

ILEAL AMINO ACIDS DIGESTIBILITY COEFFICIENTS OF FISH SILAGE, FISH MEAL AND SOYABEAN MEAL IN BROILER CHICKENS

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Abstract

The objective of this study was to assess the amino acid digestibility of fish silage prepared from minced Indian oil sardines (*Sardinella longiceps*) using inorganic acid (3% hydrochloric acid) compared with fishmeal and soyabean meal for feeding broiler chickens. Three protein sources were evaluated using six replicates of four birds per cage at 19 day of age. Cages were located in an environmentally controlled room maintained under conditions suitable for birds at this age with a photo-period of 23h in every 24h. Diets and water were offered on *ad libitum* basis. On the fourth day after the adaptation to the experimental diets, feed troughs were removed from every cage for 1 h and then reintroduced for 2 h. The ileal digesta sample were collected into vessel and frozen prior to being freeze-dried. The results indicate that the digestibility coefficients of amino acids of fishmeal were significantly lower ($P<0.01$) than those of soyabean meal and fish silage. Fish silage had significantly higher ($P<0.01$) digestibility coefficients for all amino acids than soyabean meal and fishmeal. For the most important essential amino acids for poultry performance, fish silage had higher digestibility coefficients of methionine 4 % more (0.94 vs. 0.90), threonine 10.2% more (0.88 vs. 0.79) and lysine 7.4% more (0.95 vs. 0.88) than soyabean meal; respectively. The lower digestibilities of amino acids for soyabean meal is related to the presence of antinutritional factors such trypsin inhibitors. In conclusion, sardines (*Sardinella longiceps*) fish silage shows a great potential to be used as protein supplements for poultry feeding.

Keywords: Sardine fish silage, Soyabean meal, Ileal Digestibility, Broiler Chicken

Introduction

A major constraint of poultry production in Oman is the high cost of feeds. One way to overcome this problem is by using local ingredients available in the Sultanate for feeding chickens. Prior to including these feed ingredients in poultry diets, their nutrition value should be evaluated. Soyabean meal by far is a valuable commodity used widely in animal nutrition in general and poultry diets in particular due to its amino acid profile composition. The protein level in soyabean meal can be variable, and this may be an indication of seed variety and or/ processing conditions involving fat extraction (Veltman *et al.*, 1986). Soybean contains a number of natural toxins and antinutritional factors, the most challenging being the trypsin inhibitor. As with most types of beans, the trypsin inhibitors will disrupt protein digestion, and its presence is characterized by compensatory hypertrophy of the pancreas (McNaughton, 1981).

In the Sultanate of Oman, there are many non-conventional feeds and by-products which if effectively utilized could improve the supply of poultry feeds. Fish by-products are the most important by-products available at reasonable prices in the Sultanate. These fish by-products have the potential to be used as a high protein supplement for farm animal and poultry feeding in Oman. Sardines in Oman are traditionally dried on the beach for several days before being used as animal feed. This type of drying reduces the quality of sardines and makes them prone to insect infestation and sand contamination. Fish silage can be an effective way for processing fish by-products and using them as a feed ingredient. In regions, where by-catch surplus catches or fish processing wastes are available, fish silage represents a possible alternative to fishmeal as a source of animal protein.

The ileal collection method has been suggested by several researchers to allow a more accurate determination of protein and amino acid digestibility (Achinewhu and Hewitt, 1979; Raharjo and Farrell, 1984; Kadim, *et al.*, 2002). It has been shown to be more sensitive in detecting small differences in the digestibility of the nutrients. In addition, such, procedures remove the effects of fermentation by the bacteria of the hindgut on undigested residue and the urinary influence on digestibility (Sibbald, 1987). The objective of this experiment was to evaluate the nutritive values of fish silage as a source of protein in broiler chicken diet.

Material and Methods

Sardines silage preparation

Batches of sardine predominantly composed of the Indian oil sardines, *Sardinella Longiceps* were purchased from the fishermen at Seeb landing beach. They were transported to the university in cool boxes then kept frozen at (-20 °C) until the time of usage. The frozen sardines were minced in the mincer (4 mm) and collected in plastic containers for acid addition. A 3% of HCL acid was added in each container and thoroughly mixed using a mixing rod fitted on a drill. Ethoxyguin (200 ppm) was added as an anti-oxidant to prevent oxidation of fish oil. The containers were covered by plastic sheets to keep the flies away and allow autolysis under ambient temperatures. The silage was stirred 3-4 times a day to maintain uniformity especially during the first two days to avoid pockets of untreated fish where spoilage bacteria can continue to grow. The pH level was daily monitored and maintained below 4 by adding additional acid when required. The fish gradually converted to semi liquid product within two to three days. After the material had liquefied, the oil, which had separated and surfaced, was skimmed off and disposed. Sardine fish silage sample was freeze-dried in an Edwards drier to a constant mass. For further chemical analysis.

Birds and Housing

One hundred and forty four newly hatched (Marshall R strain) broiler chickens were housed in suspended grower cages. The cages were located in an environmentally controlled metabolism room maintained at 35°C on day 1 and reduced by 1 °C per day until 22 °C was reached. Birds had free access to water and feed; lighting was maintained at photo-period of 23 h in every 24 h. Birds were Initial allocation to replicate cage was at day 13, with live weights of birds in a replicate differing by no more than 10 g. Birds were fed on a commercial broiler diet from day one to day 18. The birds were 19 d old at the commencement of the ileal digestibility assay.

Experimental diets and procedure

Three test ingredients (soybean meal, fish meal and dried fish silage) were ground using a laboratory hammer mill fitted with a 3 mm screen and then incorporated into semi-synthetic diets at one rate of inclusion (0.50 as proportion) as the only component containing protein/amino acids (Table 1). Other raw materials, added sequentially while the mixer was on slow speed (to ensure effective homogenisation of all ingredients), were the inert internal marker titanium dioxide, a vitamin/mineral premix, vegetable oil and 50:50 mixture of purified maize starch and glucose (in amounts to make diets up to 1000 g/kg). Each of the 3 experimental diets was evaluated with six replicates of a cage of eight birds in a cage. Experimental diets were fed *ad libitum* for 4 days from 19 to day 23 of age.

On day 23, birds were starved for one hour then fed for two hours to ensure sufficient gut fill for digesta sample collection. Birds were then killed by an intra-cardial injection of sodium pentobarbitone. Following dissection of the lower small intestine, digesta sample was gently flushed with distilled water and collected into a collection vessel. Samples from birds in a cage were pooled in order to provide enough samples for chemical analysis following the procedure described by Wiseman *et al.* (2006).

Calculations

The titanium and amino acid data were used to calculate the coefficient of apparent amino acid digestibility using the following equation as described by Wiseman *et al.* (2006):

$$1 - (aa_{dig} * marker_{feed}) / (aa_{feed} * marker_{dig})$$

where

aa_{dig} = amino acid concentration in digesta;

$marker_{feed}$ = titanium concentration in the diet;

aa_{feed} = amino acid concentration in the diet; and

$marker_{dig}$ = titanium concentration in the digesta.

Chemical analysis

Samples of test ingredients and ileal digesta samples used for laboratory analysis were ground to pass through a 1mm mesh in a micro-Wiley meill. Samples of ileal contents were freeze dried prior to grinding. Analysis was always carried out in duplicate. Duplicate determinations of dry matter, crude protein, ether extract, crude fiber, ash and gross energy content were made on test ingredients samples according to AOAC (2000). Amino acid contents of duplicate of test ingredients and ileal digesta samples were carried out at Massey University Analytical Laboratory in New Zealand. Amino acids contents were determined, by using a Waters ion-exchange

HPLC system, utilizing post-column ninhydrin derivatisation and fluorescence detection, following hydrolysis in 6M glass-distilled hydrochloric acid containing 0.1 % phenol for 24 h at 110 + 2 °C in evacuated sealed tubes. Lysozyme was used as external standard for the amino acid analysis. Trypsin inhibitor activity (TIA) was measured according to the method established by Kakade *et al.* (1974). Titanium (the inert internal marker) was analysed using a modified version of the AOAC method (Short *et al.*, 1996).

Results

The chemical composition and amino acid content of the soyabean meal, fishmeal and fish silage is summarized in Table 2. Crude protein was higher by 33% and 29% in fishmeal and fish silage than in soyabean meal; respectively. Both fishmeal and fish silage had 80% higher fat content, determined as ether extract, than for soyabean meal. Gross energy content of the soyabean meal, fishmeal and fish silage were 14.29, 15.88 and 19.9 MJ kg⁻¹, respectively, which indicates that both fishmeal and fish silage had 28% and 10% more gross energy than the soyabean meal.

Both fishmeal and fish silage had a similar content of total essential amino acids. Soyabean meal had lower level of sulphur-containing amino acids than fishmeal and fish silage. For the most important essential amino acids for poultry performance; fish silage contained 66.1 % more methionine (16.91 vs. 5.73 g/kg DM); 24.1% more threonine and 34 % more lysine (47.3 vs. 31.23 g/kg DM) compared to soyabean meal.

Mean digestibility coefficients of the individual amino acids of essential and non-essential amino acids determined in the ileum for soyabean meal, fishmeal and fish silage are shown in Table 3. The overall mean amino acids digestibility coefficients of fishmeal were considerably lower than those of soyabean meal and fish silage. Fish silage had higher digestibility coefficients for all essential amino acids and non-essential amino acids than soyabean meal and fish meal. For the most important essential amino acids for poultry performance, fish silage had 4 % more methionine (0.94 vs. 0.90), 10.2% more threonine (0.88 vs. 0.79) and 7.4% more lysine (0.95 vs. 0.88) than soyabean meal; respectively.

Discussion

Soyabean meals are of major importance worldwide as a plant protein constituent of diets for poultry. It is well acknowledged that limitations to their use are associated with relatively modest concentrations of protein and nutritionally essential amino acids and there is a considerable interest in selecting cultivars with improved nutritional quality (Clarke and Wiseman, 2000). However, a further area of primary significance is the presence of anti-nutritional factors, such as trypsin inhibitor, as it interferes with protein digestion. Trypsin inhibitor is heat liable and is reduced below levels likely to cause problems, although necessary processing is associated with increased cost. The trypsin inhibitor of soybean meal used in this study is 5 mg/g (Table 3) and is above the recommended trypsin inhibitor threshold of 4 mg/g (Clarke and Wiseman, 2005). The lower digestibility of amino acids of soybean meal can be attributed to the presence of anti-nutritional factor as it can affect protein digestibility in the digestive tract of the birds. Trypsin inhibitor level varies with source of bean and processing conditions (Nitsan and Nir, 1977; Clarke and Wiseman, 2000). Many authors reported negative effects of feeding soyabean meal high in trypsin inhibitor levels to broiler chicks (Leeson and Atteh, 1996; Zhu *et al.*, 1996 ; Zollitsch *et al.*, 1993). It is well-known that processing conditions can markedly influence the digestibility of amino acids in soyabean meal (Parsons *et al.*, 1991). Therefore, the wide variation in processing conditions of soyabean meals means that the actual amount of trypsin inhibitor ingested by poultry will vary considerably between batches.

In the current study, the amino acid digestibility assays were based on the ileal collection method that involved using an indigestible marker, which was included in the diet at a 0.05% concentration. This was implemented to avoid uncertainty introduced by the contamination of droppings by feathers, scurf, spelt feed and the activities of microflora in the hindgut (Parsons *et al.*, 1982). All the amino acid digestibility coefficients presented in this study are apparent since no account was taken of endogenous amino acid losses. As mentioned previously, the amino acids digestibilities in fish silage were consistently higher than those in soyabean meal (Table 3). For the most important essential amino acids for poultry performance, fish silage had 4 % more methionine (0.94 vs. 0.90), 10.2% more threonine (0.88 vs. 0.79) and 7.4% more lysine (0.95 vs. 0.88) than soyabean meal; respectively.

The utilization of fish waste and catch surplus of sardine fish offers a great potential to utilize these wastes as a protein source in poultry feeding. Fish silage has various industrial applications. It is friendly to the environment, safer, technologically simpler, and more economical than the manufacture of fishmeal (Gildberg, 1993). Fishmeals are produced from many types and varieties of fish and the various ranges of raw materials may account much of the difference in this foodstuff (Ravindran and Blair, 1993). However, the results of this study indicate that fish silage shows a great potential to be used as protein supplements for poultry feeding replacing partially the soyabean meal. A further growth study will be conducted to evaluate the effect of use of different levels of fish silage on performance and meat quality characteristics in broiler chicken.

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Table 1. Formulation of experimental diets for experiment 1 (ileal digestibly assay).

Raw material (g/kg)	Diet 1	Diet 2	Diet 3
Soyabean Meal	500	---	---
Fish Meal	---	500	---
Fish Silage	---	---	500
Vegetable oil	50	50	50
Vitamin and mineral premix*	50	50	50
Titanium dioxide	5	5	5
Starch:glucose (50:50)	395	395	395
TOTAL	1000	1000	1000

* Designed to meet the requirements of the young broiler chicken.

Table 2. Chemical composition, amino acid profile and anti-nutritional contents in soybean meal, fish meal and fish silage.

Chemical composition (g/kg DM)	Soybean Meal	Fish Meal	Fish Silage
Dry matter	923.2	949.5	290
Crude protein	446	667.5	631
Ether extract	21.4	109.0	117.0
Crude Fiber	68.6	-	-
Ash	115.7	190.1	157.9
Gross energy (kj/ g DM)	142.9	158.8	199.9
Essential amino acids (g/kg DM)			
Threonine	18.45	24.43	24.31
Valine	23.83	32.94	32.51
Methionine	5.73	13.94	16.91
Isoleucine	21.54	23.44	25.00
Leucine	37.12	44.51	40.76
Phenylalanine	25.74	23.94	23.18
Histidine	14.46	19.24	22.72
Lysine	31.23	43.25	47.30
Arginine	36.77	44.41	36.20
Total	214.9	270.1	269
Non-essential amino acids (g/kg DM)			
Aspartic acid	59.24	55.44	54.06
Tyrosine	17.23	18.37	19.66
Serine	22.14	25.10	22.12
Glutamic acid	91.96	87.40	76.05
Proline	25.33	42.49	26.38
Glycine	21.19	60.26	40.66
Alanine	23.12	47.21	39.42
Total	260.2	336.3	278.4
Overall Mean	29.7	37.9	39.4
Anti Nutritional factors (mg/g DM)			
Trypsin Inhibitor	5	-	-

Table 3. Mean coefficients of apparent ileal (CAID) amino acids digestibility of in soyabean Meal, fish meal and fish silage.

	Protein source				
Amino Acid	Soyabean Meal	Fish Meal	Fish Silage	SEM	Significance
Essential amino acids					
Threonine	0.79	0.71	0.88	0.017	**
Valine	0.82	0.74	0.92	0.015	***
Methionine	0.90	0.75	0.94	0.019	**
Isoleucine	0.84	0.73	0.92	0.019	**
Leucine	0.85	0.75	0.93	0.020	**
Phenylalanine	0.86	0.75	0.91	0.019	**
Histidine	0.85	0.74	0.92	0.019	**
Lysine	0.88	0.76	0.95	0.018	***
Arginine	0.90	0.79	0.92	0.015	**
AVG-Essential amino acids	0.85	0.75	0.92	0.018	
Non-Essential amino acids					
Aspartic acid	0.84	0.64	0.87	0.025	***
Serine	0.80	0.69	0.87	0.013	***
Glutamic acid	0.87	0.73	0.88	0.021	**
Proline	0.83	0.74	0.84	0.014	**
Glycine	0.80	0.74	0.85	0.014	**
Alanine	0.85	0.77	0.92	0.016	**
Tyrosine	0.86	0.74	0.92	0.016	***
AVG-Non essential amino acids	0.84	0.72	0.88	0.017	
AVG-all amino acids	0.85	0.74	0.90	0.018	

AVG = Average digestibility; ** = P<0.01; *** = P<0.001.