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Physico-chemical, Proximate and Fatty Acid Analysis of Animal Feeds Marketed in Sindh, Pakistan

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Abstract. In this study, the proximate, physico-chemical and fatty acid analysis were carried out to evaluate the quality of animal feeds (compound and seed cake) commercially available in Sindh, Pakistan. The proximate composition of feed was found as follows: moisture (5.0-12%), ash (0.5-3.2%), crude protein (9.19-2.47%), fibre (49.8-72.81%), carbohydrate (34.03-67.79%), energy value (1392.05-1679.23 KJ/mol) and oil content (0.8-10.5%). Official AOCS methods were used to evaluate the quality of animal feed oil. Higher free fatty acids (2.5-59%) and peroxide value (0.4-12 meq/Kg) were determined in feed oil. The fatty acid analysis revealed that saturated and unsaturated fatty acids in animal feed oil were found in the range of 2.44 to 57.12% and 3.5 to 40.97%, respectively. Comparatively, compound animal feed showed better quality characteristics than seed cake. The proximate and chemical analysis of animal feed showed that oil present in animal feed supports good quality except for peroxide value and free fatty acid, whereas proximate composition still needs improvement.

Keywords: animal feed, proximate composition, oil quality, fatty acids, FT-IR

Introduction

The animal feed provides nutrients to animals and may be part of the plant or any other source. It contains important nutrients such as minerals, vitamins, fat, proteins, carbohydrates and water absorbed by the body of animals. The industry related to the feed is one of the main viable businesses in the agricultural sectors. The American Feed Industry Association reported that $20 billion worth of feed ingredients are purchased each year (Varma *et al*., 2018). For animals, feed grains play an important role in global animal feeds. Animal feeds are classified as fodder and forage. Still, fodder is further classified as concentrates and roughages (crops, silage, dry forage, fresh-cut forage, straw and root) are examples of roughages, whereas grains, legumes and by-products of processing are called concentrates. The animals consumed plants directly, such as crop residue pasture and immature cereal crops which is called forage. The forages are commonly found in a major portion of the animal feeds in our country (Kaithwas *et al*., 2020) and used for animal feed as straw, husk, stover are very poor in nutrition. These usually fulfill only the hunger of the animals (Manoj *et al*., 2020). The food given to animals comprises one or more mixed materials known as animal feed (Danieli *et al*., 2019). Many types of ingredients used for the preparation of feed contain one or more nutrients.

Mixed feeds are also produced from different feed materials combined to achieve a particular nutritional quality called mash or pellets (Danieli *et al*., 2019). Feed compounders manufacture the compound feed as pellets, meal-type or crumbles (Sullivan and Bernard, 2004). The compound feed can be sold as premixes containing minerals, antibiotics, vitamins, preservatives and other necessary components for mixing commercial rations (Konar *et al*., 2019). Animal feeds are important for both feeds manufacturers and animal producers and policymakers, regulators, processors and consumers of the end-products. This is because animal feed is an essential component of the food supply chain, ensuring the effective and profitable processing of high quality, nutritious food. As a result, the feed protection is important for food safety. Stakeholders involved in providing nutritious foods must be vigilant about the quality of animal feeds. Research evidence shows the risks that may increase with the consumption of contaminated feeds. Several spates traceable to animal feeds in different countries have made the demands for safe animal feed even more serious in recent times worldwide (Kabeer *et al*., 2021). It isn't easy to control the safety levels of concentration and acceptance of

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living organisms and animals (Kamal *et al*., 2020). The present study aimed to determine the proximate composition and physico-chemical parameters of oil and the fatty acid composition of commercially available animal feeds in the local market and villages.

Materials and Methods

Chemicals and reagents. In the present study, all the reagents and chemicals were used by E-Merck (Darmstadt, Germany), such as potassium hydroxide, sodium hydroxide, n-hexane, sodium-thiosulphate, potassium iodide, hydrochloric acid, methyl alcohol, carbon tetrachloride, anhydrous sodium sulphate, ethyl alcohol, sulphuric acid, nitric acid, phenolphthalein, pure iodine and starch.

Sample collection. A total of ten animal feeds samples (triplicate) were collected from local villages and Jacobabad city, Sindh, Pakistan. Feed samples were coded for local villages as sunflower seed cake (SFSC), cottonseed cake (CSC), linseed seed cake (LSC), rapeseed cake (RSC) and mustard seed cake (MSC). In contrast, samples collected from the city were coded as mixed feed (MF), L.S. Minerals (LSM), newtramn (NT), white gold (WG) and high milker (HM).

Moisture content. The moisture content of the animal feed was quantified by applying the official method as reported by (Laghari *et al*., 2018). Approximately 10 g of animal feed was taken in the Petri dish and kept in an oven (Memmerts Schwabach, Germany) at 105 °C for 180 min.

Oil extraction. Oil from animal feeds was extracted as reported earlier by (Laghari *et al*., 2018) using hexane as a solvent. Around 10 g of animal feeds were put into a cellulose thimble and placed inside the extractor then 300 mL of hexane was added to the round bottom flask. The temperature was set at 70 °C and the process continued for about 4 h. After complete extraction, the solvent was evapourated by using a rotary evaporator. The extracted oil was kept in the refrigerator at 4 °C for further analysis.

Ash content. For ash content, approximately 2 g of animal feeds were ignited in a muffle furnace (Fenwal 550 single point) at 600±15 °C for 2 h, as reported by (Laghari *et al*., 2018).

Protein content. For the determination of total protein content in animal feeds, the Kjeldahl method was used as described earlier (Laghari *et al*., 2018).

Fiber content. The fiber content of the animal feed was determined by decomposing fatty substances with dilute base, whereas dilute acid for starch and protein. Following filtering, the residual material was burned at 600 °C in the muffle furnace for 3 h, as described by (Laghari *et al*., 2018).

Carbohydrate content. Carbohydrate content in animal feed was determined by calculating the difference between total percentage and sum of mean values of moisture, ash, oil and protein and carbohydrate = [100 – (moisture + protein + ash + oil content)].

Dry matter. The part of animal feed which is free from water is known as dry matter. The following formula calculated the percent dry matter: DM = 100% - moisture% (Khoddami *et al*., 2014).

Energy value. The energy value of feeds was counted in megajoules and determined by applying the relevant factors of fat, protein and carbohydrate 37.7%, 16.7% and 16.7%, correspondingly Laghari *et al.* (2018).

Nitrogen free extract. Nitrogen-free extract in animal feed samples was determined according to the following formula reported earlier (Laghari *et al*., 2018).

NFE% = 100 – (ash% + crude protein% + crude fiber% + free fatty acid% + moisture contents%).

Iodine value (IV). The iodine value of animal feed oil was determined using the standard AOCS method (2013) Cd 1-25. Briefly, 1 g oil was taken in the 250 mL of conical flask and added 10 mL of CCl_4 and 25 mL of Wijis solution and kept in the dark for 30 min. After that, 10% of KI and 100 mL of deionized water added in to the solution. At the end, the mixture was titrated with sodium thiosulphate $Na_2S_2O_2$ solution (0.1 N) by using starch as an indicator. Similarly, the blank test carried out by the same procedure in the absence of oil. The following formula used for the calculation of the IV.

IV (gI_2 / 100 g) = [{(Blank reading - sample reading)mL × N of sodium thiosulphate × 12.69} / weight of sample (g)]

Peroxide value (PV). The standard official AOCS method (2013) Cd 8-53 used to measure the PV. About 2 g of animal feed oil was kept in a 250 mL conical flask containing a mixture of chloroform: glacial acetic acid (v/v) in the ratio of 2:3. The flask's content

was titrated with a standardized solution of sodium thiosulphate 0.1 N using a 1% starch solution as an indicator. The PV of animal feed oil was calculated using the following formula.

PV (meq/Kg of oil) = [{(blank reading – sample reading)mL × N of sodium thiosulphate} / weight of sample (g)]

Saponification value (SV). The standard AOCS official method (2013), Cd 3-25 was applied to estimate the SV. About 2 g of animal feed oil was put into a round bottom flask and refluxed for 1 h in the presence of 25 mL of ethanolic potassium hydroxides. The titration was accomplished with a standard solution of 0.5 M hydrochloric acid in the presence of a phenolphthalein indicator. The SV was measured by using the following formula.

SV (mg of KOH/g of oil) = [{(blank reading – sample reading)mL × 56.1}/weight of sample (g)]

Free fatty acid (FFA). The quantity of FFA in animal feed oil was determined by the AOCS (2013) Aa method 6-38. Approximately 1 g of animal feed oil was placed in a 250 mL conical flask and added 20 mL hot ethanol. The content of the flask was titrated against sodium hydroxide solution 0.1 N in the presence of a phenolphthalein indicator. FFA value of animal feed oil was measured by applying the following formula.

FFA% = [{(28.2 × mL × (NaOH) × N(NaOH)}/weight of sample (g)]

Fatty acid composition. Fatty acid methyl esters of animal feed oil were prepared according to the official method 2.302 (IUPAC, 1979). Agilent 6890 N gas chromatograph (Agilent technologies, little fall, NY, USA) coupled with Agilent MS-5975 detector was used to separate, identify and quantify fatty acids. Instrumental parameters of GC-MS are as follows: HP-5MS column (5% phenyl methyl siloxane) with 30 m × 0.25 mm ID × 0.25 µm film thickness, starting oven temperature was 150 °C and holding time 2 min, final temperature 230 °C, ramp rate 4 °C/min, helium as the carrier gas with 0.8 mL/min flow rate, injector temperature 240 °C, detector temperature 260 °C, 1 µL sample inserted as a split mode with a ratio 50:1. The detected fatty acids in animal feed oil were compared with those available in the NIST and Wily libraries.

FT-IR Spectroscopy. Infrared spectra of animal feed oil were recorded using FTIR (Thermo Nicolet IS10). The instrument was controlled with OMINIC software, and the other parameters used for the characterization are spectral range (4000-650 cm^{-1}), resolution (4 cm^{-1}), scan (32), sampling accessory (SB-ATR), crystal (diamond) and detector (DTGS).

Calculation and statistical analysis. The data obtained from different parameters were put into the Minitab16 USA software and analyzed by analysis of variance (ANOVA) followed by the Tukey test $P \leq 0.05$ (probability value).

Results and Discussion

Proximate analyses. The composition of feed contains added minerals and vitamins, which are necessary for the performance of animals. The quantity supplied to animals should be available in the right proportions as deficiency or excess in these essential constituents may lead to diseases and intoxication in the organisms (Jonge and Jackson, 2013). So, that proximate analysis of animal feeds must be done using the different parameters. Table 1 shows the proximate analysis of the animal feed.

Moisture content. In this study, high moisture content was found in WG 12±0.21, but a low amount was present in the CSC 5.8±0.12. The moisture content of CSC 5.8±0.12 was low compared with a reported value of 7.0–11.0% (Robbins and Firman, 2006). The CSC contains very high dry matter, which is advantageous because it reduces microbial activities and saves from oxidation reactions. Compared to CSC, the WG sample showed lower dry matter and had low quality.

Oil content. The amount of oil in feeds increases energy and growth rates and increases feed efficiency (Robbins and Firman, 2006). Fat is very important in the diet as it promotes the absorption of fat soluble vitamins and provides high energy nutrients. The oil content results show a high amount was analyzed in HM 88±1.0 but a lower amount was found in SFSC 0.8±0.03%.

Ash value. Ash contents play an essential role in promoting the balanced growth of animals. It has been reported that high ash contents increase with the degree of maturity of plants (Ahmed *et al*., 2013). The ash indicates high inorganic matter that could be retained in the body. In the present study, high ash was found

Table 1. Proximate analysis of animal feed

Physical parameters	SFSC	CSC	LSC	RSC	MSC	MF	L.SM	NT	WG	HM
MC (%)	6.5±0.10 cd	5.8±0.12 d	10.2±0.15 b	10.57±0.41 ab	10.3±0.16 b	5.0±1.0 d	11.1±0.10 ab	10±1.0 b	12±1.01 a	7.6±0.20 c
OC (%)	0.8±0.03 g	3.23±00.1 e	7.2±0.2 c	5.93±0.02 d	10.5±0.01 b	1.97±0.015 f	6.0±0.21 d	1.77±0.01 gf	1.79±0.015 gf	88±1.0 a
AV (%)	2.1±0.2 b	1.0±0.06 cde	1.3±0.1 c	3.2±0.1a	1.0±0.24 cd	0.5±0.2 e	0.9±0.43 de	2.0±0.12 b	3.1±0.10 a	1.1±0.1 cd
PC (%)	30.2±0.1 c	18.4±0.2 e	17.8±0.11 f	30.1±0.1 c	18.3±0.20 e	34.54±0.5 b	9.19±0.02 h	24.5±0.1 d	11.8±0.1 g	52.4±0.2 a
FC (%)	60.57±0.41 e	71.59±0.2 b	63.5±0.1 h	49.8±0.1 f	59.8±0.02 f	57.9±0.44 g	72.8±0.1 a	61.7±0.1 d	61.3±0.01 d	71.3±0.02 b
CC (%)	48.1±0.2 f	63.19±0.01 b	52.26±0.4 e	39.8±0.65 g	58.51±0.01 c	54.59±0.02 d	67.71±0.2 a	57.7±0.6 c	67.8±0.03 a	34.0±1.0 h
DM (%)	93.5±0.1 ab	94.5±0.2 ab	89.8±0.1 c	89.1±0.1 c	87.8±0.2 c	95±2.0 a	88.9±0.4 c	90±1.0 c	88±1.0 c	92.5±0.1 b
EC(KJ/Kg)	13.7±0.1 j	144±1.0 i	1442±2.0 g	1392±1.0 H	1679±0.8 a	1562±0.23 b	1510±0.12 d	1489±0.18 e	1480±0.28 f	1548±1.13 c
NF (%)	53.6±0.1 b	47.5±0.3 c	7.46±0.01 J	35.73±0.02 F	9.56±0.01 i	21.56±0.40 g	62.71±0.1 a	44.7±0.01 e	45.82±1.1 d	12.73±0.01 h

a-j=different letters indicate significant difference (P≤0.05) in different animal feeds samples such as sunflower seed cake (SFSC); Cottonseed cake=CSC; Linseed seed cake=LSC; Rapeseed cake=RSC; Mustard seed cake=MSC; Mixed feed=MF; L.S. Minerals=LSM; Newtramn=NT; White gold=WG and High Milker=HM

at 3.1±0.10% in WG among all analyzed feed samples, while a lower amount of ash was found in MSC at 1.0±0.24%. The highest amount of ash shows the high value of metals in the animal feed, which may increase the value of nutrients in animal feed and increase the quality of animal feed. According to the previous study (Manoj *et al*., 2020), a high value of ash content was reported in feeds in the range of 9 to 13%. The higher values of ash content may also cause the harmful effects on the health of animals.

Protein content. A low amount of protein in animal feeds reduces the flavour of feeds, so the animals consume a low amount of feed and do not grow properly. These types of feed are not suitable for livestock development. The healthy development of livestock and animal feed is significantly essential, which contain a high amount of proteins (Ahmed *et al*., 2013). Animals are healthier in the season when animal feed is rich in protein contents. The health of animals may be decreased when the animal feed has a low amount of protein because the high amount of protein provides a high amount of amino acids to both animals and men (Sullivan *et al*., 2004). The protein content results indicated that a high amount of protein was found in HM 52.4±0.2. On the other hand, a lower protein content, 17.8±0.11%, was observed in the LSC sample. The highest amount of protein shows the high value of nitrogen which may increase the value of protein and nutrients in animal feeds and improve the quality of animals.

Fiber content. (Williams *et al*., 2001) reported that water soluble fiber might cause a high viscosity and could help explain the effects for pelleting characteristics of animal feed. It has also been reported that dry fiber in animal feed has much importance for animals (Molist *et al*., 2014; Mateos *et al*., 2006). A high amount of fiber is essential for dietary nutrition, increasing intestinal digestion and reducing the risk of constipation, cancer and colon diseases (Arisa *et al*., 2017). The high fiber content was found 72.8±0.1 in LSM. In contrast, a low amount of fiber 49.8±0.1% was observed in the RSC sample.

Carbohydrate content. Carbohydrate provides energy to animals through feed components. It is composed of carbon, oxygen and hydrogen. In animal feeds, it should be about 75%. Animal's body remains warm due to carbohydrates that produce heat and the daily diet

required for the animals (Poorkasegaran and Yansari, 2014). In this study, higher carbohydrate was observed 67.8±0.03% in WG, while a lower carbohydrate was present in the RSC sample 39.8±0.65%.

Dry matter. The remaining part of any substance without water is known as dry matter. In the present study, the dry matter was found in the range of 87.8 to 94.5. A high and low amount of dry matter was observed in WG and CSC samples, respectively.

Iodine value. The iodine value shows the degree of un-saturation within the molecules of oil and fats. In the present study, the iodine value in animal feed oil was determined by using the Wijis method. The highest iodine value was found at 111±1.0 gI_2/100 g in the RSC sample and the lowest was found in the LSM sample 87.8±0.06 gI_2/100 g. The higher iodine value shows the higher level of un-saturation which may increase the value of animal feed.

Peroxide value. The peroxide value is used to measure the peroxides formed in animal feed oil due to oxidation (Babalola and Apata, 2011). In animal feed oil, the peroxide value was found in the range of 0.4 to 12 meq/Kg. A lower peroxide value was observed in SFSC and a higher value in the MF sample.

Saponification value. Saponification value represents the number of mg of potassium hydroxide required to saponify 1 g oil. It indicates the number of carbons in the fatty acids which are present in animal feed oil. Those fatty acids with many carbons have low saponification value because they have smaller carboxylic functional groups per unit mass than short chain fatty acids. The highest saponification value was found 245±1.9 mg KOH/g in the CSC sample, while the lower value was found 177±2.1 mg KOH/g in the NT sample.

Free fatty acid. The essential quality parameter of oil is the free fatty acid value produced due to oil hydrolysis (Khoddami *et al*., 2014). After processing, some oils have been reported to contain minimal amounts of FFA but can be increased due to the long storage (Babalola and Apata, 2011). Among the analyzed samples, comparatively, a lower quantity of FFA was found in the LSM sample 11±2.0%. In contrast, the highest amount of FFA was found at 59±2.1 in the MSC sample (Table 2).

Fatty acid composition of animal feed oil. To check the fatty acid composition in animal feed oil, first prepared fatty acid methyl esters and then quantified fatty acid composition by GC-MS. The results of total saturated fatty acids are shown in Table 3A. Saturated fatty acids in animal feed oil were found in the range of 11.26-89.88%. Among the detected saturated fatty acids, the higher percentage of octadecanoic acid (C18:0) 57.12±0.1% was found in NT sample, whereas a lower percentage of pentadecanoic acid (C15:0) 1.84±0.02% was found in the RSC sample. The results of un-saturated fatty acids are shown in Table 3B. The total un-saturated fatty acids in animal feed oil were found in the range of 10.14-88.74%. Among the detected un-saturated fatty acids in feed oil samples, the higher percentage of octadecenoic acid (C 18:1) 66.59±0.06% was found in the RSC sample and a lower percentage of docosenoic acid (C 22:1) 0.79±0.2% was found in the HM sample.

FTIR characterization of feed oil. The extracted feed oil samples were further analyzed by FTIR spectroscopic method with an SB-ATR accessory. The animal feed oil was characterized in terms of band intensity (frequency) of different functional groups by FTIR and the results are shown in Table 4A and 4B, respectively. It was observed that the intensity of different functional groups varied among different samples, which clearly shows the diverse nature of feed ingredients.

Table 2. Physio-chemical characteristics of animal feed oil

Chemical parameters	SFSC	CSC	LSC	RSC	MSC	MF	LSM	NT	WG	HM
IV (gI_2/100 g)	85±0.02 cd	65.1±0.1 f	87.8±0.06 c	111±1.0 a	112±2.0 a	87±0.5 c	77.9±0.2 e	82.1±0.1 d	93±1.0 b	88±1.1 c
PV (meq/Kg)	0.4±0.1 e	4±1.0 d	8.0±1.9 abcd	8.1±2.0 abcd	9±1.0 abc	12±2.0 a	9±1.0 abc	11±1.0 ab	5±2.0 cd	7±2.2b cd
SV (mg KOH/g)	238±1.0 b	245±1.9 a	207±1.2 d	196±1.0 e	203±2.1 d	206±2.3 d	192±2.0 e	177±2.1 f	179±1.53 f	221±1.3 c
FFA (%)	2.5±0.1 g	13±1.2 ef	2±2.0 b	10±1.3 f	59±1.0 a	35±2.0 c	11±2.0 f	16±1.0 e	26±2.0 d	24±1.0 d

a-f=different letters indicate significant difference (P≤0.05) in different animal feeds samples such as sunflower seed cake=SFSC; Cottonseed cake=CSC; Linseed seed cake=LSC; Rapeseed cake=RSC; Mustard seed cake=MSC; Mixed feed=MF; L.S. Minerals=LSM; Newtramn=NT; White gold=WG; High Milker=HM

Table 3A. Composition of saturated fatty acids in animal feed oil

Fatty acid	SFSC	CSC	LSC	RSC	MSC	MF	LSM	NT	WG	HM
					Mean±SD					
Dodecanoic acid (C12:0)	-	-	10.0±0.2 b	-	5.79±0.1 c	-	7.31±0.3 b	2.16±0.1 d	2.17±0.1 d	12.13±0.8 a
Tetradecanoic acid (C14 :0)	3.0±0.01 c	-	29.16±0.04 a	-	-	-	3.05±0.1 c	-	4.16±0.2 c	34.16±0.01 a
Pentadecanoic acid (C15:0)	4.44±0.1 c	-	13.15±0.2 d	1.84±0.02 c	-	-	-	-	-	-
Hexadecanoic acid (C16:0)	16±0.2 c	8.82±0.02 a	3.27±0.11 b	3.76±0.1 d	46.28±0.01 a	23.24±0.3 a	34.36±0.3 c	20.3±0.4 a	26.13±0.01 a	17.12±0.10 a
Heptadecanoic acid (C17:0)	-	-	5.42±1.3 e	-	3.27±0.1 b	1.13±0.01 c	-	-	-	9.23±1.0a
Octadecanoic acid (C18:0)	6.31±0.2 e	2.44±0.1 g	-	24.4±0.1 c	-	4.15±0.21 f	6.0±0.5 e	57.12±0.1 a	33.99±0.02 b	11.12±0.05 d
Eicosanoic acid (C20:0)	2.99±0.01 c	-	-	-	9.30±1.8 a	1.66±0.1 d	-	-	-	6.12±0.13 b
ΣSFA	32.74	11.26	61	30	64.64	30.18	20.72	79.58	66.45	89.88

Table 3B. Composition of unsaturated fatty acids in animal feed oil

Fatty acid	SFSC	CSC	LSC	RSC	MSC	MF	L.SM	NT	WG	HM
					Mean±SD					
Octadecanoic acid (C18:1)	30.99±0.08 c	5.42±0.13 g	26.56±0.4 d	66.59±0.06 a	1.0±0.01 i	45.43±0.01 b	-	13.15±1.1 f	14.14±0.5 e	1.63±0.07 h
Octadecadinoic acid (C18:2)	36.36±0.31 b	20.18±0.8 d	7.0±0.1 f	3.5±0.1 g	29.16±0.07 c	14.41±0.9 e	40.97±0.11 a	-	19.44±0.4 d	-
Eicosenoic acid (C20:1)	-	63.14±0.13 a	2.44±0.01 g	-	4.20±0.1 f	9.13±0.01 b	8.31±0.09 c	5.13±0.21 e	-	5.59±0.06 d
Eicosadienoic acid (C20:2)	-	-	0.56±0.01 c	-	-	0.89±0.01 b	-	2.16±0.1 a	-	2.13±0.06 a
Docosenoic acid (C22:1)	-	-	2.44±0.1 a	-	1.0±0.01 b	-	-		-	0.79±0.2 c
ΣUSFA	67.35	88.74	39	69.59	35.36	69.86	49.28	20.44	33.58	10.14

Table 4A. FTIR band intensity of animal feed oil (sample 1 to 5)

Frequency (cm^{-1})	Intensity					
	SFSC	CSC	LSC	RSC	MSC	Observation
3007	0.0225	0.0237	0.0313	0.0246	0.0348	C-H stretching vibration of the *cis*-double bond (=CH)
2922	0.196	0.202	0.194	0.186	0.196	CH_2 Asymmetrical stretching
2855	0.135	0.141	0.136	0.128	0.139	CH_2 Symmetrical stretching
1744	0.157	0.157	0.0755	0.178	0.0681	C=O stretching (ester)
1708	0.075	0.071	0.200	0.0658	0.208	C=O stretching (acid)
1650	0.0094	0.0094	-	0.0121	0.0093	C=C
1455	0.0670	0.067	0.0708	0.0674	0.0710	CH_2 Scissors
1366	0.0347	0.0347	0.0394	0.0423	0.0364	Bending vibration of CH2 groups
1235	0.0558	0.05	0.0657	0.0661	0.0631	Vibrations of the C-O ester groups
1160	0.110	0110	0.0700	0.127	0.0652	
1118	-	0.0589	0.0443	0.0729	0.0391	
1089	0.0651	0.0651	-	0.0750	0.0376	
944	0.025	0.0258	0.049	0.0313	0.0496	CH=CH (cis) bending out of plane
846	-	-	0.0247	0.0219	0.0293	$=CH_2$ Wagging
719	0.0830	0.030	0.0790	0.084	0.0846	Overlapping of the CH_2 rocking vibration and the out-of-plane vibration of *cis*-disubstituted olefins

Table 4B. FTIR band intensity of animal feed oil (sample 6 to 10)

Frequency (cm^{-1})	Intensity					Observation
	MF	L.SM	NT	WG	HM	
3007	0.0245	0.0235	0.0255	0.0277	0.02767	C-H stretching vibration of the *cis* double bond (=CH)
2922	0.229	0.194	0.193	0.196	0.196	CH_2 Asymmetrical stretching
2855	0.166	0.134	0.135	0.137	0.136	CH_2 Symmetrical stretching
1744	0.0989	0.169	0.148	0.124	0.135	C=O stretching (ester)
1708	0.159	0.0635	0.0958	0.128	0.112	C=O stretching (acid)
1366	0.0533	0.0378	0.0373	0.0369	0.0369	Bending vibration of CH_2 groups
1235	0.0813	0.0598	0.0601	0.0601	0.0595	Vibrations of the C-O ester groups
1160	0.0940	0.118	0.108	0.0954	0.101	
1118	0.014	0.0643	0.0594	0.0534	0.0559	
1089	0.0634	0.0694	0.0627	0.0558	0.0594	
944	0.0620	0.0268	0.0306	0.0251	0.0324	CH=CH (cis) bending out of plane
846	0.0332	0.0147	0.0304	0.0229	0.0321	$=CH_2$ Wagging
719	0.0754	0.0807	0.0826	0.0816	0.08827	Overlapping of the CH_2 rocking vibration and the out-of-plane vibration of *cis*-disubstituted olefins

Conclusion

The improvements in livestock productivity are generally associated with improved biological efficiency used for the animals. Determining nutrients alone is insufficient to assess the quality of feeds used for animal nutrition, determining the digestibility and energy content is essentially vital. In this study, the protein, carbohydrate and fiber content in compound feed meet the minimum required range of nutrients than seed cake. Among oil parameters, comparatively less free fatty and iodine value except higher peroxide value found in compound feed than seed cake which support a good quality of oil present in the compound feed.

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References

Ahmed, K., Shaheen, M., Mirzaei, F., Khan, Z.I., Gondal, S., Fardous, A., Hussain, A., Arshad, F., Mehmood, T. 2013. Proximate analysis relative feed values of various forage plants for ruminants investigated in a semi-arid region of Punjab, Pakistan. *Agricultural Sciences*, **4:** 302-308. dio:10.4236/as.2013.46043

Arisa, N.U., Adelekan, A., Malomo, O.O., Dudu, O.E. 2017. Physico-chemical and sensory properties of blends from beniseed, trifoliate yam and wheat flours. *African Journal of Food Science and Technology*, **5:** 067-073. dio: 10.14303/ajfst. 2017.024

Babalola, T.O., Apata, D.F. 2011. Chemical and quality evaluation of some alternative lipid sources for aqua feed production. *Agriculture and Biology Journal of North America*, **2:** 935-943. https://doi.org/10.5251/ abjna.2011.2.6.935.943

Danieli, P., Lussiana, C., Gasco, L., Amici, A., Ronchi, B. 2019. The effects of diet formulation on the yield proximate composition and fatty acid profile of the black soldier fly (*Hermetia illucens* L.) prepupae intended for animal feed. *Animals*, **9:** 178. https://doi.org/10.3390/ani9040178

Firestone, D. 2013. *Official Methods and Recommended Practice of the American Oil Chemist's Society* (AOCS), 6th edition, AOCS press, Urbana, Illinois, USA.

Jonge, L.H., Jackson, F.S. 2013. The feed analysis laboratory: establishment and quality control. Setting up a feed analysis laboratory and implementing a quality assurance system compliant with ISO/IEC 17025:2005 FAO, Roma (Italia). https://www.fao.org/publications/card/fr/c/ bf25b34e-c860-5a66-b971-43680caf90b6/

Kabeer, M.S., Hameed, I., Kashif, S.U.R., Khan, M., Tahir, A., Anum, F., Raza, S. 2021. Contamination of heavy metals in poultry eggs: a study presenting relation between heavy metals in feed intake and eggs. *Archives of Environmental Occupational Health*, **76:** 220-232. dio:10.1080/19338244. 2020. 1799182

Kaithwas, M., Singh, S., Prusty, S., Mondal, G., Kundu, S.S. 2020. Evaluation of legume and cereal fodders for carbohydrate and protein fractions, nutrient digestibility, energy and forage quality. *Range Management and Agroforestry*, **41:** 126-132. https://www.researchgate.net/publication/344150891

Kamal, M.T., Hashem, M.A., Al Mamun, M., Hossain, M.M., Razzaque, M.A., Ritu, J.H. 2020. Investigating the quality of commercial beef cattle feeds and feed ingredients used in Bangladesh. *SAARC Journal of Agriculture*, **18:** 197-208. https://doi.org/10.3329/sja.v18i1.48393

Khoddami, A., Che Man, Y.B., Roberts, T.H. 2014. Physico-chemical properties and fatty acid profile of seed oils from pomegranate (*Punicagranatum* L.). *European Journal of Lipid Science and Technology*, **116:** 553-562. https://doi.org/10.1002/ejlt.201300416

Konar, M., Qiu, S., Tougher, B., Vause, J., Tlusty, M., Fitzsimmons, K., Cao, L. 2019. Illustrating the hidden economic, social and ecological values of global forage fish resources. *Resources, Conservation and Recycling*, **13:** 151-156. https://doi.org/10.1016/j.resconrec.2019.104456

Laghari, Z.H., Mahesar, S.A., Sherazi, T.H., Memon, S.A., Sirajuddin, Mugheri, G.A., Shah, S.N., Panhwar, T., Chang, A.S. 2018. Quality evaluation of pomegranate waste and extracted oil. *International Food Research Journal*, **25:** 1295-1299. http://www.ifrj.upm.edu.my/ ifrj-2018-25-issue-http://www.ifrj.upm.edu.my/ifrj-2018-25-issue-3.html

Manoj, K.N., Shekara, B.G. 2020. Agronomic management practices for enhancing quality and productivity of fodder: a review. *Indian Journal of Pure Applied Bioscience*, **8:** 403-410. http://dx.doi.org/10.18782/2582-2845.8335

Mateos, G.G., Martin, F., Latorre, M.A., Vicente, B., Lázaro, R. 2006. Inclusion of oat hulls in diets for young pigs based on cooked maize or cooked rice. *Animal Science*, **82:** 57-63. DOI:https://doi.org/10.1079/ASC20053

Molist, F., Van Oostrum, M., Pérez, J.F., Mateos, G.G., Nyachoti, C.M., Van Der Aar, P.J. 2014. Relevance of functional properties of dietary fibre in diets for weanling pigs. *Animal Feed Science and Technology*, **189:** 1-10. https://doi.org/10.1016/j.anifeedsci.2013.12.013

Paquot, C. 1979. *Standard Methods for the Analysis of Oils, Fats and Derivatives Part 1 (Section I and II)*, pp.103-108, Pergamon Press, Oxford, UK.

Poorkasegaran, S., Yansari, A.T. 2014. Effects of different sources of carbohydrates on intake, digestibility, chewing and performance of Holstein dairy cows. *Journal of Animal Science and Biotechnology*, **5:** 1-11. doi:10.1186/2049-1891-5-6

Robbins, D.H., Firman, J.D. 2006. Evaluation of the metabolizable energy of poultry by-product meal for chickens and turkeys by various methods. *International Journal of Poultry Science*, **8:** 753-758. doi:10.3923/ ijps.2006.753.758

Sullivan, H.M., Bernard, J.K. 2004. Performance of lactating dairy cows fed whole cotton seed with elevated concentrations of free fatty acids in the oil. *Dairy Science*, **87:** 665-671. https://doi.org/10.3168/jds.S0022-0302(04)73209-9

Varma, V.S., Shabtay, A., Yishay, M., Mizrahi, I., Shterzer, N., Freilich, S., Laor, Y. 2018. Diet supplementation with pomegranate peel extract altered odorants emission from fresh and incubated calves' feces. *Frontiers in Sustainable Food Systems*, **2:** 33-40. https://doi.org/10.3389/fsufs. 2018.00033

Williams, B.A., Verstegen, M.W., Tamminga, S. 2001. Fermentation in the large intestine of single-stomached animals and its relationship to animal health. *Nutrition Research Reviews*, **14:** 207-227. https://doi.org/10.1079/ NRR200127