

## SUPPLEMENTATION AND CARCASS CHARACTERISTICS OF *ABERGELLE* GOATS IN TIGRAY, ETHIOPIA

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### ABSTRACT

The feeding trial was conducted at Abergelle Agricultural Research Center breed evaluation and distribution site which is located in central zone of Tigray, North Ethiopia. The objective of this study was to determine the optimum supplementation option that can promote better animal performance and carcass parameters. Twelve yearling uncastrated male growing Abergelle goats were purchased from the local market. The average initial weight of the purchased goats was  $14.2 \pm 1.09$  kg. Randomized complete block design was employed and goats randomly assigned to any of the three feeding options (dietary treatments). The three experimental rations were composed of different industrial by-products made as treatment one (43% wheat bran + 35% cotton seed cake + 20% molasses), treatment two (43% wheat bran + 35% noug seed cake + 20% maize grain) and treatment three (33% wheat bran + 45% dried brewery grain + 20% molasses). The supplementary feeds were formulated according to the growth requirements of the experimental animals considering their body weight. The experiment was conducted for 90 days of feeding and 14 days of adaptation trials. Grass hay and clean water was offered ad libitum to each animal. At the end of the trial, the goats were slaughtered and soon measured data on carcass parameters following appropriate procedures. The collected data were subjected to one way analysis of variance (ANOVA) with least significance difference mean separation. Most of the carcass parameters didn't significantly ( $p > 0.05$ ) respond to the different treatment diets. But, slaughter weight was significantly affected by the supplementation feeds with higher for goat group in treatment diet two. Hence, animal producers are advised to use supplementation option treatment two also based on the local availability of the feeds.

**KEYWORDS:** Abergelle goats, Carcass parameters, concentrate supplementation.

### INTRODUCTION

Livestock is central to the Ethiopian economy, contributing 15 to 17% of the national GDP and 35 to 49% of agricultural GDP, and 37 to 87% of the household incomes (Samuel *et al.*, 2010). Meat production is the most important function of these animals in the country. The annual Ethiopian goat meat production is 67, 580 tons which contributes 16.98% of the total ruminant livestock meat output (FAOSTAT, 2010). Annual meat production is estimated 8–10 kg per slaughtered goats. Thornton (2010) stated that there was an increase of total annual meat production (4.6%) in Ethiopia. However, this development was entirely based on an enlargement of the total number of livestock; as productivity growth rates during this period were nearly zero (Asfaw *et al.*, 2010). Apart from the low productivity, the animal outputs such as meat quality attributes do not satisfy the demand of the consumers.

Because of the slow growth rate, the animals become old before they reach the desired live weight for sale and this could degrade the meat quality. However, there is a significant potential for feeding livestock from occasional surplus grains such as sorghum and agro-industrial by-products like cotton seed cakes, noug seed cake, dried brewery grains, wheat bran, molasses and maize grains (Adugna, 2007). Increasing the current level of productivity is essential to provide meat to the ever-increasing human population, to increase export earnings and household income thereby improving the living standard of smallholders (Markos, 2006). Thus, in order to increase the productivity, the quality and quantity of the nutrient input must increase or management practices where external stresses such as diseases, parasites, feed limitations should be minimized to provide an environment that tends to enhance net output (Kassahun, 2000).

Feeding system impacts growth and subsequent carcass traits. Increasing concentrates in meat-goat diets also increased live-harvest weights, as well as carcass weights. Goats fed high-concentrate diets appear to have more muscling and high meat yield (Asfaw, 2008). According to Ameha *et al.* (2007), most of the Ethiopian indigenous goats have not been evaluated and characterized in terms of growth and their carcass yield, showing little information on whether the growth and carcass characteristics of Ethiopian indigenous goats are differently influenced by nutritional regimes. With this cognizant, this study was made to focus on evaluating carcass characteristics of *Abergelle* goats fed different supplementation feeding options.

## MATERIALS AND METHODS

### Study area description

The feeding trial was conducted at *Abergelle* Agricultural Research Center breed evaluation and distribution (BED) site (Figure 1), which is located at 13° 14' 06" N latitude and 38° 58' 50" E longitude. The area is categorized as hot to warm sub-moist lowland (SM1 - 4) sub-agro ecological zone with an altitudinal range of 1300 - 1800 m.a.s.l. The mean annual rainfall ranges from 300 to 650 mm which is characterized by low and erratic nature. The area is designated as Mono-modal that is dominated by single maximum rainfall pattern in the wet season (June to September). The mean annual temperature ranges from 28 - 42°C.

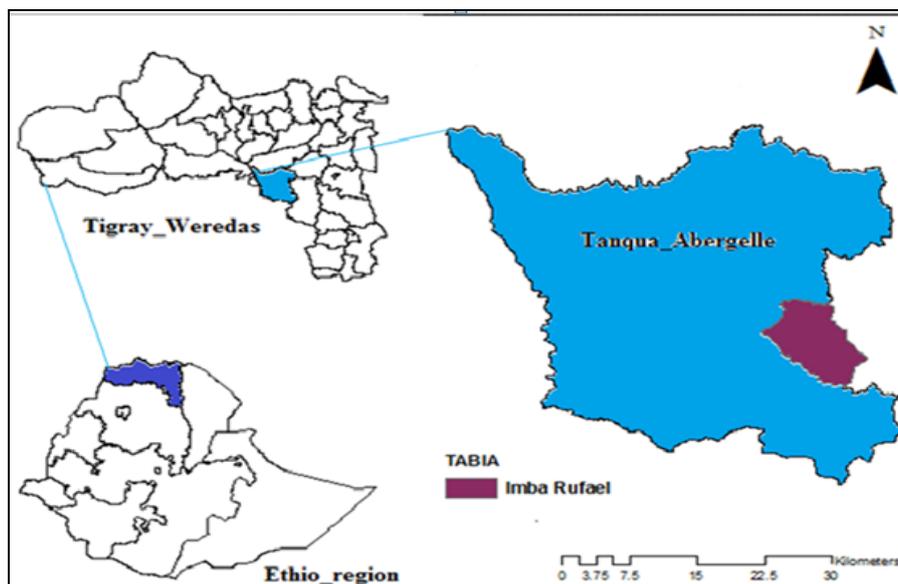


Figure 1: Location map of the study area.

According to the Wereda office of agriculture (2011) the total land coverage of the area is about 144,564 ha (1,444.64 km<sup>2</sup>), of which 29,466 ha is cultivable land, 15,381.7 ha is enclosed and the remaining 99,716.3 ha is uncultivated (includes bare lands, marginal lands, rocky, roads and very steep and unproductive land). The district has a total of 264,596 goats, 78,244 sheep, 81,649 cattle, 15,732 equines, 104,496 poultry and 11,220 hives of honey bees. Hence, the district is well noted for its high population potential in small ruminants.

### Management of experimental animals

Twenty-four yearling uncastrated male growing *Abergelle* goats were purchased from the local market based on their dentition and information obtained from the owners. The animals were then drenched with a broad spectrum anti helmentic (Albendazol) drug against internal parasites and sprayed against external parasites and vaccinated against common diseases like anthrax, and goat sheep pox. The experimental animals were fed hay grass and clean water on free choice, while, different supplementation feeds and salt were offered daily in their individual pens. The experimental animals were adapted

to the feeds, feeding schedule and pen environment for about 14 days prior to the beginning of the experiment. Moreover, animals were closely observed for the occurrence of any ill health and disorders during the experimental period. In general, the animals were kept for 104 days on the experimental feeds with individual housed pens for 90 days and 14 days of adaptation period.

### Experimental design and treatments

The experiment was designed in a randomized complete block design (RCBD) with three treatments and eight replications. The experimental animals were blocked into three blocks of four animals each based on their initial body weight ( $14.2 \pm 1.09$  kg). Treatment diets were randomly assigned to each animal and each animal in a block was getting equal chance to receiving one of the treatment diets. The supplemental feed mixture offered approximate value of 63% of energy sources, 35% of protein sources and 2% of salt on DM base per head per day. The supplement feeds were offered in two equal portions twice a day at 10:00 am and 4:00 pm after the

animals well fed the basal feed and taking clean water through all the experimental period.

The experimental animals were considered to be fed according to their initial body weight and increase the amount of the feed depending on daily body weight gain within the recommendation of NRC (1985) for each experimental animal. The diets were furnished according to the growth requirements of the experimental animals based on the recommendations of the NRC (1985) and by considering their body weight and the expected

weight gain. Therefore, to set the feed allowance a maximum daily weight gain of 100g of small mature weight goats was considered. In addition to this, the ration was formulated as iso-nitrogenous. This does mean that each experimental animal had received approximate equal CP amount. Thus, feed supplementation provision was adjusted fortnightly depending on live weight increment of the animals based on weighing weekly measurement. Treatment combinations of the experiment are presented in the table 1 below.

**Table 1: Treatment diets used in the experiment.**

Ingredients	Treatments		
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Grass hay (GH)	Adlibitum	Adlibitum	Adlibitum
Wheat bran (WB)	151 g (43 %)	151 g (43%)	119 g (33%)
Cotton seed cake (CSC)	123 g (35%)	-	-
Noug seed cake (NSC)	-	123 g (35%)	-
Maize grain (MG)	-	70 g (20%)	-
Dried brewers grain (DBG)	-	-	158 g (45%)
Molasses	70 g (20%)	-	70 g (20%)
Salt	7 g (2%)	7 g (2%)	7 g (2%)

**Experimental feed preparation**

The basal diet (grass hay) and molasses were obtained from Abergelle agricultural research center breed evaluation and distribution (BED) site. The supplement feeds like, cotton seed cake (CSC), noug seed cake (NSC), maize grain (MG), salt and wheat bran (WB) used for the experiment were purchased from the nearby local market. However, the dried brewery was purchased from Raya brewery factory. After preparing all the required supplementation inputs, the feeds were thoroughly mixed at the given proportion after proper grinding.

**Carcass parameters**

A total of twelve goats were slaughtered for carcass parameters evaluation following the standard procedure of animal slaughtering after overnight fasting. Slaughter weight and hot carcass weight were measured directly by spring balance before and after the animal slaughtered, respectively. Empty body weight was calculated as the difference between slaughter body weight and weight of gut fill content as follows:

$$\text{Empty Body Weight (EBW) (kg)} = \text{Slaughter Weight (kg)} - \text{Gastro-Intestinal Tract (kg)}$$

.....Equation 1

Therefore, the dressing percentage of the experimental animals was calculated by both slaughter body weight base and empty body weight bases using the following formula:

$$\text{Dressing Percentage (Slaughter body weight base)} =$$

$$\frac{\text{Hot carcass weight}}{\text{Slaughter bodyweight}} * 100 \dots\dots \text{Equation 2}$$

And

$$\text{Dressing Percentage (Empty body weight base)} =$$

$$\frac{\text{Hot carcass weight}}{\text{Empty body weight}} * 100 \dots\dots \text{Equation 3}$$

The rib eye area (cm<sup>2</sup>) was calculated by taking traced on transparency paper between thoracic ribs of 11<sup>th</sup> and 12<sup>th</sup> after chilled at 4<sup>0</sup>C overnight. The rib-eye area, which is an indirect estimate of body musculature or lean meat of the body, indicates the muscular development of the animal (Galal *et al.*, 1979). The rib-eye area was taken as the mean of the two sides of the ribs. Total edible offal component (TEOC) was computed as the sum of tongue, liver, kidneys, empty gut, visceral fat (kidney fat and omental fat), and tail. However, total non-edible offal component (TNEOC) was calculated as the sum of lung, testicles, spleen, pancreas, head, skin, penis, esophagus, trachea, gall bladder, total gut fill, blood and feet. Finally, the total usable product (TUP) was used as the summation of hot carcass weight, TEOC and skin. In general, taking different offal's as TEOC and TNEOC may be differing from place to place based on the feeding custom of the society.

**Statistical analysis**

The measured carcass parameters were subjected for analyses of variance (ANOVA) using the General Linear Models (GLM) procedure (SAS, 1998). Significant differences were determined using LSD mean separation. Mean differences were considered significant at P < 0.05. Results were summarized and presented using tables. Relationship between the dependent variables was examined using Pearson's correlation function. The

statistical model used for the analysis of all parameters was;

$$Y_{ij} = \mu + a_i + b_j + e_{ij} \dots \dots \dots \text{Equation 4}$$

Where:  $Y_{ij}$  = response variable (carcass parameters),  $\mu$  = overall mean,  $a_i$  =  $i^{\text{th}}$  treatment effect (diet),  $b_j$  =  $j^{\text{th}}$  block effect and  $e_{ij}$  = random error

## RESULTS AND DISCUSSIONS

### Carcass characteristics

The smallest slaughter weight (15.8 kg) was recorded from supplementation of molasses, wheat bran and cotton seed cake mixtures and this was not even significantly different from supplementation of molasses, wheat bran and dried brewery mixtures. However, feeding the concentrate mixture of maize grain, wheat bran and Noug seed cake has brought a statistically significant difference ( $p < 0.05$ ) slaughter weight over the other treatments. Unlike the slaughter weight, there was no statistically significant difference in empty body weight (EBW), hot carcass weight (HCW), dressing

percentage (DP) and rib-eye muscle area (REA) of the experimental goats between treatments (Table 2). The dressing percentage (SW base) ranges between 37.6 to 38.9 % which is similar with the result of Abebe (2006) dressing percentage of 38.4 - 45.6% at for Arsi Bale sheep weighing 14.6 - 24.4 kg at slaughter. The DP values found in this study are lower than to the average of 43 and 55% reported for Afar, Long-eared Somali and Central highland goats (Ameha *et al.*, 2007) and comparable with 39.5 to 41.8% reported for Omani goat breeds (Kadim *et al.*, 2003).

The reason for variation in DP between treatments in the present study was because of the difference in SBW and EBW and partly due to variation in proportion of non-carcass components including contents of GIT. The response of the experimental goats to hot carcass weight and empty body weight under different supplementations were statistically the same. The rib eye area of the experimental goats was between the ranges of 6.33 to 7.5  $\text{cm}^2$  (Table 2).

**Table 2: Carcass characteristics of Abergelle goats fed different concentrates.**

S/N	Variables	Treatments			SEM	LS
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>		
1	SW (kg)	15.8 <sup>b</sup>	17.3 <sup>a</sup>	16.2 <sup>b</sup>	0.33	*
2	HCW (kg)	6	6.5	6.3	0.27	NS
3	EBW (kg)	10.82	11.56	11.4	0.63	NS
4	Rib eye area ( $\text{cm}^2$ )	6.75	7.5	6.33	0.25	NS
5	Dressing percentage	-	-	-	-	-
	Slaughter weight base (%)	38	37.6	38.9	0.01	NS
	Empty weight base (%)	55.5	56.2	55.3	0.02	NS

Significant at ( $P < 0.05$ ) = \*, ( $P < 0.01$ ) = \*\*, ( $P < 0.001$ ) = \*\*\*, SW= slaughter weight, HCW= hot carcass weight, EBW=empty body weight, SEM= standard error of mean

Comparable results to this study were reported by Asnakew (2005), from 5.2 to 8.8  $\text{cm}^2$  for Hararghe goats and Hirut (2008) recorded 3.7- 8.4  $\text{cm}^2$  rib eye area for Hararghe Highland sheep, but, this was lower than Mulu (2005) who reported 13 to 19.5  $\text{cm}^2$  rib eye area for Wogera sheep.

Though the rib eye area was higher (7.5  $\text{cm}^2$ ) at the second treatment (T<sub>2</sub>), it was statistically similar with the other treatments ( $p > 0.05$ ). Similar to this result, Saikia *et al.* (1996) reported that rib-eye muscle area was not affected in goats supplemented with low, medium and high energy feeds. Shahjalal *et al.* (2000) also reported that lower and higher protein supplement to goats had no effect on the rib-eye muscle area. However, (Mulu, 2005; Emebet and Ermias, 2008) on the other side, reported that supplementation significantly affects for rib eye area of goats. According to Wolf *et al.*, (1980), rib-eye muscle area is mostly used as a tool to indicate the proportion of carcass lean meat or an expression of carcass desirability and hence, according to this author it is likely to respond similarly to the different treatments.

Dressing percentage values on empty body weight basis were higher than slaughter weight basis, which implies the influence of digesta (gut fill) on dressing percentage. Ingesta constitute a large portion of the body weight even when the animals are fasted for long hours (Gibbs and Iving, 1993). Then from this point of view, it is more meaningful to express dressing percentage as the proportion of empty body weight than slaughter weight base. The DP estimated for most of the indigenous goats in Ethiopia are between 42 and 45% on slaughter body weight basis and 53 and 55% on empty body weight basis which is comparable with the current study (37.6-38.9 % and 55.3 - 56.2 %), respectively. Wondwosen *et al.* (2010) also reported similar results for Sidama goat with 51.6% dressing percentage. The figures are within the range of 38 to 56% for other goat breeds in the tropics (Hag and Shargi, 1996; Dhanda *et al.*, 1999).

### Edible and non-edible offal's

Heart, liver with gallbladder, empty gut (reticulo-rumen + omaso-abomasum + small intestine + large intestine), visceral fat (kidney fat + omental fat), tail, head with tongue, and kidneys are considered as edible offal's in

the area (Table 3). In the current study, of all the edible offal only reticulo-rumen has shown statistically significant difference between treatments ( $p < 0.05$ ). The highest reticulo-rumen (498.75g) was obtained through feeding concentrate mixture of maize grain, wheat bran and noug seed cake ( $T_2$ ), while the remaining two treatments were statistically the same ( $p > 0.05$ ). The total edible offal components (TEOC) were not significantly different ( $p < 0.05$ ) between treatments, however, relatively the highest TEOC was obtained through supplementation of treatment two (2.7 kg). Moreover, there was no significant difference in visceral fat between treatments ( $p > 0.05$ ) which implies the attribute of fat deposition between the concentrate mixtures is similar. Contrary to this result, Galal *et al.*

(1997) reported heavier visceral fat and tail weight due to supplementation of concentrate feeds. However, Kirton *et al.* (1995) pointed that when animals fail to fulfill their nutrient requirements, body fat reserves could be used particularly during dry season and hence, decrease the fat storage.

The highest total edible offal content (30.3%) on the basis of total edible part was obtained from treatment two followed by treatment one (27.7%) and treatment three (26.8%). Similarly, the total edible portion of goats fed treatment diet two was the highest (59.7%) compared to the results of treatment diet one (55.7%) and treatment three (55.51%) on the basis of total offal weight.

**Table 3: Edible offal components of Abergelle goats fed different concentrates.**

S/N	Variables	Treatments			SEM	LS
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>		
1	Liver (g)	186.5	181.5	196.67	8.49	NS
2	Kidney (g)	41.75	39.75	41	1.71	NS
3	Tongue (g)	38.75	38	43.67	1.51	NS
4	VIS (g)	13.25	52	42.46	7.56	NS
5	Tail (g)	24	19.75	20	2.64	NS
6	Head (g)	864	968.75	811	33.77	NS
7	Heart (g)	69	81.75	74	3.22	NS
8	Abomasum + Omasum (g)	87	132.75	200.67	20.58	NS
9	Rumen + Reticulum (g)	316.75 <sup>b</sup>	498.75 <sup>a</sup>	205.67 <sup>b</sup>	40.05	***
10	SILI (g)	484	639.5	468	35.74	NS
	<b>TEOC (kg)</b>	<b>2.1</b>	<b>2.7</b>	<b>2.1</b>	<b>0.11</b>	<b>NS</b>

Significant at ( $P < 0.05$ ) = \*, ( $P < 0.01$ ) = \*\*, ( $P < 0.001$ ) = \*\*\*, T<sub>1</sub> = hay + Wheat bran (43%) + cotton seed cake (35%) + Molasses (20%) + salt (2%); T<sub>2</sub> = hay + Wheat bran (43%) + Noug seed cake (35%) + Molasses (20%) + salt (2%); T<sub>3</sub> = hay + Wheat bran (33%) + dried brewery grain (45%) + Molasses (20%) + salt (2%), SEM = standard error of mean, SILI = small and large intestine, TEOC=total edible offal contents, LS = level of significance

This was similar with the results of Banerjee *et al.* (2000) for indigenous goats in the tropics. It was also similar with the results of Asnakew and Berhan (2007) who

reported total edible offal ranging between 50 and 62% (on the basis of total offal weight) for Hararghe highland goats supplemented with different proportion of hay and concentrate diets. The Lung, trachea and esophagus, blood, spleen, pancreas, skin, feet, gut contents, testicles and penis are considered as non-edible offal (Table 4). The non-edible offal such as urinary bladder, feet, skin, gall bladder, penis and testicles significantly ( $p < 0.05$ ) responded to the different concentrate supplementations. On the contrary, the spleen and total gut fill were not significantly different between the treatments ( $p > 0.05$ ). Generally, the TNEOC was not significantly affected by the supplement concentrate mixture feeds ( $p > 0.05$ ).

**Table 4: None edible offal components of Abergelle goats fed different concentrates.**

S/N	Variables	Treatments			SEM	LS
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>		
1	Spleen (g)	20.00	23.50	25.67	1.33	NS
2	Total gut fill (kg)	4.75	3.94	4.99	0.26	NS
3	Urinary Bladder (g)	8.25 <sup>b</sup>	21.25 <sup>a</sup>	8.33 <sup>b</sup>	2.07	***
4	Feet (kg)	0.36 <sup>b</sup>	0.42 <sup>a</sup>	0.34 <sup>b</sup>	0.01	*
5	Testicles (g)	85.50	74.75	50.33	12.79	NS
6	Esophagus + Lung + Trachea (g)	181.00	170.75	202.67	10.90	NS
7	Skin (kg)	0.91 <sup>b</sup>	1.34 <sup>a</sup>	0.96 <sup>b</sup>	0.07	**
8	Gall bladder (g)	9.00 <sup>a</sup>	10.25 <sup>a</sup>	4.33 <sup>b</sup>	0.88	***
9	Blood (g)	485.50	444.75	530.33	23.85	NS
	<b>Total NEOC (kg)</b>	<b>6.8</b>	<b>6.5</b>	<b>7.1</b>	<b>0.21</b>	<b>NS</b>

Significant at ( $P < 0.05$ ) = \*, ( $P < 0.01$ ) = \*\*, ( $P < 0.001$ ) = \*\*\*,  $T_1$ = hay + Wheat bran (43%) + cotton seed cake (35%) + Molasses (20%) + salt (2%);  $T_2$ = hay + Wheat bran (43%) + Noug seed cake (35%) + Molasses (20%) + salt (2%);  $T_3$ = hay + Wheat bran (33%) + dried brewery grain (45%) + Molasses (20%) + salt (2%), SEM = standard error of mean, NEOC = none edible offal contents and LS = level of significance

Even though it is not statistically significant, there is relatively lower total gut fill content in treatment two which probably contributed to the expected higher rate of digestion and faster passage rate of the diet through the digestive tract due to consumption of more digestible feed. However, animals on poor feed are forced to fill their gut with less digestible roughage and have proportionally bigger gut content Van Soest (1994) and Pond *et al.* (1995). The weight of the skin supplemented with treatment two ( $T_2$ ) concentrate mixture were significantly higher ( $p < 0.05$ ) than the other treatments. This also made treatment two to have the highest total usable product (TUP) as it is the summation of hot carcass weight, TEOC and the skin. According to Lawrence and Fowler (1997), the increasing trend of the external offal (skin) might be due to an increase in

subcutaneous fat deposition in the skin. Similar results were also reported by Mulu (2005) on Wogera sheep fed hay and supplemented with brewery dried grain.

#### Carcass parameters and offal components

The correlation analysis revealed that slaughter weight was positively and significantly ( $p < 0.05$ ) correlated with empty body weight and dressing percentage (DP) as slaughter weight base (Table 5). Even though it was not significant, hot carcass weight, visceral fats, blood, skin and SILI were also positively correlated with slaughter weight. The rib eye area was positively and significantly correlated with the slaughter weight. Sendros (1993) presented similar results on local Menz sheep and their crosses under different feeding regimen. The total gut fill, SW, EBW, HCW, head and REA exhibited positive insignificant ( $p > 0.05$ ) correlations with VF (visceral fat). Skin was positively and significantly ( $p < 0.05$ ) correlated with total gut fill and rib eye area.

Besides, blood was significantly and positively correlated with hot carcass weight and total gut fills. The rib eye area with total gut fills, head with slaughter weight and skin with hot carcass weight have recorded the highest negative insignificant correlations.

**Table 5: Correlation of carcass parameters with edible offal components of Abergelle goats.**

Variables	Correlations									
	SW	HCW	EBW(kg)	Vis	BL	TGF	Sk	Hd	SILI	REA(cm)
SW	1									
HCW	0.39	1								
EBW	0.94**	0.53	1							
Vis	0.36	0.19	0.36	1						
BL	0.18	0.72*	0.25	0.19	1					
TGF	-0.15	0.57	-0.20	-0.22	0.71*	1				
Sk	0.02	-0.46	0.002	0.48	0.66	0.77**	1			
Hd	-0.46	0.15	-0.32	0.42	0.25	0.03	0.30	1		
SILI	0.40	0.17	0.39	0.28	-0.01	0.31	0.34	0.27	1	
REA	0.49*	0.17	-0.01	0.47	-0.34	0.51	0.73**	0.55	0.05	1

Significant at ( $P < 0.05$ ) = \*, ( $P < 0.01$ ) = \*\*, ( $P < 0.001$ ) = \*\*\*, SW= slaughter weight, HCW = Hot carcass weight, EBW = empty body weight, BL = blood, SK = skin, HD = head, SILI = small and large intestine, REA = rib eye area, TGF = total gut fill, VIS = visceral fats

#### CONCLUSIONS

The smallest slaughter weight was recorded from supplementation of molasses, wheat bran and cotton seed cake mixtures and this was not even significantly different from supplementation of molasses, wheat bran and dried brewery mixtures. However, feeding the concentrate mixture of maize grain, wheat bran and noug seed cake has brought a statistically significant difference in slaughter weight over the other treatments. Unlike the slaughter weight, most of the carcass characteristics and offal components were not affected by the supplementation options. Both the total edible offal components (TEOC) and total non-edible offal components (TNEOC) were not significantly different ( $p < 0.05$ ) between treatments, however, relatively the

highest TEOC was obtained through supplementation of treatment diet two.

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