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Ileal Digestibility of Amino Acids in Fish Meal-Based Diets for Broiler Starters Using Regression Technique

Research Article

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ABSTRACT

An experiment was conducted to determine apparent ileal digestibility of crude protein (CP) and amino acids (AA) and digestible CP and AA of fish meal (FM) in 21-d-old broiler chickens using the regression technique. Four diets containing 0, 1, 2, and 3% FM in place of cornstarch were formulated with FM as the sole contributor of CP and AAs in the sequential diets. Eighty 14-d-old broiler chicks were weighed, and sorted by body weight and randomly allotted to the experimental diets with 4 replicates per diet and 5 chicks per replicate. Titanium dioxide was added to the diets at the rate of 5 g/kg diet as an indigestible marker. They were fed the experimental diets till d 21 post hatch when they were killed by asphyxiated with CO_2 and digesta from the last two-third of ileum collected. The concentrations of CP and AA in the diets increased as dietary FM increased. Apparent digestibility of CP and AA significantly (P<0.05) increased with dietary FM inclusion but with a decrease in the 3% FM diet. The digestibility values of CP, lysine and methionine estimated as slopes of the regression of the amount of CP or AA digested dry matter intake dry matter intake (DMI) at the terminal ileum against CP or AA intake dry matter intake (DMI) were 88.7, 97.2 and 97.0% respectively. The digestible amounts of CP, lysine and methionine of FM were 63.63 g, 5361.04 mg and 1801.45 mg. Results of the study suggest a maximum level of 2% inclusion of FM was adequate for broiler starter chicks as higher levels may induce higher endogenous CP and AA flow and hence reduced digestibility of these nutrients.

KEY WORDS amino acids, broiler starters, fish meal, ileal digestibility, regression technique.

INTRODUCTION

The major concern of the nutritionists has been how to ensure that amino acid (AA) requirements of fast growing broilers are met. However, manipulation of AA of undigested proteins by hind-gut microbes is of a great concern. Circumventing the intervention of these microbes to improve efficiency of AA utilization in birds has necessitated the measurement of AA flow at the terminal ileum. Similarly, in terms of feed evaluation it has been argued that the measurement of AA flow at the terminal ileum is a more reliable measure of the value of the AAs to chickens than the measurement of total AA excretion (Ravindran *et al.* 1999; Rodehutscord *et al.* 2004).

A large volume of published data on the AA digestibility of raw materials for poultry is available, but different techniques have been shown to lead to great differences in the estimate of endogenous losses (Ravindran and Bryden, 1999; Donkoh and Moughan, 1999; Jansman *et al.* 2002; Rodehutscord *et al.* 2004; Ravindran *et al.* 2005; Hoehler *et al.* 2006; Adedokun *et al.* 2007; Adedokun *et al.* 2008; Adedokun *et al.* 2011), and all techniques are subject to certain limitations and criticism (Adedokun *et al.* 2011). Thus, approaches that do not depend on a separate determination of endogenous losses appear advantageous for the purpose of feed evaluation (Rodehutscord *et al.* 2004).

Fish meal (FM) is among the best sources of high-quality protein for animals with excellent AA profile. High-quality fish meal normally contains between 65% and 72% crude protein, 5.57% lysine and 1.87% methionine (Agboola, 2011). FM has been widely used as a supplemental protein for many years primarily for monogastrics (Lemme *et al.* 2001). The aim of this study therefore, was to estimate the AA digestibility of FM in broilers using a linear regression approach.

MATERIALS AND METHODS

Experimental diets and management of animals

Four diets were prepared containing 0, 1, 2 and 3% each of FM. Diet 1 with no FM served as the control (Table 1).

Table 1 Gross composition (g/100 g DM) of experimental broiler diets with varying levels of fish meal (n=4 replicates of 5 birds each)

Test Ingredient	Fish Meal (%)			
Ingredient	0	1	2	3
Cornstarch	35.00	34.00	33.00	32.00
Wheat offal	0.00	-	-	-
Fish meal (72% CP)	0.00	1.00	2.00	3.00
Rice bran	11.50	11.50	11.50	11.50
Maize	10.00	10.00	10.00	10.00
Soyabean meal	15.00	15.00	15.00	15.00
Groundnut cake	24.00	24.00	24.00	24.00
Bone meal	2.00	2.00	2.00	2.00
Oyster shell	1.50	1.50	1.50	1.50
Titanium dioxide	0.50	0.50	0.50	0.50
Broiler premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00

Titanium dioxide was included as a dietary marker at a level of 5 g/kg. Eighty broiler chicks (Arbor Acre strain) were brooded for 2 weeks in a standard poultry house. They were fed a standard broiler starter diet and given water *ad libitum*. On day 14, the birds were randomly allotted to the 4 treatment diets by body weight and their weights equalized across the pens. Each dietary treatment had 4 replicates of 5 birds each. The birds were fed their respective experimental diets *ad libitum* for a further 7 days.

Collection of digesta

On day 21, the birds were weighed after which they were killed by asphyxiation in carbon dioxide. They were immediately cut open and the terminal two thirds of the section between Meckel diverticulum and 2 cm anterior to the ileo-caeco-colonic junction were severed. The contents were flushed out with distilled water, pooled according to replicates and frozen. The frozen samples were then freeze-dried and milled for further analysis.

Digestibility calculations

Apparent ileal CP and AA digestibility were calculated using the following equation.

$$D_{CP} \text{ or } _{AA}(\%) = [1 - (TiO_{2diet}/TiO_{2digeta}) \times (CP \text{ or } AA_{digesta} / CP \text{ or } AA_{diet})] \times 100$$

Where:

D_{CP} or $_{AA}$: percentage apparent ileal crude protein or amino acid digestibility.

TiO_{2diet}: concentration of titanium dioxide in the diet (%).

$TiO_{2digesta}$: concentration of titanium dioxide in the digesta (%).

CP or $AA_{digesta}$: concentration of crude protein or amino acid in the digesta (%).

CP or AA_{diet}: concentration of crude protein or amino acid in the diet (%).

The digestibility values of CP or AA in the diets was used to calculate the amount of CP (g/d) or AA (mg/d) digested up to the terminal ileum. The CP (g/d) or AA (mg/d) intake was calculated from the data of feed intake and CP or AA concentrations in the diets.

The digested CP or AA was then plotted against the CP or AA intake using Graphpad Prism 4.0. The slope of the graph represented the digestibility of CP or AA in FM. The digestible CP or AA in the test ingredients was then calculated using the formulae:

$$\text{Digestible CP} / AA_{FM} = \text{Amount of CP} / AA_{FM} \times \text{digestibility coefficient}_{FM}$$

Chemical analysis

The proximate composition of the diets and digesta was determined according to methods of AOAC (2000). The concentrations of titanium dioxide in feed and digesta were estimated by photometric technique of Brandt and Allam (1987). Amino acid analysis was determined by HPLC (AOAC, 2000; Method 982.30).

Statistical analysis

Data were analyzed as a completely randomized design using the GLM procedure of SAS (2006). Means were separated using Tukey HSD method and regression techniques using GraphPad Prism 4.0.

RESULTS AND DISCUSSION

Amino acid digestibility of broilers fed graded levels of fish meal-based diets

The results of analyzed CP and AA concentrations of experimental diets are shown in Table 2.

Table 2 Analyzed crude protein (g/100 g) and amino acids (g/100 CP) of experimental diets (n=4 replicates of 5 birds each)

Item	Control (0%)	1% FM	2% FM	3% FM
Dry matter	90.00	91.30	90.40	90.00
Crude protein	19.30	21.57	22.15	32.89
Essential amino acids				
Arginine	2.38	2.79	2.93	3.72
Histidine	0.68	0.83	0.90	0.86
Isoleucine	1.02	1.35	1.54	1.51
Leucine	2.00	2.50	2.82	2.75
Lysine	1.21	1.82	2.26	2.32
Methionine	0.32	0.52	0.71	0.75
Phenylalanine	1.65	1.32	1.74	1.62
Threonine	0.86	1.16	1.37	1.35
Tryptophan	0.29	0.35	0.38	0.35
Valine	1.21	1.61	1.82	1.82
Non-essential amino acids				
Alanine	1.20	1.65	1.97	1.99
Aspartic acid	2.97	3.63	3.99	3.77
Cysteine	0.32	0.36	0.38	0.35
Glutamic acid	4.43	5.26	5.66	5.33
Glycine	1.32	1.77	2.05	2.07
Hydroxyproline	0.07	0.06	0.14	0.21
Ornithine	0.02	0.02	0.04	0.05
Proline	1.30	1.53	1.76	1.58
Serine	1.22	1.44	1.62	1.47
Tyrosine	0.89	1.06	1.15	1.06

FM: fish meal.

Concentrations of CP and most of the AA increased in sequential diets with increase in FM. The results of apparent ileal digestibility of dry matter, crude protein and AA in broilers fed FM diets are shown in Table 3. Amount of dietary FM significantly ($P<0.05$) increased apparent ileal CP and AA digestibility across the diets. An ileal digestibility value of AA was greater than 90% except for threonine in diet 4 containing 3% FM.

A level of 3% dietary FM (diet 4) resulted in lower apparent AA digestibility, perhaps due to higher endogenous AA flow in birds on these diets. The amount of digestible CP, lysine and methionine calculated from the regression of their digested amounts (g or mg/d DMI) against ingested amounts (g or mg/d DMI) as represented in Figures 1, 2 and 3 respectively were 63.63g, 5361.04 mg and 1801.45 mg.

Amino acid digestibility of broilers fed graded levels of fish meal-based diets

The level of essential AA analyzed for fish meal diets was highest in diet containing 2% fish meal except for lysine and methionine and least in birds fed the control diet which had 0% FM. Fish meals are produced from many types and varieties of fish and the diverse range of raw materials may account for much of the variations in the feedstuff (Ravindran and Blair, 1993).

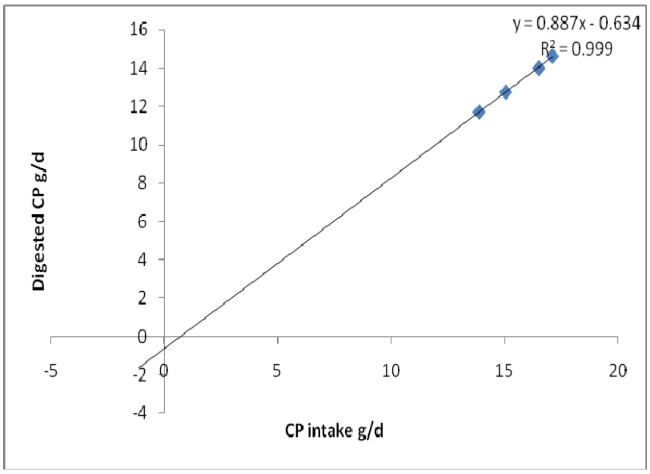

Figure 1 Relationship between amounts of crude protein ingested and digested by broiler chickens fed fish meal diets
Crude protein (CP) in fish meal (FM)= 72.45 g
The digested CP was plotted against the CP intake using regression technique (Graphpad Prism 4.0). The slope of the graph represented the digestibility of CP in FM.
The digestible CP in FM was then calculated using the formulae:
Digestible CP_{FM}= amount of CP_{FM} × digestibility $coefficient_{FM}$
Y= 72.45 × 0.887 - 0.634= 63.63 g
Y= mX + c;

Where:
Y: digestible CP content in FM
m: regression slope (% digestibility)
c: intercept
X: CP in FM

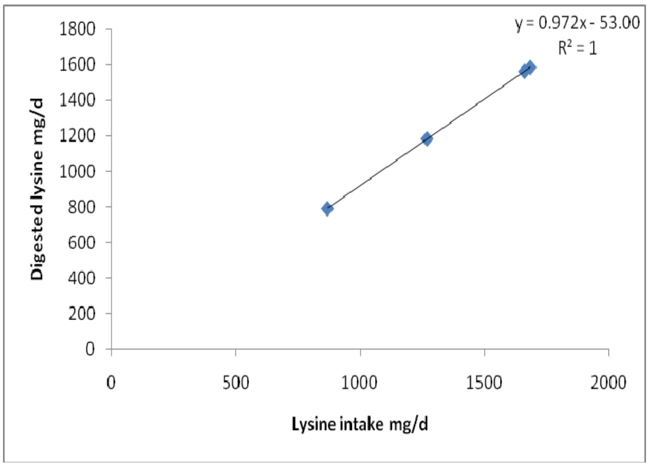

Figure 2 Relationship between amounts of lysine ingested and digested by broiler chickens fed fish meal diets
Lysine in fish meal (FM)= 5570 mg
The digested lysine (mg/d) was plotted against the lysine intake (mg/d) using regression technique (Graphpad Prism 4.0). The slope of the graph represented the digestibility of lysine in FM
The digestible lysine in FM was then calculated using the formulae:
Digestible $Lysine_{FM}$= amount of $Lysine_{FM}$ × digestibility $coefficient_{FM}$
Y= 5570 × 0.972 - 53.00= 5361.04 mg

Y= mX + c;

Where:
Y: digestible lysine content in FM
m: regression slope (% digestibility)
c: intercept
X: lysine in FM

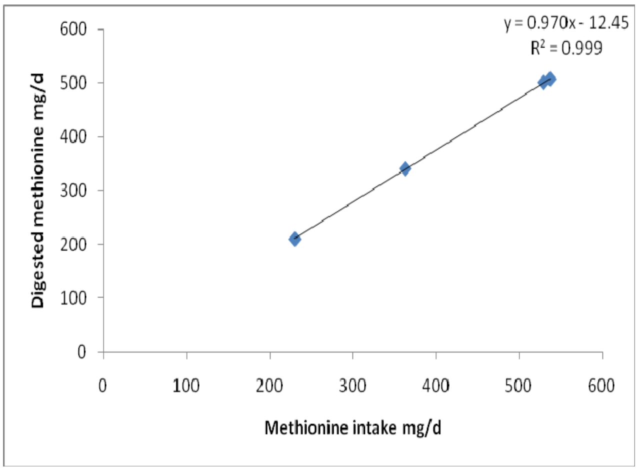

Figure 3 Relationship between amounts methionine ingested and digested by broiler chickens fed fish meal diets
Methionine (Met) in fish meal (FM)= 1870 mg
The digested Met (mg/d) was plotted against the Met intake (mg/d) using regression technique (Graphpad Prism 4.0). The slope of the graph represented the digestibility of Met in FM
The digestible Met in FM was then calculated using the formulae:
Digestible Met_{FM}= Amount of Met_{FM} × digestibility $coefficient_{FM}$
Y= 1870 × 0.970 - 12.45= 1801.45 mg
Y = mX + c

Where:
Y: digestible Met content in FM
m: regression slope (% digestibility)
c: intercept
X: Met in FM

Digestibility of AA at the end of the ileum is currently widely considered a suitable measure of feed protein quality in birds, as postileal fermentation may have variable contribution to AA excretion (Ravindran *et al.* 1999).

Fish meal belongs to a short list of excellent feedstuffs that provide essential nutrients with high digestibility. The apparent ileal AA digestibility increased with increasing amount of FM in the diets. The decreased amino acid digestibility for the 3% dietary FM can be attributed to the increased endogenous AA flow (Adedokun *et al.* 2007). According to Adedokun *et al.* (2011), two reasons can be adduced for this. A possible explanation is increased mucin production and concentration of the digesta resulting from increased mucus hydrolysis in the presence of high levels of dietary CP. Another reason could be due to increase in secretion of digestive enzymes, also in response to increased protein and peptide in the gut. Mucine production has been reported to contain high concentrations of glutamic acid, aspartic acid, leucine, threonine, proline and serine (Lemme *et al.* 2004; Ravindran and Hendriks, 2004; Adedokun *et al.* 2011) and low amounts of methionine and histidine (Adedokun *et al.* 2011). Since FM was the ingredient solely responsible for increase in the amount of CP and AA in the diets (Table 2) the increase in digestibility was due to the sequential increase in FM. These results agree with those of Rodehutscord *et al.* (2004) who reported increased AA digestibility in broiler chickens fed diets containing 60, 120, 180, 240 and 300 g/kg rapeseed meal. Also, Rezvani *et al.* (2008) reported increased digestibility of amino acids in cecectomized laying hens when they were fed 0, 15 or 30% toasted soybean or corn gluten meal. The reports of (Adedokun *et al.* 2007; Adedokun *et al.* 2011) of increased AA concentrations in diets containing increasing amounts of casein from 0 to 150 g/kg and hence increase in standardized ileal digestibility of AA in broiler chicks and turkey poults support results of this study of increased amino acids digestibility in response to increase in dietary FM. According to Rodehutscord *et al.* (2004) and Rezvani *et al.* (2008), increased apparent AA digestibility is in response to amino acid intake, which in turn is linearly related to the increase in the test ingredient in the diets when the regression is method is used. The high digestibility values of the AA in FM can be attributed to the adequate AA balance in FM since it has been reported that protein in FM has a high proportion of essential AA in a highly digestible form, particularly methionine, cysteine, lysine, threonine and tryptophan. The higher digestibility of essential AAs in FM observed in this study might be due to relatively high concentrations of lysine, arginine and sulfur amino acids in the meal. Another possibility may be that selective absorption occurring from AA mixtures significantly modifies the pattern of AA availability and utilization.

A comparison of ileal digestibility of the essential and non-essential AAs in FM revealed considerable variations. This variation might reflect the differences in amino acid composition, structure and distribution of protein in the test feedstuff because it has been shown that apparent AA digestibility depends on the protein and amino acid contents in the experimental diet (Fan and Sauer, 1994; Angkanaporn *et al.* 1997). The regression method, which was employed in this study, has been reported to be useful in estimating the amount of digested nutrient in a feed ingredient (Fan *et al.* 1995; Fan *et al.* 2001; Rodehutscord *et al.* 2004). According to Rodehutscord *et al.* (2004), when the amount of CP or amino acid digested (DMI) up to the terminal ileum is regressed against the intake (DMI) the deviation of the slope from 1 is caused by the amount of unabsorbed CP or AA and the endogenous CP or AA related to the test ingredient.

Table 3 Apparent ileal digestibility (%) of crude protein and amino acids of experimental diets in broiler chicks (n=4 replicates of 5 birds each)

Item	Control (0%)	1% FM	2% FM	3% FM	SEM	P-value
Dry matter	71.48^{d}	73.33^{c}	78.34^{b}	79.10^{a}	0.096	< 0.0001
Crude protein	84.14^{d}	84.54^{c}	84.63^{b}	85.34^{a}	0.003	< 0.0001
Essential amino acids						
Arginine	96.24^{c}	96.72^{a}	96.52^{b}	96.06^{d}	0.004	< 0.0001
Histidine	90.71^{b}	92.56^{a}	92.53^{a}	92.00^{b}	0.021	< 0.0001
Isoleucine	90.03^{d}	92.53^{b}	92.74^{a}	92.22^{c}	0.004	< 0.0001
Leucine	91.23^{d}	93.14^{b}	93.28^{a}	92.67^{c}	0.004	< 0.0001
Lysine	91.02^{c}	93.20^{b}	94.03^{a}	94.02^{a}	0.021	< 0.0001
Methionine	91.22^{d}	94.02^{c}	94.71^{a}	94.53^{b}	0.005	< 0.0001
Phenylalanine	92.76^{c}	94.08^{a}	94.05^{a}	93.13^{b}	0.013	< 0.0001
Threonine	86.55^{d}	90.73^{b}	91.34^{a}	89.98^{c}	0.038	< 0.0001
Tryptophan	92.67^{c}	93.43^{b}	94.84^{a}	93.19^{b}	0.068	< 0.0001
Valine	89.31^{c}	92.17^{b}	92.47^{a}	92.21^{b}	0.023	< 0.0001
Non-essential amino acids						
Alanine	89.95^{d}	92.96^{c}	93.55^{a}	93.39^{b}	0.003	< 0.0001
Aspartic acid	91.53^{d}	93.34^{a}	93.24^{b}	92.56^{c}	0.003	< 0.0001
Cysteine	82.28^{d}	86.19^{a}	86.08^{b}	84.38^{c}	0.003	< 0.0001
Glutamic acid	93.53^{d}	94.54^{a}	94.42^{b}	94.08^{c}	0.003	< 0.0001
Glycine	87.58^{d}	91.03^{b}	91.65^{a}	90.93^{c}	0.015	< 0.0001
Hydroxyproline	60.84^{d}	80.72^{c}	88.22^{b}	88.93^{a}	0.004	< 0.0001
Ornithine	49.38^{d}	63.87^{c}	76.18^{b}	82.28^{a}	0.013	< 0.0001
Proline	88.71^{d}	91.63^{b}	91.83^{a}	90.87^{c}	0.004	< 0.0001
Serine	90.95^{d}	92.97^{b}	93.13^{a}	92.42^{c}	0.004	< 0.0001
Tyrosine	93.15^{d}	94.17^{b}	94.29^{a}	93.24^{c}	0.003	< 0.0001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.
FM: fish meal.
P Anova (P=0.05).

Thus, the slope of the graph represents the amount of digested CP or AA in the ingredient. The value of the slope when the regression method is used is closer to the true digestibility of CP or AA since both basal and specific endogenous losses are accounted for. In the current study, the slopes of the curves were 88.7, 97.2 and 97.0% for CP, lysine and methinone respectively.

These values represent the true digestibility of these nutrients in FM and as expected are higher than the apparent digestibility values (Table 3) with the difference accounted for by the endogenous flow.

Reports of other workers using different techniques (Ravindran and Hendriks, 2004; Adedokun *et al*. 2007; Kim *et al*. 2012) agree with these results.

Furthermore, with the true digestibility values the amount of digested CP and lysine and methionine were determined to be 63.63 g, 5361.04 mg and 1801.45 mg respectively. These results are in agreement with results from other studies on nitrogen or AA flow at the terminal ileum (Short *et al*. 1999; Rodehutscord *et al*. 2004).

The regression method earlier proposed and validated by Fan *et al*. (2001) in pigs, Rodehutscord *et al*. (2004) in broilers and Rezvani *et al*. (2008) in cecetomized laying hens proves to be an effective tool for estimating digestible nutrients.

Based on the premise that the amount of digested CP or amino acids is a function of their incremental intake, this method is a suitable measure of estimating the true CP and AA digestibility and hence digestible amounts of the nutrients in FM which is an expensive ingredient in poultry feeds and whose inclusion in diet has be precise for cost effectiveness and avoidance of excess N being passed to the environment.

CONCLUSION

The apparent digestibility of CP and AA was increased with sequential dietary FM levels. A linear relationship of CP or AA intake and CP or AA digested was established and hence the true CP and AA digestibility were estimated by the regression method. The digestible CP, lysine and methionine of FM was calculated to be 63.63 g, 5361.04 mg and 1801.45 mg. Due to increased endogenous loss from high dietary CP, results of this study suggest that dietary inclusion of FM for broiler chicks should not exceed 2% as higher levels may induce higher endogenous AA loss and consequently reduced amino acid digestibility.

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