Replacement of commercial premix with composite leaf meal: effects on growth and reproductive performance of peri-pubertal pigs

Samuel Olanrewaju Aro*
**corresponding author : soaro@futa.edu.ng*

Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria

ABSTRACT

The effects of replacing the dietary premix in commercial pig's growers diets with different levels of composite feed meal (CLM) on growth and reproductive performance of peri-pubertal pigs was investigated in an eight-week experiment. Thirty-six weanling pigs of mixed sexes were randomly distributed into six treatment groups with six diets, replicated six times with one animal per replicate. Each group and treatment comprised three males and three females. Diet 1 had no inclusion (0 %) of CLM while diets 2,3,4,5 and 6 included CLM at 1 %, 2 %, 3 %, 4 % and 5 % of the diets respectively.

The CLM thus replaced the commercial premix at 0, 20, 40, 60, 80 and 100% of the six treatment diets respectively. The results showed significant ($p < 0.05$) differences in weight gain (WG), feed conversion ratio, (FCR), protein efficiency ratio (PER) and final weight of the pigs.

The gonadal morphometry of the female reproductive tract revealed enhanced length of the oviduct and uterine horn up to 2% CLM level of inclusion which thereafter declined as inclusion level increased. The seminal vesicles of the male pigs in Diet 2 to Diet 6 were inferior to those of pigs fed the control diet. The histopathology of the testes revealed intact seminiferous tubules, germinal epithelium and basement membranes in pigs fed CLM up to 3 % inclusion level beyond which there was a loss of their architectural and cellular integrity.

Conclusively, the use of CLM in the diets of growing pigs would enhance growth performance up to 5 % level, but could threaten the breeding capacity of both the male and female pigs if used beyond 3% level.

Keywords: Composite leaf meal, gonads, growth performance, histopathology, swine

INTRODUCTION

The quest for alternative feedstuffs for livestock especially the monogastric has led to a series of research on the utilization of non-conventional feed ingredients for monogastric nutrition. The monogastric animals are relatively costlier to feed when compared with the ruminants because of their heavy dependence on soluble carbohydrate sources, good quality proteins, essential amino acids, mineral and vitamins all of which must be provided in their diets for optimum performance. Most of these ingredients used for feeding the monogastric are directly or indirectly used as food for humans, thus creating a form of competition between man and his livestock and the concomitant shortage and high prices of these feed ingredients. This heavy dependence of the pig enterprise on high energy, protein and vitamin/mineral requirements have prompted the evaluation and use of some neglected forages and under-utilized herbs as composite leaf meals in pigs' feed. Different authors have reported the nutritional values edible herbs as leaf meals in Nigeria (Nwaogu and Udebuani, 2010; Sallau et al., 2012; Aro and Ajiboye, 2015). Some of these herbs have high crude protein contents in addition to a good balance of vitamin and mineral constituents often supplied in the feed as vitamin/mineral premixes.

These premixes are not only expensive in the diets, their preparation is not well attuned to existing local technologies. There is therefore the need to seek readily available and affordable alternatives to these commercial premixes through the use of some under-utilized herbs reputed to contain these vitamins and minerals (Jacqueline, 2011; Odoemelam et al., 2013; Ohanaka et al., 2018). These vitamins and minerals can either be packed singly in one herb or combined in two or more of the herbs as leaf composites.

The use of these under-utilized herbs as composite leaf meal to replace the costly vitamin/mineral premix in livestock diets has to be viewed alongside its effect on growth and reproductive performance of farm animals. According to Gbadamosi and Egbunike (1999), reproductive inefficiency is the costliest and most limiting constraint to efficient animal production. Much has also been said about the anti-nutritional factors that these plants contain (Bonsi et al., 1995; Ajibade et al., 2006; Wood et al., 2008). If the composite leaf meal from these plants recommends them as a replacement for the commercial premix for pigs, there is the need for its validation through animal experimentation. The aim of this experiment was to investigate the effects of replacing the commercial grower pigs' premix with composite leaf meal (CLM) on growth and reproductive performance of peri-pubertal pigs.

MATERIAL AND METHODS

The experiment was carried out at the piggery unit of the livestock section of Teaching and Research Farm, Department of Animal Production and Health, School of Agriculture an Agricultural Technology, Federal University of Technology, Akure, Nigeria. Akure is located on latitude7.15o N and longitude 5.12o E in the hot, wet equatorial climate with high temperature and humidity throughout the year. The mean annual rainfall is about 1500 mm with a bimodal distribution pattern and relative humidity of about 75 %. The mean annual temperature is about 27o C.

Preparation of composite leaf meal (CLM): The CLM was prepared from leaves harvested from five different plants. These plants are; Cassava, (Manihot esculentum), Bitter leaf (Vernonia amygdalina), Basil (Ocimum gratissimum), Drum stick tree (Moringa oleifera) and Fluted gourd or Fluted pumpkin (Telfaria occidentalis). The leaves were chopped, air-dried for about five days to a moisture content of between 10-13 %, ground and mixed together in equal ratios (as composite leaf meal) and incorporated into the swine diets at graded levels of inclusion. The Table 1 gives the phyto-chemical and anti-nutrient composition of one of the herbs used in this study

Table 1. Phytochemical composition and anti-nutrients of Moringa oleifera from Lafia, Nasarawa State, Nigeria.(Source: Ogbe et al. , 2011)

Phytochemical/anti-nutrients	Mean values (% ±SD)
Phytates	2.59 ± 0.13
Oxalates	0.45 ± 0.01
Saponins	1.60 ± 0.05
Tannins	21.19 ± 0.25
Trypsin inhibitors	3.00 ± 0.04
Hydrogen cyanide (HCN)	0.10 ± 0.01

Experimental layout

A total of 36 pigs of mixed sexes with an average initial liveweight of 17.50 kg were used. The animals were randomly allotted to six experimental treatments (six animals per treatment) using a completely randomized design. Six standard pig's diets were formulated containing 0, 1, 2, 3, 4 and 5 % of composite leaf meal designated as Diet 1 (the control diet), Diets 2, 3, 4, 5 and 6 in which CLM replaced the commercial pigs' premix at 0, 20, 40, 60, 80 and 100% respectively (Tables 2, 3 and 4).

Ethical approval for the use of the experimental animals was obtained from the Ministry of Agriculture and Natural Resources of the Federal Republic of Nigeria under the Criminal Code Edict of 1964, CAP 30, Chapter 50 (Cruelty to Animals), paragraph 495-499, pp. 183-185. Each of the treatments was replicated six times at one animal per replicate. Each of the treatments was made of three male and three female pigs. The animals were fed the experimental diets at 5 % of their body weight adjusted weekly for a period of 56 days (8weeks).

Table 2. Experimental diets

Items	Diet 1 0.0 % CLM	Diet 2 1.0 % CLM	Diet 3 2.0 % CLM	Diet 4 3.0 % CLM	Diet 5 4.0 % CLM	Diet 6 5.0 % CLM
Basal Ingredients	100	99.0	98.0	97.0	96.0	95.0
Composite Leaf Meal	0	1.0	2.0	3.0	4.0	5.0
Total	100	100	100	100	100	100
Calculated composition of Composite Leaf Meal (CLM)						
Crude protein (%)		18.34				
Crude fibre (%)		10.62				
ME (MJ/Kg)		7.87				

CLM = Composite leaf meal; ME = Metabolizable energy.

Data were collected on initial and final weight of the pigs, daily feed intake, weekly weight gain, feed conversion ratio (FCR) and protein efficiency ratio (PER). The PER was calculated as ratio between WG and PI, where WG = Weight gain and PI = Protein intake (i.e. Total feed Intake × % Crude Protein in the diet).

All the animals were sacrificed at the end of the feeding period to determine the gonadal morphometry of both the male and female pigs and gonado-somatic indices and histo-pathology of the testes of the males. Tables 2 and 3 show the ration formulation for the weaner's and grower's phases of the experiment.

Histo-pathological study

The right testes were removed and prepared for histological study. The tissues were mechanically and biologically stabilized in a fixative (10 % formaldehyde in phosphate buffered saline [PBS]). The samples were thereafter immersed in multiple baths of progressively more concentrated ethanol to dehydrate the tissue, followed by immersion in a clearing agent (xylene) and were finally impregnated in hot molten paraffin wax. The wax was allowed to cool and thereafter placed in cold water to harden completely. The embedded tissues were sectioned into very thin sections (2-8 micrometer) using a microtome.

Table 3. Composition (g/100g) of weaners' pig diets

Ingredients	T1	T2	T3	T4	T5	T6
Maize	56.00	55.05	54.10	53.15	52.20	51.25
Soybean meal	9.00	9.00	9.00	9.00	9.00	9.00
Groundnut cake	10.00	10.00	10.00	10.00	10.00	10.00
Palm kernel cake	9.00	9.00	9.00	9.00	9.00	9.00
Brewer's dried grains	13.50	13.50	13.50	13.50	13.50	13.50
Bone meal	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	0.50	0.50	0.50	0.50	0.50	0.50
Premix	0.25	0.20	0.15	0.10	0.05	0.00
Composite leaf meal	0.00	1.00	2.00	3.00	4.00	5.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition						
Crude protein	20.10	20.21	20.30	20.40	20.51	20.60
Crude fibre	5.47	5.56	5.64	5.72	5.81	5.90
ME KJ/kg	13.16	13.10	13.05	12.99	12.94	12.88

T1 = Diet with 0% inclusion of CLM and 100% commercial premix (control); T2 = Diet with 1% inclusion of CLM that replaced 20% commercial premix; T3 = Diet with 2% inclusion of CLM that replaced 40% commercial premix; T4 = Diet with 3% inclusion of CLM that replaced 60% commercial premix; T5 = Diet with 4% inclusion of CLM that replaced 80% commercial premix; T6 = Diet with 5% inclusion of CLM that replaced100% commercial premix; SEM = Standard Error of the Mean; CLM = Composite Leaf Meal; 1% CLM = 20% commercial premix.

Table 4. Composition (g/100g) of growers' pig diets

Ingredients	T1	T2	T3	T4	T5	T6
Maize	46.00	45.05	44.10	43.15	42.20	41.25
Soybean meal	6.00	6.00	6.00	6.00	6.00	6.00
Palm kernel cake	18.00	18.00	18.00	18.00	18.00	18.00
Brewer's dried grains	27.50	27.50	27.50	27.50	27.50	27.50
Bone meal	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	0.50	0.50	0.50	0.50	0.50	0.50
Premix	0.25	0.20	0.15	0.10	0.05	0.00
Composite leaf meal	0.00	1.00	2.00	3.00	4.00	5.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition						
Crude protein	18.01	18.10	18.18	18.25	18.32	18.40
Crude fibre	6.82	6.91	6.99	7.07	7.16	7.25
ME (KJkg-1)	12.13	12.10	12.06	12.01	11.97	11.93

T1 = Diet with 0% inclusion of CLM and 100% commercial premix (control); T2 = Diet with 1% inclusion of CLM that replaced 20% commercial premix; T3 = Diet with 2% inclusion of CLM that replaced 40% commercial premix; T4 = Diet with 3% inclusion of CLM that replaced 60% commercial premix; T5 = Diet with 4% inclusion of CLM that replaced 80% commercial premix; T6 = Diet with 5% inclusion of CLM that replaced 100% commercial premix; SEM = Standard Error of the Mean; CLM = Composite Leaf Meal; 1% CLM = 20% commercial premix.

The slides were then made ready for staining. Because sections are usually stained by aqueous dyes, the paraffin wax was removed and replaced with water. Sections were therefore treated with a wax solvent, then with alcohol to remove the wax solvent. As more efficient removal of reagents the dilution was repeted; at least 2 changes of wax solvent and alcohol were incorporated for efficient hydration. Similarly, after staining with Haematoxylin and Eosin (H&E), the sections were returned to the wax solvent (xylene) before mounting in a non-water-soluble mounting medium for subsequent dehydration.

Gonadal morphometry

Both the male and female pigs were dissected and their gonads excised and measured as appropriate. The testes were weighed singly and in pairs to determine the right and left testicular weight and paired testes weight respectively. The testicular volume was determined by displacement in saline water using the Archimedes principle of floatation while the testicular density was calculated as follows:

Testicular density = Mass of testis ÷ Volume of testis

The length of seminal vesicles, Cowper's gland and length and width of the testes were measured to the nearest millimeters with Vernier calipers while the length of the penis and pelvic urethra was measured using the measuring tape graduated to the nearest millimeters. From these measurements, the gonado-somatic indices were calculated as:

Gonado-somatic index = Weight of gonad ÷ Body weight × 100

Also, the entire reproductive tracts of the female pigs were weighed while the right and left uterine horns with the oviducts, the vagina and the cervix were measured with measuring tape to determine their respective lengths.

Statistical analysis

The data obtained from the experimental study were analyzed using the Analysis of Variance Model for Completely Randomized Design with SPSS version 24 (2016) statistical package, while the means were separated using Duncan Multiple Range Test of the same statistical package.

RESULTS AND DISCUSSION

Performance parameters of the experimental animals: Table 5 shows the performance parameters of the experimental pigs. There were no

($p > 0.05$) differences in the feed intake across the treatments. However, there were differences ($p < 0.05$) in the final weight, total weight gain, feed conversion ratio and protein efficiency ratio across treatments. Pigs fed T2 were the heaviest (52.67 kg), followed by T6 (52.33 kg), while T5 had the lowest final weight (48.67 kg). Animals in T2 had the best weight gain (34.67 kg), followed by T6 (34.00 kg), while T5 had the lowest weight gain (30.34 kg). Pigs on the T2 diet had the best feed conversion ratio (2.19) while it was poorest (2.50) in pigs fed the T5 diet. The highest value (2.38) for protein efficiency ratio (PER) was recorded in T2 followed by T6 (2.30) while T5 had the lowest value (2.06). No mortality was recorded throughout the duration of the experiment.

Table 5 did not reflect any negative influence of the inclusion of composite leaf meal (CLM) on the performance parameters such as the feed intake, final weight, feed conversion ratio (FCR), protein efficiency ratio (PER) and mortality. The replacement of the commercial mineral and vitamin premix with CLM was still able to support the growth of the pigs even better than the control diet at 100 % replacement of the premix with CLM.

This could be as a result of the relatively lower crude fibre content (10.62 % in Table 2) of the CLM in the current study than those reported for Ocinum gratissimum by Nworgu (2016) and for cassava leaf meal by Ravindran (1995). High levels of crude fibre in most of the leaf meals processed from tropical herbages have been implicated in low digestibility and poor growth in livestock (Ohanaka et al., 2018). The composite nature of the feed meal could have also led to the improvement in the other performance indicators like the FCR, PER and the absence of morbidity and mortality in the experimental animals. For instance, these five leaf meals that were combined in the same ratios in the CLM are known to have varied nutrient, anti-nutrient, vitamin and mineral composition and would have worked synergistically and complementarily to improve overall digestibility, absorption, livability and general wellbeing of the pigs. The intestinal modulation of the gut microbiota by Ocinum gratissimum and the blood forming potentialities of Telfaria occidentalis have been attested to (Ijeh et al., 2004; Ajibade et al., 2006)

Gonadal morphometry of the male pigs. The result of the gonadal morphometry of boarlings fed graded levels of composite leaf meal based diets is presented in Table 6. There were significant ($p < 0.05$) differences in all the parameters considered except the weight of right and left epididymides, length of pelvic urethra, weight of prostate gland and length of penis. Pigs fed T1diet had the highest value for the weight of the seminal vesicles while T6 had the lowest value. Length of seminal vesicles was also highest in T1 while T2 had the lowest value. The control diet also recorded the heaviest penile weight while it was lightest in T2. The T1diet had the heaviest weight of pelvic urethra while the lightest value was recorded T2.

Male pigs fed on T2 had the heaviest right and left testes, while the lightest for both parameters were observed in T5. The length of right and left testes varied ($p<0.05$) among the different dietary treatments. The width of the right and left testes also varied ($p<0.05$) across the treatments. Pigs on T1 had the heaviest Cowper's gland while the least value was recorded in T2. Animals fed on T4 had the longest Cowper's, while those fed the T2 diet had the shortest.

Boarlings that were fed T1 had the heaviest weight of seminal vesicles when compared with the boarlings fed CLM diets. The heavier vesicular gland in pigs that were fed T1 is an indication of greater fluid volume and concomitantly higher constituents of seminal nutrients, buffers and biomarkers to ensure optimum motility and fertilizing capabilities of the sperm cells. (Sankhala et al., 2012; Kumar and Swamy, 2017). Smaller seminal vesicles in the pigs fed the CLM diets relative to the control could therefore compromise the breeding efficiency of boars especially when used at 5% level as observed in the current study.

Aro and Awoneye (2012) reported similar observation in pigs fed graded levels of microbially fermented cassava peel (MFCP) at higher levels of inclusion. The heavier vesicular gland in boarlings that were fed T1 is an indication of the secretion of significant proportion of the fluid that ultimately becomes semen.

About 50-70 % of seminal fluid in humans originates from the seminal vesicles (Kierszenbaum, 2002) and about 78 % in the boar (Davies et al., 1975). Fructose, fibrinogen, ascorbic acid, prostaglandins, phosphate and carbonate buffers are normal constituents of vesicular gland secretions which have been reported to nourish sperm cells and offer protection against shift in the pH of semen (Chughtai et al., 2005). These vital metabolic functions may be compromised in the boarlings fed CLM especially at 5 % level.

The Cowper's gland was heaviest in T1 relative to the CLM diets. The lower weight of Cowper's gland in CLM-fed pigs may be as a result of the anti-nutritional factors presents in the CLM (Egbunike et al., 1999; Aro, 2010).

The lower weights of the Cowper's gland observed in pigs fed CLM-based diet could mean that less seminal fluid would be produced in these animals that would have ensured the formation of post ejaculatory cervical plug thus resulting in a subsequent reduction in semen volume.

Table 5: Growth performance of weanling pigs fed graded levels of composite leaf meal-based diets in replacement of commercial mineral/vitamin premix.

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**± SEM**
Initial weight (kg)	17.67	18.00	17.67	18.00	18.33	18.33	0.54
Final weight (kg)	50.33b	52.67a	49.67bc	50.33b	48.67c	52.33a	0.84
Feed intake (kg)	75.57	75.92	75.83	75.62	75.72	75.85	0.06
Weight gain (kg)	32.67ab	34.67a	32.00ab	32.33ab	30.34b	34.00ab	0.51
FCR	2.31ab	2.19b	2.37ab	2.34ab	2.50a	2.23b	0.09
PER	2.27ab	2.38a	2.19ab	2.21ab	2.06b	2.30ab	0.04
Mortality	0.00	0.00	0.00	0.00	0.00	0.00	0.00

a, ab, b, bc, c = Means on the same row but with different superscripts are statistically (p<0.05) significant.
FCR = Feed Conversion Ratio; PER = Protein Efficiency Ratio; T1 = Diet with 0 % inclusion of CLM and 100% commercial premix (control); T2 = Diet with 1 % inclusion of CLM that replaced 20 % commercial premix; T3 = Diet with 2 % inclusion of CLM that replaced 40 % commercial premix; T4 = Diet with 3 % inclusion of CLM that replaced 60 % commercial premix; T5 = Diet with 4 % inclusion of CLM that replaced 80 % commercial premix; T6 = Diet with 5 % inclusion of CLM that replaced 100 % commercial premix; SEM = Standard Error of the Mean; CLM = Composite Leaf Meal; 1 % CLM = 20 % commercial premix.

Table 6: Gonadal Morphometry of boarlings fed graded levels of Composite Leaf Meal based diets in replacement of commercial mineral/vitamin premix.

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**± SEM**
Weight of seminal vesicle (g)	41.00a	26.00 b	33.00ab	25.00b	30.00ab	17.50b	8.38
Length of seminal vesicle (cm)	8.40a	4.00b	6.10ab	6.20ab	6.30ab	5.05ab	0.49
Weight of penis (g)	52.00a	31.00c	36.00bc	35.50bc	47.00ab	32.50c	2.56
Length of penis (cm)	38.50	29.40	30.20	31.05	32.00	29.10	11.41
Weight of pelvic urethra (g)	33.50a	21.00b	30.00a	29.00a	32.00a	26.00a	2.36
Length of pelvic urethra (cm)	13.20	11.40	12.20	13.55	14.50	13.65	3.35
Weight of prostate (g)	19.00	15.00	16.00	16.50	19.00	14.50	5.49
Weight of right epididymis (g)	25.00	19.00	19.00	20.50	19.00	20.50	7.00
Weight of left epididymis (g)	25.50	20.00	21.00	21.00	19.00	20.50	7.14
Weight of right testis (g)	99.00ab	106.00a	80.00bc	96.00ab	70.00c	95.50ab	4.07
Length of right testis (cm)	8.40ab	8.90a	7.90bc	8.95a	7.50c	8.90a	0.18
Width of right testis (cm)	4.85ab	4.30b	4.60ab	5.15a	4.40b	4.70ab	0.10
Weight of left testis (g)	99.00a	115.00a	81.00bc	104.00a	72.00c	97.50ab	4.56
Length of left testis (cm)	8.70b	9.20ab	7.60c	9.00ab	7.90c	9.50a	0.22
Width of left testis (cm)	4.95a	5.00a	4.60b	5.00a	5.10a	4.90a	0.05
Weight of Cowper's gland (g)	53.00a	20.00b	30.00ab	46.00ab	40.00ab	34.00ab	4.08
Length of Cowper's gland (cm)	8.75a	7.00b	7.80ab	8.95a	8.00ab	7.45ab	0.24

a, ab, b, bc, c = means on the same rows but with different superscripts are statistically ($p < 0.05$) significant

T1 = Diet with 0 % inclusion of CLM and 100 % commercial premix (control); T2 = Diet with 1 % inclusion of CLM that replaced 20 % commercial premix; T3 = Diet with 2 % inclusion of CLM that replaced 40 % commercial premix; T4 = Diet with 3 % inclusion of CLM that replaced 60 % commercial premix; T5 = Diet with 4 % inclusion of CLM that replaced 80 % commercial premix; T6 = Diet with 5 % inclusion of CLM that replaced 100 % commercial premix; SEM = Standard Error of the Mean; CLM = Composite Leaf Meal; 1 % CLM = 20 % commercial premix.

The reduced weights of the accessory sex glands (Cowper's gland, vesicular glands and prostate gland) in boarlings fed CLM diets in comparison with the control diets would therefore result in decreased seminal volume and hence reduced ovum fertilizing capacity of the seminal plasma of the pigs. (Strzeżek, 2002; Aro and Awoneye, 2012).

The weight, length and width of both right and left testes were all significantly different ($p < 0.05$) among all the dietary treatments. Though, the testicular parameters relative to the inclusion of CLM in the diets did not follow a well-defined trend, however, the similarity in testicular parameters between T1 and T6 showed that the total replacement of commercial premix with CLM as observed in the current study would not compromise the testicular-size-related parameters like spermatogenic efficiency, daily sperm production and gonadal sperm reserves (Aro et al., 2015).

Gonado-somatic parameters of the boarlings. Table 6 shows the gonado-somatic parameters of the peri-pubertal male pigs fed composite leaf meal in replacement of commercial premix.

All parameters considered in the gonado-somatic indices of the young male pigs fed varied levels of composite leaf meal diets were significantly different ($p < 0.05$) with the exception of prostate glands and right and left epididymides.

Pigs fed the T2 diet had the lowest relative weights of seminal vesicles, penis and pelvic urethra. The heaviest seminal vesicles were observed in T1 (0.09 %), for penis in T1 and T5 (0.13 %) and for pelvic urethra in T5 (0.09 %). The heaviest right and left testes were recorded in T2 and T4 (0.25 and 0.27 %) respectively while the lowest values were recorded in T5 (0.19 and 20%). The relative body weights of the Cowper's gland had the highest and lowest value in T1 while T2 respectively.

The accessory sex glands (seminal vesicles, prostate and Cowper's glands) are very important because the components of the secretions from these glands are essential factors for the nutrition of the sperm cells and as buffers for the maintenance of acid-base balance of the seminal plasma. The size of these accessory sex organs relative to the live body weight (Table 6) was only significantly depressed in the CLM diets in comparison with the control diet when the relative size of the seminal vesicles is taken into consideration.

Generally, at the peri-pubertal stage of sexual development especially in male animals, any categorical statement about the size of their gonads relative to the live body weight could be misleading. This is because this stage is usually a stage of disproportionate growth of the gonads in comparison with the entire body growth (Sosa et al., 2002; Aro et al., 2011).

Table 6. Gonado-somatic parameters of peri-pubertal male pigs fed varied levels of composite leaf meal diets in replacement of commercial mineral/vitamin premix.

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**± SEM**
Seminal vesicles	0.09a	0.07b	0.08b	0.07b	0.08b	0.05b	0.02
Penis	0.13a	0.07b	0.09b	0.09b	0.13a	0.08b	0.01
Pelvic urethra	0.08ab	0.05d	0.08ab	0.08b	0.09a	0.07c	0.01
Prostate gland	0.05	0.03	0.04	0.05	0.05	0.04	0.00
Right epididymis	0.06	0.04	0.05	0.06	0.05	0.05	0.00
Left epididymis	0.06	0.05	0.05	0.06	0.05	0.05	0.00
Right testis	0.24a	0.25a	0.20b	0.25a	0.19b	0.24a	0.01
Left testis	0.24a	0.27a	0.20b	0.27a	0.20b	0.24a	0.01
Cowper's gland	0.13a	0.05b	0.08ab	0.12a	0.11ab	0.09ab	0.01

a,ab,b,c,d = means on the same rows but with different superscripts are statistically ($p < 0.05$) significant;
T1 = Diet with 0% inclusion of CLM and 100 % commercial premix (control); T2 = Diet with 1% inclusion of CLM that replaced 20 % commercial premix; T3 = Diet with 2 % inclusion of CLM that replaced 40 % commercial premix; T4 = Diet with 3 % inclusion of CLM that replaced 60 % commercial premix; T5 = Diet with 4 % inclusion of CLM that replaced 80 % commercial premix; T6 = Diet with 5 % inclusion of CLM that replaced 100 % commercial premix; SEM = Standard Error of the Mean; CLM = Composite Leaf Meal; 1 % CLM = 20 % commercial premix.

Table 7. Gonadal morphometry of peripubertal gilts fed varied levels of composite leaf meal diets in replacement of mineral/vitamin premix.

Parameters	T1	T2	T3	T4	T5	T6	± SEM
Length of vaginal (cm)	17.00	17.00	18.25	18.00	16.50	16.00	0.33
Length of cervix (cm)	4.00	4.50	4.74	4.00	4.50	4.00	0.20
Weight of entire reproductive tract (g)	121.00c	144.50a	149.50a	118.00c	121.00c	132.00b	4.74
Length of left oviduct plus uterine horn (cm)	36.00ab	42.90a	40.00ab	33.50bc	32.50bc	27.20c	1.68
Length of right oviduct plus uterine horn	36.50ab	42.50a	39.05a	35.50ab	33.50ab	27.00c	1.67

a, ab, b, bc, c = means on the same rows but with different superscripts are statistically ($p < 0.05$) significant.
T1 = Diet with 0 % inclusion of CLM and 100 % commercial premix (control); T2 = Diet with 1 % inclusion of CLM that replaced 20 % commercial premix; T3 = Diet with 2 % inclusion of CLM that replaced 40 % commercial premix; T4 = Diet with 3% inclusion of CLM that replaced 60 % commercial premix; T5 = Diet with 4 % inclusion of CLM that replaced 80 % commercial premix; T6 = Diet with 5 % inclusion of CLM that replaced 100 % commercial premix; SEM = Standard Error of the Mean; CLM = Composite Leaf Meal; 1 % CLM = 20 % commercial premix.

Gonadal morphometry of peri-pubertal gilts. The gonadal morphometry of peri-pubertal gilts fed varying levels of composite leaf meal diets in replacement of commercial mineral/vitamin premix is shown in Table 7.

There were no differences ($p > 0.05$) in lengths of vagina and cervix while there were differences ($p < 0.05$) in the weight of the entire reproductive tract and the lengths of right and left oviducts of the peri-pubertal gilts fed varied levels of composite leaf meal diets. The weight of entire reproductive tract was heaviest in T3 (149.50 g) while the lowest value was observed in T4 (118.00 g). The highest values for the left and right oviducts with their uterine horns were observed in T2 (42.90 cm and 42.50 cm) while the lowest values (27.20 cm and 27.00 cm) were recorded in T6 respectively. The CLM Diets fed to the pre-pubertal gilts did not influence the length of both the vagina and cervix. It was reported that the range of length of the vagina in different livestock species is between 25-30 cm (in cow and mare) specifically, while it is between 10-15 cm in sow, doe and ewe (Schinckel et al., 1983). These values correspond with the range of values observed in this experiment. The longer the vagina, the better the reproduction process, as semen is deposited into the vagina which is connected to the cervix during natural mating. The weight of the entire reproductive tract differed significantly among dietary treatments but the trend of values obtained in the parameter showed that the use of CLM as a replacement for commercial mineral/vitamin premix did not reveal any adverse manifestation as the values obtained at 4 and 5 % CLM inclusion still compared with the control.

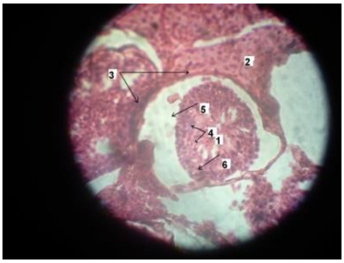
The length of the right and left oviducts with the uterine horns were significantly different. The observed trend was a significant decrease in the length of the oviducts and uterine tubes beyond the 3 % CLM level. The reduction in length of right and left oviduct plus uterine horns in T5 and T6 may not be unconnected with probable higher levels of phyto-estrogens in the two dietary treatments. Most herbs are reputed to have high content of phyto-estrogens (Kennelly et al., 2002)). For instance, the Moringa oleifera leaves used in the present study is said to be rich in kaempferol – a class of phyto-estrogens (Muhammad and Khan, 2016). Phyto-estrogens are potent disruptors of estrogens that could work negatively to the expression of the endogenous estrogens tasked with the function of maintaining the normal development and maintenance of the ovaries and oviducts (Fortune and Yang, 2011).Therefore, treatments T2 (1 % inclusion of CLM) to T4 (3 % inclusion of CLM) would not compromise the growth and development of the oviducts of peripubertal gilts, but an increment beyond 4 % level of CLM in the diets and hence a higher level of Moringa oleifera could have negative effects on their reproductive performance especially on their oviductal and uterine capacity to carry implanted foetuses to term. This could lead to reduced litter size and weight in breeding gilts and sows fed with more than 3 % inclusion of CLM in their diets.

Histopathological examination of the testis: Figure 1 to 6 shows the transverse sections of the seminiferous tubules of the testes of the peri-pubertal boarlings fed treatment 1 to 6 respectively. There were normal and distinct seminiferous tubules in Figure 1 comprising Sertoli cells and germ cells. The relatively small lumen at the centre showed that the boars had not yet attained full sexual maturity, depicting that the germ cells are still at the proliferative stages of their development. In the testis of boarlings fed the T2 diet (Figure 2), clear interstitial cells of Leydig interspersed between the seminiferous tubules were observed. The germ cells were prominent within the seminiferous tubules. The absence of a clear cut lumen is also a confirmation of the peri-pubertal state of the boarlings testicular architecture. In the testis of boarlings fed T3, (Figure 3), there was an apparent disruption of interstitial cells, and also of the myofibroblasts. The architectural structures of the lumen of the seminiferous tubules were not clearly observed.

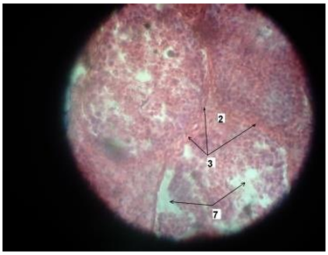
In Figure 4 that showed the histological examination of the testis of boarlings fed the T4 diet, there was a slight disruption of the myofibroblasts of the seminiferous tubules. The histological microstructures of the testis of boarlings fed T5 had no clear-cut features. There was heavy disruption of the lumen of seminiferous tubules with massive erosion of the peritubular tissue. There was degeneration of the interstitial tissues of Leydig and spermatogenic cells. In Figure 6, more vacuolation can be seen in the germinal epithelium while the basement membranes had lost their integrity in the three seminiferous tubules observed in the Figure 6.

Histological examination of the testes (Figure 1-6) of boarlings fed dietary inclusion of composite leaf meal diets-based (CLM) showed that testicular architecture and integrity were intact in the control and up to 3 % of CLM inclusion in the diets. This testicular integrity was represented by intact germinal epithelium, myofibroblasts, and proliferative germ cells at various stages of spermatogenesis and interstitial cells of Leydig interspersed between adjacent seminiferous tubules.

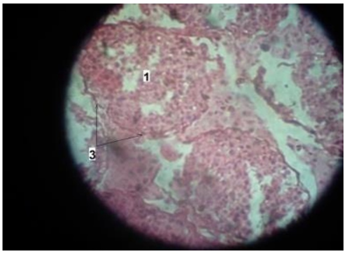
Figures 5 and 6 obtained from boarlings fed 4 % and 5 % CLM inclusion in the diets however showed that testicular architecture as represented by intact interstitial cells, germinal epithelium and concentric arrangement of spermatogenic cells have all been disrupted. The disruption of the testicular structure may be as a result of higher levels of toxigenic factors present in the CLM at these levels of inclusion (Ijehet al., 2004; Aro et al., 2015). This is in line with the changes observed in the testicular histology of boars fed 60% inclusion of microbially fermented cassava peel diets (Aro and Falowo, 2011) and also in accordance with the differences observed in spermatogenesis due to inclusion of gossypol (Haider et al., 1985; Porat, 1990), Tyotergium walfordii (Qian et al., 1986), Achillea milletolium (Montanari et al., 1998) in mice diets which are considered to be anti-spermatogenic agents. There was total disruption and sloughing off of the seminiferous tubules, peritubular tissues and interstitial cells of the testes of animals fed T6 as compared to those fed T1 to T4.



T1 (Figure 1)



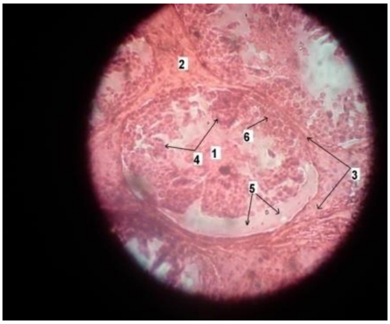
T2 (Figure 2)



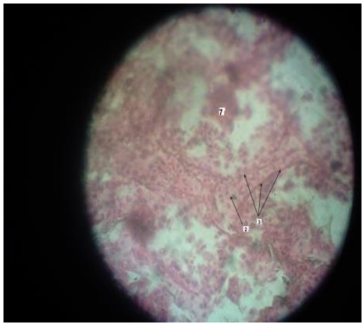
T3 (Figure 3)

Figures 1-3: Cross section of the seminiferous tubules of boarlings fed Composite Leaf Meal based-diets 1. Lumen of seminiferous tubule; 2. Leydig cells; 3. Peritubular tissues/Myofibroblast; 4. Germ cells; 5. Developing myofibroblast; 6. Germinal epithelium; 7. Total disruption of the germinal epithelium; T1 = Cross section of testis of boarlings fed the control diet (0 % CLM and 100 % commercial premix); T2 = Cross section of testis of boarlings fed 1 % CLM that replaced 20 % commercial premix; T3 = Cross section of testis of boarlings fed 2 % CLM that replaced 40 % commercial premix; CLM = Composite Leaf Meal. Magnification X100 (H&E)

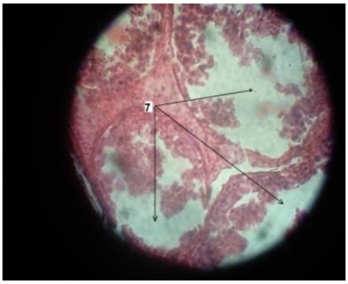
The result obtained in this research was in line with the report of Guttroff et al. (1992), and was also similar to the massive sloughing of the seminiferous epithelium in rats as reported by Fukuoka et al., (1990) and Chapin and Ku (1994). The cellular components of the seminiferous tubules were heavily disrupted and displaced in T5 and T6. The seminiferous tubule integrity determines the spermatogenic efficiency, daily sperm production and number of sperm cell produced /gram testis (Aro, 2012) and also spermatid maturation in relationship with the luminal surface (Bryant et al., 2008).



T4 (Figure 4)



T5 (Figure 5)



T6 (Figure 6)

Figures 4 – 6. Cross section of the seminiferous tubules of boarlings fed Composite Leaf Meal based-diets -1. Lumen of seminiferous tubule; 2. Leydig cells; 3. Peritubular tissues/Myofibroblast; 4. Germ cells; 5. Developing myofibroblast; 6. Germinal epithelium; 7. Total disruption of germinal epithelium with loss of basement membrane integrity with evidence of sloughing; T4 = Cross section of testis of boarlings fed 3 % CLM that replaced 60 % commercial premix; T5 = Cross section of testis of boarlings fed 4 % CLM that replaced 80 % commercial premix; T6 =Cross section of testis of boarlings fed 5 % CLM that replaced 100 % commercial premix; CLM = Composite Leaf Meal. Magnification X100 (H&E)

Spermatozoa produced through spermatogenesis are found in the lumen of the seminiferous tubules (Bryant et al., 2008), therefore, the observed progressive loss of integrity of seminiferous tubules occur because of the presence of anti-nutritional factors in the CLM in diets T3 to

T4 while the disruption of the seminiferous tubule architecture in the testes of animals fed diets T5 and T6 may be as a result of the corresponding increase in the amount of the anti-nutritional factors in the diets as a direct consequence of the increase in the dietary levels of the CLM. The disruption and total loss in the seminiferous tubules may decrease sperm production and hence compromise the reproductive capacity of the male animals fed on such diets.

CONCLUSIONS

The inclusion of composite leaf meal (CLM) in the diets of pigs after weaning even up to 5 % level did not depress growth performance indicators like feed intake, weight gain, feed conversion ratio and protein efficiency ratio. Reproductive indices like the weight of penis, weight seminal vesicles and length of seminal vesicles were depressed in the male pigs at CLM level higher than 3 %. The gonado-somatic parameters of the male pigs revealed that the use of CLM in replacement of commercial mineral/vitamin premix depressed the size of the seminal vesicles, penis and pelvic urethra especially at 5 % CLM relative to the control. In the female pigs, the length of the oviduct and uterine horns was compromised at levels above 3 % inclusion of CLM. Histological examination of the testes of the males also revealed evidence of sloughing off of the seminiferous tubules and loss of its architectural integrity at levels above 3 % inclusion of CLM. Within limits of this experiment, it could be recommended that breeder pigs should not use CLM inclusion higher than 3 % as this may depress the reproductive indices of the pigs. Pigs designated for fattening and slaughter could however be raised using CLM even at 5 % level without any deleterious effects on their growth and performance characteristics.

REFERENCES

Ajibade, S.R., Balogun, M.O., Afolabi, O.O., Kupolati, M.D. 2006. Sex differences in biochemical contents of *Telfairia occidentalis* Hook F. *Journal of Food Agriculture and Environment,* 4 (1), 155-156.

Aro, S.O. 2010. Growth and reproductive response of Swine fed fermented cassava tuber wastes. Ph. D. Thesis. University of Ibadan, Nigeria. pp. 1-176.

Aro, S.O., Awoneye, O.O. 2012. Reproductive Performance of Swine Fed Graded Levels of Fermented Cassava Peel-based Diets. In: I. I. Bitto., F. G. Kaankuka., S. Attah (eds). Proceedings of the 37th Annual Conference of the Nigerian Society for Animal Production, Federal University of Agriculture, Makurdi, Nigeria. pp. 121-123.

Aro, S.O., Falowo, A.B. 2011. Growth performance, gut morphometry and histology of grower pigs fed graded levels of microbially fermented cassava peel-based diets. In: A. O. Adukwu., T. Oluwagbemi., S. O. Aribido., S. I. Daikwo., O. J. Saliu (eds). Proceedings of the 16th Annual Conference of Animal Science Association of Nigeria (ASAN), Kogi State University, Anyigba, Kogi State, Nigeria. pp. 192-196.

Aro, S.O., Ajepe, O.A., Adejumo, D.O. 2011. Prediction of body weight and progression of sexual development from scrotal traits of pigs. In: A.A. Adeniji., E.A. Olatunji., E.S. Gana (eds). Proceedings of the 36thAnnual Conference of the Nigerian Society for Animal Production, University of Abuja, Nigeria. pp. 238-241.

Aro, S.O., Ajiboye, A. 2015. Effects of Feeding Composite Leaf Meal (CLM) on the Body Temperature of Growing Pigs. Advances in Environmental and Agricultural Science. ISBN: 978-1-61804-270-5, pp: 169-178

Aro, S.O., Falowo, A.B., Awoneye, O.O., Aletor, V.A. 2015. The Use of Microbial Biotechnology in Value Addition to Cassava Wastes as Feed for Pigs: Prospects and Challenges. *Advances in Microbiology,* 5, 28-39.

Bonsi, M.L.K., Osuji, P.O., Tuah, A.K. 1995. Effect of supplementing teff straw with different levels of leucaena or sesbania leaves on the degradabilities of teff straw, sesbania, leucaena, tagasaste and vernonia and on certain rumen and blood metabolites in Ethiopia Menz sheep, *Animal Feed Science and Technology* 52, 101 – 129.

Bryant, B.H., Yamasak, I.H., Sandrof, M.A., Boekelheide, K. 2008. Spermatid head retention as a marker of 2, 5-hexanedione-induced testicular toxicity in the rat. *Toxicology and Pathology*, 36, 552-559.

Chapin, R.E., Ku, W.W. 1994. The reproductive toxicity of boric acid. *Environmental Health Perspective,* 102 Suppl 7(Suppl 7), 87-91DOI10.2307/3431969

Chughtai, B., Sawas, A., O'malley, R.L., Naik, R.R., Ali, K.S., Pentyala, S. 2005. A Neglected Gland: A review of Cowper's gland. *International Journal of Andrology*. 28, 74-77.

Davies, D.C., Hall, G., Hibbitt, G., Moore, H.D. 1975. The removal of the seminal vesicles from the boar and the effects on the semen characteristics. *Journal of Reproduction and Fertility*,43(2), 305-12.

Egbunike, G.N., Dawodu, M.O., Eboreime, A.O.A. 1999. Effects of gossypol acetate on sperm production and fertility on the rat. *Tropical Animal Production and Investigation,* 2, 111-117.

Fortune, J.E., Yang, M.Y. 2011. Effects of exposure to phytoestrogens and environmental estrogens on ovarian follicular number and initiation of follicular growth in bovine fetal ovarian cortex *in vitro*. Available from Society for the Study of Reproduction (http://www.ssr.org/11Abstracts.shtml), Abstract #305.

Fukuoka, M., Zhau, Y., Tanaka, A., Ikemoto, I., Machida, T. 1990. Mechanism of testicular atrophy induced by di-n-butyl phalata in rats. Part 2. The effects on some testicular enzymes. *Journal of Applied Toxicology,* 10, 285-293.

Gbadamosi, A.J., Egbunike, G.N. 1999. Studies on cock semen (I): Effects of frequent ejaculation and breed on physical characteristics. *Tropical Journal of Animal Science*, 1, 157-164.

Guttroff, R.F., Cooke, P.S., Hass, R.A. 1992. Blind-ending tubules and branching patterns of the rat ductuli efferentes. *Anatomical Records,* 232, 423-431.

Haider, S.G., Passia, D., Chen, K.Q., Stumpf, W.E. 1985. Reversible changes in rat spermatogenesis induced by an anti-fertility substance (gossypol). A histochemical report. *Acta Histochem*istry, 77, 185-91.

Ijeh, I.I., Njoku, O.U., Ekenza, E.C. 2004. Medicinal evaluation of *Xylopia aethiopica* and *Ocimum gratissimum*: *Journal of Medicinal Aromatics Science*, 26 (1): 44-47.

Jacqueline, A.S. 2011. Father Kino's Herbs: Growing and using them Today.Tierra del Sol Institute Press, Tucson, AZ. 55, 357-363

Kennelly, E.J., Baggett, S., Nuntanakom, P., Ososki, A.L., Mori, S.A., Duke, J., Coleton, M., Kronenberg, F. 2002. Analysis of 13 populations of black cohosh for fomononetin. *Phytomedicine*, 9(5), 461-467

Kierszenbaum, A.L. 2002. Histology and cell biology: An introduction to pathology. St. Louis [u.a]: Mosby. Pp. 558 ISBN 0-323-01639-1.

Kumar, C.S., Swamy, M.J. 2017. Modulation of chaperone-like and membranolytic activities of major horse seminal plasma protein HSP-1/2 by L-carnitine. *Journal of Biosciences,* 42(3), 469–479.

Montanari, A., Chen, J., Widmer, W. 1998. Citrus flavonoids: A review of past biological activity against disease. J.A. Man they., B.S. Buslig (eds.), Flavonoids in the Living System, Plenum Press, New York, pp. 103-113

Muhammad, H.I., Khan, N.A.K. 2016. A review of promising phytochemical, nutritional and glycemic control studies of *Moorings oleifera* Lam. in tropical and sub-tropical regions. *Asian Pacific Journal of Tropical Biomedicine*, 6(10), 896-902.

Nwaogu, L.A., Udebuani, A.C. 2010. Effect of processing on the nutritional and toxicological components of *Cleome rutidosperma* seed. *African Journal of Biotechnology*, 9, 183-186.

Nworgu, F.C. 2016. Effect of basil leaf (*Ocimum gratissimum*) supplement on performance and carcass characteristics of growing pullets. *Sustainable Agriculture Research,* 5(3), 24-31, ISSN 1927-050X E-ISSN 1927-0518

Odoemelam, V.U., Etuk, I.F., Ndelekwute, E.K., Iwuji, T.C., Ekwe, C.C. 2013. Herbs and spices: Option for sustainable animal production. *Journal of Biology, Agriculture and Healthcare*, 3(7), 116-123.

Ogbe, A.O. and John, P. A. (2011). Proximate study, mineral and anti-nutrient composition of *Moringa oleifera* leaves harvested from Lafia, Nigeria: Potential benefits in poultry nutrition and health. *Journal of Microbiology, Biotechnology and Food Sciences.* 1 (3) 296-308.

Ohanaka, A.U.C., Duruanyim, V.O., Ukonu, E.C., Anyanwu, V.C., Okoli, I.C., Ekenyem, B.U. 2018. Carcass Characteristics of Laying Birds Fed *Chromolaena odorata* Leaf Meal in their Ration. In: I. C. Okoli., I.P. Ogbuenwu., N. J. Okeudo (eds). Proceedings of the 43rd Annual Conference of the Nigerian Society for Animal Production, Federal University of Technology, Owerri, Nigeria. pp, 176-179

Porat, O. 1990. Effects of gossypol on the motility of mammalian sperm. *Molecular Reproduction and Development*, 25(4), 400-8

Qian, S.Z., Zhong, C.Q., Xu, Y. 1986. Effects of *Trypterigium wilfordii* on the fertility of rats. *Contraception.* 33, 105-10.

Ravindran, V. 1995. Preparation of cassava leaf products and their use as animal feeds. In: Roots, tubers, plantains and bananas in animal feeding, FAO Animal Production and Health Paper, No 95. pp. 11-116.

SPSS 2016. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY:

Sallau, A.B., Mada, S.B., Ibrahim, S., Ibrahim, U. 2012. Effect of Boiling, Simmering and Blanching on the Antinutritional Content *of Moringa oleifera* Leaves. *Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.* E-Mail: sbmada@abu.edu.ng;

Schinkel, A., Johnson, R.K., Pumfrey, R.A and Zimmerman, D.R. 1983. Testicular growth in boars of different genetics line and its relationship to reproductive performance. *Journal of Animal Science*. 56: 1065-1076.

Sankhala, R.S., Kumar, C.S., Singh, B.P., Arangasamy, A., Swamy, M.J. 2012. HSP-1/2, a major protein of equine seminal plasma, exhibits chaperone-like activity. *Biochemical and Biophysical Research Communications.* 427(1), 18-23 DOI 10.1016/j.bbrc.2012.08.120

Sosa, J.M., Senger, P.L., Reeves, 1.J. 2002.Evaluation of American Wagyu sires for scrotal circumference by age and body weight. *Journal of Animal Science,* 80, 19-22.

Strzeżek, J. 2002. Secretory activity of boar seminal vesicle glands. *Reproductive Biology*. 2(3), 243-266.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I. Whittington, F.N. 2008. Fat deposition, fatty acid composition and meat quality: A review. *Meat Science,* 78, 343-358.