# Determination of prececal nutrient digestibility of raw, protease-supplemented shrimp meal and fish meal in broilers using regression approach

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**Abstract** This study was carried out to measure the prececal protein and ether extract digestibility of shrimp and fish meal using the regression approach. A total of 280 Cobb 500 day-old broilers (mixed sexes) were randomly allotted to 7 treatment groups (4 replicates of 10 broilers each) in a completely randomized design. Feed intake was higher in the birds that received protease-supplemented shrimp meal and fish meal compared to the 4% raw shrimp meal and basal diets (P<0.05). Average daily gain, feed conversion ratio, final body weight, relative weight of the digestive organs, length of ileum and dressing percentage were not affected by the treatments. The effect of treatments on apparent digestibility of ether extract, organic matter, and dry matter was significant. The highest ether extract apparent digestibility was observed in the group that received 4% raw shrimp meal. Prececal digestibility of protein and ether extract was not different among raw shrimp meal, protease-supplemented shrimp meal and fish meal diets.

Keywords: broiler, fish meal, digestibility, performance, shrimp meal

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# Introduction

Shrimp meal is the dried waste of the shrimp industry. A large amount of waste is produced because a large proportion of shrimp consists of the waste parts including the heads, exoskeleton, and soluble components (Gernat, 2001). About 45-48% by weight of shrimp raw material is discarded as waste depending on species (Kandra et al., 2012). Shrimp waste is also a serious environmental pollutant (Ibrahim et al., 1999); however, it is a good source of protein, fat, and minerals, with an amino acid profile comparable to that of fish meal (Nargis et al., 2006). Shrimp meal was shown to have the potential to replace the conventional protein sources in the layer (Gernat, 2001) and broiler diets (Rosenfeld et al., 1997; Oduguwa et al., 2004). Fish meal is the most common but expensive animal protein feed ingredient, and its replacement with other by-products of economical and adequate nutritional value can reduce the feed cost in poultry (Aktar et al., 2011).

The use of shrimp by-product presents several problems that limit its use as a feed ingredient. Chitin physically blocks the access of the digestive enzyme to proteins and lipids (Septinova et al., 2010). Oduguwa et al. (2004) showed that a high level of shrimp meal, could decrease the growth rate and feed efficiency in broilers. Ngoan et al. (2000) showed that amino acid composition of shrimp meal was fairly balanced, but the low methionine content could limit its value for monogastric animals. Other factors, such as high calcium content, could limit the amount of shrimp meal in monogastric diets (Septinova et al., 2010). The digestive tract of broiler does not produce enough chitinase (Mahata et al., 2008); therefore, shrimp meal needs to be processed to improve its nutritional quality in poultry diets. Hydrolysis and fermentation treatments can be applied to improve shrimp meal nutritional value but very little data have been published on the effect of hydrolyzed shrimp meal in broilers. An objective of this study was to determine the effect of substituting protease-supplemented shrimp meal with fish meal on broiler performance.

Estimation of endogenous protein and amino acids in digesta collected from the distal ileum is important for estimation of the prececal digestibility that is digestion before the ceca. This method was introduced to avoid the possible effect of cecal microbes on digestibility. Several methods have been developed to correct the endogenous losses including the regression analysis (Fan and Sauer, 1997). In the regression analysis, the estimates of the slopes are not affected by the endogen-

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ous nutrient losses, which are considered in the estimated intercept of the regression model (Rezvani et al., 2008a). Although the apparent nutrient digestibility of shrimp meal was reported (Khempaka et al., 2006), little research has been done concerning protein and ether extract digestibility of shrimp meal using regression approach; therefore, the present study also aimed at determining raw and protease supplemented shrimp meal protein and ether extract prececal digestibility and comparing them with fish meal by using the regression approach.

# Materials and methods

#### Birds and experimental treatments

The experiment was conducted at the Experimental Re-

search Station of Animal Science, College of Agriculture, Shiraz University. A total of 280 day-old Cobb 500 broilers (mixed sex) were randomly allotted to 7 treatment groups (4 replicates of 10 broilers each) in a completely randomized design. The birds were maintained under similar management conditions. Sun-dried shrimp meal was grounded and used as raw or proteasesupplemented meal (XAP; Avizyme 1505, Danisco Animal Nutrition, Marlborough, Wiltshire, UK), using 200 mg enzyme per kg shrimp meal (Oxenboll et al., 2011). Shrimp meal contained 7.87% of moisture, 45.12% of crude protein and 5.14% of crude fat based on analyze (Nutrition Lab of Animal Science Department, College of Agriculture, Shiraz University) and 9.26% of calcium, 1.01% of available phosphorus and 1870 kcal/kg AMEn based on literature (Aktar et al., 2011).

**Table 1.** Ingredients and chemical composition of the basal, raw shrimp meal (RSM), protease-supplemented shrimp meal (PSM) and fish meal (FM) diets fed to broilers from 28 to 42 d of age.

	Basal diet	RSM 4%	RSM 8%	PSM 4%	PSM 8%	FM 4%	FM 8%	
Ingredients (%)								
Corn grain	62.93	62.93	62.93	62.93	62.93	62.93	62.93	
Soybean meal	22.76	22.76	22.76	22.76	22.76	22.76	22.76	
Biofin <sup>®</sup> concentrate <sup>1</sup>	2.50	2.50	2.50	2.50	2.50	2.50	2.50	
Fish meal	0.00	0.00	0.00	0.00	0.00	4.00	8.00	
Shrimp meal	0.00	4.00	8.00	4.00	8.00	0.00	0.00	
Corn starch	8.00	4.00	0.00	4.00	0.00	4.00	0.00	
CaCO <sub>3</sub>	0.96	0.96	0.96	0.96	0.96	0.96	0.96	
Dicalcium phosphate	1.41	1.41	1.41	1.41	1.41	1.41	1.41	
NaCl	0.46	0.46	0.46	0.46	0.46	0.46	0.46	
<sup>2</sup> Premix/Vitamin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	
<sup>2</sup> Premix/mineral	0.25	0.25	0.25	0.25	0.25	0.25	0.25	
DL-Methionine	0.22	0.22	0.22	0.22	0.22	0.22	0.22	
Threonine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	
Rice hull	0.05	0.05	0.05	0.042	0.034	0.05	0.05	
L-Lysine HCl	0.16	0.16	0.16	0.16	0.16	0.16	0.16	
Enzyme <sup>3</sup>	0.00	0.00	0.00	0.008	0.016	0.00	0.00	
$Cr_2O_3$	0.20	0.20	0.20	0.20	0.20	0.20	0.20	
Calculated analysis (as fed basis)								
ME (kcal/kg)	2979.5	2916.0	2852.4	2916.0	2852.4	2944.4	2909.2	
Crude protein	16.09	17.89	19.69	17.89	19.69	18.66	21.23	
Ca (%)	0.77	1.14	1.51	1.14	1.51	0.92	1.06	
Available P (%)	0.38	0.42	0.46	0.42	0.46	0.48	0.57	
Methionine	0.48	0.59	0.71	0.59	0.71	0.56	0.63	
Lysine	0.46	0.63	0.80	0.63	0.80	0.67	0.87	
Methionine +Cysteine	0.75	0.83	0.92	0.83	0.92	0.85	0.96	
Threonine	0.41	0.58	0.75	0.58	0.75	0.52	0.63	
Tryptophan	0.21	0.24	0.28	0.24	0.28	0.24	0.27	
Na	0.19	0.20	0.21	0.20	0.21	0.22	0.24	

<sup>1</sup>Biofin<sup>®</sup> concentrate Provided per kg: ME, 2949.2 kcal/kg, CP, 18.5%, Ca, 0.65%, Available P, 0.40%, Cl, 0.16, Na, 0.14%, Lysine, 1.10, Methionine, 0.49, Methionine +Cysteine, 0.80%, Threonine, 0.72, Tryptophan, 0.20.

<sup>2</sup> Premix: Provided per kg: vitamin A, 7350 IU; vitamin D<sub>3</sub>, 2200 ICU; vitamin E, 8 IU; riboflavin, 5.5 mg; d-pantothenic acid, 13.0 mg; niacin, 36 mg; choline, 500 mg; vitamin B<sub>12</sub>, 0.02 mg; menadione, 2 mg; folic acid, 0.5 mg; thiamine mononitrate, 1.0 mg; pyridoxine, 2.2 mg; d-biotin, 0.05 mg; Cu, 6.0 mg; Fe, 54.8 mg; I, 1.0 mg; Mn, 65.3 mg; Se, 0.3 mg; Zn, 55.0 mg.

<sup>3</sup> Protease: XAP; Avizyme 1505, Danisco Animal Nutrition, Marlborough, Wiltshire, UK (200 mg enzyme per kg shrimp meal).

Until 28th d of age all birds received the same commercial broiler diet, and from days 28-42 seven experimental finisher diets were fed based on Cobb 500 Manual, 2014 recommendation (Table 1). The experimental diets were, T<sub>1</sub>: basal diet (0% shrimp meal and 8% corn starch), T<sub>2</sub>: basal diet in which 4 % raw shrimp meal was substituted for corn starch, T<sub>3</sub>: basal diet in which 8 % raw shrimp meal was substituted for corn starch, T<sub>4</sub>: basal diet in which 4 % protease-supplemented shrimp meal was substituted for corn starch, T<sub>5</sub>: basal diet in which 8 % protease-supplemented shrimp meal was substituted for corn starch,  $T_6$ : basal diet in which 4 % fish meal was substituted for corn starch, T7: basal diet in which 8 % fish meal was substituted for corn starch in the basal diet. Chromic oxide (Cr<sub>2</sub>O<sub>3)</sub> was added to the diets (0.2%) as an indigestible marker for measuring the prececal digestibility.

Body weight and feed intake were measured weekly on a pen basis, and average daily gain, feed intake, and feed conversion ratio (FCR) calculated. After the birds had been slaughtered at 42<sup>nd</sup> d of age the gizzard, ileum, and pancreas of one bird per pen were dissected out and weighed for calculation of their relative weight. The digesta of all birds in each pen were obtained by flushing the last two-third segment of the intestine, between the Meckel's diverticulum and 2 cm anterior to the ileoceca-colonic junction, using distilled water (Rezvani et al., 2008b). The digesta were frozen at -20°C immediately, and then vacuum-dried and ground before analysis. The dried digesta and diets were analyzed for dry matter (DM), ash, ether extract (EE) and crude protein (CP), according to AOAC (1990). The content of  $Cr_2O_3$ was measured in the diets and digesta using atomic absorption spectrophotometry (Shimadzu, AA 670, Tokyo, Japan) according to Williams et al. 1962 (Vries et al., 2014). Apparent prececal nutrient (N) digestibility of the diets was calculated using the following equation:

$$\begin{array}{l} Digestibility~(\%) = 100 - 100 \times \left[(Cr_2O_{3Diet} \times N_{Digesta})/(Cr_2O_{3Digesta} \times N_{Diet})\right] \end{array} \tag{1}$$

in which,  $Cr_2O_{3Diet}$  and  $Cr_2O_{3Digesta}$  represent the concentrations of  $Cr_2O_3$  in the diet and digesta samples (g/kg DM) and N<sub>Diet</sub> and N<sub>Digesta</sub> stand for the concentrations of nutrients in the diet and digesta samples (g/kg DM), respectively.

#### Statistical analysis

Digestibility and performance data were analyzed by one-way ANOVA, using the General Linear Model procedure of SAS (2004). Linear regression analysis, as suggested by Rodehutscord et al. (2004), was used to determine the prececal protein and ether extract digestibility, where digested protein and ether extract were regressed on the protein and ether extract intakes, respectively. As basal endogenous loss was included through the intercept ( $\alpha$ ), no correction was made for basal endogenous losses. The following model was applied to determine the prececal protein and ether exctract digestibility of test meal (Rezvani et al., 2008a and 2008b):

$$Y_{tij} = \alpha + \beta_{ti} X_{ti} + e_{tij},\tag{2}$$

where,  $Y_{tij}$  = the amount of daily digested protein from test meal-containing diet (mg/d),  $\alpha$  = intercept,  $\beta_{ti}$  = prececal digestibility of protein and ether extract originating from test meal,  $X_{ti}$  = daily intake of protein and ether extract originating from test meal, and  $e_{tij}$  = error term with mean 0 and normal distribution. Means were compared using the Duncan's multiple range test (P<0.05).

### Results

No significant differences were observed in broiler performance except for feed intake when protease-supplemented shrimp meal and fish meal was substituted with corn starch in the basal diet. Feed intake was higher in birds that received protease-supplemented shrimp meal and fish meal compared to the 4% raw shrimp meal diet and the basal diet (P<0.05; Table 2). No significant differences among treatments were observed for carcass percentages, relative weight of digestive organs, and ileal length (Table 2).

The effect of diets on apparent nutrient digestibility is shown in Table 3. Apparent protein and ash digestibility were not affected but apparent ether extract digestibility in birds receiving 4% raw shrimp and fish meal was higher than those receiving 8% raw shrimp meal, 4 and 8% protease-supplemented shrimp meal, 8% fish meal and the basal diet. Apparent organic matter digestibility in birds fed 4 and 8% raw shrimp meal, fish meal and 4% protease-supplemented shrimp meal was higher than those of the basal diet. Apparent organic matter digestibility in the birds that received 4% protease-supplemented shrimp meal was higher than that of the group fed with 8% protease-supplemented shrimp meal, i.e. shrimp meal supplementation of the diet decreased apparent organic matter digestibility. A significant increase (P<0.05) in dry matter digestibility was observed with raw and protease-supplemented shrimp meal and fish meal at 4% replacement compared to the basal diet. Overall, inclusion of shrimp meal and fish meal at 4% replacement improved the apparent ether extract, organic matter and dry matter digestibility

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Measurement	Basal Diet	RSM	RSM	PSM	PSM	FM	FM	SEM	P valve
	Busul Blot	4%	8%	4%	8%	4%	8%	5 Lin	1 (41)0
Feed intake (g/bird/d)	114.8	105.4	120.0 <sup>bc</sup>	133.0 <sup>a</sup>	141.1 <sup>a</sup>	131.9 <sup>ab</sup>	133.4 <sup>ab</sup>	5.81	0.008
Average daily gain (g/bird/d)	116.4	113.7	109.6	122.5	111.1	114.4	124.1	9.36	NS
Feed conversion ratio	0.98	0.93	1.16	1.13	1.34	1.17	1.07	0.12	NS
Final body weight (g)	2015.6	1909.3	1959.9	2084.3	1869.7	1977.2	2066.0	70.89	NS
Dressing percentage (%)	61.9	59.8	62.3	62.9	67.4	60.1	58.1	2.07	NS
Gizzard (%)	2.9	2.5	2.6	2.1	2.4	2.6	2.4	0.19	NS
Pancreas (%)	0.42	0.46	0.41	0.44	0.33	0.47	0.42	0.04	NS
Ileum <sup>1</sup> (%)	13.1	11.6	12.3	13.3	10.9	15.1	12.3	1.38	NS
Ileal length <sup>1</sup>	69.6	71.6	73.	77.9	73.8	76.0	73.4	2.46	NS

**Table 2.** Effect of substituting shrimp meal and fish meal on performance, carcass percentages, relative weight of digestive organs, and ileal length in Cobb 500 broilers

<sup>a-c</sup> Within each row, means with common superscript (s) do not differ (P < 0.05).

<sup>1</sup>From Meckel's diverticulum to ileo-ceca-colonic junction.

Raw shrimp meal (RSM, 4%, and 8%), protease-supplemented shrimp meal (PSM, 4% and 8%) and fish meal (FM, 4% and 8%) were substituted the corn starch in the basal diet.

**Table 3.** Effect of substituting shrimp meal and fish meal on apparent prececal digestibility of crude protein (CP), ether extract (EE), organic matter (OM), dry matter (DM), ash and prececal digestibility of crude protein and ether extract using the linear regression approach in Cobb 500 broilers diet

Apparent digestibility (%)	Basal	RSM 4%	RSM 8%	PSM 4%	PSM 8%	FM 4%	FM 8%	SEM	P value
СР	77.2	77.2	77.1	77.5	77.4	77.9	77.5	0.45	NS
EE	69.6 <sup>c</sup>	81.4 <sup>a</sup>	74.0 <sup>b</sup>	74.0 <sup>b</sup>	72.6 <sup>bc</sup>	80.5 <sup>a</sup>	73.1 <sup>bc</sup>	1.43	< 0.0001
OM	71.2 <sup>d</sup>	75.2ª	73.0 <sup>b</sup>	74.4 <sup>ab</sup>	72.5 <sup>cd</sup>	75.4ª	74.3 <sup>ab</sup>	0.51	< 0.0001
DM	70.3°	71.6 <sup>ab</sup>	70.5 <sup>bc</sup>	72.3ª	70.6 <sup>bc</sup>	71.6 <sup>ab</sup>	71.4 <sup>abc</sup>	0.43	0.03
Ash	49.0	49.4	49.5	50.6	47.9	49.8	49.1	0.87	NS
Prececal digestibility	Fish meal Protea			supplemente	ed shrimp	Dow chrimp mool			
(%)	FISH mean		meal			Kaw Shi	mp mear		
СР		77.1		76.9		76	5.6	0.01	NS
EE	8	33.5		84.5		84	l.1	0.03	NS

<sup>a-d</sup>Within each row, means with common superscript (s) do not differ (P < 0.05).

Raw shrimp meal (RSM, 4%, and 8%), protease-supplemented shrimp meal (PSM, 4% and 8%) and fish meal (FM, 4% and 8%) substituted the corn starch in the basal diet.



Figure 1. Prececal digestibility of protein using the regression approach in Cobb 500 broilers diet

in comparison to the basal diet. There were no differences among raw, protease-supplemented shrimp meal and fish meal in prececal protein and ether extract digestibility (Table 3; Figure 1).

#### Discussion

In this study, no significant differences were observed in broiler performance except for feed intake when protease-supplemented shrimp meal and fish meal were substituted with corn starch in the basal diet. These data are in agreement with results found by Rosenfeld et al. (1997) and Ilian et al. (1985) who included shrimp meal in the broiler diet. Shrimp meal up to 10% in broiler diets had no any negative effects on broiler performance (Ilian et al., 1985). In the present study, a significant improvement (P<0.05) in feed consumption was observed with 4 and 8% protease-supplemented shrimp meal compared to the raw shrimp meal. These might be attributed to the reduced level of chitin binding proteins by protease. However, Mahata et al. (2008) showed that feed intake was not affected by shrimp meal inclusion in the diet. Because of the non-significant differences in performance and carcass attributes among the treatments, it is concluded that shrimp meal can substitute the fish meal in the broiler diet without an adverse effect.

Data on carcass traits confirmed the findings of Okonkwo et al. (2012) and Mahata et al. (2008) that reported no significant differences in the relative weight of the digestive organs when shrimp meal was included in broiler diet.

Prececal digestibility measurements are useful in evaluating the feed quality, because of preventing the influence of post-ileal microbial activity. In this experiment, substitution of shrimp meal for fish meal in the broiler diet had no adverse effect on apparent prececal dry matter digestibility, but Khempeka et al. (2006) showed that shrimp meal decreased dry matter digestibility as result of the presence of chitin. Such discrepancy may be due to our shorter duration of feeding in the present study. Protease-supplemented shrimp meal did not improve apparent ether extract digestibility probably due to the kind of enzyme in the current experiment.

In this study, the regression method was used to determine prececal digestibility in shrimp and fish meal because this method can correct endogenous losses of protein and ether extract (Rezvani et al., 2008a). If discrepancies in the basal endogenous gut losses between the experimental diets had existed, they would not have been related to the digestibility values calculated for the protein sources (Rodehutscord et al., 2004). The regression approach proposed by Rodehutscord et al. (2004) in broilers and Rezvani et al. (2008a) in cecetomized laying hens can be a suitable method for estimating the protein digestibility. Data on prececal ether extract digestibility are scarce. According to our findings, prececal digestibility of crude protein and ether extract of shrimp meal and fish meal was the same. Therefore, it can be concluded that shrimp meal can be used as a suitable protein source in broiler diets when included up to 8%; with performance traits being more in line with prececal digestibility calculated based on regression rather than apparent digestibility.

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# تعیین گوارش پذیری پیش سکومی مواد غذایی پودر پسماندهی میگوی خام، فرآوری شده با آنزیم پروتیاز و پودر ماهی با استفاده از روش رگرسیون در جوجههای گوشتی

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چکیده این پژوهش به منظور اندازه گیری گوارش پذیری استاندارد پیش سکومی پروتین و چربی خام برای پودر ماهی و پس مانده ی پودر میگو با استفاده از روش رگرسیون انجام شد. شمار ۲۸۰ قطعه جوجه گوشتی سویهی کاب ۵۰۰ از مخلوط هر دو جنس به شیوه تصادفی به ۷ گروه تیمار (۴ تکرار به ازای هر تکرار ۱۰ پرنده) در یک طرح کاملا تصادفی تقسیم شدند. اثر تیمار پر مصرف خوراک معنیدار بود. به طوری که مصرف خوراک روزانه در تیمارهای دارای پودر پسمانده ی فرلاوری شده با آنزیم و پودر ماهی نسبت به جیره ی دارای ۴ درصد پودر میگوی خام و جیره ی پایه بیشتر بود. اثر تیمار بر افزایش وزن روزانه، ضریب تبدیل خوراک، وزن نهایی پرنده، وزن نسبی اندامهای گوار شی، طول ایلئوم و در صد لاشه معنیدار نبود. اثر تیمار بر گوارشپذیری ظاهری چربی خام، ماده آلی و ماده خشک معنیدار بود. بیشترین محمد کوارش پذیری ظاهری چربی خام در تیمار دارای چهار در صد پسمانده ی میگوی خام و دیره شد. گوارشپذیری استاندارد پیش سکومی پروتین و چربی خام برای پسمانده ی میگوی خام و دیره شد. گوارشی طول ایلئوم در صد گوارش پذیری ظاهری چربی خام در تیمار دارای چهار در صد پسمانده ی میگوی خام دیده شد. گوارش پذیری استاندارد پیش سکومی پروتین و چربی خام برای پسمانده میگوی خام و میده بر تازیم پروتناز و ماهی خام تفاوت معنی داری نداشت.

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