a	13.46 ^a	1.054

Means within each row with different superscripts are significantly different (p<0.05).

used low cost technology for processing PBPM in the rendering plants of the area, such low PER and NPR values for the product are not unexpected. The effect of heat on protein quality of animal and plant protein sources has been extensively studied by Carpenter (1973). The results of his experiments indicated that overheating cause a decrease in protein quality through the formation of bond between the amine group of basic amino acids such as lysine and arginine and reducing sugars like glucose and ribose. Oxidation of lipids by heat also forms products with carbonyl groups. These chemical groups can react with the basic amino acids and make them unavailable to animal. Heat enhances isopeptide reactions which reduce protein quality. In these reactions, aspartic acid and glutamic acid react with free amine group of basic amino acids and reduce protein quality (Carpenter, 1973). Long term storage also causes the degradation of amino acids by bacterial activities and production and accumulation of biogenic amines which decrease the protein quality of final product and these toxic compounds adversely affect the animal performance (Tamim and Doerr, 2003).

As the assay length increased from 6 to 10 days, PER and NPR values decreased. It is probably due to increasing the amino acids requirements for maintenance and growth as age increased. Jahanian et al. (2007) and Johnson and Parsons (1997) reported that PER and NPR values decreased with increasing of assay length. Protein

quality ranking, with PER, among three PBPM samples is the same in two period assays (Table 5). Decreasing of assay length showed better differences of protein quality PBPM samples. This finding suggest that assay length can be reduced to 6 days and following that cost of protein quality assay will be decreased. With increasing CP level, PER and weight gain was increased but NPR was not affected by CP level. Scott et al. (1982) stated the greater sensitivity of chickens to differences in protein quality at lower levels. At the present study, sensitivity of chickens to differences in protein quality at 6 % CP also was higher than 10 % CP (0.73 vs. 0.45, NPR values differences between # 1 sample and # 3 sample at two levels CP study, respectively). As expected, NPR values were higher than PER ones, because NPR evaluates the protein quality at maintenance and growth levels (Jansen, 1978).

Tibia ash and breast percent were not affected by PBPM samples. Among blood serum metabolites, only uric acid values were significantly different in PBPM samples. Uric acid is the final product which resulted from nitrogen metabolism in birds and net balance between the tissue synthesis and degradation of purines. The uric acid of the blood serum would be increased by increasing the consumption of protein or by decreasing its quality and it is expelled by excreta. Higher blood uric acid in sample B is due to its low protein quality. The lowest PER and NPR value were obtained in sample B. There were

no significant differences between calcium and phosphorus of the blood serum in PBPM samples. The concentration of the blood calcium and phosphorus is controlled mainly by hormones like parathyroid and calcitonin, adrenal glands and vitamin D and its derivations and is maintained in physiologic extent.

The results of this research showed that average values of CP, EE, Ash and DM in the PBPM samples have a considerable difference with the data of NRC (1994). The studied PBPM was in rich of available energy ($TME_n = 3696$ kcal/ kg). PER and NPR values of PBPM samples were influenced by PBPM samples, level of CP and length of assay. Our results indicate that chick PER and NPR assays could be shortened to 6 days without substantially reducing the sensitivity of the assay.

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