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Determination of Chemical Composition, Mineral Contents, and Protein Quality of Poultry By-Product Meal

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Abstract: In order to study the chemical composition, mineral contents and protein quality of poultry by-product meal (PBPM), 10 composed samples of PBPM produced in Iran were provided during two months sampling period from rendering units of three industrial poultry slaughter-houses. The proximate analysis showed that the average dry matter (DM), ether extract (EE), crude protein (CP), crude fiber (CF) and ash of the PBPM samples were 94.8, 23.4, 60.5, 0.90, and 9.3 percent, respectively. The average gross energy (GE) value for the PBPM samples was 5645 kcal kg^{-1}. The average values of major elements including Ca, P, Na, K, Cl, Mg, and S were 3.51, 1.88, 0.52, 0.31, 0.74, 0.06, and 0.99 percent, respectively. The average values of trace elements including Fe, Cu, Mn, Zn, and Se were 623.2, 9.3, 16.5, 47.8, and 0.73 mg kg^{-1}, respectively. Biological evaluation of protein quality was done by chicks fed a nitrogen-free basal diet (as negative control) or chicks fed semipurified diets containing 10 percent crude protein from the PBPM or Kilka fish meal (as positive control) as the sole source of dietary protein. The values of protein efficiency ratio (PER) and net protein ratio (NPR) showed significant differences ($p<0.05$) among the PBPM samples and varied between 1.45 to 2.05 and 2.31 to 2.87, respectively. The PER and NPR values for the PBPM samples were significantly lower ($p<0.05$) than that of Kilka fish meal.

Key words: Poultry by-product meal, chemical composition, protein quality

Introduction

Recycling of wastes from poultry slaughter-houses is of economical, biological and environmental importance (Cai *et al*., 1994; Steffens, 1994). Poultry by-product meal (PBPM) is one of the by-products resulting from poultry slaughter-houses and produced by processing of the inedible parts of poultry carcasses including heads, feet, and viscera, with the exception of feathers (Bohnert *et al*., 1999; Daghir, 1975; Dale *et al*., 1993; Escalona *et al*., 1987; Senkoylu *et al*., 2005). PBPM has a proper profile of available essential amino acids and is rich in calcium, phosphorus and vitamin B_{12} (NRC, 1994; Waldroup and Adams, 1994). This by-product is used as a protein source in diets of monogastric animals like pig (Zier *et al*., 2004), Poultry (Aimiuwu and Lilburn, 2006; Escalona *et al*., 1987) and aquatic animals (Steffens, 1994) and also extensively used as a ruminally undegradable protein source in ruminant diets (Bohnert *et al*., 1999). The chemical composition, mineral contents and protein quality of PBPM can vary greatly depending on the raw material source (Johnson and Parsons, 1997; Johnson *et al*., 1998), storage time of raw materials prior to rendering (Tamim and Doerr, 2003), processing method (Robbins and Firman, 2006), processing pressure and temperature (McNaughton *et al*., 1977), and ash content (Johnson and Parsons, 1997) and needs to be evaluated continuously. Determination of the chemical composition of PBPM is important in estimating its metabolizable energy content (Dale *et al*., 1993; NRC, 1994; Pesti *et al*., 1986) and measurement of its mineral content especially calcium and phosphorus is of significance to include PBPM in the balanced diets (Leeson and Summers, 2001).

Currently, biological assays including protein efficiency ratio (PER) and net protein ratio (NPR) are extensively used to evaluate the protein quality of various animal protein sources for poultry (Douglas *et al*., 1997; Escalona *et al*., 1986; Johnson and Parsons, 1997) and rainbow trout (Anderson *et al*., 1993). The classical PER and NPR assays are conducted for 28 days with rats (Jansen, 1978) and usually last for 17 days with poultry (Douglas *et al*., 1997; Johnson and Parsons, 1997), but a study has been conducted to determine whether the PER and NPR assays can be reduced in length, and thus, make them more timely to detect differences in protein quality among animal protein meals (Johnson and Parsons, 1997).

At present, there are 37 industrial poultry slaughter-houses in Iran that produce PBPM by processing waste materials from slaughtering of broilers, and spent layers and breeders which are mostly used in poultry and cold water fish diets. Although many studies have been conducted on protein quality changes and other nutritional characteristics of PBPM in several countries (Aimiuwu and Lilburn, 2006; Bhargava and O'Neil, 1975; Dale *et al*., 1993; Dozier *et al*., 2003; Escalona *et al*.,

1986, 1987; Herro *et al.*, 1988; Johnson and Parsons, 1997; Johnson *et al.*, 1998; McNaughton *et al.*, 1977; Wang and Parsons, 1998), but the chemical composition, mineral contents and protein quality of the PBPM produced in Iran has not been described quantitatively. Therefore, the present study was conducted with the aim of determining the chemical composition, mineral contents and protein quality of the PBPM produced in Iran.

Materials and Methods

Sample collection: The PBPM samples were collected during two months daily sampling period from rendering units of three industrial poultry slaughter-houses in Iran. The samples collected in each six days were mixed together and 10 composed samples were prepared so that samples #1 to #5 were taken from plant A, samples #6 to #8 were taken from plant B and samples #9 and #10 were taken from plant C. The raw material source of all the PBPM samples included heads, feet, viscera, spent liver and gizzard, feather, blood and birds found dead on arrival at the processing plants. After grinding and mixing the samples, all composed samples were stored at -20°C until further analysis.

Chemical analysis: The dry matter (DM), ether extract (EE), crude protein (CP), crude fiber (CF), gross energy (GE) and ash of the PBPM samples were determined according to AOAC (1984) procedures. Calcium, magnesium and all trace elements including iron, copper, manganese, zinc, and selenium were determined by atomic absorption, phosphorus by spectrophotometry, sodium and potassium by flame photometry, chlorine by titration, and sulfur by turbidometry methods (AOAC, 1984).

Biological evaluation: In order to evaluate the protein quality of the PBPM samples, 400 day-old Ross male broiler chicks were purchased and housed in thermostatically controlled starter batteries placed in an environmentally regulated room. The chicks were fed a commercial corn-soybean meal starter diet (3200 kcal kg^{-1} metabolizable energy and 23% CP) during the first 7 days posthatching. Following an overnight feed withdrawal, the chicks were individually weighed, separated into groups of five chicks of similar body weight, wing banded, and randomly allotted to dietary treatments. Light was provided 24 h daily. Feed and water were supplied for *ad libitum* consumption.

To determine the protein quality of the PBPM samples using PER and NPR assays, a nitrogen-free cornstarch-glucose basal diet was prepared (Table 1). The experimental diets consisted of a nitrogen-free basal diet (as negative control) and eleven semipurified diets, each contained one of the 10 PBPM samples and one sample of Kilka fish meal (as positive control) as the sole source of dietary protein. The eleven semipurified diets were formed by replacing each of the ten PBPM samples or one sample of Kilka fish meal as the sole source of dietary protein with a portion of cornstarch and glucose in the nitrogen-free basal diet to provide 10% CP containing diets. Each experimental diet was fed *ad libitum* to four groups of five male chicks from 8 to 17 days posthatching. The feed intake and body weight of each experimental unit was determined following an overnight feed withdrawal at 6 and 9 days on test (14 and 17 day of age, respectively). The PER and NPR values were calculated as follow:

Table 1: Feed ingredients and composition of nitrogen-free basal diet

Ingredients	Percent
Corn Starch[1]	57.52
Glucose	28.76
Corn Oil	8.00
Calcium Carbonate	1.28
Dicalcium Phosphate	3.31
Common Salt	0.64
Vitamin Premix[2]	0.25
Mineral Premix[3]	0.25
Antioxidant	0.01
Calculated Analysis	
Metabolizable Energy (kcal kg^{-1})	4000.00
Crude Protein (%)	0.00
Calcium (%)	1.25
Phosphorus	0.56
Calcium to Phosphorus Ratio	2.23

[1]The cornstarch: glucose was 2:1. The other eleven semipurified diets were made by replacing each of the ten composed PBPM samples or one sample of Kilka fish meal as the sole source of dietary protein with a portion of cornstarch and glucose in the nitrogen-free basal diet to provide 10% CP containing diets, [2]Vitamin premix provided the following per kilogram of diet: vitamin A, 5500 IU; vitamin D_3, 1100 ICU; vitamin E, 13 IU; thiamine, 2.2 mg; riboflavin, 6.6 mg; Ca pantothenate, 12mg; nicotinic acid, 44 mg; choline Cl, 550 mg; vitamin B_{12}, 8.8µg; vitamin B_6, 2.2 mg; menadione, 1.3 mg; folic acid, 0.72 mg; d-biotin, 0.11 mg; ethoxyquin, 125mg, [3]Mineral premix provided the following per kilogram of diet: manganese, 66 mg; zinc, 50 mg; iron, 30 mg; copper, 5 mg; iodine, 1.5 mg

PER = body weight gain (g)/CP intake (g)

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

Statistical analysis: The data were analyzed in a completely randomized design using GLM procedure of SAS (1999). Comparison of means was conducted by Duncan's multiple range test. Comparison of the average of chemical composition and mineral contents of the PBPM samples with NRC data was conducted using two-sided t-test (Zar, 1996).

Univariate and Corr procedures of SAS were used to determine the descriptive statistical parameters and

Table 2: Chemical composition (%), gross energy (kcal kg^{-1}), major elements (%), and trace elements (mg kg^{-1}) contents of the PBPM samples (as is)

	Plant A					Plant B		
Sample No.	1	2	3	4	5	6	7	8
Chemical Composition								
Dry matter	93.70	94.10	93.50	94.90	96.10	94.90	95.00	95.00
Ether extract	21.50	22.70	22.60	23.10	24.60	24.70	25.40	26.40
Crude protein	63.40	60.50	61.80	62.50	58.50	61.40	61.90	60.60
Crude fiber	0.85	0.91	0.98	0.81	0.84	0.75	0.73	0.78
Gross energy	5671.00	5526.00	5558.00	5680.00	5584.00	5814.00	5891.00	5876.00
Ash	7.90	10.30	9.50	8.20	12.20	7.40	7.00	7.30
Major Elements								
Calcium	3.36	3.69	3.54	3.42	4.25	3.15	2.95	3.04
Phosphorus	1.85	1.90	1.87	1.86	2.01	1.83	1.79	1.81
Sodium	0.52	0.47	0.48	0.49	0.56	0.51	0.48	0.46
Potassium	0.30	0.30	0.29	0.28	0.36	0.28	0.26	0.27
Chlorine	0.77	0.65	0.71	0.74	0.80	0.77	0.68	0.65
Magnesium	0.06	0.05	0.05	0.06	0.06	0.06	0.06	0.05
Sulfur	1.04	0.87	0.83	0.96	1.21	0.87	0.98	0.88
Trace Elements								
Iron	689.00	480.00	451.00	543.00	606.00	650.00	486.00	442.00
Copper	11.40	8.90	8.60	8.10	12.30	11.40	6.90	7.10
Manganese	14.80	15.40	15.20	15.00	16.40	14.30	13.40	13.70
Zinc	43.50	51.30	47.30	46.70	55.30	43.70	43.30	43.30
Selenium	0.70	0.76	0.75	0.74	0.80	0.71	0.66	0.63

	Plant C			Standard		NRC
Sample No.	9	10	Average	deviation	CV[1] (%)	(1994)
Chemical Composition						
Dry matter	95.10	95.70	94.80	0.82	0.86	93.00**
Ether extract	21.10	21.60	23.40	1.81	7.70	13.00**
Crude protein	56.50	57.50	60.50	2.26	3.70	60.00
Crude fiber	1.15	1.21	0.90	0.17	18.90	1.50**
Gross energy	5428.00	5425.00	5645.00	171.70	3.00	–
Ash	11.70	11.90	9.30	2.06	22.20	–
Major Elements						
Calcium	3.82	3.91	3.51	0.41	11.70	3.00**
Phosphorus	1.93	1.96	1.88	0.07	3.70	1.70**
Sodium	0.60	0.62	0.52	0.06	11.50	0.40**
Potassium	0.40	0.40	0.31	0.05	16.10	0.55**
Chlorine	0.82	0.83	0.74	0.07	9.50	0.54**
Magnesium	0.07	0.07	0.06	0.01	16.70	0.22**
Sulfur	1.10	1.15	0.99	0.13	13.10	0.51**
Trace Elements						
Iron	945.00	940.00	623.20	187.80	30.10	440.00**
Copper	8.90	9.10	9.30	1.85	19.90	14.00**
Manganese	23.80	23.30	16.50	3.80	23.00	11.00**
Zinc	52.00	52.00	47.80	4.48	9.40	120.00**
Selenium	0.77	0.78	0.73	0.05	6.90	0.75

**Indicating a highly significant difference (P<0.01) between the average value in our PBPM samples and that reported by NRC (1994), [1]CV = Coefficient of Variation

correlation coefficients between chemical composition and protein quality of the PBPM samples.

Results and Discussion

The chemical composition and mineral contents of the PBPM samples and their correlation coefficients are shown in Table 2 and 3, respectively. The average DM, EE, CP, CF and ash were 94.8, 23.4, 60.5, 0.90, and 9.3 percent, respectively. The average GE was 5645 kcal kg^{-1}. The coefficient of variation of CF and ash contents were 18.9 and 22.2 percent, respectively that were higher than the values for other chemical composition of the PBPM samples. The average DM of the PBPM samples (94.8%) was significantly higher (p<0.01) than that reported by NRC (1994). Pesti *et al.* (1986) reported a value of 94.6 percent for the average DM of eight PBPM samples. Also the average EE of the PBPM samples (23.4%) was significantly higher (p<0.01) than that

Table 3: The correlation coefficients between chemical composition and protein quality of the PBPM samples

	EE	CP	Ash	GE	PER	NPR
EE	1	0.253	-0.527	0.829	0.650	0.658
		(0.4804)*	(0.1174)	(0.0030)	(0.0419)	(0.0387)
CP		1	-0.822	0.631	0.702	0.691
			(0.0035)	(0.0503)	(0.0235)	(0.0269)
Ash			1	-0.891	-0.835	-0.842
				(0.0005)	(0.0026)	(0.0023)
GE				1	0.809	0.817
					(0.0046)	(0.0039)
PER					1	0.997
						(0.0001)
NPR						1

*The values in parenthesis are significance levels

reported by NRC (1994). That's why, fat is not removed from final product in rendering units producing PBPM in Iran. The high fat content may be the cause of the relatively higher DM percentage in our samples as compared with NRC (1994). Although the presence of high fat content can be beneficial in providing energy for animal, however it may facilitate the deterioration of product and reduce its nutritional value. Dale *et al.* (1993) also reported an average of 32.2 percent for EE in twenty two PBPM samples which is significantly higher than that reported by NRC (1994) or that obtained in the present study. The average CP of the PBPM samples (60.5%) was not significantly different from NRC (1994). The average CF of the PBPM samples (0.90%) was significantly lower ($p<0.01$) than that of NRC (1994). The origin of CF in PBPM is mostly mixing of crop and gizzard contents with other raw materials used in PBPM production. Poultry gizzard is used for human consumption in Iran and processed separately, therefore its contents is not mixed with other raw materials and this can be the reason for lower CF percentage in our samples as compared with NRC (1994). Wisman *et al.* (1958) also reported the values of 0.6 and 1 percent CF for two PBPM samples which is lower than that of NRC (1994). The average GE value of the PBPM samples was 5645 kcal kg^{-1}. Johnson and Parsons (1997) also reported a value of 5652 kcal kg^{-1} GE for one low ash PBPM sample. Pesti *et al.* (1986) reported a value of 4842 kcal kg^{-1} for the average GE of eight PBPM samples and this value is significantly lower than that obtained in the present study. The lower GE values for the PBPM samples reported by Pesti *et al.* (1986) as compared with our values is due to the lower EE and higher ash contents of samples analyzed by Pesti *et al.* (1986). The average ash content of the PBPM samples was 9.3 percent. Bhargava and O'Neil (1975) also reported a value of 9.15 percent for the average ash content of 201 poultry by-product and hydrolyzed feather meal samples. The high coefficient of variation for ash content as compared with that of other chemical composition indicating the use of different proportions of skeletal tissues in the PBPM samples examined in the present study. The correlation coefficients among ash, CP, EE, and GE are shown in Table 3. The CP, EE and GE values of the PBPM samples decreased as ash content increased. It seems that the ash content is a good indicator for prediction of other chemical composition of final product.

The major and trace element contents are also shown in Table 2. The average calcium and phosphorus contents of the PBPM samples (3.51 and 1.88 percent, respectively) were significantly higher ($p<0.01$) than those reported by NRC (1994). Pesti *et al.* (1986) reported the values of 5.14 and 2.36 percent, respectively, for the average calcium and phosphorus contents in eight PBPM samples whereas Dozier *et al.* (2003) reported the values of 5.17 and 2.50 percent for the average calcium and phosphorus contents in twenty six PBPM samples, respectively, which are significantly higher than those reported by NRC (1994). The lower calcium and phosphorus contents of the samples examined in the present study as compared with those reported by Pesti *et al.* (1986) or Dozier *et al.* (2003), may be due to the lower proportion of skeletal tissues in the raw material used in this study. The organs such as feet and head are used as a human food by low income families in Iran and therefore are less included in the final product. The average sodium and chlorine contents of the PBPM samples (0.52 and 0.74 percent, respectively) were significantly higher ($p<0.01$) than those reported by NRC (1994). Sodium and chlorine are the major elements of extracellular fluids and blood. After primary cutting of organs and tissues used for production of PBPM, blood and fluids present in them are released and mixed with the other materials and this seems to be the cause of higher sodium and chlorine contents in the samples evaluated in the present study as compared with that of NRC (1994). In addition, blood is also used for production of PBPM in Iran whereas the sodium and chlorine values reported by NRC (1994) are for the blood-free PBPM samples and these two factors are probably responsible for the higher sodium and chlorine contents in our samples. Dozier *et al.* (2003) reported a value of 0.67 percent for the average sodium content in twenty six PBPM samples which is higher than that of NRC (1994). The average potassium content of the PBPM samples (0.31%) was significantly lower ($p<0.01$) than that of NRC (1994). Potassium is one of the major intracellular elements and found in high amount in parts of viscera such as heart, liver and gizzard which are used for human consumption in Iran and this is probably the cause of the lower potassium level in the samples used in this study as compared with that reported by NRC (1994). The average magnesium content in the PBPM samples (0.06%) was significantly lower ($p<0.01$) than that of NRC (1994). Similar to calcium and phosphorus, magnesium is found in high amount in skeletal tissues such as feet and heads which are used for human consumption in

Table 4: Protein quality values for the PBPM samples during 6 days[1] assay period[2]

Protein Quality Index	Kilka Fish Meal	Plant A 1	Plant A 2	Plant A 3	Plant A 4	Plant A 5	Plant B 6	Plant B 7	Plant B 8	Plant C 9	Plant C 10	S.E.
PER[3]	2.35^{a}	2.00^{ef}	2.10^{cd}	2.04^{de}	1.95^{f}	1.83^{g}	2.17^{bc}	2.22^{b}	2.13^{bc}	1.75^{g}	1.76^{g}	0.03
NPR[4]	2.86^{a}	2.74^{bc}	2.80^{ab}	2.74^{bc}	2.66^{c}	2.53^{d}	2.85^{a}	2.85^{a}	2.82^{ab}	2.46^{d}	2.45^{d}	0.03
	Relative protein quality of the PBPM samples to Kilka fish meal (percent)											
PER	100.00	85.1	89.4	86.8	83.0	77.9	92.3	94.5	90.6	74.5	74.9	
NPR	100.00	95.8	97.9	95.8	93.0	88.5	99.7	99.7	98.6	86.0	85.7	

$^{a-g}$Means within each row with different superscripts are significantly different ($p<0.05$), [1]Assay length of 6 d = 8 to 14 d posthatching. [2]Means of four groups of five male chicks, average initial body weight at the start of assay was 114 g, [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) . S.E.: Standard Error

Table 5: Protein quality values for the PBPM samples during 9 days[1] assay period[2]

Protein Quality Index	Kilka Fish Meal	Plant A 1	Plant A 2	Plant A 3	Plant A 4	Plant A 5	Plant B 6	Plant B 7	Plant B 8	Plant C 9	Plant C 10	S.E
PER[3]	2.65^{a}	1.77^{ef}	1.92^{cd}	1.84^{de}	1.70^{f}	1.55^{g}	2.01^{bc}	2.05^{b}	1.98^{bc}	1.45^{h}	1.47^{gh}	0.03
NPR[4]	3.28^{a}	2.62^{ef}	2.75^{cd}	2.69^{de}	2.55^{f}	2.41^{g}	2.86^{b}	2.87^{b}	2.84^{bc}	2.34^{g}	2.31^{g}	0.04
	Relative protein quality of the PBPM samples to Kilka fish meal (percent)											
PER	100.00	66.8	72.5	69.4	64.2	58.5	75.9	77.4	74.7	54.7	55.5	
NPR	100.00	79.9	83.8	82.0	77.7	73.5	87.2	87.5	86.6	71.3	70.4	

$^{a-h}$Means within each row with different superscripts are significantly different ($p<0.05$), [1]Assay length of 9 d = 8 to 17 d posthatching. [2]Means of four groups of five male chicks, average initial body weight at the start of assay was 114 g, [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). S.E.: Standard Error

Iran and this may be the cause of the lower magnesium level in our samples as compared with that of NRC (1994). Dozier *et al.* (2003) reported a value of 0.15 percent for the average magnesium content in twenty six PBPM samples which is lower than that of NRC (1994) by about 32%. The average sulfur content of the examined samples (0.99%) was significantly higher ($p<0.01$) than the value reported by NRC (1994) and this is due to the presence of feather in the raw materials used for production of our samples. Feather contains high amount of sulfur amino acids such as cystine, whereas the sulfur value reported by NRC (1994) is for PBPM samples which are free of feather. The average iron content in our samples (623.2 mg kg^{-1}) was significantly higher ($p<0.01$) than that of NRC (1994). Blood which contains high amount of iron has been used for production of our PBPM samples, whereas the iron value reported by NRC (1994) is for blood-free PBPM samples. Dozier *et al.* (2003) also reported a value of 1830 mg kg^{-1} for the average iron content in twenty six PBPM samples which is significantly higher than that of NRC (1994) and the value obtained in this study. The average copper content in studied samples (9.3 mg kg^{-1}) was significantly lower ($p<0.01$) than that reported by NRC (1994). Copper is found in high amount in liver which is used for human consumption in Iran and this is probably why the copper level is lower in our samples. The average manganese content of the PBPM samples (16.5 mg kg^{-1}) was significantly higher ($p<0.01$) than that reported by NRC (1994). Dozier *et al.* (2003) also reported a value of 19 mg kg^{-1} for the average manganese content in twenty six PBPM samples which is significantly higher than that of NRC (1994) and the value obtained in our study. The average zinc content in our PBPM samples (47.8 mg kg^{-1}) was significantly lower ($p<0.05$) than the value reported by NRC (1994). Dozier *et al.* (2003) also reported a value of 94 mg kg^{-1} for the average zinc content in twenty six PBPM samples which is higher than that obtained in our study but it is lower than that of NRC (1994) by about 22%. Manganese and zinc are found in different proportions in all body tissues and their variations in each body organs and tissues used in production of PBPM is probably the cause of changes in their level among the PBPM samples. The average selenium content of the PBPM samples (0.73 mg kg^{-1}) was not significantly ($p>0.05$) different with NRC (1994) value.

The results of evaluating protein quality of the PBPM samples during the 6 or 9 days assay periods are shown in Table 4 and 5, respectively. The PER values of the PBPM samples were significantly lower ($p<0.05$) than that of Kilka fish meal in both periods. The content and digestibility of sulfur containing amino acids, lysine, tryptophan, threonine, phenylalanine, valine, leucine, isoleucine, and histidine in fish meal is higher than that of PBPM, therefore its protein quality is expected to be higher than that of PBPM (NRC, 1994). In addition, PBPM is deficient in sulfur containing amino acids, tryptophan and lysine (Main and Daghir, 1981, 1982; Wang and Parsons, 1998). Also, the PER values of our studied samples were significantly ($p<0.05$) different with each other in both periods. The average PER value

determined in the 6 days assay period was 2.00 with a range from 1.75 to 2.22. The average PER value determined in the 9 days assay period was 1.77 and its range varied from 1.45 to 2.05. The average PER value of the PBPM samples in the present study was lower than that reported by Escalona *et al.* (1986); Herro *et al.* (1988); Douglas *et al.* (1997) and Johnson and Parsons (1997). The range of PER for our samples was lower than that reported by Herro *et al.* (1988) for PBPM and Douglas *et al.* (1997) for spent hen meal. In the case of NPR, the values for the most PBPM samples were significantly lower than that of Kilka fish meal in the first 6 days, but in the 9 days assay period, all the NPR values were significantly lower ($p<0.05$) than that of Kilka fish meal. In addition, in each assay period there were significant differences ($p<0.05$) in the NPR values among the PBPM samples. The average NPR value for the PBPM samples in this study was lower than that reported by Escalona *et al.* (1986), Douglas *et al.* (1997) and Johnson and Parsons (1997). The variation of the NPR values for our samples was lower than that reported by Douglas *et al.* (1997) for spent hen meal.

As expected, the NPR values for the PBPM samples were higher than that of the PER, because NPR evaluates the protein quality at both maintenance and growth levels, whereas PER evaluates the protein quality only at the growth level (Jansen, 1978). The relative values of protein quality for the PBPM samples to Kilka fish meal are shown in Table 4 and 5. As noted, in the 6 days assay period, the average relative values of PER and NPR for the PBPM samples as compared with Kilka fish meal were 84.9 and 94.1 percent, respectively and these were reduced to 67.0 and 80.0 percent, respectively in the 9 days assay period. These results indicated that although the relative protein quality for the PBPM samples is higher in the 6 days assay period, however these values decreased in the 9 days assay period probably due to increasing the amino acids requirements for maintenance and growth as age increased. As shown in Table 3, a significant positive correlation coefficient (0.997) was found between PER and NPR values and significant negative correlation coefficients were obtained between ash and PER values (-0.837) and ash and NPR values (-0.843). Parsons *et al.* (1997) also found a significant negative correlation coefficient (-0.80) between ash and PER values for sixteen meat and bone meal samples. With regard to these results, it seems that the ash content can be used as an index for evaluating the protein quality of PBPM.

The exact reason of changes in protein quality among the PBPM samples used in this study is unclear. It is suggested that the variation in protein quality of the PBPM samples may be related to factors such as ash content (Johnson and Parsons, 1997; Johnson *et al.*, 1998), composition of the raw material (Johnson and Parsons, 1997; Johnson *et al.*, 1998), temperature, pressure, and duration of cooking (McNaughton *et al.*, 1977), method of processing (Robbins and Firman, 2006) and duration of the raw material storage prior to rendering (Tamim and Doerr, 2003). The ash content of an animal protein source is an indicator of its bone content. The presence of bone decreases the protein quality of product. Bone approximately contains 25 to 30 percent crude protein and its protein is reportedly made up of 83% collagen, which is devoid of tryptophan and is deficient in most other essential amino acids especially sulfur containing amino acids (Johnson and Parsons, 1997; Leeson and Summers, 2001). The temperature used in the cooking tank is one of the other factors that highly influences the protein quality (McNaughton *et al.*, 1977; Opstvedt *et al.*, 1984). An increase in cooking temperature is accompanied with a decrease in protein quality and digestibility of amino acids such as lysine and cysteine (McNaughton *et al.*, 1977; Opstvedt *et al.*, 1984). Cysteine is much more sensitive to heat than lysine. Overheating causes the formation of disulfide bonds and consequently increases the passage rate of protein through the gastrointestinal tract (Opstvedt *et al.*, 1984). 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 via the formation of bond between the amine group of basic amino acids such as lysine and arginine and reducing sugars like glucose and fructose. 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). The change in pressure is the other factor that reduces protein quality and amino acids digestibility of PBPM. An increase in the pressure from 15 to 45 pounds per square inch (PSI) during the cooking period decreases the availability of lysine (McNaughton *et al.*, 1977). The storage time of the raw materials prior to rendering is another factor that influences the protein quality of PBPM. Long term storage causes the degradation of amino acids by bacterial activities and production and accumulation of biogenic amines that decrease the protein quality of final product and these toxic compounds adversely affect the animal performance (Tamim and Doerr, 2003).

Conclusions: In summary, the results showed that the DM and EE contents of studied PBPM samples were different and the CP content was similar to NRC (1994) value. The GE content of the PBPM samples used in this study was higher than that reported in literature which may be due to the higher EE and lower ash in our

samples. The negative correlation coefficients found between the ash content and either EE, CP and GE value which may be used to predict the chemical composition of the PBPM samples. The mineral contents of our PBPM samples except of selenium were different from NRC (1994). The PER and NPR values for the PBPM samples during the 6 and 9 days assay periods were lower than that of Kilka fish meal. Also, the PER and NPR values for the PBPM samples during the 6 and 9 days assay periods were different. The negative correlation coefficients were obtained between the ash and PER and ash and NPR values that may be used to predict the protein quality of the PBPM samples. In general, the results of the present study indicate that the chemical composition, mineral content and protein quality of PBPM may vary greatly and needs to be evaluated continuously.

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