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Determination of Chemical Composition, Mineral Contents, and Protein Quality of Iranian Kilka Fish Meal

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Abstract: In order to study the chemical composition, mineral contents and protein quality of Iranian Kilka fish meal, 6 samples of Kilka fish meal were provided from three commercial rendering plants. The proximate analysis showed that the average Dry Matter (DM), Ether Extract (EE), Crude Protein (CP), Crude Fiber (CF) and ash content of the Kilka fish meal samples were 94.5, 22.9, 59.1, 0.62, and 13.2 percent, respectively. The average Gross Energy (GE) value for the Kilka fish meal samples was 5574 kcal kg^{-1}. The average values of major elements including Ca, P, Na, K, Cl, Mg, and S were 3.97, 2.61, 0.83, 0.52, 0.64, 0.27, and 0.39 percent, respectively, and these values for trace elements including Fe, Cu, Mn, Zn, and Se were 229.3, 6.2, 3.7, 74.5, and 1.58 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 Kilka fish meal or Peruvian fish meal or Chile fish meal (both as positive controls) 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 Kilka fish meal samples and varied between 2.41 to 3.41 and 3.07 to 3.97, respectively. The PER and NPR values for the Kilka fish meal samples were significantly lower ($p<0.05$) than that of Peruvian fish meal and Chile fish meal.

Key words: Kilka fish meal, chemical composition, protein quality, broilers

Introduction

Fish meal can be produced from whole fish or wastes from the use of fish prepared for human consumption (Ponce and Gernat, 2002), but currently fish meal is mostly produced from smaller oily fish caught specifically for fish meal production (Leeson and Summers, 2001, 2005). High quality fish meal has excellent amino acid balance (Rand *et al*., 1960; Ponce and Gernat, 2002) and is rich in protein, calcium, phosphorus, iron, vitamin B_{12}, choline, niacin, pantothenic acid and riboflavin (Grau and Williams, 1955; Rand *et al*., 1960; Miller, 1970; Waldroup and Adams, 1994; NRC, 1994; Ponce and Gernat, 2002). In addition, fish meal is a good source of energy (Ponce and Gernat, 2002) and omega-3 fatty acids (Hulan *et al*., 1989; Burke *et al*., 1997). Fish meal is used as a source of protein and unidentified growth factors in diets of monogastric animals like pig (Kim and Easter, 2001), Poultry (Sibbald and Wolynetz, 1984; Wu *et al*., 1984; Ponce and Gernat, 2002) and aquatic animals (Anderson *et al*., 1993; Steffens, 1994) and also extensively used as a ruminally undegradable protein source in ruminant diets (Petit and Flipot, 1992; Petit and Castonguay, 1994; Burke *et al*., 1997; Walz *et al*., 1998). The chemical composition, mineral contents and protein quality of fish meal can vary greatly depending on the species of fish used (Wu *et al*., 1984; Ponce and Gernat, 2002), freshness of the raw materials (Anderson *et al*., 1993), conditions and length of storage (Kellenbarger, 1961; Anderson *et al*., 1993) amount of residual oil (Anderson *et al*., 1993), processing method and handling condition (Grau and Williams, 1955; Kellenbarger, 1961; Smith and Scott, 1965; Schumaier and McGinnis, 1969; Whitacre and Latshaw, 1982; Anderson *et al*., 1993), drying method and temperature (Anderson *et al*., 1993; Rosselot *et al*., 1996), and whether the meal is made from whole fish or the waste from some other processing operation (Anderson *et al*., 1993), thus fish meal needs to be evaluated continuously. Determination of chemical composition of fish meal is important in estimating its metabolizable energy content (Sibbald and Wolynetz, 1984; NRC, 1994) and measurement of its mineral content especially calcium and phosphorus is of significance to include fish meal in the balanced diets (NRC, 1994; Leeson and Summers, 2001, 2005).

Currently, mathematical equations 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 (Johnston and Coon, 1979; Douglas *et al*., 1997; 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).

In Iran, the Kilka fish (*Clupeonella engrauliformis*) is obtained from the Caspian Sea in Mazandaran and Gilan provinces. The most part of the catch is processed into fish meal by different processing methods. Now, there are 9 rendering plants in Iran that produce Kilka fish meal by processing whole Kilka fish, 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 fish meal in several countries (Grau and Williams, 1955; Rand *et al*., 1960; Smith and Scott, 1965; Schumaier and McGinnis, 1969; Miller, 1970; Sibbald and Wolynetz, 1984; Anderson *et al*., 1993; Ponce and Gernat, 2002), but the chemical composition and protein quality changes of Kilka fish meal 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 Iranian Kilka fish meal.

Materials and Methods

Sample collection: Kilka fish meal samples were collected from three commercial rendering plants in Iran so that samples #1 and #2 were taken from plant A, samples #3 and #4 were taken from plant B and samples #5 and #6 were taken from plant C. The raw material source of all Kilka fish meal samples was whole Kilka fish. After grinding and mixing the samples, all samples were stored at -20^{o}C until further analysis.

Chemical analysis: The Dry Matter (DM), Ether Extract (EE), Crude Protein (CP), Crude Fiber (CF), Gross Energy (GE) and ash contents of the Kilka fish meal 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 Kilka fish meal samples, 300 day-old Ross male broiler chicken were purchased and housed in an environmentally controlled 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. The chicks were housed in thermostatically controlled starter batteries placed in an environmentally regulated room. Light was provided 24 h daily. Feed and water were supplied for *ad libitum* consumption.

To determine the protein quality of the Kilka fish meal 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 eight semipurified diets, each contained one of 6 Iranian Kilka fish meal samples, one sample of Peruvian fish meal and one sample of Chile fish meal (both as positive controls) as the sole source of dietary protein. The semipurified diets were formed by replacing each of six Kilka fish meal samples, Peruvian fish meal sample or Chile fish meal sample as the sole source of dietary protein with a portion of the cornstarch and glucose in the nitrogen-free basal diet to provide 10% CP containing diets. Each of the experimental diets was fed 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 PER = body weight gain (g)/CP intake (g), and 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

Table 1: Feed ingredients and nutrients 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 eight semipurified diets were formed by replacing each of six Kilka fish meal samples and one sample of Peruvian fish meal and one sample of Chile fish meal as the sole source of dietary protein with a portion of the 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

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

	Kilka fish Meal					
	Plant A		Plant B		Plant C	
Sample No.	1	2	3	4	5	6
Chemical Composition						
Dry Matter	94.3	95.2	93.8	93.5	95.5	94.8
Ether Extract	23.3	21.5	24.4	25.5	20.3	22.3
Crude Protein	60.6	56.1	60.1	61.1	57.1	59.4
Crude Fiber	0.57	0.74	0.52	0.49	0.76	0.64
Gross Energy	5539.00	5431.00	5682.00	5897.00	5362.00	5531.00
Ash	12.8	14.2	12.3	12.1	14.4	13.6
Major Elements						
Calcium	3.86	4.25	3.69	3.61	4.33	4.08
Phosphorus	2.52	2.76	2.45	2.49	2.81	2.63
Sodium	0.75	0.92	0.71	0.73	0.99	0.86
Potassium	0.48	0.58	0.46	0.44	0.61	0.55
Chlorine	0.66	0.71	0.54	0.52	0.73	0.68
Magnesium	0.23	0.30	0.25	0.24	0.32	0.28
Sulfur	0.46	0.26	0.42	0.49	0.33	0.38
Trace Elements						
Iron	211.0	253.0	206.0	194.0	272.00	240.00
Copper	5.7	7.3	4.9	4.6	7.9	6.8
Manganese	3.5	4.3	3.3	2.9	4.4	3.7
Zinc	72.2	83.7	66.5	58.3	89.2	77.2
Selenium	1.62	1.71	1.41	1.32	1.79	1.63

Sample No.	Average	Standard Deviation	CV[1] (%)	NRC Data for Anchovy Fish Meal	NRC Data for Herring Fish Meal	NRC Data for Menhaden Fish Meal
Chemical Composition						
Dry Matter	94.5	0.79	0.84	92**	93**	92**
Ether Extract	22.9	1.91	8.3	5**	10**	9.4**
Crude Protein	59.1	2.02	3.42	64.2**	72.3**	60.05
Crude Fiber	0.62	0.11	17.7	1**	0.7	0.7
Gross Energy	5574.0	192.1	3.45	–	–	–
Ash	13.2	0.98	7.4	–	–	–
Major Elements						
Calcium	3.97	0.30	7.6	3.73	2.29**	5.11**
Phosphorus	2.61	0.15	5.7	2.43**	1.7**	2.88**
Sodium	0.83	0.11	13.3	0.65**	0.61**	0.65**
Potassium	0.52	0.07	13.5	0.69**	1.09**	0.65**
Chlorine	0.64	0.09	14.1	0.6	0.9**	0.6
Magnesium	0.27	0.04	14.8	0.24	0.15**	0.16**
Sulfur	0.39	0.09	23.1	0.54**	0.69**	0.45
Trace Elements						
Iron	229.3	30.4	13.3	220	140**	440**
Copper	6.2	1.34	21.6	9**	6	11**
Manganese	3.7	0.58	15.7	10**	5**	33**
Zinc	74.5	11.31	15.2	103**	132**	147**
Selenium	1.58	0.18	11.4	1.36**	1.93**	2.1**

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

average of chemical composition and mineral contents of the Kilka fish meal samples with NRC data was conducted using two-sided t-test (Zar, 1996).

Univariate and Corr procedures of SAS were used for determination of descriptive statistical parameters and correlation coefficients between chemical composition and protein quality of the Kilka fish meal samples.

Results and Discussion

The chemical composition and mineral contents of the Kilka fish meal samples and their correlation coefficients are shown in Table 2 and 3, respectively. The average DM, EE, CP, CF, and ash were 94.5, 22.9, 59.1, 0.62, and 13.2 percent, respectively. The average gross energy was 5574 kcal kg^{-1}. The coefficient of

Table 3: The correlation coefficients between chemical composition and protein quality of the Kilka fish meal samples

	EE	CP	Ash	GE	PER	NPR
EE	1	0.857	-0.978	0.962	0.838	0.842
		(0.0290)*	(0.0007)	(0.0021)	(0.0372)	(0.0352)
CP		1	-0.911	0.796	0.885	0.885
			(0.0116)	(0.0582)	(0.0190)	(0.0192)
Ash			1	-0.909	-0.850	-0.853
				(0.0120)	(0.0319)	(0.0307)
GE				1	0.818	0.821
					(0.0469)	(0.0452)
PER					1	0.997
						(0.0001)
NPR						1

*The values shown in parenthesis under the correlation coefficients are significance levels

variation of EE, CF and ash were 8.3, 17.7 and 7.4 percent, respectively that were higher, as compared with the coefficient of variation of other chemical composition of the Kilka fish meal samples. The average DM of the Kilka fish meal samples (94.5%) was significantly higher ($p<0.01$) than those reported by NRC (1994) for Anchovy, Herring and Menhaden fish meals. Ponce and Gernat (2002) also reported a value of 94.60 percent for the DM of one tilapia by-product meal sample, whereas Anderson *et al*. (1993) reported a value of 95.45 percent for the average DM of six fish meal samples which are significantly higher than those reported by NRC (1994) for Anchovy, Herring and Menhaden fish meals. Also, the average EE of the Kilka fish meal samples (22.9%) was significantly higher ($p<0.01$) than those reported by NRC (1994) for Anchovy, Herring and Menhaden fish meals, because fat is not removed from final product in rendering units producing Kilka fish meal in Iran. The high fat content may be the cause of the relatively higher DM in our Kilka fish meal samples as compared with those reported by 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. The average CP of the Kilka fish meal samples (59.1%) was significantly lower ($p<0.01$) than those reported by NRC (1994) for Anchovy and Herring fish meals but not from Menhaden fish meal. Miller (1970) reported a value of 64.48 percent for the average CP of eight fish meal samples, whereas Ponce and Gernat (2002) reported a value of 63.50 percent for the CP of one tilapia by-product meal sample. The average CF of the Kilka fish meal samples (0.62%) was significantly lower ($p<0.01$) than that reported by NRC (1994) for Anchovy fish meal but not from those of Herring and Menhaden fish meals. Similarly, Ponce and Gernat (2002) reported a value of 0.58 percent for the CF of one tilapia by-product meal sample, whereas Sibbald and Wolynetz (1984) reported a value of 0.72 percent for the average CF of fifteen Menhaden fish meal samples. The average GE value of the Kilka fish meal samples was 5574 kcal kg^{-1}. Sibbald and Wolynetz (1984) reported a value of 4667 kcal kg^{-1} for the average GE of fifteen Menhaden fish meal samples and this value is significantly lower than that obtained in the present study. The lower GE values reported by Sibbald and Wolynetz (1984) as compared with our values may be due to the lower EE and higher ash contents of samples analyzed by Sibbald and Wolynetz (1984). The average ash content of the Kilka fish meal samples was 13.2 percent. Anderson *et al*. (1993) reported a similar value of 12.85 percent for the average ash content of six fish meal samples. As shown in Table 3, the CP, EE and GE values of the Kilka fish meal 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 of samples are also shown in Table 2. The average calcium content of the Kilka fish meal samples (3.97%) was not significantly different from that reported by NRC (1994) for Anchovy fish meal, but was significantly higher ($p<0.01$) than that of Herring fish meal and lower ($p<0.01$) than that of Menhaden fish meal value. Waldroup *et al*. (1965) reported a value of 4.27 percent for the average calcium content of four fish meal samples, whereas Miller (1970) reported a value of 4.50 percent for the average calcium of eight fish meal samples. The average phosphorus content of our samples (2.61%) was significantly higher ($p<0.01$) than those reported by NRC (1994) for Anchovy and Herring fish meals but was significantly lower ($p<0.01$) than that of Menhaden fish meal. Miller (1970) also reported a value of 2.56 percent for the average phosphorus content of eight fish meal samples, whereas this value was reported 2.57 percent by Waldroup *et al*. (1965) for four fish meal samples, which are higher than those reported by NRC (1994) for Anchovy and Herring fish meals but lower than that of Menhaden fish meal. The average sodium content of the Kilka fish meal samples (0.83%) was significantly higher ($p<0.01$) than those reported by NRC (1994) for Anchovy, Herring and Menhaden fish meals. Anderson *et al*. (1993) also reported a value of 0.89 percent for the average sodium content of six fish meal samples which is higher than those reported by NRC (1994). The average potassium content of the Kilka fish meal samples (0.52%) was significantly lower ($p<0.01$) than those reported by NRC (1994) for Anchovy, Herring and Menhaden fish meals. Anderson *et al*. (1993) reported a value of 0.81 percent for the average potassium content of six fish meal samples which is higher than those reported by NRC (1994) for Anchovy and Menhaden fish meals but lower than that of Herring fish meal value. The average chlorine content of examined samples (0.64%) was not significantly different from those reported by NRC (1994) for Anchovy and Menhaden fish meals but was significantly lower ($p<0.01$) than that of Herring fish meal. The average magnesium content of the Kilka fish

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

Protein Quality Index	Peruvian Fish Meal	Chile Fish Meal	Kilka Fish Meal: Plant A 1	Plant A 2	Plant B 3	Plant B 4	Plant C 5	Plant C 6	Standard Error
PER	3.95[a]	3.63[b]	2.96[d]	2.29[g]	2.61[e]	3.17[c]	2.35[g]	2.48[f]	0.03
NPR	4.36[a]	4.07[b]	3.44[d]	2.84[g]	3.11[e]	3.64[c]	2.86[g]	2.99[f]	0.04
			The relative protein quality of the Kilka Fish meal samples to Peruvian fish meal (percent)						
PER	100.00	91.9	74.9	58.0	66.1	80.3	59.5	62.8	
NPR	100.00	93.3	78.9	65.1	71.3	83.5	65.6	68.6	
			--- The relative protein quality of the Kilka Fish meal samples to Chile fish meal (percent) ----						
PER	108.8	100.0	81.5	63.1	71.9	87.3	64.7	68.3	
NPR	107.1	100.0	84.5	69.8	76.4	89.4	70.3	73.5	

[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 posthatch.
[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)

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

Protein Quality Index	Peruvian Fish Meal	Chile Fish Meal	Kilka Fish Meal: Plant A 1	Plant A 2	Plant B 3	Plant B 4	Plant C 5	Plant C 6	Standard Error
PER	4.25[a]	3.86[b]	3.18[d]	2.41[h]	2.80[e]	3.41[c]	2.50[g]	2.65[f]	0.03
NPR	4.71[a]	4.36[b]	3.76[d]	3.07[g]	3.42[e]	3.97[c]	3.14[g]	3.28[f]	0.03
			The relative protein quality of the Kilka Fish meal samples to Peruvian fish meal (percent)						
PER	100.00	90.8	74.8	56.7	65.9	80.2	58.8	62.4	
NPR	100.00	92.6	79.8	65.2	72.6	84.3	66.7	69.6	
			--- The relative protein quality of the Kilka Fish meal samples to Chile fish meal (percent) ----						
PER	110.1	100.0	82.4	62.4	72.5	88.3	64.8	68.7	
NPR	108.0	100.0	86.2	70.4	78.4	91.1	72.0	75.2	

[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 posthatch.
[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)

meal samples (0.27%) was not significantly different from that reported by NRC (1994) for Anchovy fish meal but was significantly higher ($p<0.01$) than those of Herring and Menhaden fish meals. Anderson *et al*. (1993) also reported a value of 0.23 percent for the average magnesium content of six fish meal samples. The average sulfur content of the Kilka fish meal samples (0.39%) was significantly lower ($p<0.01$) than NRC (1994) values for Anchovy and Herring fish meals but was not significantly different from that of Menhaden fish meal. The average iron content of our samples (229.3 mg kg^{-1}) was not significantly different from that reported by NRC (1994) for Anchovy fish meal but was higher ($p<0.01$) than Herring fish meal value and lower ($p<0.01$) than that of Menhaden fish meal. Anderson *et al*. (1993) reported a value of 164.5 mg kg^{-1} for the average iron of six fish meal samples which is higher than that reported by NRC (1994) for Herring fish meal but is lower than those of Anchovy and Menhaden fish meals. The average copper content of the Kilka fish meal samples (6.2 mg kg^{-1}) was significantly lower ($p<0.01$) than NRC (1994) values for Anchovy and Menhaden fish meals but was not significantly different from that of Herring fish meal. Anderson *et al*. (1993) reported a value of 5.1 mg kg^{-1} for the average copper content of six fish meal samples which is lower than those reported by NRC (1994) for either Anchovy, Herring or Menhaden fish meals. The average manganese content of our studied samples (3.7 mg kg^{-1}) was significantly lower ($p<0.01$) than those reported by NRC (1994) for Anchovy, Herring and Menhaden fish meals. Anderson *et al*. (1993) reported a value of 11.2 mg kg^{-1} for the average manganese content of six fish meal samples which is also different from NRC (1994) values. The average zinc content of the Kilka fish meal samples (74.5 mg kg^{-1}) was significantly lower ($p<0.01$) than those reported by NRC (1994) for Anchovy, Herring and Menhaden fish meals. Similarly, Anderson *et al*. (1993) reported a value of 93 mg kg^{-1} for the average zinc content of six fish meal samples which is lower than those of NRC (1994) values. The average selenium content of the Kilka fish meal samples (1.58 mg kg^{-1}) was significantly higher ($p<0.01$) than that of NRC (1994) value for Anchovy fish meal but was significantly lower ($p<0.01$) than those for Herring and Menhaden fish meals.

The results of evaluating protein quality of the Kilka fish meal samples during 6 or 9 days assay periods are shown in Table 4 and 5, respectively. In both assay periods, the PER values of the Kilka fish meal samples were significantly lower ($p<0.05$) than those of Peruvian fish meal and Chile fish meal. In the 6 days assay period, the PER values of the Kilka fish meal samples, except of samples #2 and #5, were significantly ($p<0.05$) different with each other, but in the 9 days assay period, the PER values of all Kilka fish meal samples were significantly ($p<0.05$) different from each other. The average PER value determined in 6 days assay period was 2.64 with the range from 2.29 to 3.17. The average PER value determined in 9 days assay period was 2.83 and its range varied from 2.41 to 3.41. The average PER value of the Kilka fish meal samples in the present study was higher than that reported by Douglas *et al*. (1997) for spent hen meal and Johnson and Parsons (1997) for poultry by-product meal. 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 poultry by-product meal, therefore its protein quality is expected to be higher (NRC, 1994). The range of PER for the Kilka fish meal samples in our study was lower than that reported by Douglas *et al*. (1997) for spent hen meal.

Also, in both assay periods, the NPR values of the Kilka fish meal samples were significantly lower ($p<0.05$) than those of Peruvian and Chile fish meals. In addition, in both assay periods, the NPR values of the Kilka fish meal samples, except of samples #2 and #5, were significantly ($p<0.05$) different with each other. The average NPR value determined in 6 days assay period was 3.15 with the range from 2.84 to 3.64, and this value was 3.44 in 9 days assay period with the range varied from 3.07 to 3.97. The average NPR value for the Kilka fish meal samples in this study was higher than that reported by Douglas *et al*. (1997) for spent hen meal and Johnson and Parsons (1997) for poultry by-product meal. The variation of the NPR values for the Kilka fish meal samples was lower than that reported by Douglas *et al*. (1997) for spent hen meal.

As expected, the NPR values for the Kilka fish meal 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). In each assay period, the relative values of protein quality for the Kilka fish meal samples to Peruvian and Chile fish meals are shown in Table 4 and 5. As noted, in the 6 days assay period, the average relative values of PER for the Kilka fish meal samples as compared with Peruvian and Chile fish meals were 66.9 and 72.8 percent, respectively, whereas in the 9 days assay period, these values were 66.5 and 73.2 percent, respectively. In the 6 days assay period, the average relative values of NPR for the Kilka fish meal samples as compared with Peruvian and Chile fish meals were 72.2 and 77.3 percent, respectively, whereas these values were 73.0 and 78.9 percent in the 9 days assay period, respectively. 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 (-0.850) and also ash and NPR values (-0.853). 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 Kilka fish meal. The exact reason of changes in protein quality among the Kilka fish meal samples used in this study is unclear. It is suggested that the variation in protein quality of the Kilka fish meal samples may be related to factors such as freshness of the raw materials (Anderson *et al*., 1993), conditions and length of storage (Kellenbarger, 1961; Anderson *et al*., 1993) amount of residual oil (Anderson *et al*., 1993), processing method and handling condition (Grau and Williams, 1955; Kellenbarger, 1961; Smith and Scott, 1965; Schumaier and McGinnis, 1969; Whitacre and Latshaw, 1982; Anderson *et al*., 1993), and drying method and temperature (Anderson *et al*., 1993; Rosselot *et al*., 1996). The temperature used in the cooking tank is one of the factors that highly influence the protein quality (Anderson *et al*., 1993; Rosselot *et al*., 1996; 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 cystein (Opstvedt *et al*., 1984). Cystein 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 causes 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). Freshness of the raw materials is another factor that influences the protein quality of fish meal. 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 (Anderson *et al*., 1993).

According to the present results, it was found that the chemical composition, mineral contents and protein quality of fish meal samples vary greatly depend on the species of fish used, conditions and length of storage, processing method and handling condition, and drying method and temperature, so needs to be evaluated continuously.

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