Exploring the Nutritional Profile of the Indigenous Feedstuff for Tilapia Fish

Dr. Fehmeeda Bibi
Assistant Professor

FACULTY OF VETERINARY SCIENCES

BAHAUDDIN ZAKARIYA UNIVERSITY, MULTAN

CONTENTS

ABBREVIATIONS

GDP = Gross domestic product

SBM = Soybean meal

CM = Canola meal

RSM = Rapeseed meal

CP = Crude protein

AA = Amino acid

AME = Apparent metaboliseable energy

TAA = Total amino acids

GE = Gross energy

DM = Dry matter

EE = Ether extract

CF = Crude fiber

ME = Metaboliseable energy

MUL = Multan

SKR = Sukkur

^{o}C = Degree Celsius

FI = Feed intake

PFD = Protein free diet

RESEARCH PROJECT SUMMARY

Principal Investigator (PI)	Dr. Fehmeeda Bibi Bahauddin Zakariya University, Multan
Co-Principal Investigator (Co-PI)	Dr. Shafqat Nawaz Qaisrani University of Veterinary and Animal Sciences, Lahore.
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INTRODUCTION

There is a tremendous increase in animal protein demands globally due to a population increase. Per capita fish consumption has been increased from, on an average, 9.9 kg in 1960 to above 20 kg in 2015, possibly due to many factors including increase in living standards and urbanization (Smith et al. 2010). Supply-chain and other improvements have also raised the share of world fish production utilized for direct human consumption to 87 percent or 146 million tonnes in 2016. It is expected that this increase in consumption will continue to grow in future because fish is an excellent source of easily digested superior quality protein containing all essential amino acids, fats, especially omega-3 fatty acids, vitamins and minerals. Fish are rich in essential fatty acids, as well as in iron, vitamin B12 and calcium. Eating fish twice per week is recommended to obtain important health benefits (Ministry of Health of Brazil, 2006; AHA, 2008). Inland fishes are important food and nutritional resources, especially rural economies in developing countries (Welcomme et al. 2010). Low-income food-deficit countries account for 80% of the total reported harvest from inland capture fisheries (Kapetsky, 2003). Over 90% of global inland capture fisheries production is used for human consumption, the majority of which is in the developing world (Welcomme et al. 2010).

Fisheries and fish farming are playing an increasingly critical role for many local economies (Silva and Lemme, 2014). There is no doubt that fish can grow and utilize plant protein diets well, provided that species specific essential amino acids (EAA) requirements are met, the palatability of the feed is guaranteed and the levels of anti-nutritional factors are low. Covering the protein requirement of animals corresponds to the increasingly well-understood requirements for specific EAA (National Research Council, 2011). This has become a priority

given the current constrains on fish meal availability, cost and the need to replace it with plant protein sources that are economical and sustainable (Ahmad, 2014). Tilapia fish are the third most important cultured fish group in the world, after carps and Salmonids. Tilapia culture is also one of the fastest growing farming activities, with an average annual growth rate of 13.4%. For optimum profitability from fish production, the cheapest possible nutritionally balanced diets containing accurate proportions of protein, energy, minerals, vitamins and essential fatty acids are prerequisite. The use of highly digestible ingredients including soybean meal in fish feeds leads to increased cost of production due to their higher price. In Pakistan, most of the fish feed ingredients; especially protein sources including soybean meal are imported from India, America, Argentina and Brazil. This import leads to a greater cost of production that can be reduced by using locally grown feed raw ingredients including guar meal, rapeseed meal, cotton seed meal and other cereals such as corn, wheat, rice tips and their by-products including wheat bran, wheat middling and rice polishing. For proper growth and economical fish production, however, it is critical to evaluate their nutritional composition and availability of amino acids and energy, from these ingredients, to fish. The composition of protein (amino acids contents) and energy levels contained in these indigenous feedstuffs are, however, unexplored until now.

The use of these indigenous feedstuffs in commercial fish diets, therefore, results in nutritionally imbalance diets that lead to a poor production performance from these animals. It is the need of hours, therefore, to characterize the indigenous feed stuffs for their nutritional composition and availability levels of these nutrients to fish. Since the greater accuracy in dietary macro and micronutrients provision not only results in an enhanced fish's performance, but also reduces the likelihood of nutrient waste posing a pollution threat to the environment, which will be an

increasingly important issue. The use of indigenous feed stuffs in fish feeds, furthermore, will reduce the huge economic burden spent over the import of dietary raw ingredients. For this purpose, two experiments will be conducted to evaluate the nutritive profile including their energy, protein and amino acid contents. The digestible protein, amino acids and energy of the tested ingredients will be quantified. The data base of their total nutritive values including protein, amino acids and energy contents of these ingredients for fish will be made. So that the economical fish production in Pakistan can be done.

As the human population continues to grow, finding means to feed those people is one of the most important challenges faced around the globe. Fish and aquatic species in general are a much healthier source of protein compared to livestock commonly consumed. Beef and chicken all have their positive attributes, but no one stood up to the positive attributes of fish (Merino et al., 2012). Fish are the most widely aqua-cultured species all over the world. It is man's most important single source of high-quality protein, providing about 16% of the animal protein consumed by the world's population (FAO, 1997). Fish supplies <10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China (FAO, 2000). Globally, around one billion people rely on fish as their primary source of animal protein (FAO, 2000). Increase in worldwide supply of fish for human consumption has outpaced population growth in the past five decades. Global total capture fishery production reached 93.4 million tonnes in 2014, whereas 81.5 million tonnes from marine waters and 11.9 million tonnes from inland waters (FAO, 2016).

Fishery plays an important role in Pakistan's economy and is considered to be a source of livelihood for the inhabitants. During 2016-17, livestock accounts 58.33 percent share in the

agriculture and 11.39 percent to GDP. The Fishing sector contribution stands at 2.12 percent in agriculture value addition and recorded a growth of 1.23 percent (Pakistan Economic Survey 2016-17). Fishing contributes 0.41 percent to GDP but has a value addition in export earnings. During 2016-17 (July-March), total marine and inland fish production was estimated at 520,000 m. tons out of which 375,000 m. tons were marine production and the remaining catch came from inland waters. In total, 103,277 m. tons of fish and fish preparations was exported during 2016-17 (Pakistan Economic Survey 2016-17), major buyers included China, Thailand, Malaysia, Middle East, Sri Lanka, Japan. Pakistan earned US $ 276.269 million. According to Economic Survey 2016-17, the exports of fish and fish preparations have increased by 12.20 percent in quantity and 15.09 percent. In Pakistan, per capita fish consumption (about 2 kg) is still very low compared with the developed world (19.9kg) per capita consumption. This consumption can be increased by promoting aquaculture production in the country.

Feed is widely recognized as the most costly component of fish farming. A cost-farm budget analysis shows that feed constitutes 60-70% of total production costs of tilapia (Bolivar et al., 2010). In Pakistan, most of the fish feed ingredients; especially protein sources including SBM are imported from various countries including India, America, Argentina and Brazil. This import leads to a greater cost of production and ultimately higher price of the end product (fish) for the consumer. One of the possible ways to reduce this greater cost of production is the use of indigenous feed stuff. This may include various protein sources including rapeseed meal (RSM), canola meal (CM), cotton seed meal (CSM) and other cereals such as corn, wheat, rice tips and their by-products including wheat bran, wheat middling and rice polishing in fish feed. Since the price of imported raw ingredients directly affects the cost of fish feed. Various protein sources are cultivated locally and are comparatively cheaper due to indigenous production.

Pakistan is producing 190000, 109000, 15000 tonnes of rapeseed, sunflower seed and canola seed, respectively (Pakistan Economic survey, 2016-2017), which can potentially replace SBM partially or completely in fish diets. The use of indigenous plant protein sources such as canola meal (Rs. 35/kg) instead of imported soybean meal (Rs.60/kg), will reduce the cost of fish production and will greatly influence the financial returns.

Statement of Problem

Soybean meal prices are high and fluctuating throughout the year because of its import. This high price directly affects the cost of fish production since it is a major protein source used in fish diets. Since soybean meal costs significantly less than most animal meals included fish meal (FM). Reducing feed cost is critical to improve efficiency and to maintain sustainable fish production. To date, the majority of researches on SBM and FM replacement with substitute protein in fish diets is focused on the use of protein derived from plant sources. Indigenous plant protein sources including CM and RSM can be used as complete or partial substitute of SBM. The composition of protein (amino acids contents) and energy levels contained in the indigenous feedstuffs are, however, unexplored until now, therefore, considered as major hurdles in their proper use in fish diet. It is the need of hours, therefore, to characterize the indigenous feed stuffs for their nutritional composition and availability levels of these nutrients to fish. The use of indigenous feed stuffs in fish feeds, furthermore, will reduce the huge economic burden spent over the import of dietary raw ingredients.

Objectives

The objectives of the study were to evaluate;

1. The nutritive profile, especially energy, protein and amino acids contents, of the indigenous feed ingredients commonly used in fish diets.
2. Apparent metaboliseable Energy (AME) of commonly used indigenous energy sources in Tilapia fish.
3. Apparent digestibility of protein and amino acids in Tilapia fish.

MATERIALS AND METHODS

1. Feed Ingredients samples collection:

In the first step, samples of the indigenous feed stuff commonly used in fish feed were collected from different geographic locations as shown in Table 1. The collected samples were labelled having named, sampling date and sample geographic location for correct and easy identification. Samples of the following ingredients were collected;

a) Plant Energy Source

1. Wheat
2. Wheat middling

b) Plant Protein Source

1. Rapeseed Meal
2. Canola Meal

Table 1: Samples collection areas

Sample Name	Punjab	Sindh
Wheat	Multan (MUL)	Sukkur (SKR)
Wheat middling	Multan (MUL)	Sukkur (SKR)
Rapeseed meal	Multan (MUL)	Sukkur (SKR)
Canola meal	Multan (MUL)	Sukkur (SKR)

2. Analysis of the samples

The chemical composition of the collected indigenous feed ingredients was analysed. The following analysis was carried out to evaluate the nutritional profile of the protein sources in the Animal Nutrition Research Laboratory at Animal Nutrition Department, UVAS, Ravi Campus, Pattoki.

Proximate analysis

Proximate analysis was carried out for the precise nutritional profile of the collected ingredients.

Dry matter (DM) contents

The samples were dried at 105 ^{0}C for four hours for DM determination. Ash contents were determined at 550 ^{0}C for 15h. (AOAC, 2000).

Ash contents were determined a

Ether extract (EE)

Crude fat or EE values were analysed according to AOAC (2000) by acid hydrolysis treatment with 3 M HCl.

Crude protein (CP) contents

Total nitrogen content and CP was determined by adopting the Kjeldhal method (AOAC, 2000).

Crude fibre (CF)

AOAC (2000) method No. 32-10 was followed to the determination of CF content of the collected samples.

Amino acid contents

Amino acid profile of collected feed ingredients was done at amino acid analysis laboratory available at UVAS, Ravi Campus Pattoki.

The collected feed ingredients were analysed for their amino acid contents. Amino acid analyzer was used for AAA that is routinely carried out by using ion-exchange chromatography. The procedure described by Official Journal of European Communities was followed.

Briefly, well ground samples up to 500 micron was sieved and used. Thereafter, oxidation of the sample was done by performic acid to protect methionine and cysteine. These amino acids were converted to cys-cystic acid and met-methionine sulphone during oxidation. Then hydrolysis of the samples was carried out with 6M hydrochloric acid/phenol for 24h and their pH was set to 2.2. The filtered samples were poured in sample vials for determination of amino acid contents in Biochrom 30+ amino acid analyser by using ion exchange chromatography.

3. Digestibility measurements

Trials for digestibility measurement were carried out at experimental facility of the Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan. Three different trials were carried out for the measurement of Apparent Metaboliseable energy of indigenous wheat, wheat middling, apparent digestibility of energy, protein and amino acids of indigenous canola and rapeseed meal used in reversed Nile Tilapia (*Oreochromis niloticus*) fish.

Digestibility experiments with live fish

Trial 1.

Apparent Metaboliseable Energy (AME) measurements of Wheat and wheat middling in Tilapia fish

A total of 320 reversed Nile Tilapia (*Oreochromis niloticus*) of about 5 g body weight were reared on commercial diet for four weeks (28 days) to gain acquired body weight on an average 30 g. The ideal temperature and dissolved oxygen was provided along with feed @ 10% of their body weight. Fish was maintained in 15 cylindrical fiber glass tanks (20 fish in each) with 450 L capacity and equipped with individual aeration system, water supply and central drainage, with 100% water renewal of at least two times per day. Tanks were used as a flow through metabolic chambers. To minimize environmental fluctuation, tanks were placed in an indoor room. Water temperature was maintained (26 to 28 ˚C) by using water submerged heaters. The dissolved oxygen was maintained by air pumps. The pH and dissolved oxygen in water were measured daily through portable pH meter and DO meter of Ohaus Company.

After four weeks, four experimental diets (Table 3) of wheat and wheat middling of different origins i.e. Multan (Punjab) and Sukkur (Sindh) from Pakistan, with one reference diets were provided to fish. Each diet was randomly assigned to three tanks with 20 fish/ tank. All the

experimental diets were offered in mash form to their corresponding tanks for seven days in order to adopt the fish to these treatments in completely randomized design.

After adaptation period, fish were off feed for 24 hours and fecal materials from each tank were completely washed. Actual digestibility trial period was 21 days and fish were offered calculated feed (5% of their body weight), three times daily, divided in equal amounts. The fecal samples were collected on daily basis in plastic container of 200 mL capacity and immediately stored in freezer. At the termination of collection period, the samples from experimental diets and dried excreta were assayed for DM and gross energy contents by using bomb calorimeter. Determination of Metaboliseable energy of feedstuff was carried out using AOAC (1999) method (Willoughby, 1999). Experimental layout of trial 1 is shown in Table 2.

Table 2. Experimental layout of apparent digestibility of energy contents in indigenous wheat and wheat middling in Tilapia

Diet	Inclusion (%)	Treatment
Ref. Diet		Experimental Diets =2
Wheat Diet (MUL)	30	Location = 2
Wheat Diet (SKR)	30	Replicates = 3
Wheat Middling Diet (MUL)	30	Fish per replicate = 20
Wheat Middling Diet (SKR)	30	2*2*3*20 = 240
		Ref. Diet = 1
		Replicate = 3
		Fish Per replicate = 20
		1*3*20 = 60
		Total fish = 300
		Total trial days = 28

Table 3: Composition of the reference and experimental diets for apparent metaboliseable energy measurements.

Ingredients (%)	RD	W (MUL)	W (SKR)	WM (MUL)	WM (SKR)
SBM	60.0	39.0	39.0	39.0	39.0
Corn	28.0	19.0	19.0	19.0	19.0
Wheat	0.00	30.0	30.0	0.00	0.00
Wheat Middling	0.00	0.00	0.00	30.0	30.0
Oil	10.0	10.0	10.0	10.0	10.0
Premix	1.00	1.00	1.00	1.00	1.00
DCP	1.00	1.00	1.00	1.00	1.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

RD = Reference Diet, W = Wheat, WM = Wheat Middling, MUL= Multan, SKR= Sukkur, DCP = Di-calcium Phosphate

B). Determination of apparent digestibility of protein and amino acids

Trial 2. Apparent Digestibility of Protein and Amino Acids of Wheat and Wheat Middling in Tilapia

The objectives of this study were to evaluate the apparent digestibility of protein and amino acids present in wheat and wheat middling in Tilapia fish. In total, 300 reversed Tilapia (*Oreochromis niloticus*) fish of about 33 gm on an average were randomly distributed among 15 tanks containing 20 fish each, on commercial diet for 03 days. The experimental diets (Table 5) comprised of wheat and wheat middling from two different origins i.e. Multan (Punjab) and Sukkur (Sindh), of Pakistan and a protein free diet were randomly assigned to 15 tanks with three replicates of each. After adaptation period of three days, fishes were fasted for 24 hours and were randomly assigned experimental and reference diet. The experimental diets were supplied at 5% live weight to all treatment groups three times daily, at equal intervals. The fecal samples were collected on daily basis in plastic bottles. The digesta of each experimental unit, consisting of 20 fish, was pooled into a plastic container of 200 mL. All the collected samples was frozen at -20 °C and then freeze-dried for 72 hours to prevent amino acid break down. The trial lasted for 14 days. Experimental layout of trial 2 is presented below (Table 4)**.**

Table 4: Experimental layout of apparent digestibility of protein and amino acids contents in indigenous wheat and wheat middling in Tilapia

Diet	Inclusion (%)	Treatment
Protein Free diet		Experimental Diets = 2
Wheat Diet (MUL)	30	Location = 2
Wheat Diet (SKR)	30	Replicates = 3
Wheat Middling Diet (MUL)	30	Fish per replicate = 20
Wheat Middling Diet (SKR)	30	2*2*3*20 = 240
		Protein free diet = 1
		Replicate = 3
		Fish Per replicate = 20
		3*20 = 60
		Total days = 14

Table 5: Dietary ingredients and calculated nutrient composition of for protein and AA digestibility in Tilapia

Ingredients (%)	RD	W (MUL)	W (SKR)	WM (MUL)	WM (SKR)
SBM	55.5	36.39	36.39	36.39	36.39
Corn	35.0	24.1	24.1	24.1	24.1
Wheat	0.00	30.0	30.0	00.0	0.00
Wheat Middling	0.00	0.00	0.00	30.00	30.0
Oil	8.00	8.00	8.00	8.00	8.00
Choline	0.50	0.50	0.50	0.50	0.50
Salt	0.01	0.01	0.01	0.01	0.01
Premix	1.00	1.00	1.00	1.00	1.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

RD = Reference, W = Wheat, WM = Wheat Middling, MUL= Multan, SKR= Sukkur.

Trial 3: Apparent digestibility of protein and amino acids of Canola and Rapeseed meal in Tilapia fish

For this purpose, 20 fish was maintained in each of 15 cylindrical fiber glass tanks with 450 L capacity and equipped with individual aeration system, water supply and bottom outflow. Tanks were used as a flow through metabolic chambers.

Four diets of protein sources (Table 7) comprised of RSM and CM of two different origins i.e Multan (Punjab) and Sukkur (Sindh) of Pakistan, and one reference protein free diet, were prepared. Chromium oxide, an indigestible marker was used in all the diets of 1%. Each diet was randomly assigned to three tanks with 20 fish/ tank. All the experimental diets were offered in mash form to their corresponding tanks.

After the adaptation period of three (3) days, fish were off feed for 24 hours and fecal materials from each tank were completely washed. Actual digestibility trial period was 10 days, excluding adaptation period and off feed time. The experimental diets were supplied at 5% live weight to all treatment groups three times daily, divided in equal amounts. The digesta of each experimental unit, consisting of 20 fish, was pooled into a sample bottle of 200 mL. Experimental layout of trial 3 is presented below in Table 6**.**

Table 6: Experimental layout of apparent digestibility of protein and amino acids contents in indigenous canola and rapeseed meal in Tilapia

Diet	Inclusion (%)	Treatment
Protein Free diet		Experimental Diets = 2
RSM (MUL)	30	Location = 2
RSM (SKR)	30	Replicates = 3
CM (MUL)	30	Fish per replicate = 20
CM (SKR)	30	2*2*3*20 = 240
		Protein free diet = 1
		Replicate = 3
		Fish Per replicate = 20
		3*20 = 60
		Total days = 14

Table 7: Dietary ingredients and calculated nutrient composition of for protein and AA digestibility in Tilapia

Ingredients (%)	RD	W (MUL)	W (SKR)	WM (MUL)	WM (SKR)
SBM	55.5	36.39	36.39	36.39	36.39
Corn	34.9	24.1	24.1	24.1	24.1
Wheat	0.00	30.0	30.0	00.0	0.00
Wheat Middling	0.00	0.00	0.00	30.00	30.0
Oil	8.00	8.00	8.00	8.00	8.00
Choline	0.50	0.50	0.50	0.50	0.50
Salt	0.01	0.01	0.01	0.01	0.01
Premix	1.00	1.00	1.00	1.00	1.00
Total	100.00	**100.00**	**100.00**	**100.00**	**100.00**

RD = Reference, W = Wheat, WM = Wheat Middling, MUL= Multan, SKR= Sukkur.

All the collected samples were frozen at -20 °C to prevent amino acid break down. The amino acid analysis of the feed stuff and digesta was carried out.

Protein and amino acid digestibility was evaluated by the following equations;

The apparent digestibility coefficients (ADCs) of energy, protein and amino for the test ingredients and diets were calculated as described by Cho and Slinger, (1979):

$$ADC = 100 \times [1 - (F/D) \times (D_i / F_i)]$$

$$ADC_1 = [ADC_T - (0.7 \times ADC_R)]/0.3$$

Where, D=% nutrient or energy of diet; F=% nutrient or energy of feces; Di=% marker (Cr_2O_3) in diet; F_i=% marker (Cr_2O_3) in feces, ADCT=% apparent digestibility coefficient of protein, amino acids or energy in test diet; ADCR=apparent digestibility coefficient of nutrient or energy in the reference diet; I=test ingredient under investigation.

4. Data analysis

The data was analysed by using PROC MIXED of SAS (version 9.2, SAS Institute Inc., Cary, NC). Following model was used for data analysis;

Following model was used for data analysis;

$$Y = \mu + I_j + O_k + I_j*Ok + \varepsilon_{jkl}$$

Y_{jkl} = Observation of dependent variable recorded on *i*th, *j*th and $_k$th treatment

μ = Population mean

O_j = Origin (*j* = MUL, SKR)

ε_{jkl} = Residual effect associated with *j*th, *k*th and *l*th treatment NID ~ 0, σ2.

RESULTS

Proximate Analysis:

The data for proximate analysis are summarized in Table 8. There were significant differences between different origins for DM. Dry matter contents were higher in CM from SKR and lowest in those from MUL, whereas DM contents in the RSM of different origins were significantly higher in SKR from MUL. There were non-significant differences between different origins for DM of wheat and wheat middling of SKR and MUL. There were non-significant differences between different origins for CF in CM, RSM, wheat and wheat middling of SKR and MUL. There were significant differences between different origins for CP. Crude protein contents were higher in CM from SKR and lowest in those from MUL, whereas CP contents in the RSM of different origins were significantly higher in SKR from MUL. There were significant differences between the CP of different origins of wheat and wheat middling of SKR and MUL. The CP contents of wheat and wheat middling of MUL regions were significantly higher from SKR. There were non-significant differences between different origins for EE in CM, RSM, wheat and wheat middling of SKR and MUL. There were significant differences between different origins for ash. Ash contents were higher in CM and RSM from MUL and lowest in those from SKR, whereas ash contents were higher in wheat and wheat middling from SKR and lowest in those from MUL respectively (Table 8).

2. Gross Energy Measurement:

The total energy contents of the indigenous protein and energy sources are presented in Table 8. It was observed that GE contents in CM, RSM, wheat and wheat middling showed no difference regardless of their origins.

Table 8: Proximate analysis (%) and energy contents (Kcal/kg) of the indigenous protein and energy sources for fish diet.

Item	CM				RSM				Wheat				Wheat Middling			
	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*- value
DM	89.7	90.1	0.3	*	89.1	90.7	0.2	*	92.3	92.4	0.21	NS	92.1	92.0	0.11	NS
CF	12.2	11.7	0.8	NS	11.9	12.6	0.4	NS	3.0	3.1	0.08	NS	3.2	3.3	0.07	NS
CP	35.4	36.8	0.5	*	36.4	37.2	1.1	NS	11.3	10.1	0.11	*	17.3	16.4	0.22	*
EE	2.4	3.1	0.7	NS	1.6	1.4	0.5	NS	5.3	5.4	0.12	NS	9.3	9.2	0.11	NS
Ash	6.9	5.8	0.4	*	9.1	8.2	0.2	*	1.71	2.21	0.13	*	6.6	5.4	0.19	*
GE	4200	4150	15.6	NS	4210	4260	20.8	NS	4179	4190	12.4	NS	4390	4430	20.3	NS

CM= canola meal. RSM=rapeseed meal. DM= dry matter. CP= crude protein. CF= crude fiber. EE= Ether extract. GE= gross energy. MUL=multan. SKR= Sakhar. SEM= standard error of mean. NS= non-significant

Apparent Metaboliseable Energy Digestibility

Digestibility of apparent metaboliseable energy of indigenous wheat and wheat middling in tilapia is presented in Figure 1. There were no differences observed in the apparent digestibility of energy for the indigenous energy sources (wheat and wheat middling) because of their origin.

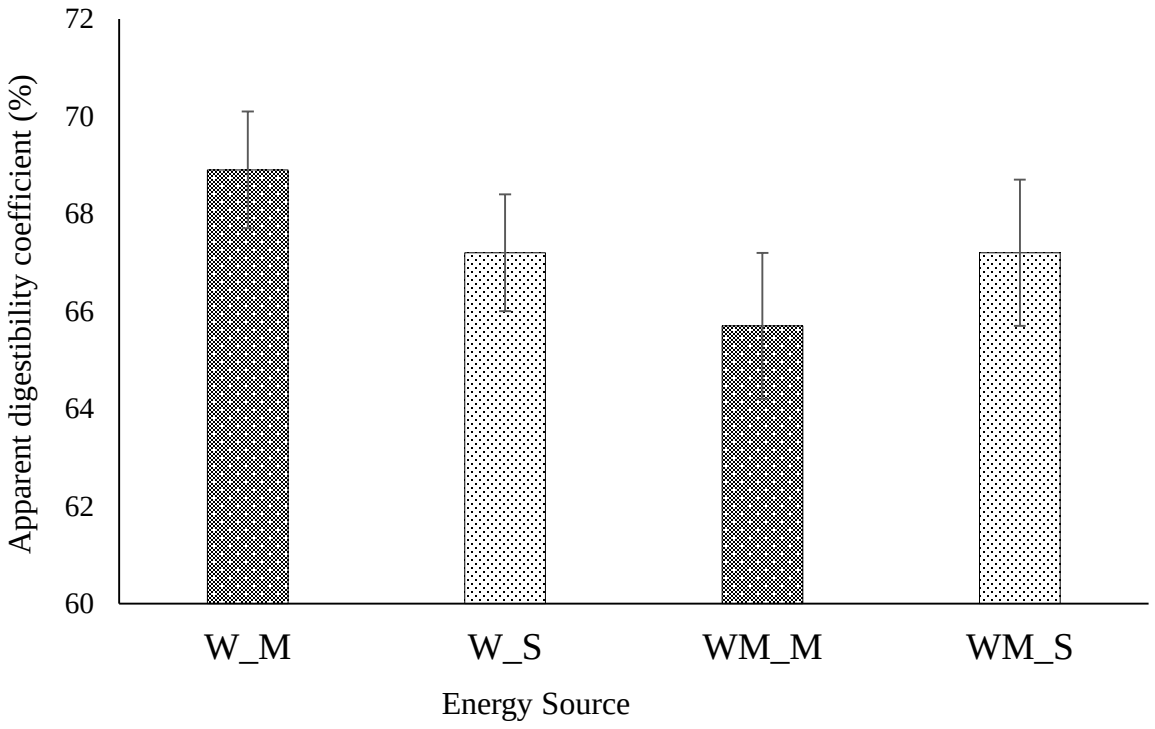

Figure 1: Apparent digestibility coefficients of indigenous energy sources of different origin.

Total Amino Acid Contents of Indigenous Energy Sources

Table 9 shows the total amino acid contents of wheat and wheat middling of indigenous energy sources of different origin, commonly used in aqua culture feed. The results revealed that despite significant differences in the CP contents of the wheat and wheat middling from two different origins, only two (02) Arg and Lys of the 09 EAA of wheat differed due to origins. Arg and Lys contents were high in SKR samples. Among wheat non-essential AA, differences were observed for Ser, Cys, Glu and Asp. The Ser contents were observed high in MUL

samples, whereas Cys, Glu and Asp contents were significantly higher in SKR origin samples (Table 9).

Despite significant difference in the CP contents of wheat middling from different origins, only 03 of the 09 AA differed due to origin. Among EAA, differences were observed for Arg, His and Val. The Arg, His and Val contents were higher in the MUL samples, whereas among non-essential AA, Gly, Ser and Glu contents were observed high in MUL samples than those SKR due to origins (Table 9).

Apparent digestibility of CP and Amino acid content of indigenous energy sources

The digestibility coefficient of CP and AA of the indigenous energy sources (wheat and wheat middling) from two different origins (SKR and MUL) are represented in Table 10. On a CP basis of wheat, differences existed across origins only for two (02) EAA (Lys and Thr; Table 10**)** were higher in MUL than SKR**,** whereas in non-essential AA of wheat, Asp was observed higher in MUL than SKR samples.

On a CP basis of wheat middling, EAA of wheat middling, Arg was significantly higher in the MUL sample than SKR, whereas Thr and Val contents were significantly higher in the SKR samples than MUL origin sample. Among non-essential AA, differences were observed for Ala, Tyr, Ser and Asp of wheat middling of two different origins. Ala, Ser and Asp contents of MUL samples were higher than SKR, whereas Tyr content were higher in the SKR samples compared with those in MUL (Table 10).

Total Amino Acid Contents of indigenous protein sources:

Table 11 shows the total amino acid contents of CM and RSM indigenous protein sources of different origin, commonly used in feed. The results revealed that despite significant differences in the CP contents of CM and RSM from two different origins, only three (03) of the 09 EAA

of CM and just 02 of the 09 EAA of RSM differed due to origins. Among CM EAA, differences were observed for Arg, Ile and Phe. Arg and Phe contents were higher in MUL samples, whereas Ile conetnts were higher in SKR origin sample. Among CM non-essential AA, differences were observed for Gly and Ser. Both were significantly higher in SKR origin samples (Table 11**)**. Among RSM, only 02 of the 09 EAA, Val and Phe contents of SKR sample origins were higher than those from MUL, whereas in RSM non-essential AA, Ser and Glu contents differences were observed. Serine content was highest in MUL sample, whereas Glu content of SKR sample were observed high (Table 11**)**.

Apparent digestibility of CP and Amino acid content of indigenous energy sources

Apparent digestibility coefficients of CP and amino acids of indigenous protein sources (CM and RSM) are presented in Table 12. The results indicates no significant differences in digestibility coefficients of CP regarding their origin in Tilapia fish.

The digestibility coefficients of CM, similarly, was not influenced by the origin in Tilapia. The digestibility coefficients of Leu was significantly greater in the samples of MUL origin compared with those from SKR in RSM diets. Similarly digestibility coefficients of Cys and Gly (Non-essential amino acids) were greater in samples of MUL origin compared with those from SKR origin in Tilapia (Table 12).

Table 9: Amino acid profile of the indigenous energy sources, with different origins, commonly fed to tilapia fish.

Item	Wheat				Wheat middling			
	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value
Essential AA								
Arginine	4.3	4.7	0.1	*	6.1	5.8	0.1	*
Isoleucine	3.1	3.2	0.2	NS	3.0	2.6	0.2	NS
Leucine	6.2	6.0	0.2	NS	5.8	5.5	0.2	NS
Lysine	2.7	3.1	0.1	*	3.7	3.5	0.2	NS
Histidine	2.0	1.9	0.2	NS	2.2	1.9	0.1	*
Methionine	1.5	1.4	0.1	NS	1.2	1.1	0.2	NS
Phenylalanine	4.3	4.0	0.2	NS	3.2	3.0	0.2	NS
Threonine	2.7	2.8	0.1	NS	3.1	2.9	0.1	NS
Valine	4.0	3.9	0.1	NS	4.2	3.8	0.1	*
Nonessential AA								
Glycine	3.5	3.3	0.1	NS	4.5	4.0	0.2	*
Alanine	3.2	3.0	0.2	NS	4.1	3.8	0.2	NS
Tyrosine	2.4	2.3	0.2	NS	2.3	2.1	0.1	NS
Serine	4.4	4.1	0.1	*	4.0	3.6	0.1	*
Cysteine	1.9	2.3	0.1	*	1.8	1.7	0.2	NS
Glutamic acid	25.6	26.3	0.2	*	17.8	16.9	0.2	*
Aspartic acid	4.8	5.6	0.1	*	6.2	6.0	0.2	NS

EAA= essential amino acids, NEE= non-essential amino acids, CM= canola meal. RSM=rapeseed meal MUL=Multan. SKR= Sukkur. SEM= standard error of mean.

Table 10: Apparent digestibility (%) of crude protein and Amino acid index of the indigenous energy sources from different origins when fed to Tilapia fish.

Item	Wheat				Wheat Middling			
	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value
CP	94.1	93.4	1.3	NS	86.4	83.1	1.2	*
Essential AA								
Arginine	89.1	86.7	1.2	NS	88.9	85.7	1.2	*
Isoleucine	94.2	95.4	1.5	NS	82.8	84.7	1.5	NS
Leucine	96.5	96.2	1.3	NS	84.3	82.1	1.4	NS
Lysine	93.4	91.8	1.1	*	85.7	84.3	1.5	NS
Histidine	94.8	93.9	1.5	NS	86.4	87.5	1.2	NS
Methionine	96.2	95.7	1.8	NS	84.0	83.5	0.9	NS
Phenylalanine	95.2	94.8	1.4	NS	81.3	83.8	1.4	NS
Threonine	93.4	90.7	1.2	*	76.8	80.2	1.3	*
Valine	93.4	95.6	1.3	NS	78.2	83.1	1.5	*
Nonessential AA								
Glycine	97.2	96.8	1.4	NS	84.1	82.2	1.3	NS
Alanine	96.4	97.2	1.7	NS	81.8	78.2	1.6	*
Tyrosine	94.3	95.1	1.5	NS	80.2	83.1	1.0	*
Serine	95.7	96.5	1.6	NS	85.3	82.1	0.9	*
Cysteine	94.5	96.8	1.2	NS	80.7	79.2	1.2	NS
Glutamic acid	97.2	95.1	1.4	NS	90.2	89.7	1.4	NS
Aspartic acid	96.8	93.1	1.7	*	84.3	81.2	1.1	*

EAA= essential amino acids, NEE= non-essential amino acids, CM= canola meal. RSM=rapeseed meal MUL=Multan. SKR= Sukkur. SEM= standard error of mean.

Table 11: Amino acid profile of the indigenous protein sources, from different origins, commonly fed to tilapia fish.

Item	CM				RSM			
	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value
Essential AA								
Arginine	6.8	6.4	0.1	*	6.1	5.6	0.2	NS
Histidine	2.8	2.6	0.2	NS	2.5	2.8	0.2	NS
Isoleucine	4.3	4.8	0.1	*	3.8	3.2	0.3	NS
Leucine	6.9	7.1	0.2	NS	6.5	6.1	0.1	NS
Lysine	5.4	5.2	0.2	NS	5.3	6.1	0.2	NS
Methionine	2.4	2.5	0.1	NS	1.98	2.21	0.1	*
Phenylalanine	5.8	5.2	0.1	*	5.6	6.2	0.1	NS
Threonine	4.6	4.4	0.2	NS	3.9	4.2	0.2	NS
Valine	5.2	5.0	0.2	NS	4.7	5.3	0.1	*
Nonessential AA								
Alanine	4.10	3.98	0.2	NS	4.2	3.8	0.3	NS
Aspartate	3.21	3.10	0.1	NS	2.1	1.9	0.1	NS
Cystine	2.43	2.48	0.2	NS	2.0	2.3	0.2	NS
Glycine	4.51	4.67	0.1	*	4.9	5.2	0.2	NS
Glutamic acid	6.42	6.18	0.3	NS	16.5	14.3	0.3	*
Serine	4.02	4.23	0.1	*	4.1	4.8	0.2	*

EAA= essential amino acids, NEE= non-essential amino acids, CM= canola meal. RSM=rapeseed meal MUL=Multan. SKR= Sukkur. SEM= standard error of mean.

Table 12: Apparent digestibility (%) of crude protein and amino acids of indigenous protein sources from different origin in Tilapia fish.

Item	CM				RSM			
	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value
CP	64.3	66.1	1.5	NS	61.3	62.8	1.3	NS
Essential AA								
Arginine	90.1	91.5	1.8	NS	89.5	87.2	1.8	NS
Histidine	90.4	88.4	2.1	NS	88.3	87.8	1.6	NS
Isoleucine	82.3	83.1	1.3	NS	79.4	77.9	2.3	NS
Leucine	85.4	85.0	1.1	NS	84.3	80.1	1.2	*
Lysine	84.3	83.7	1.2	NS	87.5	85.4	2.4	NS
Methionine	88.4	88.0	0.9	NS	84.1	83.2	2.0	NS
Phenylalanine	86.1	85.9	1.8	NS	80.3	77.9	1.7	NS
Threonine	85.7	85.2	2.1	NS	81.8	79.5	2.1	NS
Valine	81.9	80.8	1.4	NS	78.9	76.4	1.3	NS
Nonessential AA								
Alanine	85.4	85.1	1.6	NS	83.4	82.4	2.4	NS
Aspartate	80.6	78.9	2.3	NS	82.1	80.7	2.1	NS
Cystine	77.8	76.9	1.8	NS	72.3	70.2	1.5	NS
Glycine	83.1	82.8	2.7	NS	80.1	76.1	1.3	*
Glutamic acid	90.7	90.1	2.0	NS	88.5	80.7	2.3	*
Serine	84.3	84.0	1.8	NS	82.7	80.7	1.8	NS

EAA= essential amino acids, NEE= non-essential amino acids, CM= canola meal. RSM=rapeseed meal MUL=Multan. SKR= Sukkur. SEM= standard error of mean.

DISCUSSION

Proximate Analysis

The proximate analysis values of wheat middling were within the range described in the literature (Ensminger and Olentine, 1980; Chu et al. 1991; Cromwell et al. 2000). Considerable variations were, however, observed between samples of wheat middling from different origins for these chemical components. The CP and Ash content were higher in wheat middling in MUL sample compared with the SKR. The higher CP concentration in MUL samples, compare with SKR samples, may be interrelated with variety, agronomic characteristics geographical locations, environmental circumstances during crop development, harvesting conditions and processing of the seed (Barthet and Daun, 2011). The proximate analysis values of CM were in close agreement with values described in the literature (Khajali and Slominski, 2012; Current canola meal feeding guide, 2015; Leeson and Summer, 2005; Selle and Ravindran, 2007; Newkirk, 2009; Rogiewicz et al. 2012). Considerable variations were, however, observed between samples of CM from different origins for these chemical components. The CP, EE and DM contents were higher, whereas CF and ash contents were lower in SKR sample compared with MUL. The average CP and EE content form SKR sample were 36.8% and 3.1%. The higher CP concentration in SKR samples, compare with MUL samples, may be interrelated with variety, agronomic characteristics geographical locations, environmental circumstances during crop development, harvesting conditions and processing of the seed and meal (Barthet and Daun, 2011; Newkirk, 2009). The CP content in RSM was not influenced by origin. In SKR sample DM content was higher compare with MUL. The CP content in wheat of MUL sample was higher than that from SKR, whereas ash content in SKR sample of wheat was higher. The difference in CP content may be attributed due to season and site of cultivation (Conan et al.

1992; Metayer et al. 1993). Nyirenda et al. (1987) provided supporting evidence of seasonal and varietal effects on CP, CF, starch and ash in grains.

Apparent Digestibility of Energy

There was no difference observed in apparent digestibility of energy wheat and wheat middling of two different locations. The digestibility values for wheat and wheat middling observed in this study were higher than reported previously (Cheng and Hardy, 2002; Brunson et al. 1997). The difference in digestibility values might be due to difference season of harvesting (Hughes et al. 1998). Moreover, the increase in non- starch polysaccharides (NSPs) also decrease the nutritive value of cereals (Annison 1991).

Contents and Apparent Digestibility of Crude Protein and Amino Acids:

The total AA content of indigenous wheat samples were in accordance with literature (Shoup et al. 1966; Ling et al. 2008; Abdel and Hucl, 2002). Arginine, Lys Asp, Cys and Glu content was higher in wheat sample from SKR, whereas in MUL sample the Ser content was higher. The higher content of AA in SKR sample might be due to application of fertilizer, since fertilizer application increases the total AA content in wheat (McNab, 1991). The digestibility of CP and EAA Lys and Thr of wheat from MUL was observed greater than those form SKR. The CP digestibility of wheat was in close relationship with the results reported by Cheng and Hardy (2002). In general, the AA digestibility observed in this study was higher and the values were in close agreement with the results reported by Allan et al. (2000). The digestibility coefficient of Lys was lowest despite a markedly high CP and TAA digestibility in wheat sample from SKR. This low total Lys content indicates that some of Lys and AA were damaged during the fermentation or drying process of grains (Widyaratne and Zijlstra, 2006).

In wheat middling from MUL, Arg, His, Thr and Val content was lower than those from SKR. For NEAA, Gly, Ser, and Glu content were also greater in wheat middling from MUL. These variations in TAA content in wheat middling samples may be interrelated with variety, agronomic characteristics geographical locations, environmental circumstances during crop development, harvesting conditions and processing of the seed (Barthet and Daun, 2011). Among all the EAA the Arg from MUL had the highest digestibilty in wheat middling. The Thr and Val digestibility in wheat middling from SKR had the greater digestibility. Low digestibility of CP and EAA in wheat middling from SKR might be due to the high CF and non-starch polysaccharides content, which increase digesta flow rate, hence reducing time of contact between enzyme and substrate (Furuya et al. 2001; Guimaraes, 2008).

The determined total amino acids (TAA) contents of indigenous CM samples were in accordance with literature (Adewole et al. 2017; Kim et al. 2012). Despite significant differences in CP content of CM from different origins, only 5 of 15 AA differ due to origin. The AA digestibility of CM showed no difference regardless of their origin. The high digestibility of CM is also reported by (Stickney et al. 1996; Forster et al. 1999). This high digestibility might be due to reduce content of erucic acid and glucosinolates in CM by applying heat treatment after oil extraction by hexane treatment (Newkirk, 2002). However, this treatment can also affect protein and amino acid digestibility (Drew et al. 2007).

Among essential AA, the differences were observed for Arg, Ile and Phe. The Met, Val and Ser content were higher in RSM from SKR, than those from MUL. These differences in AA content may be due to the high temperature in order to reduce the oil content which might reduce the AA content in RSM (Gonzalez-Vega et al. 2011). The total AA profile of indigenous RSM samples was in agreement with the values already reported in literature (Kasparzak et al. 2016;

Nadeem et al. 2005; Ullah et al. 2016). The EAA Ile and NEAA Gly and Glu showed higher digestibility values in RSM from SKR. The values of RSM digestibility were in agreement with the values reported by Zhou et al. (2004). The reduced digestibility of RSM than CM regardless of their origin might be due to anti-nutritional factors in RSM (Zhou et al. 2004). The higher CF content and ANFs including erucic acid ang glucosinolates RSM might decrease the digestibility of AA and CP (Khajali and Slominski, 2012). The higher pectin, hemicellulose and cellulose in cell wall of rapeseed hulls may bind with AA released during protein digestion and thereby decreases the AA absorption in the small intestine (Howard et al. 1986; Bjergegaard et al. 1991).

Conclusions:

The results of the present study indicated various nutritional differences in the ingredients from different origins. However, these variations were small in most of the nutrients. The variations were also observed in the digestibility values of different nutrients. A data base has been generated regarding commonly used indigenous energy as well as protein sources used in the Tilapia diets. This database can be helpful for nutritionist formulating diets for Tilapia and other fish species.

REFERENCES

Allan GL, Parkinson S, Booth MA, Stone DAJ, Rowland SJ, Frances J, Smith RW. 2000. Replacement of fish meal in diets for Australian silver perch, Bidyanus bidyanus: I. Digestibility of alternative ingredients. Aquaculture. 186: 293–310.

AHA (American Heart Association), 2008. Fish and omega-3 fatty acids. http://www.americanheart.org/presenter.jhtml?identifier=4632

Abdel-Aal ESM, Hucl P. 2002. Amino Acid Composition and In Vitro Protein Digestibility of Selected Ancient Wheats and their End Products. Journal of Food Composition and Analysis. 15: 737-747.

Drew MD, Borgeson TL, Thiessen DL. 2007. A review of processing of feed ingredients to enhance diet digestibility in finfish. Animal Feed Science and Technology. 138:118–136.

Ministry of Health of Brazil, 2006. General Department of Food and Nutrition Policy, Department of Basic Attention, Secretariat of Health Care. Food Guide for the Brazilian population: promoting healthy eating. 2006.

Bolivar RB, Cruz EV, Jimenez EB, Sayco RM, Argueza RL, Ferket PR, Stark CR, Malheiros R, Ayoola AA, Johnstone WM, Picha ME. Feeding reduction strategies and alternative feeds to reduce production costs of tilapia culture. Technical Reports: Investigations 2007-2009, AquaFish Collaborative Research Support Program. Oregon State University; 2010.

Barthet VJ, Daun JK. 2011. Seed morphology, composition, and quality. Pages 125-145 in Canola: Chemistry, Production, Processing, and Utilization. J. K. Daun, N. A. M. Eskin, D. Hickling, eds. AOCS Press, Urbana, IL.

Bjergegaard C, Eggum BO, Jensen SK, Sørensen H. 1991. Dietary fibers in oilseed rape: Physiological and antinutritional effects in rats of isolated IDF and SDF added to a standard diet. Journal of Animal Physiology and Animal Nutrition. 66:69−79.

Leeson, S., and J. D. Summers. 2005. Commercial Poultry Nutrition. 3rd Ed. University Books, Guelph, ON, Canada.

Newkirk R. 2009. Canola Meal Feed Industry Guide 4th Edition. Canola council.

Newkirk RW. 2002. The Effects of Processing on the Nutritional Value of Canola Meal for Broiler Chickens. Ph.D. Thesis. University of Saskatchewan, Ssakatoon, SK, Canada.

Cho, C.Y., Slinger, S.J., 1979. Apparent digestibility measurement in feedstuffs for rainbow trout. In: Halver, J., Tiews, K. (Eds.), Proceedings of World Symposium on fish Nutrition and Fish feed Technology vol. 2. Heenemann, Berlin, pp. 239 – 247.

Chu KS, Han IK, Won TH, Park, BC. 1991. Studies on nutrient availabilities of feed ingredients in Israeli sheep. Asian Austurlasin Journal of Animal Sciences. 4: 263-274.

Cromwell GL, Cline TR, Crenshaw JD, Crenshaw TD, Easter RA, Ewan RC, Hamilton CR, Hill GM, Lewis AJ, Mahan DC, Nelssen JL. 2000. Variability among sources and laboratories in analyses of wheat middling. Journal of Animal Sciences. 78:2652–2658.

Conan L, Metayer JP, Lessire M, Widiez, JL. 1992. Metabolizable energy content of cereal grains in poultry. Recent yearly surveys in France. Productions Animales 5: 329-38.

Cheng ZJ, Hardy RW. 2002. Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middling, measured in vivo using rainbow trout (Oncorhynchus mykiss). Aquaculture Nutrition. 8:271-277.

Furuya WM, Pezzato LE, Pezzato AC, Barros MM, de Miranda CE. 2001. Digestibility coefficients and digestible amino acids values of some ingredients for Nile tilapia (Oreochromis iloticus). Brazilian Journal of Animal Science 33(4):1143–1149.

González-Vega JC, Kim BG, Htoo JK, A. Lemme A, Stein HH. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. Journal of Animal Science. 89:3617–3625.

Guimaraes IG. 2008. Nutrient Digestibility of Cereal Grain Products and By-products in Extruded Diets for Nile Tilapia. Journal of the World Aquaculture Society. 39.6.

Howard P, Mahoney RR, Wilder T. 1986. Binding of amino acids by dietary fibers and wheat bran. Nutrition Republica International. 34: 135−140.

Khajali F and Slominski BA. 2012. Factors that affect the nutritive value of canola meal for poultry. Poultry Science. 91:2564–2575.

Ling JX, Chun T, Zhi H, Dong Z. 2008. Protein Content and Amino Acid Composition in Grains of Wheat-Related Species. Agricultural Sciences in China. 7(3): 272-279.

Metayer JP, Grosjean F, Castaing J. 1993. Study of variability in French cereals. Animal Feed Science and Technology 43: 87-108.

McNab JM. (1991). Factors affecting the nutritive value of wheat for poultry. HGCA Project Report No. 43. Home Grown Cereals Authority.

Nyirenda DBG, Vohra P, Ingebretson KH. 1987. Nutritional evaluation of some varieties of sorghums (Sorghum bicolor Moench). Cereal Chemistry 64: 413-417.

Nadeem MA, Gilani AH, Khan AG, Nisa M. 2005. Amino Acids Availability of Poultry Feedstuffs in Pakistan. International Journal of Agriculture & Biology. 1560–8530/2005/07–6–985–989.

J. E. Pettigrew, T. L. Veum, and J. T. Yen Ensminger ME, Olentine CG Jr. 1980. Composition Table of Energy Feeds. In: Feeds & Nutrition, Complete. The Ensminger Publishing Company, Clovis, CA.

Rana KJ, Siriwardena S, Hasan MR. Impact of rising feed ingredient prices on aquafeeds and aquaculture production. Food and Agriculture Organization of the United Nations (FAO); 2009.

Merino, G., Barange, M., Blanchard, J.L., *et al.* (2012) Can marine fisheries and aquaculture met fish demand from a growing human population in a changing climate? *Global Environmental Change*. 22: 795-806.

Ravindran V, Abdollahi MR, Bootwalla SM. Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. Poultry science. 2014 Sep 20; 93(10):2567-77.

Wilson RP. Amino acid requirements of finfish and crustaceans. In: D´MELLO, J.P.F. (Ed.). Amino acid in farm animal nutrition. Wallingford: CAB International, 2003. p. 427-447.

Furuya WM, Botaro D, Macedo RMG. et al. Aplicação do conceito de proteína ideal para redução dos níveis de proteína em dietas para tilápia-do-Nilo (*Oreochromis niloticus*). Revista Brasileira de Zootecnia, v.34, n.5, p.1433-1441, 2005.

Welcomme RL, Cowx IG, Coates D, Béné C, Funge-Smith S, Halls A, Lorenzen K. 2010. Inland capture fisheries. Phil. Trans. R. Soc. Lond. Ser. B, Biol. Sci. 365(1554): 2881-2896.

Kapetsky, J.M. 2003. Review of the State of World Fishery Resources: Inland Fisheries. Rome.

Rogiewicz A, Nurnberg L, Slominski BA. 2012. The effect of prepress-solvent exctraction on the chemical and nutritive composition of canola meal. Proc. 24th World's Poultry Congress. Salvador, Brazil.

Shoup FK, Pomeranz Y, Deyoe CW. 1966. Amino Acid Composition of Wheat Varieties and Flours Varying Widely in Bread-Making Potentialities. DOI: 10.1111/j.1365-2621.1966.tb15420.x.

Selle PH, and Ravindran V. 2007. Microbial phytase in poultry nutrition. Animal Feed Science and Technology. 135:1–41.

Shahzad KH, Salim MU, Asad FA. 2006. Evaluation of apparent digestibility coefficient of corn, wheat and feather meal for Labeo rohita. Pakistan Journal of Zoology. 38(2):125.

Widyaratne GP, Zijlstra, RT. 2006. Nutritional value of wheat and corn distiller's dried grain with solubles: Digestibility and digestible contents of energy, amino acids and phosphorus, nutrient excretion and growth performance of grower-finisher pigs. Canadian Journal of Animal Science.

Ullah Z, Rehman Z,Yulong Y, Stein HH, Hayat Z, Ahmed G, Nisa M, Akhtar M, Sarwar M. 2017. Comparative ileal digestibility of amino acids in 00-rapeseed meal and rapeseed meal fed to growing male broilers. Poultry Sciences. 0:1–7.

Zhoua QC, Tanc BP, Maib KC, Liu YJ. 2004. Apparent digestibility of selected feed ingredients for juvenile cobia Rachycentron canadum. Aquaculture. 241: 441-451.