EXPLORING THE NUTRITIVE POTENTIAL OF INDIGENOUS PROTEIN SOURCES AND THEIR DIGESTIBILITY INDEXES IN BROILERS

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ABBREVATIONS

GDP = Gross domestic product

SBM = Soybean meal

CM = Canola meal

SFM = Sunflower meal

GM= Guar meal

RSM = Rapeseed meal

CP = Crude protein

AA = Amino acid

AME = Apparent metabolizable energy

AMEn= Apparent metabolizable energy corrected for nitrogen

TAA = Total amino acids

AIA = Apparent ileal amino acids

SIAA = Standardized ileal amino acids

ANF = Anti nutritional factors

GE = Gross energy

DM = Dry matter

EE = Ether extract

CF = Crude fiber

TI = Trypsin inhibitor

TIU = Trypsin inhibitor unit

ME = Metabolizable energy

BWP = Bahawalpur

MUL = Multan

SKR = Sukkur

^{o}C = Degree Celsius

FI = Feed intake

BWG = Body weight gain

PFD = Protein free diet

MCDP = Mono calcium di-phosphate

EAA = Essential amino acids

NESS Non-essential amino acids

RESEARCH PROJECT SUMMARY

Principle Investigator (PI)	**Dr. Shafqat Nawaz Qaisrani**
Title of Research Project	**Exploring The Nutritive Potential Of Indigenous Protein Sources And Their Digestibility Indexes In Broilers**
Duration	**04 Months**
Department	**Animal Nutrition**
University/Institute	**University of Veterinary and Animal Sciences, Lahore**

CHAPTER 1
INTRODUCTION

Increasing human population, purchasing power and urbanization are strong drivers of poultry growth. Global population is estimated to be 7.5 billion and will reach up to 8 billion till 2023 (United Nations, 2017). This rapid population growth has resulted in increased demand for animal derived food (FAO, 2009). To cope with this huge demand in a short time, new, better and cheaper food resources are under investigation. The major animal source of protein among the food consumed worldwide is the poultry meat and eggs, providing proteins, energy and essential nutrients to the human beings in relatively short time period with rapid production cycles (Mottet et al. 2017). Over 23 billion poultry exist in the world sharing 3 birds per person on the planet which is 5 times greater than 50 years ago. United States is on top position regarding production of the poultry/chicken meat which is about 20 million tons per year, China is at second producing 18 million tons followed by the Brazil and EU with 13 million tons per year (FAOSTAT, 2016). In developed countries, per capita meat consumption is about 41 kg and 300 eggs per year, whereas in Pakistan it is about 5 kg meat and 51 eggs per year (PPA, 2013). World Health Organization (WHO) recommends that daily animal protein requirements of the adult body is 27 g per person, on an average, whereas in Pakistan daily consumption is about 17 g of the protein from the routine diet (Memon, 2012). The poultry meat only shares 5 g out of this 17 g protein, creating to a gap of 10 g per person per day (Hussain et al. 2015). Since, the commercial poultry in Pakistan was established half century ago and this sector is now making tremendous contribution in providing of good quality animal protein for human. According to economic survey (2016-2017) of Pakistan, poultry is contributing 1.4% in national GDP and 12.2% in livestock and 7.1% in agricultural GDP. Pakistan is ranked 11th in poultry production globally with the production about 1.02 billion broilers annually (Figure1). Feed cost accounts for 70% of total cost of poultry production (Figure 2). This high feed cost is the major issue faced by the poultry industry today and is expected to increase in upward

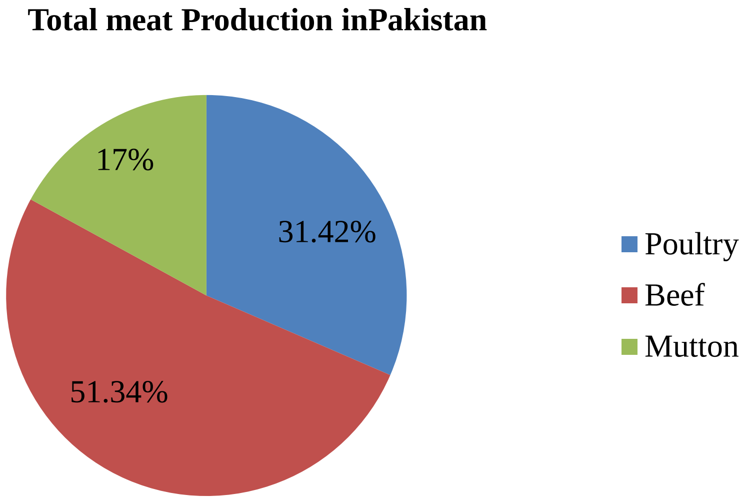

Figure 1. Total meat production and share of poultry meat in Pakistan.

direction due to the import and inadequate supply of the feed ingredients (Conolly, 2012; Lepleaideur, 2004). Poultry feed is composed of cereal grains as energy sources and meals as protein sources.

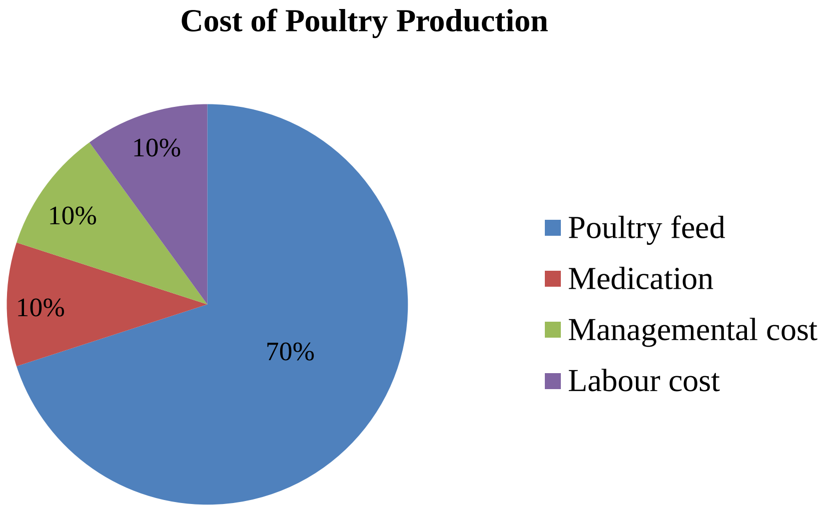

Figure 2. Poultry production cost.

In Pakistan, livestock and poultry sector are importing considerable amount of high profile protein ingredient including soybean meal (SBM). The SBM has maximum digestibility (70-

75%) along with excellent digestible amino acid profile but it is not cultivated in Pakistan. It is imported from other countries like Brazil, Argentina and India, which increases cost of feed. Pakistan has imported more than 700 metric tonnes SBM during 2015-2016. The price of SBM directly affects the cost of feed since it is a major protein ingredient in poultry diets. The higher cost of SBM is encouraging nutritionists to find out its alternate plant protein sources.

To overcome this problem, the use of indigenous dietary ingredients can be a good option. Plant protein sources mainly used in poultry diets includes canola meal (CM), sunflower meal (SFM), rapeseed meal (RSM) and guar meal (GM). These protein sources are cultivated locally and are comparatively cheaper. Pakistan is producing about 190000, 109000, 15000 tonnes of rapeseed, sunflower seed and canola seed, respectively, (Economic survey 2016-2017), which can potentially replace SBM partially or completely. 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 poultry production and will greatly influence the financial returns. The unknown composition and nutritional profile of these mentioned indigenous protein sources, however, is the major hurdle in their use to formulate nutritionally balanced poultry diets. Since protein quality and nutritional composition of these meals depends on many factors including processing conditions (Gonzalez-Vega et al. 2011), characteristics of soil and oil seeds origin (Mateos et al. 2011). Environmental conditions and genetics, additionally, influence plants composition including their crude protein (CP) and total amino acid (TAA) concentrations. In Pakistan, usually follow international standards of ingredient composition (NRC, 1994; CVB 2016; Fundacion Española Desarrollo Nutricion Animal, 2010; Rostagno, 2011) to formulate the poultry diets even by using the indigenous feed stuff. The unknown complete nutritional profile and unavailability of digestible values of energy, CP and AA may leads to formulation of nutritionally imbalanced diets resulting in either poor performance from the birds or environment pollution.

It is the need of hours to evaluate nutritional profile along with digestible values of indigenous plant protein sources. Measurement of total amino acid (AA) profile, moreover, is the best way to evaluate digestible AA content of the different ingredients (Rostagno et al. 1995). The modern day nutritionists are endorsing to formulate poultry diets on digestible AA basis because diet formulated on the basis of digestible amino acid (DAA) are more accurate. In addition to reduction of excreted nitrogen in poultry houses, ultimately improvement of the health of birds and humans, also have lower cost (Alagawany et al. 2014). Some data is present on total amino acids digestibility, however, the adequate literature on SIAAD for indigenous plant protein sources still required. This might certainly increase the reliability of the available literature, besides, the probability of using this data in diet formulation by nutritionists. The objectives of this project were to evaluate and compare nutritional profile, metabolizable energy and standardized ileal amino acid digestibility (SIAAD) contents of different indigenous plant protein sources cultivated in Pakistan.

Balanced and least cost poultry diet formulation has become a challenging due to higher cost of good quality feed ingredients and their reduced availability. Non-conventional oil seeds, therefore, have prime importance in poultry and livestock feed industry. Plant protein sources mainly used in poultry diets are soybean meal (SBM), canola meal (CM), sunflower meal (SFM), rapeseed meal (RSM) and guar meal (GM). Soybean meal and CM are the most commonly used protein source in poultry diets due to their high concentration of protein 44 to 49% and 34-38%, respectively, and their excellent amino acid profile. Canola meal, however, may have a poor nutritional values because of the presence of anti-nutritional factors (ANF) and increased level of non-starch polysaccharides. Inclusion level of CM in poultry feed is limited due to the presence of these ANF including phytic acid, tannins, glucosinolates and erucic acids. Regardless of the fact that, CM is a rich source of methionine and cysteine, the arginine content of CM is nearly two-third (2.08 vs. 3.14%) that of SBM (NRC, 1994).

Different varieties of canola are being processed having low level of erucic acid and glucosinolates in canola oil and meal (Bell, 1993). Erucic acid level in canola is less than 2% with glucosinolates < 30 µmol/g (Khajali and Slominski, 2012).

Guar meal (GM) is such an ingredient with better nutritional profile and higher availability (Ahmed, 1998). Guar meal is produced during the extraction process of guar gum from endosperm (Conner, 2002; Lee et al. 2005) having 75% hull and 25% germ. Nutritional composition of GM contains 40.7% CP on dry matter (DM) basis (Nadeem et al. 2005), 92% DM (Ahmed, 1998), 4406 Kcal/kg gross energy (GE) (Nadeem et al. 2005), 5.5% ash concentration (Nagpal et al. 1971), ether extract (EE) concentration 4.8% (Nadeem et al. 2005). Guar meal contains 1.7% lysine, methionine 0.36%, threonine 1.51% and 4.03% arginine (Lee et al. 2004). Its bitter taste and ANFs, including trypsin inhibitor (TI) (1.35mg/g) limits its use in poultry diets (Ahmed, 1998). Trypsin inhibitors negatively influence the growth performance (Couch et al. 1966), body weight and feed efficiency (Thakur and Pradhan, 1975). Extraction process, heat treatment and methionine supplementation reduce the TI activity (Cheeke and Shull, 1985). Heat treatment for 60 minutes might reduce the 80% TI activity in GM (Couch et al. 1966).

Sunflower meal obtained after the extrusion of oil from sunflower seed is a good quality plant protein source for poultry. Higher level of fiber, however, in SFM constrained its usage in poultry diet (Ravindran and Blair, 1992). The higher level of fiber can be reduced through decortication process (McDonald et al. 2011; Alagawany et al. 2015). Higher level of fiber in SFM negatively influence the CP content (Mushtaq et al. 2006). Higher level of SFM in broiler diet might reduce the availability of lysine in broilers (Mushtaq et al. 2009). Sunflower meal inclusion up to 300g did not show any harmful effect in broilers of 14 days age (Mushtaq et al. 2006). Although the magnitude of improvement in AA digestibility was consistently lesser in canola meal.

Canola meal is another plant protein source which is an improved variety of RSM. Concentration of Sulphur containing amino acids are more in RSM than SBM (Khajali and Slominski, 2012). Protein quality of RSM might be higher than animal protein sources and other plant protein sources having higher amount of essential amino acids (Friedman, 1996). Anti-nutritional factors including, erucic acid, glucosinolates and fiber content are higher in RSM compared with CM (Kasprzak et al. 2016). Canola meal has low erucic acid (<2%) and glucosinolates (<30umol/g) in defatted meal (Maison and Stein, 2014). This improved variety of RSM in Europe, is called as “double zero” and in Australia and North America known as “canola” (Newkirk, 2009). A study was conducted to calculate the prececal AA digestibility in broiler by Kluth and Rodehutscord (2006) by providing 300g/kg of RSM and SBM in each diet. These later authors reported the 2.4% enhanced CP digestibility in RSM based diet.

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 broiler production since it is a major protein source used in poultry diets. Indigenous plant protein sources including canola meal, sunflower meal, guar meal and rapeseed meal, can be used as partial substitute of SBM. The unknown nutritional profile, specially total and digestible energy and amino acid contents of these ingredients are major hurdles in their proper use in poultry diets.

Objectives

The study was, therefore, designed to evaluate;

1. The nutritional profile, including gross energy and crude protein, of indigenous plant protein sources.
2. Apparent metabolizable energy (AME), apparent metabolizable energy corrected for nitrogen (AMEn) in broilers.
3. Standardized ileal digestibility of crude protein (SIDCP) and amino acids (SIDAA) in broilers.

CHAPTER 3
MATERIALS AND METHODS

1. Collection of ingredients samples:

In total, four different indigenous plant protein sources, commonly used in poultry diets, were collected from two different geographical locations of Pakistan (Figure 3) Punjab (Multan) and Sindh (Sukkur). All the samples were labelled, showing the name, date of sampling and the origin of the samples. Samples of the following ingredients were collected;

1. Canola meal
2. Rapeseed meal
3. Guar meal
4. Sunflower meal

Table 3.1: Samples collection areas

Sample Name	Punjab	Sindh
Sunflower meal	Bahawalpur (BWP)	Sukkur (SKR)
Rapeseed meal	Multan (MUL)	Sukkur (SKR)
Guar meal	Multan (MUL)	Sukkur (SKR)
Canola seed	Multan (MUL)	Sukkur (SKR)

Figure 3. Geographical locations of sample collection areas.

2. Analysis of the samples:

The collected ingredients were examined for proximate analysis in the Animal Nutrition Research laboratory at Animal Nutrition Department, UVAS, Ravi Campus, Pattoki.

Proximate analysis

To get the precise nutritional profile of the collected ingredients, their proximate analysis was done.

Dry matter (DM)

For the determination of DM values, samples were dried at 105 ^{0}C for four hours, whereas Ash determination was done at 550 ^{0}C for 15h. (AOAC, 2000).

Ether extract (EE)

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

Crude protein (CP)

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

Crude fibre (CF)

Analysis of the collected ingredients for CF was done according to the methods mentioned in (AACC, 2000).

Amino acid analysis

Amino acid profile of collected ingredients was carried out at Prof. Dr. Muhammad Yaqoob Malik amino acid analysis laboratory, department of animal nutrition UVAS, Ravi campus Pattoki by adopting techniques of amino acid analyser. The method mentioned by Official Journal of European Communities were used.

Concisely, well ground sample up to 500micron was used, afterwards, the ground samples were oxidized with performic acid in order to protect methionine and cysteine. After oxidation, these aforementioned amino acids were changed to met-methionine sulphone and cys-cystic acid. Thereafter, 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 the digestibility determination were executed at poultry bio-digestibility laboratory, equipped with metabolic cages and also have tools for collection of digesta, at University of Veterinary and Animal Sciences, Ravi Campus, Pattoki.

Digestibility experiments with live birds

Trial 1.

Exploring the Apparent Metabolizable Energy (AME) and AME Corrected for Nitrogen of different protein sources including Canola meal, Guar meal, Rapeseed meal and Sunflower meal in Broilers.

A total of 240 male (Ross 308) broilers at one day of age were reared on commercial diet for 21 days. The ideal temperature and light was provided according standard practices of strain (Ross 308), along with *ad libitum* feed and water. After three weeks of age, ten experimental diets were prepared including two reference diets and eight experimental diets. Thereafter, birds were randomly allotted to ten experimental diets, each containing three cages as replicates with eight birds/cage. All the experimental diets were offered in mash form to their corresponding cages for 72 hours in order to adopt the birds to these treatments in completely randomized design. After adaptation period, birds were off feed for 24 hours and excreta trays from each cage were be totally cleaned. Actual digestibility trial period was from 26 to 28 days and birds were offered calculated feed. Feed intake and body weight gain of the birds were measured after 96 hours of feeding and excreta was collected on daily basis in plastic containers and dried. At the termination of collection period, the samples from experimental diets and dried excreta were assayed for DM percentage and gross energy content by using bomb calorimeter.

The AME and AMEn (Kcal/Kg) of the samples were calculated by given formulas;

$$\mathrm{AME} = \frac{(\text{Feed GE} \times \text{feed intake g}) - (\text{Excreta GE} \times \text{excreta in grams})}{(\text{feed intake g})}$$

$$\mathrm{AMEn} = \frac{((\text{Feed GE} \times \text{feed intake g}) - (\text{Excreat GE} \times \text{excreta in grams})) - (\mathrm{K} \times \mathrm{NR})}{\text{Feed intake g}}$$

In this equation, Nitrogen Retention is abbreviated as NR which is supposed to (20 percent of BWG/ loss) /6.25, K in the equation is constant number which is equals to 8.21 kcal/kgN.

Following equation was used for AME;

Ingredient AME = (diet AME) – ((ref. corn AME) – (ref. SBM AME))

Table 3.2: Experimental layout for AME and AMEn.

Diets	Origin	Inclusion Rate (%)	Treatments
Corn SBM based Reference Diet 1	Reference Diet 1	Corn = 81 SBM = 15	Ingredients = 4 Origin = 2 Treatments = 4*2 = 8 Replicates = 3 Birds/Replicate = 8 4*2*3*8 = 192 Ref. diets = 2 Replicates = 3 Birds/Replicate = 8 2*3*8 = 48 Total = 240 Male Ross broilers
Corn SBM based Reference Diet 2	Reference Diet 2	Corn = 51 SBM = 45	
Canola Meal	MUL	15	
	SKR		
Rapeseed Meal	MUL	15	
	SKR		
Sunflower Meal	BWP	15	
	SKR		
Guar Meal	MUL	15	
	SKR		

Table 3.3: Dietary ingredients and calculated nutrient composition of for AME and AMEn trial.

Ingredients (%)	T1 Ref. diet 1	T2 Ref. diet 2	T3 Test diet 1	T4 Test diet 2	T5 Test diet 3	T6 Test diet 4
Corn	81.00	51.00	66.00	66.00	66.00	66.00
Soybean meal	15.00	45.00	15.00	15.00	15.00	15.00
Canola meal 1	0.00	0.00	15.00	0.00	0.00	0.00
Rapeseed meal 2	0.00	0.00	0.00	15.00	0.00	0.00
Guar meal 3	0.00	0.00	0.00	0.00	15.00	0.00
Sunflower meal 4	0.00	0.00	0.00	0.00	0.00	15.00
MCDP	1.650	1.650	1.650	1.650	1.650	1.650
Limestone	1.600	1.600	1.600	1.600	1.600	1.600
Salt	0.400	0.400	0.400	0.400	0.400	0.400
Premix	0.200	0.200	0.200	0.200	0.200	0.200
Choline Chloride	0.100	0.100	0.100	0.100	0.100	0.100
Maduramicin	0.050	0.050	0.050	0.050	0.050	0.050
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Trial 2.

Exploring the Standardized Ileal Crude Protein and Standardized Ileal Amino Acids Digestibility of Indigenous Sources including Canola meal, Guar meal, Rapeseed meal and Sunflower meal in Broilers.

A total of 216 male (Ross 308) broilers of one day of age were reared on commercial diet for 21 days. The ideal temperature and light were provided according standard practices of strain, along with *ad libitum* feed and water. After three weeks of age, nine experimental diets were prepared including one protein free diet and eight experimental diets. Protein free diet was offered to determine the ileal endogenous losses in broilers. Thereafter, birds were randomly allotted to nine experimental diets, each containing four cages as replicates with six birds/cage. All the experimental diets were offered in mash form to their corresponding cages for 72 hours in order to adopt the birds to these dietary treatments in completely randomized design. After the adaptation period of three days, birds were off feed for one day in order to clean their gastro intestinal tract. Chromium oxide, an indigestible marker was used in every diet with inclusion level of 0.3%. After one day of off feeing, birds were provided experimental diets again, thereafter, 4h after feeding, four birds from each replicate were slaughtered to collect ileal digesta. The collected samples from experimental diets and ileal digesta were dried and stored for the analysis of crude protein, amino acids contents and ash. Calculation of ileal protein and digestibility of amino acid on dry matter basis by the equation given below.

$$\mathrm{SIDP}\ (\%) = \frac{\mathrm{CPd/AIAd\ CPi/AIAi} + \mathrm{CPe/AIAe} \times 100}{\mathrm{CPd/AIAd}}$$

SIDCP is abbreviated as standardized Ileal digestibility of CP

CPd = Dietary crude protein level

CPi = Ileal digesta having CP concentration

CPe = Endogenous losses having CP content

AIAd = Apparent ileal amino acid level in diet

AIAi = Apparent ileal amino acid level in Ileal digesta

AIAe = Apparent ileal amino acid content in endogenous losses

$$\mathrm{SIDAA}\ (\%) = \frac{\mathrm{AAd}/\mathrm{AIAd}\ \mathrm{AAi}/\mathrm{AIAi} + \mathrm{AAe}/\mathrm{AIAe}}{\mathrm{AAd}/\mathrm{AIAd}}$$

SIDAA is abbreviated as standardized Ileal amino acid digestibility

AAd = Dietary AA level

AAi = Ileal digesta containing AA level

AAe = Endogenous losses containing AA content

AIAd = Dietary level of AIA

AIAi = AIA content in Ileal digesta

AIAe = AIA content in endogenous losses

Table 3.5: Experimental layout for protein and amino acid estimation trial

Diets	Origin	Inclusion Rate (%)	Treatments
Protein Free Diet (PFD)		Corn starch = 55.2 Dextrose = 32.2	Ingredients = 4 Origin = 2 Treatments = 4*2 = 8 Replicates = 4 Birds/Replicate = 6 4*2*4*6 = 192 PFD = 1 Replicates = 4 Birds/cage = 6 1*4*6 = 24 Total = 216 Male Ross broilers
Canola Meal	MUL	15	
	SKR		
Rapeseed Meal	MUL	15	
	SKR		
Sunflower Meal	BWP	15	
	SKR		
Guar Meal	MUL	15	
	SKR		

Table 3.6: Dietary ingredients and calculated nutrient composition of for protein and amino acid digestibility estimation trial.

Ingredients (%)	T1 Protein free diet	T2 Test diet 1	T3 Test diet 2	T4 Test diet 3	T5 Test diet 4
Corn starch	55.2	48.4	48.4	48.4	48.4
Dextrose	32.2	28.0	28.0	28.0	28.0
Soybean oil	4.00	0.0	0.0	0.0	0.0
Canola meal 1	0.0	15.0	0.0	0.0	0.0
Rapeseed meal 2	0.0	0.0	15.0	0.0	0.0
Guar meal 3	0.0	0.0	0.0	15.0	0.0
Sunflower meal 4	0.0	0.0	0.0	0.0	15.0
Paper Pulp	4.3	4.3	4.3	4.3	4.3
MCDP	1.65	1.65	1.65	1.65	1.65
Limestone	1.60	1.60	1.60	1.60	1.60
Salt	0.40	0.40	0.40	0.40	0.40
Premix	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.10	0.10	0.10	0.10	0.10
Maduramicin	0.05	0.05	0.05	0.05	0.05
Chromium Oxide	0.30	0.30	0.30	0.30	0.30
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Data Analysis

For the data analysis, PROC Mixed model of SAS (version 9.2, SAS Institute Inc., Cary, NC) was used. Following model was used for statistical analysis;

$Y_{ijkl} = \mu + PS_i + O_j + \varepsilon_{ijkl}$

Where,

Y_{ijkl} = Observation of dependent variable recorded on i^{th}, j^{th} and k^{th} treatment

μ = Population mean

PS_i = Protein source (i = CM, GM, RSM, SFM)

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

ε_{ijkl} = Residual effect associated with i^{th}, j^{th} and k^{th} treatment NID ~ 0, σ^2

CHAPTER 4
RESULTS

1. Proximate Analysis:

The data for proximate analysis are summarized in Table 4.1.The results indicated that there were significant ($P<0.05$) differences between the DM, CP and Ash contents of CM from different origins. Crude protein and DM contents were higher in CM from SKR, whereas CF and EE contents in CM samples from SKR and MUL showed no difference. There were also significant ($P<0.05$) differences in CP, DM and Ash contents of RSM from different origins. Crude protein and DM contents were higher in RSM from SKR, whereas Ash contents in RSM from MUL were higher. Non-significant differences were observed in EE and CF contents of RSM samples form SKR and MUL. Significant ($P<0.05$) differences were observed in CP and CF contents of GM from SKR and MUL. Crude protein and CF contents were higher in GM from MUL, whereas no significant differences were observed in DM, EE and ash of GM samples from MUL and SKR. Due to origin, significant ($P<0.05$) effects were observed in CP and CF contents of SFM. The SFM from SKR had higher CP and CF contents, whereas no significant differences were observed in DM, EE and ash contents of SFM from both origins.

2. Gross Energy, AME and AMEn measurement:

The total energy contents of the indigenous protein sources are presented in Table 4.3. It was observed that there was no difference ($P > 0.05$) in GE content of CM from both origins. In RSM, no difference ($P > 0.05$) were found in GE value of SKR and MUL sample. The GE value of GM form SKR was greater ($P < 0.05$) than those from MUL. The SFM was determined to have no difference ($P > 0.05$) in GE values form both origins.

Table 4.1: Proximate analysis (%) and energy contents (Kcal/kg) of the indigenous ingredients from different origins

Item	CM				RSM				GM				SFM			
	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value	BWP	SKR	SEM	*P*- value
DM	89.7	90.1	0.3	*	89.1	90.7	0.2	*	94.3	95.1	0.5	NS	93.2	92.4	0.3	NS
CF	12.2	11.7	0.8	NS	11.9	12.6	0.4	NS	13.1	11.2	0.2	*	21.2	24.6	0.5	*
CP	35.4	36.8	0.5	*	36.1	37.2	0.3	*	43.1	40.4	0.4	*	28.1	30.8	0.5	*
EE	2.4	3.1	0.7	NS	1.6	1.4	0.5	NS	5.41	5.04	0.3	NS	0.84	0.78	0.2	NS
Ash	6.9	5.8	0.4	*	9.1	8.2	0.2	*	5.61	5.11	0.8	NS	6.42	5.87	0.4	NS
GE	4200	4150	15.6	NS	4210	4260	20.8	NS	4400	4515	18.7	*	4050	4070	15.4	NS

CM= Canola Meal, GM= Guar Meal, RSM= Rapeseed Meal, SFM= Sunflower meal, MUL= Multan, SKR= Sukkur, BWP= Bahawalpur, *$P < 0.05$, NS= Non significant

Table 4.2: Apparent Metabolizable energy and AMEn for broilers of different indigenous protein sources, (Kcal/Kg).

Protein Source	Origin	AME	AMEn	SEM	*P*-value
Canola meal	MUL	2571	2397	20	NS
	SKR	2521	2367		
Guar meal	MUL	2311	2165	15	NS
	SKR	2325	2180		
Sunflower meal	MUL	2690	1697	22.5	NS
	BWP	2677	1686		
Rapeseed meal	MUL	2130	1980	17.3	NS
	SKR	2145	1984		

AME= Apparent metabolizable energy, AMEn= Apparent metabolizable energy corrected for nitrogen, SEM= Standard error of mean, CM= Canola meal, GM= Guar meal, RSM= Rapeseed meal, SFM= Sunflower meal, MUL= Multan, SKR= Sukkur, BWP= Bahawalpur, *P < 0.05, NS= Non significant

2. AME and AMEn

There was no difference ($P > 0.05$) in AME and AMEn contents of CM from MUL and SKR. The AME and AMEn contents in GM samples from both region SKR and MUL showed no difference ($P > 0.05$). There was no difference ($P > 0.05$) between AME and AMEn contents of SFM and RSM samples from their respective origins. The SFM was determined to have the lowest values of AME and AMEn compared with other protein sources. The average AMEn values of CM, GM, SFM and RSM were 2382, 2172, 1691 and 1982 kcal/kg, respectively.

3. Total Amino Acid Content:

Table 4.3 shows the amino acid contents of different indigenous protein sources of different origin, commonly used in poultry feed. Despite significant ($P < 0.05$) difference in the CP content of GM samples from different origins, only 1 out of 15 analyzed AA differ due to origin. Among essential AA, no differences ($P > 0.05$) were observed for arginine (Arg), lysine (Lys), methionine (Met) and threonine (Thr) contents in the SKR and MUL samples. Among non-essential amino acids, cysteine (Cys) is considered as semi essential amino acid for poultry. Cysteine content was similar in sample from MUL and SKR. In non-essential AA the amount of alanine (Ala) was higher ($P < 0.05$) in sample of SKR, whereas the content of all other non-essential AA showed no differences ($P > 0.05$) in sample form MUL and SKR. The sample of RSM from MUL was having relatively higher ($P < 0.05$) concentration of essential AA including Arg and Leu , whereas Lys, phenylalanine (Phe) and Val contents were higher ($P < 0.05$) in sample from SKR. The semi essential AA, Cys content was not influenced ($P > 0.05$) by origin in RSM. Sample from MUL had higher concentration ($P < 0.05$) of aspartae (Asp) and glutamic acid (Glu), whereas serine (Ser) contents was higher ($P < 0.05$) in SKR sample. The concentration of essential AA was not influenced ($P > 0.05$) by origin in SFM from BWP and SKR, whereas, total non-essential AA content of Gly and Glu was higher in ($P < 0.05$) SKR SFM than that of BWP. The CM sample from MUL had higher ($P < 0.05$) Arg and Phe content, whereas Ile content was higher ($P < 0.05$) in SKR CM. No differences ($P > 0.05$) were observed for Arg, Lys, Met, Thr content in CM from both regions. The non-essential AA including Ala, Asp, Cys and Ser contents were higher ($P < 0.05$) in MUL CM than that of SKR.

4. Amino Acid Digestibility and Digestible AA contents:

In Table 4.4 shows standardized ileal digestibility of crude protein and amino acids. The standardized ileal digestibility of all AA were not influenced by the origin of CM. Digestible contents of essential AA including Arg, Phe, Val and Thr were higher ($P<0.05$) in the MUL sample to that from SKR. No differences ($P>0.05$) existed for non-essential AA between MUL and SKR sample of CM in terms of digestibility. The digestible contents of Lys, His, Ile, Phe in the GM samples from MUL and SKR was similar, whereas the MUL GM sample had higher ($P < 0.05$) digestible content of Arg, Met and Thr. The digestible content of semi essential AA Cys was higher ($P < 0.05$) in MUL GM sample than that in GM from other origin. No differences ($P > 0.05$) were observed in digestibility of non-essential AA between the GM samples from both origins. The essential AA His and Met digestibility was higher ($P < 0.05$) in MUL RSM and Val digestible content was greater in SKR RSM. No differences existed ($P > 0.05$) between MUL and SKR RSM in the digestible content of Arg, Lys, Thr, Ile, Phe and Leu. The digestible contents of Asp and Cys in the MUL RSM sample were higher ($P < 0.05$) than there in RSM from other origin. Trends for digestibility of Arg, Met and Val were observed higher ($P < 0.05$) in SFM from BWP sample of SFM, whereas His digestible content was higher ($P < 0.05$) in SKR SFM than there of SFM from BWP.

Table 4.3. Total amino acid contents (% of CP) of indigenous plant protein sources commonly used in poultry feed.

	CM				GM				RSM				SFM			
Items	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value	SKR	BWP	SEM	*P*-value
EAA																
Arginine	6.8	6.4	0.1	*	4.38	4.29	0.2	NS	6.1	5.6	0.2	*	2.18	2.27	0.2	NS
Histidine	2.8	2.6	0.2	NS	0.89	0.82	0.1	NS	2.5	2.8	0.2	NS	0.69	0.58	0.5	NS
Isoleucine	4.3	4.8	0.1	*	1.18	1.04	0.2	NS	3.8	3.2	0.3	NS	1.15	1.07	0.3	NS
Leucine	6.9	7.1	0.2	NS	2.05	2.16	0.1	NS	6.5	6.1	0.1	*	1.75	1.81	0.4	NS
Lysine	5.4	5.2	0.2	NS	1.49	1.67	0.1	NS	5.3	6.1	0.2	*	0.94	0.90	0.3	NS
Methionine	2.4	2.5	0.1	NS	0.43	0.39	0.1	NS	1.98	2.21	0.2	NS	0.61	0.57	0.2	NS
Phenylalanine	5.8	5.2	0.2	*	1.40	1.35	0.1	NS	5.6	6.2	0.1	*	1.28	1.19	0.1	NS
Threonine	4.6	4.4	0.2	NS	1.12	1.23	0.2	NS	3.9	4.2	0.2	NS	1.12	1.04	0.5	NS
Valine	5.2	5.0	0.1	NS	1.33	1.21	0.1	NS	4.7	5.3	0.1	*	1.51	1.44	0.6	NS
NEAA																
Alanine	4.8	4.1	0.2	*	1.63	1.92	0.1	*	4.2	3.8	0.3	NS	1.18	1.27	0.1	NS
Aspartate	2.7	2.1	0.1	*	3.54	3.21	0.2	NS	2.1	1.9	0.1	*	2.58	2.26	0.1	NS
Cysteine	3.1	2.6	0.1	*	0.48	0.32	0.1	NS	2.0	2.3	0.2	NS	0.37	0.31	0.3	NS
Glycine	5.3	5.2	0.2	NS	2.14	2.53	0.2	NS	4.9	5.2	0.2	NS	1.62	1.41	0.1	*
Glutamic acid	13.4	14.3	0.2	*	7.12	6.71	0.2	NS	16.5	14.3	0.4	*	5.53	5.06	0.2	*
Serine	5.1	4.6	0.1	*	1.64	1.34	0.3	NS	4.1	4.8	0.2	*	1.07	1.19	0.3	NS

CM= Canola meal, GM= Guar meal, RSM= Rapeseed meal, SFM= Sunflower meal, MUL= Multan, SKR= Sukkur, BWP= Bahawalpur, *$P < 0.05$, NS= Non significant, EAA= Essential amino acids, NEAA= Non-essential amino acids

Table 4.4: Coefficients of Standardized ileal digestibility of protein and amino acids of commonly used indigenous protein sources, from different origin, in broilers.

	CM				GM				RSM				SFM			
Items	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value	MUL	SKR	SEM	*P*-value	BWP	SKR	SEM	*P*-value
CP	0.68	0.65	0.02	*	0.64	0.69	0.01	*	0.66	0.63	0.01	*	0.70	0.64	0.02	*
EAA																
Arginine	0.85	0.81	0.01	*	0.76	0.73	0.01	*	0.86	0.85	0.01	NS	0.90	0.82	0.02	*
Histidine	0.84	0.79	0.02	NS	0.64	0.65	0.02	NS	0.82	0.78	0.01	*	0.76	0.81	0.01	*
Isoleucine	0.80	0.85	0.01	NS	0.57	0.59	0.02	NS	0.78	0.76	0.02	NS	0.82	0.77	0.02	NS
Leucine	0.82	0.81	0.01	NS	0.59	0.57	0.01	NS	0.80	0.79	0.01	NS	0.80	0.79	0.01	NS
Lysine	0.81	0.77	0.01	NS	0.58	0.56	0.01	NS	0.71	0.73	0.02	NS	0.76	0.75	0.01	NS
Methionine	0.89	0.86	0.02	NS	0.64	0.52	0.02	*	0.84	0.80	0.01	*	0.90	0.84	0.02	*
Phenylalanine	0.84	0.75	0.02	*	0.61	0.58	0.02	NS	0.80	0.79	0.01	NS	0.77	0.73	0.02	NS
Threonine	0.73	0.80	0.02	*	0.53	0.46	0.02	*	0.70	0.74	0.02	NS	0.71	0.68	0.01	NS
Valine	0.79	0.71	0.02	*	0.55	0.61	0.01	*	0.72	0.76	0.01	*	0.77	0.70	0.01	*
NEAA																
Alanine	0.83	0.86	0.01	NS	0.50	0.48	0.02	NS	0.79	0.74	0.02	NS	0.77	0.71	0.01	*
Aspartate	0.77	0.74	0.02	NS	0.64	0.61	0.02	NS	0.71	0.65	0.02	*	0.76	0.82	0.02	*
Cysteine	0.76	0.77	0.01	NS	0.49	0.43	0.02	*	0.74	0.70	0.01	*	0.67	0.60	0.02	*
Glycine	0.75	0.74	0.02	NS	0.55	0.51	0.02	NS	0.71	0.68	0.01	NS	0.58	0.61	0.01	NS
Glutamic acid	0.86	0.84	0.01	NS	0.63	0.75	0.01	NS	0.83	0.79	0.02	NS	0.85	0.80	0.01	NS
Serine	0.74	0.71	0.02	NS	0.61	0.58	0.01	NS	0.71	0.68	0.01	NS	0.67	0.61	0.02	*

CM= Canola meal, GM= Guar meal, RSM= Rapeseed meal, SFM= Sunflower meal, MUL= Multan, SKR= Sukkur, BWP= Bahawalpur, *$P < 0.05$, NS= Non significant, EAA= Essential amino acids, NEAA= Non-essential amino acids

CHAPTER 5

DISCUSSION

The present study was conducted to evaluate the nutritional profile of indigenous protein sources including CM, SFM, RSM and GM collected from two different geographical locations of Pakistan. The general objective of the project was to fully characterize these protein sources in terms of proximate analysis and to describe their AME, AMEn and standardized ileal digestibility (SID) of CP and AA in broilers.

Proximate Analysis

The proximate analysis of CM were within the range described in the literature (Khajali and Slominski, 2012; Current canola meal feeding guide, 2015; Leeson and Summers, 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 positively influenced by origin. In SKR sample CP content was higher compre with MUL. Canola mal and RSM from yellow-seeded varieties have greater concentration of oil and CP, and less CF than meal obtained from black-seeded varieties (Slominski et al. 1994; Trindade Neto et al. 2012; Slominski et al. 2012). Compared with GM form other origin, the sample from MUL had higher content of CP, EE and CF. The CP content in GM was in agreement with value with the findings reported by Singh et al. (2008). Higher CP content 48% in GM was reported by Vatandousti et al. (2010). These variations can be attributed to different varieties of guar seed, alterations of processing techniques, and variation in analytical procedures among laboratories (Lee et al. 2004). Ash values determined in this study was in agreement with the values reported by (Nagpal et al. 1971; Verma and McNab, 1984). Guar meal is mixture of 1% germ and 3% hulls. The presence of hulls not only increases the CF content but also negatively influences the nutrients (Dilger et al. 2004). Crude protein contents of SFM from SKR was higher than those from BWP. The CP content of SFM ranged from 29 to 45%. In literature, difference in chemical composition of SFM has been reported Pinheiro et al. (2002), this may be attributed to different grain processing methods, as mentioned by Pinheiro et al. (2002). The higher content of CF 24.6% in sample from SKR may be because of varieties of sunflower seeds containing 25 to 30% hulls. The higher level of CF content which depends on de-hulling and oil extraction process, has inverse relation with the CP content (Moghaddam et al. 2012).

Apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen (AME and AMEn)

Apparent metabolizable energy is the difference between gross energy is the diet and the GE of excreta (NRC, 1994). Apparent metabolizable corrected for nitrogen is usually considered to be more accurate to convert all data to a basis of N equilibrium for comparative purposes (Leeson and Summers, 2001; MacLeod, 2002). When nitrogen is retained in the body, it yields energy-containing compounds with metabolites that are excreted in the urine. Nitrogen correction is adjusted to reduce the differences of AME for protein retention in birds (Leeson et al., 1977; Lopez and Leeson, 2007). No variation was observed in AME and AMEn of CM and RSM from MUL and SKR. The values observed for CM in this study are in close agreement with the results of (Toghyani et al. 2014) and RSM values were in accordance with results of (Nadeem et al. 2005). Lower hemicellulose content in CM may also be the reason of lower AME, since, hemicellulose could partially digested by Hcl and contribute energy to birds (Leeson and Summers, 2001). Additionally, high fiber content in CM and RSM samples may also speed up passage time of digesta and decrease the ability of the gut microbiota to digest complex carbohydrates, which could increase higher energy output in excreta. The low values of AME and AMEn of SFM in this study may be related to its higher CF content. Protein and many other nutrients are “encapsulated” to variable degrees inside fibrous structures, and they remain less available for digestion by the proteases and other endogenous enzymes of the bird. These effects may decrease the AMEn value of seed meals (Moghaddam et al. 2012).

Contents and Digestibility of Crude Protein and Amino Acids:

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 8 of 15 AA differ due to origin. Among essential AA, the differences were observed for Arg, Ile and Phe. Cysteine has disulphide bond in its structure, that is the most heat labile AA of all (Wall, 1971). Greater Cys content in MUL sample suggests that processing conditions may be optimal in MUL processing plants. In current study, the variations among CM in the SID of CP and AA were expected because of the variance in growing, processing and high dietary fiber fractions. In general, standardized ileal CP and AA digestibility of CM determined for broilers in the current experiment agree with the published literature (Adedokum et al. 2008; Woyengo et al. 2010; Kim et al. 2012). The total AA and CP content were higher in RSM from SKR, than those with greater content of AA. The Lys and sulphur containing AA were higher in RSM from SKR. 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). Variability in SID of CP and AA was observed in samples of RSM from SKR and MUL. Digestibility values of CP and AA in RSM were in accordance with previously published literature (Lemme et al. 2004;

Woyengo et al, 2010). The MUL RSM had higher CP digestibility compared with SKR. Sulphur containing AA, Met and Cys were more digestible in RSM MUL. 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). Similarly, Eklund et al. (2015) in his study demonstrated the linear relationship between SID of CP and AA and the content of NDF and glucosinolates in RSM fed to pigs. The analyzed total AA content in GA were in accordance with literature (Lee et al. 2004; Ullah et al. 2017). The standardized ilead digestibility of CP and AA was lowest of all ingredients in the current study. The digestibility measurements in GM samples were in accordance with the results reported by Ullah et al. (2017). The low digestibility values compared with those reported by Nadeem et al. (2005) might be due to difference in birds species and source of sample (Wang and Parsons, 1998) or the chemical characteristics, differences in environmental and soil conditions (Ravindran et al. 2014) and processing technology may also influence the ingredients digestibility. The anti-nutritional factor in GM including, β-mannans and gums may reduce the digestive enzymes function and enhance viscosity of digesta by forming complex in tract (Ullah et al. 2017).

The TAA content in SFM sample collected from BWP and SKR were in accordance with the values reported previously (Lie et al. 2015; Villamide and San Jua, 1998), whereas, lower than those reported by Nadeem et al. (2005). The decreased values may be attributed due to climatic conditions, geographical impact and use of fertilizers in crops (Piper and Boote, 1999). The SID of CP and AA was markedly higher in SFM from BWP compared with these sample from SKR. Despite high total digestibility in SFM from BWP, no difference in digestibility of Lys and Thr was observed, whereas, sulphur containing amino acids had higher digestibility value in SFM from BWP. The difference in AA digestibility between SFM origins may be due to the higher content of CF and NDF, which negatively influences the AA digestibility (Lenis et al. 1996). Differences in heating temperature probably also caused the variation of SID values of CP and AA, resulting in Maillard reaction which decreased the digestibility of AA (Pahm et al. 2008). The interesting findings of the current experiment is the lack of any correlation between CP content and nutritive quality of any protein source. Data shown in Table 4.1 and 4.4 shown that CP content of any of ingredient was not correlated with either SDI digestibility of CP, AA and AME. The data highlight that selection and pricing of protein sources must not be on CP content. Over the years, Pakistan is importing huge tons of SBM from other countries, because of the lack of nutritional matrices of the indigenous protein sources. The present data demonstrate the nutritional profile of indigenous protein sources including proximate, metabolizable energy and SDI of CP. Moreover, these values also demonstrate differences in digestible contents of CP, AA, AME and AMEn among the origins.

CHAPTER 6
SUMMARY

1. Background

The study was planned to find out the nutritional potential of indigenous plant protein sources and their digestibility indexes in broilers.

2. Methodology

In total, four different indigenous plant protein sources CM, RSM, SFM and GM commonly used in poultry diets were collected from two different geographical locations i.e. Multan (Punjab) and Sukkur (Sindh) of Pakistan and analyzed for proximate, gross energy and amino acid contents. Trials for the digestibility determination was executed at poultry bio digestibility laboratory at UVAS, Ravi Campus, Pattoki, where metabolic cages to collect the digesta for various traits are available. During first trial, a total of 240 male (Ross 308) broilers at one day of age were reared on commercial diet for 21 days. After three weeks of age, ten experimental diets were prepared including two reference diets and eight experimental diets. Thereafter, birds were randomly allotted to ten experimental diets, each containing three cages as replicates with eight birds/cage. All the experimental diets were offered in mash form to their corresponding cages for 72 hours in order to adopt the birds to these treatments. After adaptation period, birds were off feed for 24 hours and excreta trays from each cage were totally cleaned. Actual digestibility trial period was from 26 to 28 days, and birds were offered calculated feed. Feed intake and body weight gain of the birds were measured after 96 hours of feeding and excreta was collected on daily basis in plastic containers and dried. At the termination of collection period the samples from experimental diets and dried excreta was assayed for DM percentage and gross energy content by using bomb calorimeter.

In second trial, a total of 216 male (Ross 308) broilers of one day of age were reared on commercial diet for 21 days. After three weeks of age, nine experimental diets were prepared including one protein free diet and eight experimental diets. Protein free diet was offered to determine the ileal endogenous losses in broilers. Thereafter, birds were randomly allotted to nine experimental diets, each containing four cages as replicates with eight birds/cage. All the experimental diets were offered in mash form to their corresponding cages for 72 hours in order to adopt the birds to these dietary treatments. After the adaptation period of three days birds were off feed for one day in order to clean their gastro intestinal tract. Chromium oxide an indigestible marker was used in every diet with inclusion level of 0.3%. After one day of off feeing, birds were provided experimental diets again, thereafter, 4h after feeding, four birds from each replicate were slaughtered to collect ileal digesta. The collected samples from experimental diets and ileal digesta were dried and stored for the analysis of crude protein, amino acids contents and ash.

Results

Nutrient composition, ileal amino acid (AA) digestibility, AME and AMEn of CM samples from SKR and MUL were compared using laboratory analysis and animal studies. There were ($P < 0.05$) differences in DM, CP ash, Arg, Ile, Phe for TAA content, whereas, the SID content of CP and AA were higher ($P < 0.05$) in CM from MUL compared to SKR. No differences ($P > 0.05$) were observed for AME and AMEn in CM samples. The nutrient composition of RSM from SKR was greater ($P < 0.05$) than those from MUL. The TAA content showed difference ($P < 0.05$) in between RSM samples. The SID of CP and AA was significantly greater ($P < 0.05$) in RSM sample from MUL, whereas AME and AMEn showed no differences ($P > 0.05$). The CF, CP and GE content showed markedly differences ($P < 0.05$) in between samples of GM from SKR and MUL, with higher content in GM from MUL. Total AA profile showed no difference ($P > 0.05$) regardless of origin in GM. The SID of EAA was higher ($P < 0.05$) in GM sample from MUL. The CF and CP content of SFM was greater ($P < 0.05$) in SKR sample than that of BWP. Total AA profile also showed no differences ($P > 0.05$) in SFM from both origin. The SID of CP and EAA was higher ($P < 0.05$) in SFM from BWP than those from SKR. The AME and AMEn showed no differences ($P > 0.05$) in SFM between its origins In conclusion, the present study showed that major differences in nutritive value do exist between CM, GM, RSM and SFM from different origins in terms of nutrient contents, and digestible CP and AA values.

CHAPTER 7

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