**Online Journal of Animal and Feed Research** 

Volume 14, Issue 5: 274-286; September 30, 2024



DOI: https://dx.doi.org/10.51227/ojafr.2024.32

#### **EFFECT OF SUPPLEMENTATION OF CACTUS (Opuntia ficus-indica)** CLADODES, Acacia saligna, WHEAT BRAN AND COTTON SEED CAKE DIGESTIBILITY, GROWTH FEED INTAKE. AND CARCASS ON CHARACTERISTICS OF GOATS

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Supporting Information

ABSTRACT: The objective of this study was to evaluate effect of supplementation of cactus (Opuntia ficusindica) cladodes, Acacia saligna, wheat bran and cotton seed cake on growth, digestibility, intake and carcass characteristics of goats. A randomized complete block design was used in the experiment with 24 yearling central highland goats with an initial body weight of 15.6 - 16.1 kg. The same amount of grass hay (GH) + 150 gDM/head/day wheat bran (WB) was given to all animals. The experimental diets consisted of 80 gDM of cotton seed cake (CSC) as treatment 1 (T1); 45 gDM of CSC + 160 gDM of cactus cladodes (CC) as T2; 45g DM of CSC + 80 gDM of Acacia saligna (AS) as T3; and 45 gDM of CSC + 80 gDM of CC + 40 gDM of AS as T4 (per day per goat). Data were gathered on the goats' growth, digestibility, intake, and carcass of major organs, edible and nonedible organs. The consumption of dry matter and organic matter was higher in goats fed T2 and T4 than in T1 group. The dry matter (DM), organic matter (OM) and crude protein (CP) digestibility. average daily body weight gain and feed conversion efficiency were higher in T4 and T3 goats when compared to T2 goats. Goats fed on T4 had higher hot carcass weight and dressing percentage on slaughter body weight basis than T2, T3, and T1 supplemented goats. Generally, the experimental diets improved goats' performance in descending order (T4 > T3 >T1 >T2). Supplementation of T4 (replacement of 35 gDM of cotton seed cake per day by 40 gDM of Acacia saligna and 80 gDM of cactus cladodes on dry matter bases) could be recommended to improve goat performance.



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Keywords: Digestion, Dry matter, Feed conversion efficiency, Goat nutrition, Protein.

## INTRODUCTION

Goats and sheep are farmers' preferred livestock in arid areas. For farmers in arid and semi-arid areas, small-scale ruminant production is their primary source of income (Nampanzira et al., 2015; Timpanaro and Foti, 2024). However, during periods of insufficient feed supply, sheep and goats raised in these locations frequently suffer from substantial nutritional deficits, resulting in low productive and reproductive performance (Salem, 2010). Feed shortage, land scarcity and cost of feeds were the major constraints that hinder sheep and goat productivity (Diriba and Kebede, 2020). According to Endalew et al. (2016), in Ethiopia, there are significant limitations on livestock to have get year-round feed supplies, and the majority of feed resources are of low quality. Therefore, it could be necessary to use plants like cactus that are adapted to dry and semi-arid environments.

Cactus (Opuntia ficus-indica) has a high water retention capacity, high values in nutrients, and well-adapted to semiarid environmental conditions (Costa et al., 2013). The chemical composition of spineless cactus cladode consists of 9.2-10% dry matter, 5.62-7.9% crude protein, 25.97-38.7% neutral detergent fiber, and 15.08-27.2% acid detergent fiber (Shruthilaya et al., 2022) as well as water content (89.9%) and energy (8.4-9.2 MJ metabolizable energy per kg dry matter), minerals, and vitamin A for inclusion in ruminant diets (Abidi et al., 2009; Costa et al., 2013; Bezerra et al., 2021; Nyambali et al., 2022). Spineless cactus are often grown by smallholder farmers in arid and semiarid regions to use for animal feed, fences for dwellings and farm plots, and fruits for human consumption (Alary et al., 2007). Spineless cactus cladodes are low in phosphorous, fiber, and crude protein (Batista et al., 2003; Kumar et al., 2018). Due to these limitations, supplementation of protein source feeds like legume browse species is required to use spineless cactus cladodes for animals as forage.

As such in arid and semi-arid regions, browse species provide as a source of nourishment for ruminants, especially during the dry season when there is an abundance of low-quality fodder and crop residues (Amole et al., 2022). Farmers commonly use Acacia saligna to feed sheep and goats during dry season. Acacia leaves and twigs from both young and mature trees are regularly collected and given to grazing animals. This is a common practice used by farmers (Meneses et al., 2012). Nevertheless, its continued use as animal feed may be restricted due to the presence of secondary plant compounds. The primary anti-nutritional substances found in some Acacia species are condensed tannins, which reduce the digestibility of organic matter, dry matter, and crude protein content (Ben Salem et al., 1999). However, drying of *Acacia saligna* reported to reduces its tannin content than offering fresh leaves (Gebreslassie et al., 2021). In lambs, dried *Acacia saligna* leaves outperformed fresh leaves in terms of apparent digestibility of dry matter, organic matter, and crude protein content (Asefa and Tamir, 2006).

On smallholder mixed crop-livestock farms, such as those predominate in Ethiopia, supplementation with concentrate is not a feasible option due to the cost and limited availability. In such case; use of forages that are locally available, well adaptive and cheap in price like cactus cladodes and *Acacia saligna* could be feasible. Previous studies conducted on effect of cactus cladodes and legume browse species on the performance of goats and sheep in varied proportions shown that: supplementation with 300 gDM/day/head cactus and browse species mix (1:1 ratio) enabled body weight gain and prevented body weight loss of Somali goats (Taddesse et al., 2014). The total substitution of corn by cactus pear (280 gDM/kg), even if it resulted in reduced weight gain, the DM intake increases and improved the ability of sheep to digest the nutrients (Costa et al., 2013). To promote better animal performance, it is recommended to replace 63% of wheat bran by spineless cactus in sugar cane-based diets because of the optimal ruminal fermentation and higher volatile fatty acids synthesis for the animal (Lins et al., 2016). According to Aranda-Osorio et al. (2008), when cactus pear inclusion was raised from 15% to 30%, DM intake increases whereas feed conversion efficiency and overall live weight gain decreased. Other studies showed.

Spineless cactus species can replace ground corn as a source of energy in diets for finishing lambs without changing the ADG, DMI, and ingestive behavior and yield of commercial cuts (de Alencar Alves et al., 2023). Additionally, in sheep fed Acacia saligna and wheat bran, the average daily body weight gain was similar to that of sheep fed cotton seed cake and wheat bran (Yirdaw et al., 2017). Despite their abundant availability little research has been done undertaken in northern Ethiopia, especially Tigray region on the effect of supplementing of Acacia saligna, cactus and concentrate, on productive performance of goats. This study was thus designed to evaluate the effect of dietary supplementation of cactus cladodes, Acacia saligna, wheat bran and cotton seed cake on feed intake, growth, digestibility and carcass characteristics of goats.

# MATERIALS AND METHODS

The study was conducted in the eastern zone of Wukro town in North Ethiopia's Tigray region, 820 kilometers north of Addis Ababa, 70 km south of Adigrat, the zonal city, and 40 km north of Mekelle, the regional capital. Its latitude is 13°47' 59.99" N and its longitude is 39°35' 59.99" E. This region is 1977 meters above sea level, with a distinct rainy season from July to September and followed by a lengthy dry season. The mean annual rainfall is 300-350 mm. The mean annual temperature in the study area varies from maximum of 31°c in May and minimum of 8.3°C in July with an overall mean range from 11.1 -28.3°C. The study area's primary farming method is a mixed crop livestock subsistence economy. In order to minimize production risk and optimize the return on their limited land capital resource, smallholder farmers integrate the production of crops and livestock (Abegaz, 2005).

#### Animal management and experimental design

To carry out the experiment, 24 yearling male Central highland goats were purchased from the local area market. Prior to the commencement of the feeding trial, the residence was maintained and cleaned. Following that, the goats spent 21 days getting used to the feed and the experimental housing. Within the experimental house individual pen was built with 0.75 meters of width and 1.2 meters of length. Goats were treated for worms and sprayed to defend against internal and external parasites during this time. They were also given antibiotics and ivermectin vaccinations to protect them from frequent diseases in the area. All of the goats were put in separate pens after the end of adaptation period.

The diets in the experiment consisted of grass hay as roughage, wheat, bran cotton seed cake, Acacia saligna, and cactus cladodes. Roughage feed was made using grass hay that was bought locally. During the feeding trial, the goats were given *ad libitum* access to the measured grass hay. The concentrate (cotton seed cake and wheat bran) purchased from a neighboring market. The closest local farmers to the study area provided fresh spineless cactus cladodes. The spineless cactus cladode was manually cut into 1-2 cm length strips by lengthwise cutting.

The chopped cactus cladodes were then dried in air in shade by being stretched out on a plastic sheet for eight days. Acacia saligna leaves were harvested from locally available trees and dried in air in shade for 4 days, by being spread out on plastic sheets until easily crushed by twisting. They were then stored in a well-ventilated, cool and dry area to prevent degradation or mold formation until the completion of the experiment. Then the feed ingredients were mixed properly in the required proportion for each treatment. A randomized complete block design was used to conduct the feed trial. Six blocks (six animals per treatment) of experimental goats were assigned based on their initial body weight of  $15.9\pm0.237$  kg. After the adaption period was ended, their weight was determined by averaging two consecutive weighings that came after an overnight fast. Random assignments were made to each goat in a block to receive one of the four dietary treatments. The experimental diets were formulated according to Kearl (1982); (Table 1) to provide 48 gCP/day.

Experimental	Basal diet+ wheat bran	Supplement feed	Supplement feed ingredients in gDM/head/day				
diets/Treatments	(WB)	CSC	AS	CC	CPI gDM/d		
1	GH+150 WB	80	0	0	48.77		
2	GH+150 WB	45	0	160	48.56		
3	GH+150 WB	45	80	0	49.864		
4	GH+150 WB	45	40	80	49.89		

# **Data collection procedures**

# Feeding trial

Before the feeding trial began, the goats in the experiment were gradually acclimated to the experimental diet and environment over a period of 21 days. Then the feeding trial continued for ninety days. The experimental goats had unrestricted access to water and basal feed (natural grass hay) during the feeding trial. Concentrate (wheat bran and cotton seed cake), *Acacia saligna* and spineless cactus cladode (*Opuntia ficus-indica*) were combined for each treatment in appropriate ratio as described in Table 1 and supplemented to goats. Additionally, salt was added to the experiment at 1% of the total experimental diet. The goats were given the experimental diet twice a day, at 8:00 a.m. and 5:00 p.m. Every day of the trial, the quantity of feed that each goat eaten and refused was recorded. Before fresh feed was supplied each morning, feed refusals were gathered, weighed, and individually recorded for every animal. To determine the chemical composition, representative samples of feed that was offered and refused by each animal were gathered.

Feed and nutrient intake, changes in body weight, and feed conversion efficiency

Using a hanging scale, the initial and final body weights of the experimental goats were determined in the morning before they were fed. The amount of feed declined was subtracted from the amount offered to determine each goat's daily feed consumption. The calculation of nutrient intake involved multiplying the feed intake by the appropriate percentage of each parameter's approximate chemical composition (dry matter, organic matter, crude protein, neutral detergent fiber, and acid detergent fiber). Throughout the trial, follow-up body weight measurements were made every ten days to know weight change. The average daily body weight growth and feed conversion efficiency were calculated using the formula below:

Average daily body weight gain =  $\frac{\text{Final body weight} - \text{Initila body weight}}{\text{Number of feeding days}}$ Feed conversion efficiency =  $\frac{\text{Average daily body weight gain in gram}}{\text{Daily dry matter intake}}$ 

# Digestibility trial

After the end of the feeding trial, a digestibility trial was conducted. To gather feces for digestibility testing, a fecal collecting bag was fastened to each goat. For three days in order to allow for adaption, the goats were left in the presence of the fecal collection bags. Following that, feces collection took place for 7-day, every morning before feeding the goats. Every day, the feces were gathered and weighed, and at the end of the trial, 20% of each goat's daily feces were collected for analysis. Each goat's feces were collected and packed in polyethylene plastic bags, which were then stored in a deep freezer at -20°C until the digestibility study was completed. Following the completion of the collecting time, the feces were well combined, and 10% of the sample was dried for 72 hours at 60 degrees Celsius, crushed through a 1 mm sieve, and then stored at -20 degrees Celsius for chemical analysis. The following Equations were used to calculate apparent digestibility (Zeng et al., 2018).

Digestibility of Nutrients =  $\frac{\text{Nutrient Intake} - \text{Nutrient excreted in feces}}{\text{Nutrient Intake}} * 100$ 

The equation for DM digestibility estimation (Somanjaya et al., 2022) was;

DM digestibility  $= \frac{DM Intake - Feces DM}{DM Intake} * 100$ 

### **Carcass characteristics**

Following an overnight fast, 20 experimental goats (five from each treatment) were slaughtered at the completion of the digestibility trial. Measurements included rib-eye muscle area (REMA), dressing percentage (DP), hot carcass weight (HCW), empty body weight (EBW), and slaughterer body weight (SBW) were done. Weighing and recording of the edible offals-heart, liver with gall bladder, kidney, empty stomach, testes, and head with tongue-were also done. Similarly, the weight and records of the inedible offals (skin with feet, lungs, trachea, esophagus, spleen, and penis) were also conducted. Each goat's empty body weight was calculated by deducting its slaughter body weight from the weight of its digesta (gut filling) (Soares et al., 2012). Whereas hot carcass weight was measured after the weight of the head, tail, thoracic, abdominal, and pelvic chambers, as well as the legs below the knee joints, were removed. Then the hot carcasses were divided along the dorsal midline. Following that, the left half of the goat carcass was dissected into normal commercial cuts, including the neck, proximal thoracic limb, proximal pelvic limb, steaks, and brisket. The hot goat carcass was placed in a freezer for 24 hours at 4°C to cold it down. Then, according to Koyuncu et al. (2007) measurements on commercial carcass cut and the REMA around the ribs, at the 11th and 12th rib positions were taken. After making the cut on the 11th and 12th ribs perpendicular to the dorsal bone, the cross-sectional area of the REMA (Longissimus dorsi) was drawn using plastic paper. Graph paper of 5 mm by 5 mm was used to trace the rib-eye muscle area once more, as measured using plastic paper. To determine the REMA in cm<sup>2</sup>, the area of the squares that felt into the tracing paper on both sides was measured, and the average of two was used. Dressing percentage was computed using the empty body weight and slaughter body weight.

Dressing percentage =  $\frac{HCW*100}{BWS}$  and Dressing percentage =  $\frac{HCW}{EBW} * 100$ 

Where HCW, SBW and EBW are hot carcass weight, slaughter body weight and empty body weight, respectively.

#### **Chemical analysis**

Feed and fecal samples were analyzed for ash, crude protein (CP; Nx6.25), and dry matter (DM) contents using the approved AOAC (1990) procedures. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined according to Van Soest et al., (1994). The ash content of the feed was ascertained by burning the samples in a muffle furnace for five hours at 550 °C, while the dry matter content was determined by drying the samples in an oven at 105 °C for overnight. Nitrogen (N) was determined by Kjeldahl method (CP = N×6.25).

#### Statistical analysis

The general linear model (GLM) procedure of SAS version 9.0 (SAS, 2002) was used to analyse the data on feed intake, digestibility, body weight increase, and carcass characteristics to the analysis of variance model for randomized complete block design. Tukey's test was used for mean separation and values were considered significant at P< 5%. The following statistical model was employed to analyze the data:

 $Yij = \mu + T_i + B_j + e_{ij},$ 

Where:  $Y_{ij}$  is the response variable (body weight gain, feed intake, digestibility, carcass characteristics;  $\mu$  = the overall mean; Ti= the i<sup>th</sup> treatment effect;  $B_j$  = the j<sup>th</sup> block effect;  $e_{ij}$  = the random error.

## RESULTS

#### Chemical composition of feed ingredients

The chemical composition of the feeds in the experiment are shown in Table 2. Cotton seed cake (CSC) contained more CP than wheat bran or *Acacia saligna*. Cactus cladode and hay had the lowest CP levels, with 6.13% and 5.38%, respectively. Cotton seed cake had 66.35% NDF and 37.62% ADF, whereas hay had 71.86% NDF and 45.87% ADF. *Acacia saligna* has a high ADL concentration.

#### Effect of concentrate, Acacia saligna and cactus cladodes on feed/ dry matter and nutrient intake of goats

Table 3 displays the results of the goats' nutritional and dry matter intake. The grass hay dry matter consumption was higher in sole concentrate (T1) and concentrate and *Acacia saligna* (T3) supplemented goats than concentrate + cactus (T2) and concentrate+ *Acacia saligna* + cactus (T4) supplemented goats. Similarly supplement dry matter intake was higher in cactus containing experimental diets (T2 and T4) supplemented goats than that of non-cactus containing diets (T3 and T1) supplemented goats. The total dry matter and organic matter intake was higher in concentrate + cactus (T2) and concentrate+ cactus + *Acacia saligna* (T4) supplemented goats than in sole concentrate (T1) supplemented goats. The reason for this could be the presence of cactus cladode, which had a lower NDF content compared to cotton seed cake and *Acacia saligna* which contains higher NDF content (Table 2). The amount of crude protein that the goats consumed across all experimental diets did not differ significantly. The NDF intake was highest in concentrate and cactus

supplemented goats (307.09 g/day) then followed by concentrate + Acacia saligna (289.45 g/day)>sole concentrate (284.36 g/day)>concentrate + Acacia saligna + cactus (266.44 g/day) supplemented goats. Acid detergent lignin intake of goats was highest in concentrate + cactus supplemented goats then followed by concentrate + Acacia saligna + cactus > sole concentrate supplemented goats.

Chemical composition	Chemical composition Feed ingredients (supplements)						
Parameters	Acacia saligna	Spineless cactus	Cotton seed cake	Wheat bran	Grass hay		
DM	90.26	95.33	93.81	92.24	89.74		
Ash	11.1	11.67	4.32	2.92	9.64		
OM	88.89	88.33	95.68	97.07	90.36		
СР	13.88	6.13	28.60	17.26	5.38		
NDF	45.05	39.44	66.35	41.04	71.86		
ADF	21.63	27.72	37.62	8.21	45.87		
ADL	9.52	3.01	6.74	1.86	6.50		

Table 3 - Goats fed on grass hay and supplemented with concentrate, cactus cladodes and Acacia saligna and their dry matter and nutrient intake

Experimental diets/Treatments Intake (gDM/d)	T1	Т2	Т3	Т4	SEM	P-value
Grass hay DMI	264.28ª	229.53 <sup>b</sup>	244.45 <sup>ba</sup>	230.45 <sup>b</sup>	5.2	0.0023
Supplement DMI	208.15°	296.63ª	258.26 <sup>b</sup>	289.45 <sup>ba</sup>	7.74	0.0001
Total DMI	472.44 <sup>b</sup>	526.16ª	502.71 <sup>ab</sup>	519.48ª	8.94	0.001
Nutrient Intake						
OMI grass hay	239.28ª	208.68 <sup>b</sup>	221.81 <sup>ba</sup>	208.14 <sup>b</sup>	4.63	0.0022
OMI supplement	191.27 <sup>b</sup>	265.20ª	237.07ª	260.59ª	7.01	0.0001
OMI Total	430.57 <sup>b</sup>	473.89ª	458.89 <sup>ab</sup>	468.74ª	8.04	0.019
CPI grass hay	<b>14.64</b> ª	13.18 <sup>b</sup>	13.36 <sup>b</sup>	12.45 <sup>b</sup>	0.25	0.0008
CPI from supplement	39.09 <sup>b</sup>	40.69 <sup>ab</sup>	43.01 <sup>ab</sup>	<b>44.19</b> ª	1.06	0.018
CPI total	53.74	53.88	56.37	56.64	1.05	0.114
ADFI grass hay	119.76 <sup>ab</sup>	125.38ª	<b>111.12</b> <sup>bc</sup>	104.15°	2.48	0.0004
ADFI from supplement	33.87 <sup>d</sup>	<b>86.18</b> ª	58.29 <sup>b</sup>	47.28°	2.26	0.0001
ADFI total	153.64°	<b>189.50</b> ª	169.42 <sup>b</sup>	151.43°	3.23	0.0001
NDFI grass hay	<b>190.63</b> ª	<b>169.78</b> <sup>b</sup>	178.28 <sup>ab</sup>	<b>166.40</b> °	3.75	0.003
NDFI from supplement	93.72°	<b>137.31</b> ª	<b>111.17</b> <sup>b</sup>	100.04 <sup>bc</sup>	4.31	0.0001
NDFI total	284.36 <sup>bc</sup>	307.09ª	289.45 <sup>b</sup>	266.44°	5.76	0.0028
ADLI grass hay	16.87 <sup>ab</sup>	<b>18.24</b> ª	15.50 <sup>bc</sup>	<b>14.60</b> °	0.34	0.0001
ADLI from supplement	5.36 <sup>b</sup>	<b>12.53</b> ª	<b>12.70</b> ª	10.83	0.54	0.0001
ADLI total	22.23d	30.78ª	28.21 <sup>b</sup>	25.43°	0.55	0.0001

a. b. c. d = means within a row not bearing a common superscript letter differ significantly(p<0.05); SEM= standard error of mean, l=Intake, DM=dry matter, OM=Organic matter, CP=Crude Protein, NDF=Neutral Detergent Fiber, ADF=Acid Detergent Fiber , ADL=Acid detergent Lignin, T1= 150g DM of WB +80g DM of CSC, T2= 150g DM of WB + 45 g DM of CSC + 160g DM of cactus, T3= 150g DM of WB + 45g DM of CSC+ 80g DM of AS, T4= 150g DM of WB +45g DM of CSC + 80g DM of AS

# Effect of concentrate, Acacia saligna and cactus cladodes on apparent dry matter and nutrient digestibility of goats

Table 4 displays the apparent dry matter and nutrient digestibility data for goats. Goats supplemented with concentrate plus *Acacia saligna* + cactus cladodes (T4) had greater digestibility of DM, OM, and CP than goats supplement with concentrate plus cactus (T2). The CP digestibility of goats was higher in *Acacia saligna* containing experimental diets (concentrate + *Acacia saligna* (T3) and concentrate + *Acacia saligna* + cactus (T4)). On the other hand, the NDF digestibility was higher in goats supplement sole concentrate (T1) than in concentrate + cactus (T2) and concentrate + *Acacia saligna* (T3). This might be due to the presence of higher amount of cotton seed cake containing high level of NDF in sole concentrate feeds. The ADF digestibility of goats was higher in T3 supplement than in concentrate + *Acacia saligna* +cactus (T4) supplement. However, the ADF digestibility of goats supplemented (T2) was the same to the ADF digestibility of goats' supplemented (T2) was the same to T2 supplement.

# Effect of concentrate, Acacla sallgna and cactus cladodes on body weight change and feed conversion efficiency of goats

Table 5 displays the body weight and feed conversion efficiency data for goats given grass hay supplemented with concentrate, *Acacia saligna*, and cactus cladode. In all experimental diets, there was no significant variation between the goats' initial and final body weights. However, numerically, the initial body weight ranged from 15.6-16 kg, while the final body weight ranged from 18.6-19.2 kg. In comparison to concentrate + cactus (T2) and sole concentrate (T1), the average daily body weight gain of goats in concentrate+ cactus+ Acacia saligna (T4) and concentrate + *Acacia saligna* (T3) revealed a significant difference (p<0.05). Goats' feed conversion efficiency varied between 0.049 to 0.79. There was significant (p<0.05) variation in the feed conversion efficiency of goats between the experimental diets; goats in T4 had a greater feed conversion efficiency than goats in T2.

## Effect of concentrate, Acacia saligna and cactus cladodes on carcass characteristics

Table 6 displays the results of the carcass parameters and commercial cuts of goats. For all experimental diets, there was a significant difference (p<0.01) in the goats' hot carcass weight and dressing percentage. Goats supplemented with concentrate+ Acacia saligna + cactus (T4) had higher hot carcass weight and dressing percentage (on slaughter body weight) than goats supplemented with concentrate+ cactus (T2), concentrate + Acacia saligna (T3), and sole concentrate (T1). Goats supplemented with T4 exhibited significantly higher hot carcass weight and dressing percentage than goats supplemented with T2, T3, and T1 supplements.

### Effect of concentrate, Acacia saligna and cactus cladodes on edible and non-edible carcass organs of goats

The results of weights of heart, kidney, small intestine, large intestine, testes, blood, and gut contents are presented in Table 7 and showed significant differences among experimental diets. The size of the heart was larger in concentrate supplemented (T1) goats than that of concentrate + *Acacia saligna* + cactus (T4) supplemented goats. The size of kidney was ranged from 62 - 73.6 grams and it was higher in T4 supplemented goats than that T1 supplemented goats. Goats supplemented with T1 had larger small and large intestine weight with its contents, compared to goats supplemented with concentration + *Acacia saligna* (T3) and concentrate + *Acacia saligna* + cactus (T4). The size of the testes of goats in this study was ranged from 121.6-150.2 grams and it was higher in T3 than in T2 and T4 supplement. The gut content of goats was ranged from 2531-3530 grams and it was higher in goats supplemented T1 than T4.

Table 4 - Apparent dry matter and nutrient digestibility of goats fed on grass hay and supplemented Concentrate,           Acacia saligna and cactus cladodes								
Experimental diets/Treatments Apparent Digestibility (g/kg)	T1	T2	тз	Т4	SEM	P-value		
DM	642.2 <sup>b</sup>	<b>611</b> .7∘	641.5 <sup>b</sup>	669.6ª	0.38	0.0003		
ОМ	608.8ª	572.2 <sup>b</sup>	609.0ª	622.7ª	0.31	<0.0001		
СР	717.6ª	672.1 <sup>b</sup>	717.8ª	736.9ª	0.42	<0.0001		
ADF	486.6ab	497.0 <sup>ab</sup>	512.6ª	471.5 <sup>b</sup>	0.67	0.031		
NDF	612.86ª	560.3°	595.3ab	582.0bc	0.46	0.0002		

<sup>a. b. c. d</sup> = means within a row not bearing a common superscript letter differ significantly (p<0.05). DM=dry matter, OM=Organic matter, CP=Crude Protein, NDF=Neutral Detergent Fiber, ADF=Acid Detergent Fiber, T1= 150g DM of WB +80gDM of CSC, T2= 150g DM of WB + 45 g DM of CSC + 160gDM cactus, T3= 150g DM of WB + 45gDM of CSC+ 80gDM of AS, T4= 150g DM of WB +45gDM of CSC + 80gDM of Cactus+40gDM of AS, SEM=standard error of mean

**Table 5 -** Goats fed grass hay and supplemented with concentrate, Acacia saligna, and cactus cladodes, and their body weight change and feed conversion efficiency.

Exper	imental diets/Treatments	T1	T2	Т3	T4	SEM	P-value
Parameter			•-	10	• •	0 E III	i valuo
IW (kg)		15.96	15.94	16.10	15.60	0.237	NS
FW (kg)		18.60	18.70	18.80	19.20	0.366	NS
BG (g/d)		<b>25.55</b> ⁵	26.22 <sup>b</sup>	28.88 <sup>ab</sup>	<b>41.11</b> ª	3.393	0.014
FCE		0.0541 <sup>ab</sup>	0.049 <sup>b</sup>	0.057 <sup>ab</sup>	0.079ª	0.006	0.019

a. b. c. d =Means with different superscripts in the same row differ significantly (P<0.05); BG=daily body weight gain; FW=final body weight; FCE = feed conversion efficiency; IW=initial body weight; SEM= standard error of mean, T1= 150g DM WB +80g DM of CSC, T2= 150g DM of WB + 45 g DM of CSC + 160g DM of cactus, T3= 150g DM of WB + 45g DM of CSC+ 80g DM of AS, T4= 150g DM of WB +45g DM of CSC + 80 g DM of Cactus +40g DM of AS

# Table 6 - Carcass characteristics and commercial cuts goats fed on grass hay and supplemented with concentrate, Acacia saligna and cactus cladodes

Experimental diets/Treatments	T1	T2	ТЗ	T4	SEM	P-value
Carcass characteristics (kg)	14	12	13	14	JEM	F-value
Final body weight	18.6	18.7	18.8	19	0.337	NS
Slaughter body weight (SBW)	17.80	17.80	17.60	18.20	0.26	NS
Hot carcass weight	5.9 <sup>b</sup>	6 <sup>b</sup>	5.84 <sup>b</sup>	6.7ª	0.13	0.002
Cold carcass weight	5.42 <sup>b</sup>	5.87 <sup>ab</sup>	5.68 <sup>ab</sup>	6.33ª	0.18	0.011
Empty Body Weight (EBW)	14.26	14.75	14.83	15.26	0.30	Ns
Rib-eye muscle area (cm <sup>2</sup> )	4.95	4.82	5.27	5.87	0.41	NS
Dressing percentage on SBW	33.09 <sup>b</sup>	33.63 <sup>b</sup>	33.11 <sup>b</sup>	36.77ª	0.66	0.001
Dressing percentage EBW	<b>41.27</b> <sup>b</sup>	40.5 <sup>ab</sup>	39.34 <sup>b</sup>	43.91ª	1.056	0.009
Neck	0.72	0.61	0.69	0.71	0.056	NS
Proximal thoracic limb	1.35 <sup>b</sup>	<b>1.26</b> °	1.42 <sup>b</sup>	<b>1.53</b> ª	0.021	<0.0001
Steaks + Brisket	1.53	1.43	1.53	1.61	0.053	NS
Lumbar + abdominal region	0.81	0.78	0.85	0.88	0.038	NS
Proximal pelvic limb	1.64 <sup>bc</sup>	<b>1</b> .55°	<b>1.7</b> ab	<b>1.81</b> ª	0.025	0.002

a. b. c. d =Means with different superscripts in the same row differ significantly (P<0.05); SEM= standard error of mean, NS=non significance, T1= 150g DM of WB +80g DM of CSC, T2= 150g DM of WB + 45 g DM of CSC + 160g DM of cactus, T3= 150g DM of WB + 45g DM of CSC + 80g DM of AS, T4= 150g DM of WB +45g DM of CSC + 80g DM of Cactus+40g DM of AS

 Table 7 - Weight of edible and non-edible carcass organs of goats fed on grass and hay supplemented Concentrate

 Acacia saligna and cactus cladodes

Treatments	T1	T2	тз	T4	SEM	P-value
Carcass organs						
Edible carcass organs (g)						
Tongue, ear, head	1252.2	1252.2	1295.4	1281.2	37.31	Ns
Heart	<b>78.8</b> ª	70.2 <sup>ab</sup>	71.8 <sup>ab</sup>	65.40 <sup>b</sup>	2.73	0.032
Liver with gallbladder	252.6	242.36	224.4	242.6	11.5	Ns
Kidney	62 <sup>b</sup>	62.6 <sup>b</sup>	63 <sup>b</sup>	73.6ª	1.9	0.003
Small and large intestine and its contents	<b>1395.4</b> ª	1316.2ab	1185.6 <sup>b</sup>	1212.6b	21.22	0.000
Testes	132.8 <sup>ab</sup>	123.8 <sup>b</sup>	150.2ª	121.6 <sup>b</sup>	4.42	0.002
Empty stomach	436.8	458.8	469.0	469.8	21.55	Ns
Blood	580 <sup>ab</sup>	680 <sup>ab</sup>	720 <sup>ab</sup>	<b>780</b> ª	41.63	0.032
Total edible carcass organs (kg)	4.18	4.20	4.14	4.24	0.085	Ns
Nonedible carcassorgans (g)						
Skin with feet, tail	2070	2062	2124	2084	58.89	Ns
Spleen	25.4	25	23.6	26	2.25	Ns
Penis	33.6	33.2	31.0	29	2.85	Ns
Lungs, trachea, esophagus	263.6	263.8	273.2	263.8	21.58	Ns
Gut content	3530ª	3041 <sup>ab</sup>	2763ab	2531 <sup>b</sup>	205.97	0.027
Total nonedible carcass organs (kg)	5.72	5.16	4.94	5.07	0.27	Ns

<sup>a, b, c, d</sup> =Means with different superscripts in the same row differ significantly (P<0.05); T1= 150g DM of WB+80gDM of CSC, T2= 150g DM of WB + 45g DM of CSC + 160gDM cactus, T3= 150gDM of WB + 45gDM of CSC + 80gDM of AS, T4= 150gDM of WB + 45gDM of CSC + 80gDM of CSC + 80

## DISCUSSION

## Chemical composition of feed ingredients

Cotton seed cake contained higher crude protein content than wheat bran and Acacia saligna. Cactus cladode and hay had the lowest crude protein levels, with 6.13% and 5.38%, respectively. Cotton seed cake had 66.35% NDF and 37.62% ADF, whereas hay had 71.86% NDF and 45.87% ADF. Acacia saligna has a high acid detergent lignin (ADL) concentration of 9.52%. The crude protein (CP) content of cotton seed cake analyzed in this particular study (28%) exhibited similarity to the value of 28% as reported by Negussie et al. (2015). But it was lower than the value of 38% reported by Al-asa et al. (2023). Such discrepancies can potentially be attributed to variances in soil fertility and the technique employed for cotton seed extraction.

Similar values of 16.2% and 17.7%, as reported by Yirdaw et al. (2017) and Negussie et al. (2015), were obtained for the CP content of wheat bran in this study. However, it was greater than the 15.1% CP value that Al-asa et al.(2023) reported. Wheat bran's NDF content was comparable to the 39.5% recorded by Negussie et al. (2015). On the other hand, wheat bran's NDF content was less than the 35.5% that Yirdaw et al. (2017) reported. Wheat bran's ADF concentration turned out to be lower than the 12.6% and 11.6% reported by Yirdaw et al. (2017) and Negussie et al. (2015), respectively. Acacia saligna's CP content was comparatively close to the 14.20% recorded by Yirdaw et al. (2017). However, it was less than the 16.4%-28.3% range number that Gebremeskel et al. (2019) reported. Acacia saligna's NDF content was less than the value 44.29% reported by Yirdaw et al. (2017). However, it was greater than the value range of 28%-36% reported by Gebremeskel et al. (2019). The present investigation found that the CP content of spineless cactus cladodes was higher than the values 3.6% and 3.26% reported by Negussie et al. (2015) and de Oliveira et al. (2022), respectively. But it was similar to the values of 5.99%, 5.86% and 6.1% reported by Bezerra et al. (2021) and Alkhtib et al. (2023), respectively. Additionally, this study's NDF content for spineless cactus cladode was greater than the values reported by Negussie et al. (2015); Bezerra et al. (2021), and Alkhtib et al. (2023) of 23.3%, 26.5%, 25.97%, and 26%, respectively. In this experiment, the ADF content of cactus cladodes was more than the reported values of 15% and 23.39% by Negussie et al. (2015); Bezerra et al. (2021). The age of the cladodes, the fertility of the soil, and the harvest season are a few possible explanations for this discrepancy. With the exception of cotton seed cake, the inclusion of feed ingredients in this study had no discernible impact on animal feed intake. Dry matter intake (DMI) was negatively affected when the neutral detergent fiber (NDF) level exceeded 60%.

### Effect of concentrate, Acacia saligna and cactus cladodes on dry matter and nutrient intake of goats

Grass hay dry matter intake was higher in sole concentrate (T1) and concentrate + Acacia saligna (T3) supplemented goats than concentrate + cactus (T2) and concentrate + Acacia saligna + cactus (T4) supplemented goats. Similarly, supplement dry matter intake of goats was higher in cactus containing diets (concentrate + cactus and concentrate + cactus + Acacia saligna) than the non-cactus containing diets (concentrate+Acacia saligna and sole concentrate). Goats supplemented cactus containing experimental diets (T2 and T4) consume more in order to fulfill their dietary needs due to the presence of cactus which contains low protein level compared to other feed ingredients. There is a direct correlation between an increase in dry matter intake and an increase in organic matter intake. In a similar manner, as organic matter intake increases intake of dry matter increases (Widyobroto et al., 2016). Goats that were supplied concentrate plus cactus or concentrate plus cactus and Acacia saligna consumed more total dry matter and total organic matter than those that were fed solely concentrate. Given that cotton seed cake has a higher NDF concentration than cactus cladode, which has a lower NDF level (Table 2). The total dry matter intake found to be similar to the values 407.3 - 647.1 g/day, 481.5- 586.3 g/d and 349-515 g/d reported by Worku and Urge (2014); Gebremariam et al. (2006) and Megersa et al. (2012) respectively. But it was lower than the value, 754-822 g/d reported by Hidosa (2017). The total organic matter intake was similar to the values 339.3- 538.7g/d, 384.8- 515.1 g/d and 316-475g/d reported by Worku and Urge (2014); Gebremariam et al. (2006) and Megersa et al. (2012), respectively. However, organic matter intake was comparatively lower than the value 741.1-827 g/day reported by Hidosa (2017). The observed disparity in intake could potentially be explained by the increased consumption of dry matter and the enhanced nutritional value of grass hay in earlier research. Their increased intake as the number of cactus cladodes grows may have been caused by factors such as the cactus pear's high palatability, low fiber content, and high passage rate (Costa et al., 2012).

The amount of crude protein consumed by goats across all experimental diets did not differ significantly. But numerically it was higher in Acacia saligna containing diets (T3 and T4) supplemented goats than goats supplemented non Acacia saligna containing diets (T1 and T2). The study found that the goats' crude protein intake was comparable to the values reported by Gebremariam et al. (2006) and Megersa et al. (2012), which were 48.6-55.5 g/d and 16-69 g/d, respectively. It was lower than the value 43.9-110 g/h/d reported by Worku and Urge (2014). The Neutral detergent fiber (NDF) intake of goats was higher in T2 (307.09 g/day) followed by T3 (289.45 g/day)>T1 (284.36 g/day)>T4 (266.44 g/day). The study's NDF consumption for goats was comparable to Gebremariam et al. (2006)'s reported value of 235.1-388.0 g/d. In line with this discovery, Sileshi et al. (2021) observed that the experimental animals' daily consumption of NDF and acid detergent lignin (ADL) decreased when dietary energy and protein levels rose. Goats' body weight, the kind and quality of the feed, the amount provided, the breed, and the feed's palatability are some of the variables that might affect how much feed they consume. Han et al. (2019) further emphasized that palatability plays a significant role in determining feed consumption in livestock. Additionally, the dietary NDF content, which is known for its slow degradation and low rate of passage through the rumen, is considered to be limiting. Small ruminants' intake and digestion of roughages are greatly influenced by the fiber content, particularly the NDF (Mertens, 2002; Harper and Mcneill, 2015). The NDF consumption of the goats in this study increased linearly with increasing cactus cladodes from 80 gDM/day to 160 gDM/day in the experimental diet, which is similar to the finding of Costa et al. (2012).

#### Effect of concentrate, Acacia saligna and cactus cladodes on apparent dry matter and nutrient digestibility of goats

The dry matter, organic matter and crude protein digestibility of goats was higher in concentrate + Acacia saligna + cactus than concentrate + cactus. This might be due to the positive associative effect of combined feed ingredients (Acacia saligna and cactus). Dry matter digestibility and organic matter digestibility of goats in this study was similar to the range value of 42.11% - 69.96% reported by Hidosa (2017). The increased crude protein digestibility of goats in T3 and T4 compared to goats in T2 can be attributed to the presence of Acacia saligna. This notion is supported by the fact that the digestibility of nutrients in ewes is enhanced by an increased level of Acacia saligna supplementation, as stated by Maamouri et al. (2011). The NDF digestibility was higher in goats supplemented T1 than in goats supplemented T2 and T4. This may be due to the presence of higher amount of cotton seed cake containing high level of NDF in T1 (Table 2). Similarly NDF digestibility of goats was higher in T3 supplemented than in T2 supplemented. The ADF digestibility of goats was higher in T3 supplemented than in T4 supplement. Gebremariam et al. (2006) observed that the digestibility coefficients for ADF, CP, and NDF decreased as the quantity of cactus increased in the diet. In a similar way, the digestibility coefficients of DM, OM, CP, and NDF decrease as the level of cactus cladodes increases from 80 g/d to 160 g/d. Dietary OM digestibility was reduced when barely replaced by cactus. Contrary to this, studies by Salem (2010) and Costa et al. (2013) showed that the digestibility coefficients of DM, OM, and CP in sheep increased linearly as the amount of cactus cladodes in the diet increased. This discrepancy can result from the cladodes' age, the fertility of the soil, or the harvesting season or the species of the animals.

# Effect of concentrate, Acacia saligna and cactus cladodes on body weight change and feed conversion efficiency of goats

There was a significant difference (p<0.05) in growth rate of goats between the concentrate + *Acacia saligna* + cactus (T4) and concentrate + *Acacia saligna* (T3) supplements compared to the sole concentrate (T1) and concentrate + cactus (T2) supplements. The combination of cactus and *Acacia saligna* in the experimental diet increase weight gain. This coincides to the finding of de Oliveira et al. (2022), who reported goats fed on a combination of spineless cactus and Leucaena hay gained weight on a daily basis at a rate that was 68.5% more than that of goats grazing only on pasture. Enhancing the ruminal microorganism population's growth performance with values of 42.84 g/day is primarily attributable to the synchronization of the crude protein of leucena hay and the energy of the spineless cactus. The range reported by Rahman et al. (2014) and de Oliveira et al. (2022) which were 30.8-43.5 g/d and 13.5-42.84 g/d, respectively, was within the range of daily growth rate found in our investigation. On the other hand, the gains were lower than the 41.67–60.65 g/d range found by Hidosa (2017).

There are reasons for the reduced growth rate of goats observed in this study. These include the small quantity of roughage feeds (natural grass hay) that are ingested, the portion of crude protein in the hay that is indigestible, and the feeds' palatability (Mulligan et al., 2001). Furthermore, the animals' actual consumption of the intended supplement feed and their possible reaction to weight growth were factors. For example, in treatment 2 (T2), leftover feed resulted in an actual consumption of just 40.69 DM g/d of crude protein, compared to the targeted intake of 48.56 DM g/d. This disparity suggests that the goats may not have eaten all of the supplement feed that was provided, which may have affected their overall nutritional intake and consequent weight increase.

When comparing cactus-containing diets, it was found that goats supplemented with T4 had a higher average daily weight gain than those supplemented with T2. This aligns with findings from Costa et al. (2013), who observed that the daily weight gain of sheep decreases as the amount of cactus supplementation increases. However, this is inconsistent with the study by Nyambali et al. (2022), which reported that heifers fed diets with 10% and 20% cactus had lower average daily gains (ADG) compared to those fed commercial diets. In the current study, the weight gain of goats was highest in those supplemented with T4 compared to goats supplemented with T1 and T2. This observation is supported by the findings of Degen et al. (2000) who suggested that the provision of *Acacia saligna* had a positive effect on body mass change in animals.

Goats supplemented with T4 had a greater feed conversion efficiency (p<0.05) than goats supplemented with T2. Goat feed conversion efficiency was found to be comparable to the range of 0.05-0.09 reported by Rahman et al. (2014); Hidosa (2017). Diets using 80 gDM cactus (concentrate + Acacia saligna+cactus) had a higher feed conversion efficiency than diets containing 160 gDM cactus (concentrate + cactus). This finding is comparable to that of Costa et al. (2013), who found that sheep's feed conversion efficiency drops when cactus levels rise. Diets containing cactus pear forage are beneficial when there is sufficient nitrogen available, as proven by Misra et al. (2006). This could be because cactus and Acacia saligna are available at the same time as a source of energy and protein, respectively, which could support ruminal microbial communities and improve animal performance. The nutritional composition of the experimental diets used in this study comparatively proved to be fairly adequate in increasing the goats' body weight. In contrast to sheep in other studies findings, goats in this study grew at a relatively slower rate than the recommended growth rate. In line with this finding Awassi lambs grow faster than Baladi kids and can get to the desirable market weight (Haddad and Obeidat, 2007). This could be because goats have different nutritional needs than sheep and consume less concentrated feed, which ultimately helps sheep perform better in feedlots (Sheridan et al., 2003).

Effect of concentrate, Acacia saligna and cactus cladodes on carcass parameters and commercial cuts of goats The hot carcass weight and dressing percentage of goats supplemented with concentrate, cactus, and Acacia saligna were significantly higher than those of goats supplemented with the other treatments. Goats with concentration + cactus + Acacia saligna (containing 80 gDM cactus) supplement had a higher hot carcass weight than goats with concentrate + cactus (containing 160 gDM cactus). This was in line with the recommendation made by Bezerra et al. (2021), who found that hot carcass weight decreased from 15.6 to 14.4(kg) with cactus supplementation to lambs increases from 51.9 gDM to 75.3 gDM. The higher hot carcass weight of goats in concentrate+ cactus + Acacia saligna might be due to the lower NDF content of cactus cladodes and Acacia saligna. Concurred to this study, Mirzaei-Alamouti et al. (2021) reported a linear increases in hot carcass weight was found by decreasing dietary NDF concentration on a quantitative level. In this study, the dressing percentage and hot carcass weight of goats were found to be lower than those reported by Worku and Urge (2014), which were 35.4%-41.1% and 47.1%-58.9% and 6-9.7 (kg), respectively. There is no significance difference in rib eye muscle area of goats among experimental diets. The rib eye muscle area in this study was similar to the value 4.3- 7.7cm<sup>2</sup> reported by Worku and Urge (2014). The larger body weight of goats, the kind of feed, the breed of animals, and the setting in which the trials were conducted in the prior study could all be contributing factors to the discrepancy. The proximal pelvic limb was varied in weight from 1.558-1.815 (kg). The proximal pelvic limb of goats in T4 was larger than in goats supplemented with T2 and T1 supplement. The proximal thoracic limb was varied in weight from 1.268 -1.533 (kg). The proximal thoracic limb of goats in T4 was larger than in T2, T3 and T4 supplement. Goats' neck and proximal pelvic leg weight had greater than those of values of 0.47 kg and 1 kg respectively reported by Atay et al. (2011). The kind of feed, age, breed, and quantity of concentrate supplementation may all have a role in this discrepancy. When goats were supplemented with T4, their carcass qualities improved. Quantitatively speaking, goats fed this experimental diet (T4) had a greater rib eve muscle area compared to goats fed the other treatments.

Effect of concentrate, Acacia saligna and cactus cladodes on edible and non-edible offal components of goats

The kidney and spleen weight of goats in this study was similar to the values, 42-63g and 59-78g reported by Worku and Urge (2014) but lower than the values of 82g and 97g respectively reported by Atay et al. (2011). Kidney weight of the experimental goats increases significantly as body weight increases. Similarly, spleen weight increases as body weight increases numerically. The testes weight of goats in this study ranged from 121.6-150.2 grams and it was higher in goats supplemented with T3 than in goats supplemented with T2 and T4. The testis weight of goats was higher than the value 80g reported by Atay et al. (2011). But it was lower than the value 155g-206.3g reported by Alemu et al. (2010) and Worku and Urge (2014). There was no significance difference in empty stomach weight of goats in all experimental diets. But numerically, it was smaller in sole concentrate supplemented goats than in goats supplemented the rest treatments. The kind of feed, particularly the amount of fiber, affected the weight and growth of reticulo-rumen (Cardoso et al., 2016; Klein et al., 1987). The goats' empty stomach weight was lower than the value, 573-795g reported by Worku and Urge (2014). The gut content of goats was ranged from 2531-3530 grams and it was higher in goats supplemented with T1 than in goats supplemented with T4. The gut content of goats was lower than the range of 4.2-5.8 kg reported by Worku and Urge (2014). The reason for the lighter weight of goat offal in this study might be due to lower quality of hay and amount of concentrate feed left, as well as the initial body weight of the animals. As recommended by Bezerra et al. (2021) up to 50 gDM of spineless cactus could be included in the diet of confined lambs. Similarly, in this study it is possible to include up 80g DM/d spineless cactus in the experimental diet to improve goat performance. Therefore, T4 (150 wheat bran, 45 cotton seed cake, 40 Acacia saligna and 80 cactus cladodes (gDM/d) could be recommended to small holder farmers to feed their animals in order to improve their performance.

# CONCLUSION

The study demonstrates that dietary supplementing goats with diets containing concentrate and browse plants improves some performance parameters. Goats supplemented cactus containing diets consume more DM and OM than those of goats which utilized non-cactus containing diets. Goats on T4 diet (concentrate + cactus + Acacia saligna) exhibited higher DM, OM, and protein digestibility than those on the T2 diet (concentrate + cactus). The study observed larger testis size in goats fed with concentrate + Acacia saligna (T3) compared to those on concentrate + cactus (T2) and concentrate + Acacia saligna + cactus (T4). Goats on the T4 diet achieves suitable performance in most cases: higher efficiency (better utilization of the feed), relatively high growth rate, high dressing percentage, and hot carcass weight, larger weights of kidney, proximal thoracic limb, and proximal pelvic limb. Since both cactus and Acacia saligna are locally available yearround, providing a reliable forage source that meets maintenance requirements of animals and boosts economic returns for producers. Further research could be done evaluation of performance of goats by supplementing minerals and vitamins on top of the diets used in this experiment or other proportions of these feed ingredients.

## DECLARATIONS

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### Data availability

The data that support the study findings are available from the corresponding author upon request.

#### Author contribution

Genet Berhe: Conceptualization; Methodology; Data curation; Formal analysis and writing original draft of the manuscript, Amsalu Sisay and Teferi Aregawi: reviewing; editing; supervision; validation.

#### **Consent to publish**

All participants have consented to the submission of the research article to the journal.

#### Acknowledgements

The authors would like to acknowledge to farmers for their assistance in collecting cactus for the experiment and next thanks also to staff members of Abergelle International Export Abattoir, Mekelle, Ethiopia, and Mekelle University Animal Science department for their assistance in slaughtering of goats.

### Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### **Ethics committee approval**

The experiment was approved by the Ethics Committee of the Tigray Agricultural Research Institute following guidelines of the European Union directive number 2010/63/EU (2010) regarding the care and use of animals for experimental and scientific purposes.

#### **Competing interests**

The authors declare no competing interests in this research and publication.

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