



The Effect of Feeding Different Levels of Sardine Fish Silage on Broiler Performance, Meat Quality and Sensory Characteristics under Closed and Open-sided Housing Systems

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ABSTRACT : Two experiments were conducted to evaluate the use of fish silage prepared from Indian oil sardines, *Sardinella longiceps*, as partial replacement of soybean meal as a sole source of protein for growing broiler chickens. The main objective of Experiment 1, an ileal digestibility assay, was to assess the nutritional value of fish silage compared with soybean meal for feeding broiler chickens. The two test ingredients, soybean meal and dried fish silage, were incorporated into semi-synthetic diets, as the only component containing protein. The ileal digestibility coefficients of amino acids of fish silage were considerably higher than those of soybean meal ($p < 0.001$). The lower digestibility of amino acids from soybean meal was related to the presence of anti-nutritional factors such as trypsin inhibitors. Fish silage had higher levels of sulphur-containing amino acids than soybean meal. The objective of Experiment 2, a growth study, was to evaluate the effect of feeding fish silage on performance and meat quality characteristics of broiler chickens raised under closed and open-sided housing systems. Four diets containing various levels of fish silage (0, 10, 20 and 30%) were evaluated. Daily feed intake, body weight gain and feed conversion ratio were measured. At the end of Experiment 2, 96 birds were randomly selected and slaughtered to evaluate meat quality characteristics. Housing type had significant effects on feed intake and body weight gain ($p < 0.01$). Birds in the open-sided house consumed 4.7% less amount of feed and gained 10.6% less than their counterparts in a closed house. Birds in both houses fed diets containing 10 and 20% fish silage gained more than birds fed 30% fish silage. The current study produced evidence that fish silage can replace up to 20% of soybean meal in broiler diets without affecting either growth performance or the sensory quality of broiler meat. (**Key Words :** Ileal Digestibility, Fish Silage, Performance, Meat Quality, Broiler Chicks)

INTRODUCTION

Oman is a sub-arid country with hot climate and negligible rainfall. This reduces its capacity to produce animal feed ingredients. Therefore, the poultry industry in Oman totally depends on imported feeds. Soybean meal is the main international source of protein in poultry feeds and Oman is no exception. However, the protein level in soybean meal can be variable, which 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, trypsin inhibitors will disrupt protein

digestion, and its presence is characterized by compensatory hypertrophy of the pancreas (McNaughton, 1981).

In Oman, there are many non-conventional feeds and by-products which if utilized effectively could improve the supply of local poultry feeds. Fish by-products are the most important by-products available at reasonable prices in Oman. These fish by-products have the potential to be used as high protein supplements for farm animals and poultry 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. The advantage of fish silage production is that the proteolytic enzymes present in the fish hydrolyse the protein and fat and this autolysis is accelerated by weak or strong acids, reaching the highest activity at pH between 2

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and 4. This creates conditions more suitable for enzyme activity, bone breakdown and inhibition of spoilage and pathogenic type bacteria and therefore, proliferation of microorganisms is avoided which improve the product safety to be used in animal feeding (Raa and Gildberg, 1982; Alwan et al., 1993). The resultant liquid can then be dried or mixed with cereals, or other carbohydrate sources, and then dried. The dried mixture is easier to manage in the preparation of diets for animal feeding (Viana et al., 1993; Vizcarra-Magaña et al., 1999; Goddard and Perret, 2005).

In regions, where by-catch surplus 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 proposed by researchers since it permits a more accurate determination of protein and amino acid digestibility (Achinewhu and Hewitt, 1979; Raharjo and Farrell, 1984; Kadim, et al., 2002; Al-Marzooqi et al., 2010). 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). Research has confirmed that fishmeal is a useful protein source for poultry (Machin et al., 1990) and swine (Green et al., 1988). However, there are a number of unfavorable characteristics, which present limiting factors in fishmeal usage (Mikulec et al., 2004). Commercial fishmeal preparation is a demanding procedure including sterilization and defatting which increases the cost and may lead to protein denaturing if not carried out properly.

The objectives of this study were to assess the digestibility coefficient of amino acids of sardine fish silage and to evaluate the effect of feeding fish silage on performance, meat quality and sensory characteristics of broiler chicken raised in closed and open-sided housing systems.

MATERIAL AND METHODS

Experiment 1-ileal digestibility assay

Sardines silage preparation : Batches of Indian oil sardines, *Sardinella longiceps* were purchased from Seeb Fish Market. They were transported to the university in cool boxes then frozen at (-20°C) until the time of usage. The frozen sardines were then thawed and minced through a 4 mm screen and collected in plastic containers for acid addition. A 3% of HCL acid was added and the mixture stirred using a mixing rod fitted on to a drill. Ethoxyquin at a level of 200 ppm was added as an anti-oxidant to prevent oxidation of fish oil. The containers were covered with plastic sheets to keep the flies away and to allow autolysis under ambient temperatures. The silage was stirred 3-4

times a day to avoid pockets of untreated fish where spoilage bacteria can continue to grow. The pH level was monitored daily and maintained below 4 by adding additional acid when required. The fish was reduced to a 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. Samples of the sardine fish silage were freeze-dried in an Edwards drier to a constant mass and used for further chemical analysis.

Birds and housing : Sixty newly hatched (Cobb 500) 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. Birds had free access to water and feed; lighting was maintained at photo-period of 23 h in every 24 h. Birds were initially allocated to replicate cages was from day 13, with live weights of birds in replicates differed by <10 g. Birds were fed 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 procedures : Two test ingredients (soybean 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 indigestible 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 1,000 g/kg). Each of the 2 experimental diets was evaluated with six replicates of a cage with 5 birds each. 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.

Table 1. Formulation of experimental diets for Experiment 1 (ileal digestibility assay)

Raw material (g/kg)	Diet 1	Diet 2
Soyabean meal	500	-
Fish silage	-	500
Vegetable oil	50	50
Vitamin and mineral premix*	50	50
Titanium dioxide	5	5
Starch:glucose (50:50)	395	395
Total	1,000	1,000

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

Samples from birds in a cage were pooled in order to provide enough samples for chemical analysis following the procedure described by Al-Marzooqi and Wiseman (2009).

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 Al-Marzooqi and Wiseman (2009):

$$1 - \frac{(aa_{\text{dig}} \times \text{marker}_{\text{diet}})}{(aa_{\text{diet}} \times \text{marker}_{\text{dig}})}$$

where

aa_{dig} = amino acid concentration in digesta;

$\text{marker}_{\text{diet}}$ = titanium concentration in the diet;

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

$\text{marker}_{\text{dig}}$ = titanium concentration in the digesta.

Experiment 2-growth study

Sardine silage corn mixture preparation : Sardine silage was prepared as described earlier. Crushed corn was selected to co-dry fish silage as it is one of the main components in chicken diets. The combination of 85% fish silage: 15% crushed corn ratio was used since it has a similar protein content as soybean meal 44% on a dry

matter basis. A thin layer of mixture was spread on plastic sheets and dried in poly tunnel supplemented with exhaust fans to ensure constant flow of air. Fish silage mixture was turned over daily to enhance drying. After drying, the fish silage and crushed corn mixture was kept in bags under room temperature for future use.

Birds and housing : Two hundred and forty 1-d-old (Cobb 500) broiler chickens were used. On the day of arrival, the chicks were individually weighed and placed into narrow weight classes. Birds of relatively low or high weights were discarded. Five birds were randomly assigned to each of 48 suspended wire cages (62×62×37 cm), so that all cages had nearly a similar average initial weight. Equal number of cages was assigned randomly into two types of houses (24 cages in each): a closed and an open-sided. Lighting of 23-h light and 1 h dark was provided.

Experimental diets : Birds were given *ad libitum* access to experimental diets and water. The compositions of the experimental diets are presented in Table 2. The 4×2 factorial arrangements of treatments involved 4 levels of fish silage-crushed corn mixture: 0, 10, 20 and 30%, substituting for soybean meal, and two types of housing: a closed and an open-sided. There were 6 replicates cages for each of the four dietary treatments, and each replicate cage contained 5 birds. Treatments/replicate combinations were

Table 2. Composition of the experimental diets used in Experiment 2-growth study

Ingredients	Fish silage (%)			
	0	10	20	30
Silage-corn (85:15)	0	10	20	30
Soybean meal (44% of CP)	40	30	20	10
Crushed corn	42.5	41	39.5	38
Wheat bran	8.2	9.1	11	11.9
Limestone	2	2	2	2
Premix ¹	0.3	0.3	0.3	0.3
Salt	0.3	0.3	0.3	0.3
Vegetable oil	0	0	0	0
Binder	4.7	5.3	4.9	5.5
Dicalcium phosphate	2	2	2	2
Chemical analysis				
Dry matter (%)	93.69	92.62	93.74	94.54
Crude protein (%)	23.03	23.08	23.10	23.50
Fat (Ether extract)	2.93	5.50	7.73	9.70
Ash (%)	3.71	4.10	5.5	6.59
Gross energy (MJ/kg)	13.79	13.82	14.15	14.19
Crude fibre (%)	2.87	2.63	2.80	3.14
Calcium	1.57	1.7	1.92	1.98
Phosphorus	0.99	0.91	0.93	0.97

¹ The vitamin and mineral premix provide the following quantities per kilogram of diet: Vitamin A, 10,300 IU; Vitamin D₃, 2,500 IU; Vitamin E, 40.00 mg; Vitamin K, 3.75 mg; Vitamin B, 1.00 mg; Vitamin B₂, 6.50 mg; Vitamin B₆, 6.00 mg; Vitamin B₁₂, 0.01 mg; Calcium pantothenate, 18.00 mg; Niacin, 30.00 mg; Folic acid, 2.00 mg; Biotin, 0.06 mg; Flovomycin, 50.00 mg; Ethoxyquin, 125.00 mg; Choline, 650.00 mg; molybdenum, 2.00 mg; Manganese, 120.00 mg; Iron, 7.00 mg; Cobalt, 1.00 mg; Zinc, 90.00 mg; Idine, 1.50 mg; Selenium, 0.15 mg.

randomly allocated.

Growth and feed intake : The birds and feed of each cage were weighed at day 0, 7, 14, 21, 28, 35. All the measurements (growth rate, feed intake and feed conversion ratio) were recorded weekly. This allowed daily gain (DG), feed intake (FI) and feed conversion ratio (FCR) to be determined at these periods.

Weight of digestive organs : On day 35, two birds from each cage from both houses were randomly selected and scarified. The weight of the live bird, carcass, total digestive tract, small intestine, proventriculus, gizzard, heart and liver plus gall bladder were recorded.

Meat quality assessment : Twelve carcasses from each treatment from both houses were randomly selected to evaluate meat quality characteristics. The selected carcasses were placed in labeled plastic bags, stored in a chiller (4°C) for 24-h, then frozen at -20°C, for further evaluation. One type of muscle *M. pectoralis* from the breast was dissected out of 96 carcasses and was evaluated for meat quality characteristics. Meat quality related measurements, ultimate muscle pH, Warner-Bratzler (WB) shear force, expressed juice, cooking loss and colour L^* , a^* , b^* were determined (Tabook et al., 2006).

Sensory evaluation : Sensory evaluation was carried out in the Sensory Laboratory equipped with individual booths which consist of a counter top with three sides walls extended from floor to ceiling. Each booth was made in such way that the panelists do not influence each other. All booths were provided with florescent lights to mask color differences between the samples. The meat samples were cut to approximately 2.5 inches cubes. Four coded samples representing the four dietary treatments were traditionally cooked in pressure pots under similar flame strength and cooking duration with no spices or additives. Samples were served twice from each experimental-group, and the serving order was randomized according to sample, replicate and assessor. Intensities of tenderness, juiciness, flavor, aroma and desirability were evaluated. Water and crackers were served for cleansing the palate between samples. The scale used for evaluation of sensory attributes ranging from the lowest intensity of each attributes (score 1) to the highest intensity (score 5). Thirty panelists participated in this sensory evaluation which is within the required range.

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 mill. Samples of ileal contents were freeze dried prior to grinding. Duplicate determinations of dry matter, crude protein, ether extract, crude fiber, ash and gross energy content were made on test ingredients (Experiment 1) and feed samples (Experiment 2) 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 6 M 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 indigestible marker) was analysed using a modified version of the AOAC method (Short et al., 1996). Chemical analyses were performed in duplicate and repeated if individual data differed by <5%.

Statistical analysis

Data were analysed by general analysis of variance using the general linear model procedure (SAS Institute Inc., Version 2 and 6, 2001). The main factors tested in the analysis of variance for Experiment 1 (ileal digestibility assay) were digestibility coefficients of amino acids. In Experiment 2 (growth study) analysis of variance was carried out on data from weight gain, feed intake, feed conversion ratio, weight of digestive organs, meat quality and sensory characteristics. The main factors tested in the analyses of variance were houses and dietary treatment. Significant differences between treatment means were assessed using the least-significant-difference procedure. Interactions between the treatments were tested using Tukey's multiple comparisons test when significant and excluded from the model when not significant ($p>0.05$).

RESULTS

Experiment 1-ileal digestibility assay

The chemical composition of the soybean meal and fish silage is summarized in Table 3. Crude protein was higher by 31% in fish silage than in soyabean meal. Fish silage had 83% higher fat content, determined as ether extract, than for soybean meal. Gross energy contents of the soybean meal and fish silage were 14.2 and 20.5 MJ kg⁻¹, which indicates that fish silage had 26% more gross energy than the soyabean meal. The amino acid composition of the soybean meal and fish silage is presented in Table 3. Soybean meal had lower levels of sulphur-containing amino acids than fish silage. For the most important essential amino acids for poultry performance; fish silage contained 66% more methionine (15.6 vs. 5.3 g/kg DM); 24% more threonine (23.5 vs. 17.8 g/kg DM) and 35% more lysine (46.9 vs. 30.4 g/kg DM) than soybean meal. Mean digestibility coefficients of the individual amino acids of essential and non-essential amino acids determined in the ileum for

Table 3. Chemical composition (on DM basis), amino acid profile and anti-nutritional contents in soybean meal and fish silage

Chemical composition (g/kg DM)	Soybean meal	Fish silage
Dry matter	918	288
Crude protein	443	641
Ether extract	20.3	119
Crude fiber	65.7	-
Ash	112	160
Gross energy (kJ/g DM)	14.0	18.9
Essential amino acids (g/kg DM)		
Threonine	17.8	23.5
Valine	22.7	31.8
Methionine	5.3	15.6
Isoleucine	22.2	25.4
Leucine	36.5	41.2
Phenylalanine	24.9	22.9
Histidine	13.8	21.9
Lysine	30.4	46.9
Arginine	35.7	35.9
Total	209.3	265.1
Non-essential amino acids (g/kg DM)		
Aspartic acid	58.7	53.5
Tyrosine	18.2	20.4
Serine	21.9	21.8
Glutamic acid	91.8	75.7
Proline	24.5	26.1
Glycine	20.9	41.1
Alanine	23.4	38.8
Total	259.4	277.4
Overall mean	29.3	33.9
Anti nutritional factors (mg/g DM)		
Trypsin inhibitor	6.5	-

soybean meal and fish silage are shown in Table 4. Fish silage had higher digestibility coefficients for all essential and non-essential amino acids than soybean meal.

Experiment 2-growth study

The feed intake (g/bird/d), daily body gain (g/bird/d) and feed conversion ratio (g FI/g Gain) for the overall period (1-35 days) and on a weekly basis are presented in Table 5. Diet had significant effects on feed intake in week 1, 2 and 3. Birds on diet 10 and 20% fish silage consumed more feed than the other groups. Daily weight gain was also significantly influenced by diet in week 2 and 4. Birds fed diet 10 and 20% fish silage gained more than the other groups. When the mean weight gain for the overall period (0-35 days) was considered, birds fed diet 10 and 20% fish silage gained more than the other groups. Mortality rate was low for all the dietary treatment across the two houses (1.85% for the closed house and 2% for the open-sided

Table 4. Mean coefficients of apparent ileal amino acid digestibility of soyabean meal and fish silage

Amino acid	Protein source		SEM	Signif.
	Soybean meal	Fish silage		
Essential amino acids				
Threonine	0.78 ^b	0.88 ^a	0.015	*
Valine	0.81 ^b	0.92 ^a	0.014	**
Methionine	0.86 ^b	0.95 ^a	0.010	**
Isoleucine	0.83 ^b	0.92 ^a	0.021	*
Leucine	0.84	0.93	0.024	NS
Phenylalanine	0.86	0.92	0.022	NS
Histidine	0.85	0.92	0.018	NS
Lysine	0.88 ^b	0.95 ^a	0.018	*
Arginine	0.90	0.93	0.017	NS
AVG-essential amino acids	0.86	0.93	0.018	
Non-essential amino acids				
Aspartic acid	0.83	0.86	0.024	NS
Serine	0.79 ^b	0.87 ^a	0.012	**
Glutamic acid	0.87	0.88	0.022	NS
Proline	0.82	0.85	0.013	NS
Glycine	0.80	0.85	0.014	NS
Alanine	0.85 ^b	0.92 ^a	0.017	*
Tyrosine	0.84 ^b	0.92 ^a	0.016	*
AVG-non essential amino acids	0.83	0.86	0.017	
AVG-all amino acids	0.85	0.89		

AVG = Average digestibility; NS = Not significant; * p<0.05; ** p<0.01.

house). The pathoanatomical findings of the birds did not indicate any connection between diet and cause of death. According to the findings, causes of death were sudden death syndrome and stunted growth.

Housing type had significant effects on feed intake and body weight gain in week 1, 2, 3, 4 and 5. Birds in the open-sided house consumed 9.1%, 2.4%, 2.7%, 8.5% and 3.5% less feed than their counterparts in the closed house in weeks 1, 2, 3, 4 and 5, respectively. When the overall feed intake (0-35 days) was considered, the birds in the open-sided house consumed approximately 4.7% less amount of feed than their counterparts in the closed house. Daily weight gain was also significantly influenced by housing type during the same period. Birds in open-sided house gained 15.6%, 9.8%, 4.7%, 18.9%, and 6.7% less weight than their counterparts in closed house in week 1, 2, 3, 4 and 5, respectively. When the mean weight gain for overall period (0-35 days) was considered, birds in the open-sided house gained approximately 10.6% less than their counterparts in the closed house. This significant reduction in daily and over all weight gain in birds reared in open-sided house was entirely related to the reduction in feed

Table 5. Feed intake (FI, g/bird/d), daily gain (DG, g/bird/d) and feed conversion ratio (FCR, g feed/g gain) of broiler chickens reared in closed and open-sided houses fed different levels of fish silage at various stages of development

Stages	Closed house				Open-sided house				SEM	Significance		
	Fish silage (%)				Fish silage (%)					House	Diet	House×diet
	0	10	20	30	0	10	20	30				
Week 1												
FI	18.30 ^b	19.26 ^a	19.41 ^a	18.46 ^b	16.63 ^d	17.18 ^c	17.21 ^c	16.56 ^d	0.087	***	***	*
DG	15.04	16.44	16.86	15.19	12.69	14.04	14.26	12.65	0.882	***	NS	NS
FCR	1.23	1.20	1.18	1.23	1.33	1.28	1.25	1.33	0.086	NS	NS	NS
Week 2												
FI	49.17	50.55	51.94	49.13	48.00	49.34	49.97	48.08	0.337	***	***	NS
DG	34.14	35.40	36.35	34.13	30.79	31.92	32.20	30.80	0.528	***	**	NS
FCR	1.44	1.43	1.43	1.44	1.56	1.55	1.55	1.56	0.022	***	NS	NS
Week 3												
FI	79.70	81.13	82.19	79.97	77.52	78.08	78.26	77.74	0.401	***	***	NS
DG	49.11	50.84	51.49	49.40	46.79	47.45	48.08	46.82	1.597	*	NS	NS
FCR	1.64	1.60	1.60	1.64	1.67	1.65	1.65	1.67	0.057	NS	NS	NS
Week 4												
FI	105.33	108.63	110.09	105.09	96.34	97.43	98.49	96.36	1.926	***	NS	NS
DG	63.35	66.30	67.32	63.50	51.32	54.11	55.21	51.46	0.999	***	***	NS
FCR	1.66	1.64	1.64	1.65	1.88	1.80	1.79	1.88	0.032	***	NS	NS
Week 5												
FI	143.83	143.09	142.59	143.62	138.25	138.19	138.37	138.54	1.710	***	NS	NS
DG	77.60	78.24	79.10	77.43	72.39	73.75	74.39	72.83	1.509	***	NS	NS
FCR	1.86	1.83	1.81	1.86	1.91	1.88	1.86	1.90	0.036	*	NS	NS
Overall												
FI	79.26	80.53	81.24	79.25	75.53	76.04	76.46 ^b	75.46	1.155	***	NS	NS
DG	47.85	49.44	50.23	47.93	42.79	44.25	44.83	42.91	0.593	***	***	NS
FCR	1.66	1.63	1.62	1.65	1.76	1.72	1.71	1.76	0.034	***	NS	NS

SEM = Standard error of means. * p<0.05; ** p<0.01; *** p<0.001.

NS = Not significant. Means with the same row with different letters were significantly different (p<0.05).

consumption, which was apparently caused by the house temperature. Feed conversion ratio was not affected by housing type during week 1 and 2. However, in week 2, 4, and 5, birds in open-sided house had 0.12, 0.22 and 0.05 (g FI/g Gain) more FCR than their counterparts in the closed house respectively.

The weights of whole digestive tract, small intestine, liver, proventriculus, gizzard and heart per unit of body weight (g/kg body weight) are presented in Table 6. Housing type had significant effect on the weight of heart. The current study suggested that reduction in feed intake may be the reason for the low weight body gain and

Table 6. Mean weight of digestive organs per unit body weight (g/kg body weight) in birds fed various levels of fish silage and raised in closed and open-sided houses

Organ (g/kg body weight)	Closed house				Open-sided house				PSE	Significance		
	Fish silage (%)				Fish silage (%)					House	Diet	House×diet
	0	10	20	30	0	10	20	30				
Whole digestive tract	12.59	13.34	13.52	12.61	12.39	13.47	13.53	12.40	0.51	NS	NS	NS
Small intestine	4.5	5.1	5.2	4.3 ^d	4.3	4.5	5.1	4.3	0.25	NS	**	NS
Liver	2.18	2.22	2.23	2.06	2.22	2.16	2.22	2.10	0.108	NS	NS	NS
Proventriculus	0.51	0.50	0.57	0.49	0.48	0.53	0.58	0.47	0.26	NS	*	NS
Gizzard	2.00	2.03	2.08	1.93	2.04	2.08	2.13	1.80	0.091	NS	NS	NS
Heart	0.555	0.591	0.612	0.536	0.450	0.511	0.545	0.431	0.026	***	**	NS

PSE = Pool standard error. ** p<0.01; *** p<0.001; NS = Not significant.

Means with the same row with different letters were significantly different (p<0.05).

Table 7. Meat quality characteristics of broiler *M. pectoralis* fed various levels of fish silage and raised in closed and open-sided houses

Meat quality characteristics	Closed house				Open-sided house				SEM	Significance		
	Fish silage (%)				Fish silage (%)					House	Diet	House×diet
	0	10	20	30	0	10	20	30				
pH	5.78	5.72	5.73	5.72	5.70	5.71	5.71	5.79	0.061	NS	NS	NS
Cooking loss (%)	21.08	19.30	19.7	19.00	21.04	20.64	19.37	19.14	1.34	NS	NS	NS
Expressed juice (cm ² /gm)	29.12	27.48	31.63	27.85	34.08	29.55	27.60	29.91	2.28	NS	NS	NS
WB-Shear force value (kg)	1.49	1.58	1.57	1.46	1.80	2.38	2.28	2.16	0.40	NS	NS	NS
Sarcomere length (µm)	1.54	1.86	1.73	1.75	1.71	1.66	1.75	1.72	0.082	NS	NS	NS
Myofibril fragmentation index (%)	83.46	80.80	82.51	82.42	80.65	82.29	82.15	81.29	0.055	NS	NS	NS
Lightness (L*)	43.52	43.26	44.56	42.73	47.64	45.21	48.33	45.16	2.68	NS	NS	NS
Redness (a*)	9.96	8.31	7.90	9.18	7.61	7.64	8.30	7.76	0.911	NS	NS	NS
Yellowness (b*)	7.50	8.38	8.83	8.10	6.68	7.06	7.84	6.45	0.894	*	NS	NS

SEM = Standard error of means. NS = Not significant, * p<0.05.

consequently may be the cause for the lower heart weight of birds reared in open-sided house. Diet had a significant effect on the weight of the small intestine, proventriculus and heart. Birds reared in the open-sided house had lower small intestine, proventriculus and heart weights.

Meat quality characteristics of broilers breast muscle (*M. pectoralis*) is presented in Table 7. Housing type has a significant effect on meat color. The b* value (yellowness) were lower for birds raised in open-sided house as compared to muscle from birds reared in closed house. The results from taste panel scores are presented on Table 8. Diet has a significant effect on juiciness and flavour. Birds fed diet of 30% fish silage had an off-flavour, which was described as “fishy” by the sensory panel.

DISCUSSION

Experiment 1-ileal digestibility assay

Soybean meal is of major importance worldwide as a plant protein and is a generally consistent feed ingredient for poultry. However, the nutritional potential of soybean meal is limited by the presence of anti-nutritional factors,

which interfere with the intake, digestion, absorption and metabolism of nutrients (Nitsan and Nir, 1977; Liener and Kakade, 1980). The main proteinaceous anti-nutritional factors present in soybean meal are trypsin inhibitors. 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 6.5 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 soybean meal high in trypsin inhibitor levels to broiler chicks (Zollitsch et al., 1993; Leeson and Atteh, 1996; Zhu et al., 1996). It is well-known that processing conditions can markedly influence the digestibility of amino acids in soybean meal (Parsons et al., 1991). Therefore, the wide variation in processing conditions of soybean meals means

Table 8. Sensory Evaluation of broiler chicken meat fed various levels of fish silage and raised in closed and open-sided houses

Test type	Closed house				Open-sided house				SEM	Significance		
	Fish silage (%)				Fish silage (%)					Diet	House	Diet×house
	0	10	20	30	0	10	20	30				
Tenderness ^a	3.43	3.57	3.33	3.73	3.77	3.20	3.20	3.33	0.162	NS	NS	NS
Juiciness ^b	3.57	3.10	3.20	2.96	3.50	3.43	3.00	2.93	0.146	*	NS	NS
Flavor ^c	3.43	3.10	3.13	2.65	3.30	3.09	2.55	2.53	0.153	*	NS	NS
Aroma ^d	3.22	3.07	3.11	3.03	3.43	3.10	3.13	3.02	0.171	NS	NS	NS
Desirability ^e	3.47	3.23	3.13	3.03	3.20	3.03	3.00	3.01	0.148	NS	NS	NS

Score range 5 (highest affirmative value) to 1 (lowest value). Total of 30 panelists participated in the evaluation.

^a Scored as 1 = extremely tough, 5 = extremely tender. ^b Scored as 1 = extremely dry, 5 = extremely juicy.

^c Scored as 1 = extremely undesirable flavor, 5 = extremely desirable flavor. ^d Scored as 1 = extremely having fish taint, 5 = no smell of fish taint.

^e Scored as 1 = extremely undesirable, 5 = extremely desirable.

SEM = Standard error of means. * p<0.05, NS = Not significant.

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., 1985). All the amino acid digestibility coefficients presented in this study are apparent since no account was taken of endogenous amino acid losses. In the present study, the digestibility coefficient of threonine, methionine and lysine in fish silage was significantly higher than in soybean meal (Table 4). Although soybeans are perhaps the most important legume crop worldwide, there are still problems relating to their nutritional value including deficiency of sulphur-containing and other amino acids. The utilization of fish waste and catch surplus of sardines offers a great potential to utilize these wastes as a protein source in poultry feeding. Fish silage production is less energy intensive, friendly to the environment, safer, technologically simpler, and more economical than the manufacture of fishmeal (Gildberg, 1993). Fishmeals are produced from many species 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 preliminary study indicate that fish silage shows a great potential to be used as protein supplements for poultry feeding replacing partially the soybean meal.

Experiment 2-growth study

In countries, where by-catch surplus catches of fish processing wastes are available, fish silage, represents a possible alternative to fish meal as a source of animal protein, which requires less technology than for the production of fishmeal (Arruda et al., 2007).

Fish silage can be described as a stable semi liquid product. Liquefaction is brought about by the naturally present proteolytic enzymes in the fish and is accelerated by reducing pH below 4 (Raa and Gildberg, 1982; Green et al., 1988). Rahmi et al. (2008) showed that when pH dropped to 4 or below, this increase of acidity created inappropriate conditions for the growth of most pathogenic organisms (causative agents of diseases) and improved the product hygiene and safety.

The results showed that replacing soybean meal with fish silage protein improved growth performance of broiler chicks. The results of the current study demonstrated that sardines, *Sardinella longiceps*, silage could replace up to 20% of soybean meal in broiler ration without affecting their performance (Table 5). The reason for this outcome

could be due to the fact that crude protein that was being supplemented by a vegetable source (soybean meal) was being replaced with higher quality protein from an animal source (fish silage). It is well known, in general, that amino acid content and protein quality of animal sources tend to be superior to those from vegetable sources, Rosenfeld et al. (1997). Santana-Delgado et al. (2008) showed that there were no differences in the feed intake and body weight gain in dietary treatments; in which Spanish mackerel silage:sorghum mix inclusion was 0%, 11% and 22%, respectively. In addition, Johnson et al. (1985) reported that inclusion of up to 10% acid fish silage meal as a protein source in diets for broiler chicks gave a similar growth performance as soybean meal or fishmeal. McNaughton et al. (1978) reported that the inclusion of fish silage in diets for broiler chicks improved growth and feed efficiency and it was concluded that fish silage provided a source of highly available amino acid. Furthermore, Skrede and Kjos (1996) showed that fish silage is a source of highly available amino acids and the digestibility of all amino acids was higher in fish silage than in fishmeal. As mentioned previously, our results demonstrate that sardine fish silage had a higher digestibility coefficient of amino acids than soybean meal (Table 4) and this could explain the improvements in production parameters of broilers observed in this study. Furthermore, the protein in this form, as hydrolysed protein, can easily be used for protein synthesis in poultry. Espe et al. (1992) who observed an improvement in white leghorn cockerels when a portion of the protein was included as hydrolysed protein suggested a similar explanation. Their study, recommended that it may be advantageous to have some of the protein in the diet in a soluble form so that it may be rapidly absorbed and bring about an early start of protein synthesis in the chickens. This suggests that fish silage is a good protein source for broiler chicks.

However, the results showed that birds in both houses fed diet 10 and 20% fish silage gain more than birds fed diet 30% fish silage. Numerically, the feed intake for birds fed diet 30% fish silage in both houses were lower than the other groups which suggest that reduced body weight gain might be due to reduced palatability when increasing dietary levels of fish silage were used. Similar results were obtained by Santana-Delgado et al. (2008) who showed that higher inclusion level of fish silage sorghum mix (33 or 44%) reduced body weight gain in broilers. Other studies found that high dietary levels of fish fat reduced growth performance of broiler chicks (Hulan et al., 1988; Machin et al., 1990).

In the current study, feed intake and body weight gain were significantly affected by type of housing (Table 5; $p < 0.01$). The daily fluctuation in ambient temperature and relative humidity is a cause of concern in the open-sided

houses due to natural air movement. In the current study birds raised in open-sided house showed classical signs of heat stress such as panting, wing lifting, and with increased respiratory rate and change of posture. The problem with temperature fluctuation is that heat production of the chickens is not constant but varies diurnally with temperature (McGovern et al., 2000). Furthermore, when temperature fluctuations occur, birds need to use more energy in an attempt to maintain their body temperature of 41-42°C. Heat loss for birds under a hot environment relies on respiratory evaporation (Hillman et al., 1985), especially when ambient temperature approaches body temperature. When air temperature rises, the breathing frequency of chickens increases (Wiernusz and Teeter, 1996), and the evaporative heat loss is significantly enhanced (Wiernusz and Teeter, 1993). Under conditions of high humidity, evaporative heat loss by bird becomes less efficient and the effect of high temperature is much more severe (Yahav, 1998).

Birds in the open-sided house consumed approximately 4.7% less amount of feed and gained approximately 10.6% less than their counterparts in closed house. This significant reduction in daily and over all weight gain in birds reared in open-sided house was entirely related to the reduction in feed consumption, which was apparently caused by the fluctuation of open-sided house temperature as mentioned earlier. A similar observation were made by Kadim et al. (2008) who reported a growth depression effects on birds raised in open-sided house. Birds reared in the open-sided house had reduced small intestine, proventriculus and heart weights. The experiment suggested that heat stressed birds (in open-sided house) consumed less feed than the non-stressed birds (in closed house) therefore; the digestion and absorption activities were less in those stressed one. Others suggested that during heat stress, the overall blood supply to the digestive tract is decreased with the largest reductions occurring in the most specialized portion of the digestive tract, the small intestine (May et al., 1998).

There were no significant differences in meat quality characteristics between dietary treatments. The results suggest that broilers chicks fed diets containing fish silage had similar meat quality characteristics as birds fed the control diet. The values of pH obtained in the current study are comparable and within the acceptable range of pH values reported in literature (Liu et al., 2004; Mehaffey et al., 2006; Kadim et al., 2009; Abdullah et al., 2010). Shear value is an indirect measurement of muscle tenderness. Lower shear values indicate more tender meat (Herring et al., 1967). In the current study, a shear force value of 1.46 to 2.38 kg would be considered tender (Simpson and Goodwin, 1974; Lyon and Lyon, 1990). Cooking loss, or weight loss during cooking, is a measurement of water-

holding capacity of the muscle. There were no differences among any of the experimental treatments. However, housing type had significant effects on meat color. The present results showed that b* value (yellowness) were lower for birds raised in open-sided house as compared to muscle from birds reared in closed house. Meat color from heat-exposed birds is pale compared to the control or cold treatments (Qiao et al., 2002).

In the present study, the sensory panel were able to detect an off-flavour which was described as "fishy" from meat of birds fed 30% fish silage (Table 8). During fish silage preparation the oil, which had separated and surfaced, was skimmed off manually and disposed. Indian oil sardines, *Sardinella longiceps*, are one of the high oil fish species. The oil composition in any fish by-product is considered to be the most critical factor influencing the flavor of the edible tissue, even if it was used to provide the same amount of total dietary protein (Fry et al., 1965; Wu and Kellems, 1984). Fat remaining in the silage after defatting may increase polyunsaturated fatty acid content in diet and broiler meat. Consequently, this may have an adverse effect on the sensory quality of the meat (Raa and Gildberg, 1982; Krogdahl, 1985). However, fishy taint may be found in the carcass when fishery products are included above certain concentrations in the diet. It is important that the incorporation of fish protein incorporated into poultry diets is carefully regulated in order to prevent adverse changes in the final edible products, especially when those products are prepared from oily species (Wu et al., 1984). Fish meal and fish silage is commonly used in poultry diets because of their protein content and beneficial amino acid profile. These feeds will provide the diets with fat rich in polyunsaturated n-3 fatty acids. Fish fat not only increases the long chain n-3 fatty acids in the meat, but also improves the n-6:n-3 ratio (Phetteplace and Watkins, 1989). However, increasing the n-3 fatty acid content in chicken carcass significantly decreases the oxidative stability of the meat (Huang and Miller, 1993; O'keefe et al., 1995). Incorporation of fish fat into the poultry diet can lead to development of serious oxidation derived off-flavours in the stored meat (Crawford et al., 1975; Opstvedt, 1984). However, susceptibility of muscle food to lipid oxidation can be controlled by anti-oxidants (Sheehy, 1994; Mielnik et al., 1995; 1996; Decker and Xu, 1998). Sheehy (1994) reported that higher concentrations of vitamin E in the animal feeds enriched the muscles with the vitamin and extended the storage period of the meat product.

The sardine fish (*Sardinella longiceps*) is the most abundant species of small pelagic fish in all regions of Oman, comprising more than 95% of the total landings (Dorr, 1991). The sardines fish are available at reasonable price (of 0.1 Biza/Kilo in Omani currency which is

equivalent to less than 0.25 Cent in US\$ currency) in the fish market in Oman. The technology of fish silage production is very simple. The silage process is fast in tropical climates and the product can be used in place. The production costs of fish silage is reduced because the silage is generally produced locally resulting in no importation or handling costs.

In general, the current study produced evidence that fish silage can replace up to 20% of soyabean meal in broiler diets without affecting performance and sensory quality of broiler meat and it would be economically profitable to include fish silage in feed mixtures for broiler production as part of their balanced diet.

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