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Full Length Research Paper

Effects of dietary inclusion of a commercially available probiotic on growth performance, cecal microbiota and small intestinal morphology in broiler chickens

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This study was conducted in order to investigate the dietary inclusion effects of a commercially available probiotic mixture and an antibiotic on the growth performance, cecal microbiota and small intestinal morphology in broiler chickens. A total of 100 broiler chickens (Cobb 500) were subjected to 35-days of study period. The broiler chickens were randomly divided into four groups named T₁ (control group), T₂ (antibiotic fed group, amoxicillin 200 gm/ton of feed), T₃ (probiotic fed group, Pro.B[®] 250 g/ton of feed) and T₄ (probiotic fed group, Pro.B[®] 500 g/ton of feed), respectively. Body weight gain and carcass yield were measured by electrical balance at seven-days-interval. The role of the used probiotic and antibiotic on intestinal microflora were evaluated by the total *E. coli* count (TEC), total *Salmonella* count (TSC), total *Lactobacillus* count (TLC) and total *Bacillus* count (TBC) whereas the intestinal morphology was determined by histological examination. The results of this study revealed that the obtained live weight gains were significantly ($p < 0.01$) higher in both probiotic fed groups during the periods of 3rd, 4th and 5th week of age as compared to the control and antibiotic fed groups. Data analysis from the cecum samples showed that the TEC was significantly higher ($p > 0.01$) in the control group as compared to both probiotic fed groups. On the other hand, the TLC and TBC were significantly higher ($p > 0.01$) in both probiotic fed groups as compared to the control and antibiotic fed groups. Both probiotic fed groups showed strong evidence in increasing the length of jejunal villi compared to the control and antibiotic fed groups. Among the probiotic fed groups, T₄ group showed a better response in every evaluated parameter included in this study. The results of this study thus revealed that the probiotic supplementation used on the T₄ group promoted the most significant and beneficial influence on the growth performance, carcass yield, bacterial antagonism and intestinal morphology.

Key words: Broilers, probiotics, antibiotic, growth performance, intestinal morphology.

INTRODUCTION

Nowadays, the efficiency of poultry to convert the feed into meat plays a key role in the poultry industry

worldwide (Arthur et al., 2003). In recent years, concerns about antimicrobial resistance have grown, but the main concerns have been focused specifically on antimicrobial resistance within the food supply (Cui et al., 2005). The use of antibiotics, including chlortetracycline as growth promoters to increase production performance and to decrease mortality, was recommended to be banned by the European Union (Perreten, 2003). This increases the microbial resistance to antibiotics and residues in chicken meat products which can be harmful to consumers (Nonga et al., 2009). Due to their several negative effects, antibiotics have gradually been replaced by other products such as probiotics in order to control intestinal pathogenic bacteria and become an alternative as growth promoters (Fuller, 1992). Probiotics are defined as live microbial supplements which beneficially affect the intestinal microbial balance of the animal host (Fuller, 1989). One of their main roles is to control the cholesterol and triacylglycerol serum levels (Lin et al., 1989; Taranto et al., 1998). Indeed, there are some evidences suggesting the reduction of the cholesterol and fatty acid composition of the broiler chicken serum by the *Lactobacillus* feed supplementation (Kalavathy et al., 2003). Due to indiscriminate use of antibiotics in the poultry industry, some bacteria have developed resistance to commonly used antibiotics that is being considered as a vital threat to the poultry production of Bangladesh (Akond et al., 2008; Hasan et al., 2011). To combat this problem probiotic can be used as a growth promoter and alternative to antibiotic in poultry industry of Bangladesh (Kabir et al., 2005; Kabir, 2009). The objectives of this study were to investigate the effects of a commercially available probiotic mixture diet supplementation on the growth performance, cecal microbiota, carcass yield and intestinal morphology of broiler chickens.

MATERIALS AND METHODS

Experimental birds

A commercially available probiotic mixture named Pro.B® (marketed by PVF Agro Limited, Bangladesh and manufactured by K.M.P Biotech Co. Limited, Thailand) was used in this study. A total of one hundred day old broiler chicks (Cobb 500 strain) were obtained from a local sale centre (CP-Bangladesh Ltd, Mymensingh, Bangladesh). Broiler chickens were divided into four groups named group T1, T2, T3 and T4 at the beginning of the experimental study. The four groups were composed of 25 broiler chickens each. The group T1 was fed without the addition of antibiotics or probiotics and was identified as the control group. The group T2 was identified as the antibiotic (Amoxicillin 200 g/ton feed) fed group. Finally, both groups T3 and T4 were identified as the probiotic (Pro.B® 250 gm/ton of feed and Pro.B® 500 gm/ton of feed, respectively) fed groups.

Feeding and drinking

The evaluated broiler chickens had ad libitum feed and water and were exposed to 24 h of lighting during the study. The evaluated birds received a starter diet (Nourish Broiler Feed, Bangladesh) from 1 to 14 days of age and a grower diet (Nourish Broiler Feed, Bangladesh) from 15 to 35 days of age. Both diets were given in a mash form using round feeders from the first week onwards whereas drinking water was supplied two times daily. One feeder and one round drinker with eight litre capacity were provided in each pen. Routinely, feeders were cleaned once a day while drinkers were washed two times daily.

Management practices

The day-old broiler chickens were immediately weighed after its arrival and randomly distributed in each pen. The broiler chickens were provided with vitamin C (1 g/5 L water) to overcome transportation stress. The room of the experimental house was partitioned into four pens using wire-net where a group of 25 broiler chickens were randomly allocated to each pen. Therefore, floor space was about 1 sq. ft for each bird to ensure comfortness of the birds. One 100-watt hanging electric bulb at the bird level for each pen was used to maintain brooding temperature. The brooding temperature and humidity was measured four times per day by an automatic digital thermo-hygrometer. A strict biosecurity program was maintained inside and outside of the experimental sheds as an effective part of the disease prevention program. A foot bath was maintained at the gate of the shed where TH4+ solution (Sogeval, France) and Povisep (Jayson Pharmaceuticals Ltd., Bangladesh) was alternatively used as disinfectants. Regarding the health status of the evaluated animals, immunizations were performed by the use of inactivated vaccines against BCRDV and Gumboro disease on 6th and 11th day of age and booster doses on 24th and 21st day of age respectively.

Pro.B®

Pro.B® marketed by PVF Agro Limited, Bangladesh and manufactured by K.M.P Biotech Co. Limited, Thailand was used in this study. A minimum of 1.0×10^{10} colony forming units (CFU)/L were present in Pro.B®. Pro.B® product is a powder preparation containing the live viable strains of naturally occurring microorganisms such as *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus*.

Antibiotic

Amoxicillin (Renamox® 30% vet, Renata Ltd., Bangladesh) which is a broad spectrum antibiotic widely used against several bacterial diseases of poultry was included in this study. Each milligram powder contains amoxicillin 300 mg as amoxicillin trihydrate BP.

Body weight

The broiler chickens were weighed by group at the beginning of the study and every seven days intervals until the end of the study. The weights were taken at the morning. The average live weight and the live weight gains of the broilers chickens on different dietary treatments were calculated on a weekly basis and at the end of the

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study.

Calculation of average daily growth, feed efficiency, feed conversion ratio (FCR) and mortality rate

Average daily growths (ADG) were calculated as the unit of body weight gain per day per broiler. The amount of feed consumed by the experimental broiler chickens of different treatment groups were calculated in a every week basis by deducting the amount retained from the amount supplied in that week.

The feed conversion ratio (FCR) was calculated as the unit of feed consumed per unit of body weight gain. The mortality rate of the broiler chickens were recorded per group and their survivability was also calculated.

Preparation of samples for bacteriological studies

During the course of the experimental study five birds from each group were randomly selected at 21 and 35 days of age and portions of cecum with their contents were obtained aseptically with a sterile scalpel and forceps for determining bacteriological status. These portions were homogenized uniformly using a mortar and pestle. From the homogenized mass a 1 g portion was transferred to a sterile tube containing 9 ml of 0.1% peptone water. Thus, 1: 10 dilution of the sample was obtained. Then, serial dilutions of each of the samples in 0.1% peptone water were made following the recommendations of the International Organization for Standardization (ISO, 1995).

Enumeration of total *E. coli* count (TEC)

In order to determine the TEC, each sample was taken in a sterile test tube and diluted with 10 ml of 0.1% peptone water. Another tube containing 9 ml of 0.1% peptone water was taken and 1 ml diluted sample from the first test tube was added and mixed well and repeated it from the first to last tube. Finally 1 ml was discarded from last tube. One hundred μ l of the diluted sample from 10 fold dilution was then inoculated into two MacConkey agar (Hi-Media, India) plates and spread with a sterile glass spreader. Then, the plates were then kept in an incubator at 37°C for 24 to 48 h. Following incubation, agar plates exhibiting colonies were counted. The average number of colonies was multiplied by the dilution factor to obtain the total *E. coli* count. The total *E. coli* count was calculated according to the ISO (1995). The results of the total bacterial count were expressed as the number of colony forming units per gram (CFU/gm) of samples.

Enumeration of total *Salmonella* count (TSC)

The procedures of sampling, dilution and streaking for the determination of TSC were similar to those followed in TEC. Only in case of TSC, xylose lysine deoxycholate (XLD) agar (Hi-Media, India) was used. The calculation for TSC was similar to that of total viable count.

Enumeration of total *Lactobacillus* count (TLC)

The procedures of sampling, dilution and streaking for the determination of TLC were also similar to those followed in TEC. Only in case of TLC, Man, Rogosa and Sharpe (MRS) agar (Hi-Media, India) was used. The calculation for TLC was similar to that of total viable count.

Enumeration of total *Bacillus* count (TBC, spore forming *Bacillus*)

To determine the total *Bacillus* count (TBC, spore former *Bacillus*), each sample was taken in a sterile test tube and diluted with 10 ml of 0.1% peptone water to make a 10 fold dilution. The diluted sample was then placed in water bath for 30 m at 80°C temperature. Then the sample was cooled and inoculated on nutrient agar (Hi-Media, India) plate and then followed the procedure of total viable count.

Preparation of samples for histological studies

During the course of the histological study five birds from each group were randomly selected at 21 and 35 days of broiler age. The portions of jejunum were collected and fixed in the Bouin's fluid for fixation of tissues for histological studies. The tissues were then dehydrated in the graded alcohol, cleared in xylene, embedded in paraffin and finally the sections were cut at 6 μ m thickness by a rotary microtome (AO Spencer No. 820 Precision Rotary Microtome, USA). The sections so prepared were stained with standard the hematoxylin and eosin method (Gridley, 1960).

Statistical analysis

The data of the experimental study on live weight and carcass yield were analyzed by applying one-way ANOVA followed by Boneferonni and Duncan tests using IBM SPSS statistics software (version 20.1).

RESULTS

The results of the live weight gains were found significantly ($p>0.01$) higher (Table 1) in experimental birds of the group T₃, and group T₄ as compared to the control and antibiotic fed groups during 3rd, 4th and 5th weeks of age. The average carcass weight was found to have yielded more in the group T₄ birds than the group T₃ birds when they were reached 3rd, 4th and 5th week of age.

Table 2 shows that the average daily growth and feed conversion ratio were found significantly ($p>0.01$) higher in group T₄ as compared to others. Data analysis from cecum samples (Tables 3 and 4) showed that the TEC was significantly higher ($p>0.01$) in the control group as compared to the probiotic fed groups. The TLC and TBC were significantly higher ($p>0.01$) in probiotic fed groups as compared to the control group. The histological study of the jejunal tissue was performed at 21 and 35 days of age. The jejunal glands (Figure 1) were much larger and prominent in Pro.B[®] fed broilers than the antibiotic and conventional fed broilers. The thickness of the jejunal wall was much wider in probiotic fed groups (T₃ and T₄) than control and antibiotic fed groups as presented in Figure 2. Among the probiotic fed groups (T₃ and T₄), T₄ group gave better response in every cases in this study.

DISCUSSION

The evidence of data analysis had shown that the

Table 1. Effects of probiotic feeding on live weight and carcass yield.

Age	Parameter (g)	Treatment				SEM	Level of significant	p-values
		T ₁	T ₂	T ₃	T ₄			
0 week (1 st day)	Live weight	56.40±2.36 ^a	56.80±1.77 ^a	55.40±2.29 ^a	58.60±1.33 ^a	0.668	NS	0.4550
	Carcass yield	-	-	-	-	-	-	-
1st week (7 days)	Live weight	162.40±2.87 ^a	162.80±3.09 ^a	163.60±2.73 ^a	163.20±2.89 ^a	0.258	NS	0.857
	Carcass yield	-	-	-	-	-	-	-
2nd week (14 days)	Live weight	464.20±15.14 ^b	480.00±9.08 ^{ab}	493.80±9.87 ^a	496.60±9.83 ^a	7.43	*	0.0163
	Carcass yield	-	-	-	-	-	-	-
3rd week (21 days)	Live weight	807.80±36.10 ^b	840.00±49.87 ^b	1000.00±28.98 ^a	1000.60±34.01 ^a	51.35	**	0.0027
	Carcass yield	463.33±27.28 ^d	503.33±8.82 ^c	582.67±27.94 ^b	632.33±14.33 ^a	38.13	**	0.0000
4th week (28 days)	Live weight	1408.00±47.05 ^b	1586.00±46.75 ^{ab}	1735.00±81.64 ^a	1800.00±106.59 ^a	87.14	**	0.0091
	Carcass yield	-	-	-	-	-	-	-
5th week (35 days)	Live weight	1700.00±13.78 ^b	1735.00±29.83 ^b	1950.00±66.56 ^a	2101.80±81.79 ^a	94.54	**	0.0003
	Carcass yield	1276.67±43.33 ^c	1200.00±52.92 ^c	1486.67±13.33 ^b	1673.33±63.60 ^a	106.89	**	0.0000

Means with different superscripts raw wise differ significantly at **p<0.01 but means with different superscripts significantly differ at *p<0.05. T₁ = Control; T₂ = Amoxicillin 200 g/ton of feed; T₃ = Probiotic (Pro.B[®] 250 g/ton of feed); T₄ = Probiotic (Pro.B[®] 500 g/ton of feed).

Table 2. Effects on growth performance in between 1 and 35 days.

Characters	Treatment feed				SEM	Level of significant	p-values
	T ₁	T ₂	T ₃	T ₄			
ADG (g/broiler/day)	48.57 ^c	49.57 ^c	55.71 ^b	60.00 ^a	2.69	**	0.000
Feed efficiency (g/broiler/day)	108.21 ^a	106.22 ^a	106.34 ^a	100.75 ^b	1.61	*	0.0440
Feed conversion ratio (FCR)	2.21 ^a	2.14 ^a	1.90 ^{ab}	1.67 ^b	0.123	*	0.0315
Mortality (%)	4.00	0.00	0.00	0.00	-	-	-

Means with different superscripts raw wise differ significantly at **p<0.01 but means with different superscripts significantly differ at *p<0.05. T₁ = Control; T₂ = Amoxicillin 200 g/ton of feed; T₃ = Probiotic (Pro.B[®] 250 g/ton of feed); T₄ = Probiotic (Pro.B[®] 500 g/ton of feed).

average live weight gains were found always on the increase in probiotic fed groups as compared to control and antibiotic fed birds on the 2nd, 3rd, 4th and 5th week of age. According to Table 2

there were significant differences (p<0.01) between the probiotic fed groups and the antibiotic and control groups in body weight gains (g) and FCR. This result is in agreement with the

previously reported findings of several works. Higher body weight gains for probiotic fed broilers were also reported by Kamruzzaman et al. (2005), Kabir et al. (2005), Islam et al. (2014), Celik et al.

Table 3. Effects on cecal microbiota (log 10 cfu/9) at 21 days.

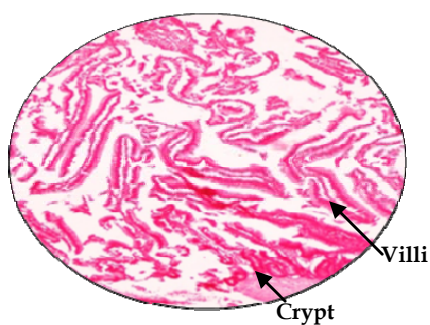
Characters	Treatment feed				SEM	Level of significant	P-values
	T ₁	T ₂	T ₃	T ₄			
<i>E. coli</i>	6.85 ^a	6.35 ^b	5.03 ^c	4.68 ^d	0.210	**	0.000
<i>Salmonella</i> spp.	6.04 ^a	6.30 ^a	6.11 ^a	6.04 ^a	0.08	NS	0.533
<i>Bacillus</i> spp.	4.43 ^d	4.68 ^c	6.03 ^b	6.30 ^a	0.190	**	0.000
<i>Lactobacillus</i> spp.	5.65 ^c	4.50 ^d	6.07 ^b	6.36 ^a	0.170	**	0.000

Means with different superscripts row wise differ significantly at ** $p < 0.01$ but means with different superscripts significantly differ at * $p < 0.05$. T₁ = Control; T₂ = Amoxicillin 200 g/ton of feed; T₃ = Probiotic (Pro.B[®] 250 g/ton of feed); T₄ = Probiotic (Pro.B[®] 500 g/ton of feed).

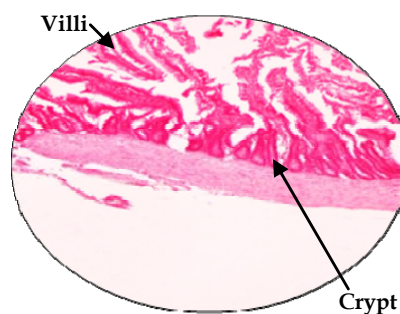
Table 4. Effects on cecal microbiota (log 10 cfu/9) at 35 days.

Characters	Treatment feed				SEM	Level of significant	P-values
	T ₁	T ₂	T ₃	T ₄			
<i>E. coli</i>	6.34 ^a	6.41 ^a	4.80 ^b	4.03 ^c	0.240	**	0.000
<i>Salmonella</i> spp.	6.38 ^a	6.56 ^a	5.00 ^b	4.81 ^b	0.190	**	0.000
<i>Bacillus</i> spp.	3.85 ^b	4.10 ^b	7.05 ^a	7.10 ^a	0.360	**	0.000
<i>Lactobacillus</i> spp.	4.00 ^c	4.44 ^c	6.05 ^b	7.01 ^a	0.290	**	0.000

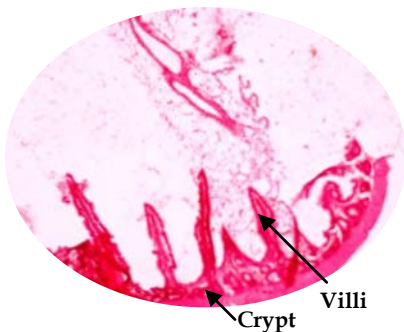
Means with different superscripts row wise differ significantly at ** $p < 0.01$ but means with different superscripts significantly at * $p < 0.05$. T₁ = Control; T₂ = Amoxicillin 200 g/ton of feed; T₃ = Probiotic (Pro.B[®] 250 g/ton of feed); T₄ = Probiotic (Pro.B[®] 500 g/ton of feed).



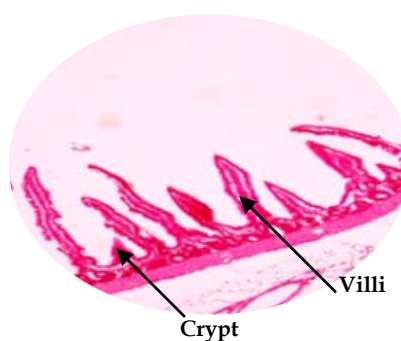
a. Histochromatography of jejunal tissue of control (T₁) group showing small growth of villus ($\times 10$, H & E)



b. Histochromatography of jejunal tissue of antibiotic fed group (T₂) showing small growth of villus ($\times 10$, H & E)

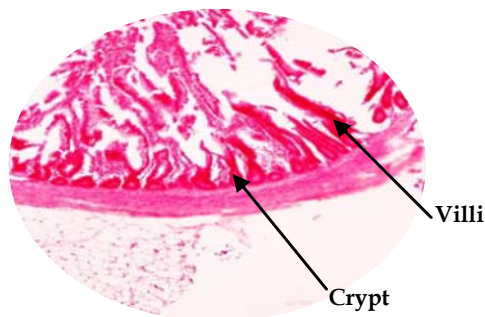


c. Histochromatography of jejunal tissue of probiotic (Pro.B[®]) fed group (T₃) showing large growth of villus ($\times 10$, H & E)

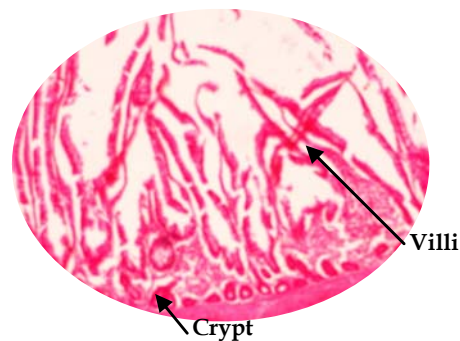


d. Histochromatography of jejunal tissue of probiotic (Pro.B[®]) fed group (T₄) showing large growth of villus (more than T₃) ($\times 10$, H & E)

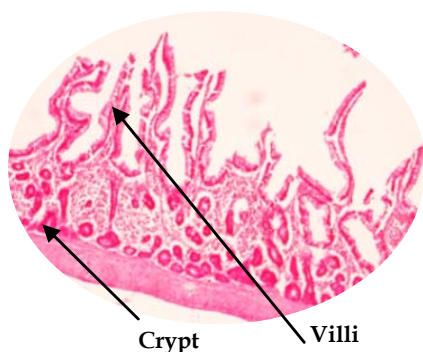
Figure 1. Histology of jejunal tissue at 21 days.



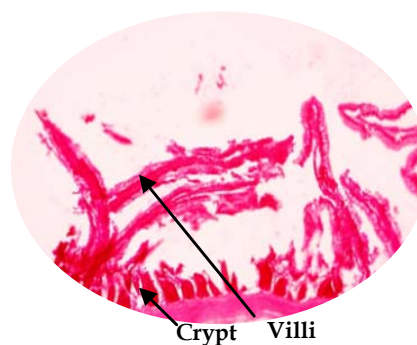
a. Histochromatography of jejunal tissue of control (T_1) group showing small growth of villus ($\times 10$, H & E)



b. Histochromatography of jejunal tissue of antibiotic fed group (T_2) showing small growth of villus ($\times 10$, H & E)



c. Histochromatography of jejunal tissue of probiotic (Pro.B[®]) fed group (T_3) showing large growth of villus ($\times 10$, H & E)



d. Histochromatography of jejunal tissue of probiotic (Pro.B[®]) fed group (T_4) showing large growth of villus (more than T_3) ($\times 10$, H & E)

Figure 2. Histology of jejunal tissue at 35 days.

(2007), Raceviciute et al. (2007), Kabir (2009), Toghyani et al. (2011) and Kral et al. (2012). Data analysis from cecum samples shown (Tables 3 and 4) that the TLC were significantly higher ($p > 0.01$) in the probiotic fed groups as compared to the control and antibiotic fed groups. Data analysis for the TSC from cecum samples has shown no significant differences among four groups (Tables 3 and 4). The TBC were significantly higher ($p > 0.01$) in the probiotic groups (Table 3 and 4) as compared to the control group. On the other hand, the TEC was significantly higher ($p > 0.01$) in the control group as compared to the probiotic fed groups. The present findings support the previously described results by Edens (2003) and Kabir et al. (2005). The thickness of the jejunal wall was much wider in group T_4 (Pro.B[®] 500gm/ton of feed) broilers than the conventional and antibiotic fed group broiler chicks at 21 days of age as presented in Figure 1. The villus growth was higher in the group T_4 at 35 days of age as compared to the other groups. The present result supports the findings previously described by Samanya et al. (2002), Kabir et al. (2005), Rehman et al. (2007) and Islam et al. (2014).

Conclusions

From this study it is clearly revealed that probiotic supplementation (Pro.B[®] 500 g/ton of feed) promoted significant influence on growth performance, carcass yield, bacterial antagonism and morphological changes of intestinal wall.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Morphological characterization of indigenous sheep population in their production system for developing suitable selection criteria in central zone of Tigray, Northern Ethiopia

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The aim of this study was to characterize the phenotype of the indigenous sheep population in central zone of Tigray. A total of 450 mature sheep were sampled randomly for collect qualitative data. Based on dentition adult sheep were classified into four age categories (from one pair of permanent incisor to four pair of permanent incisor). Coat colour, tail type, ear orientation and presence of wattle were significant ($p < 0.05$) between districts studied. Among the three districts only within Tanqua-Abergelle district attributes of coat colour type, tail form, horn and wattle were found to be significant ($p < 0.05$). Majority of the sheep population in Tanqua-Abergelle district had a dark red coat colour type (50.2%), while in Kola-Tembien and Adwa districts sheep were dominated by gray coat colour type (30.1 and 40.8%) respectively. Most of the sampled sheep population across the three districts were short fat tailed followed by short thin and rumped fat tail type. Multiple correspondence analysis indicated that the sampled sheep population in the study districts were clustered into two groups based on their unique characteristics; Kola-Tembien and Adwa sheep together as one group while Tanqua-Abergelle district sheep as the other group, based on their unique characters. Based on the present result on characterization of sheep in the study area one may develop selection criteria and productivity schemes of the local sheep.

Key words: Adwa, Kola-Tembien, Tanqua-Abergelle, qualitative traits, suitable selection criteria.

INTRODUCTION

Ethiopia is endowed with 29.33 million sheep head (CSA, 2015) with diversified genetic pools adapted to a wide range of agro-ecologies. Environmental pressure also maintains a wide range of genotypes, each adapted to a

specific set of circumstances (Getachew et al., 2010). At least 9 sheep breeds and 14 traditional sheep populations are found in Ethiopia (Gizaw et al., 2007). Although the production system and marketing are

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almost traditional (Legesse et al., 2008), sheep contribute substantial amounts to income, food (meat and milk), and non-food products like manure, skins and wool. They also serve as a means of risk mitigation during crop failures, property security, financial saving and investment in addition to many other socioeconomic and cultural functions (Tibbo, 2006).

Sheep productivity in Ethiopia is constrained by lack of technical capacity, scarce feed, diseases, insufficient infrastructure and market information resulting in varying sheep distribution and marketing across space and time (Samson and Frehiwot, 2014) and inadequate utilization of the indigenous sheep genetic resources. In spite of the several constraints, sheep in Ethiopia have been able to contribute significantly to the income of the small holder farmers/ pastoralists and also help in poverty alleviation schemes (Kosgey and Okeyo, 2007).

Genetic variation within a population provides information on the potential response of a population to adapt to the environment and selective genetic improvement (Abegaz et al., 2011). Therefore, all the breed improvement programs are based on effective utilization of genetic variation among the animals. Traits such as body weights and rate of gain that can be easily measured are among traits having the most economic importance. Improvement in live weight through indirect selection on linear size traits is possible both under nucleus and village-based breeding programs (Gizaw et al., 2008). Identification and defining the production system of any breed is the bases for breed improvement strategies and scheme.

Genetic improvement of the local livestock through appropriate techniques or selection and breeding programme is the need of the day especially under production constraints (Yakubu, 2010). The usefulness of system and breed characterization of the indigenous livestock in general and sheep in particular is unquestionable. Hence characterization, inventory and monitoring of animal genetic resources (AnGR) are essential to their sustainable management and facilitate effective planning of how and where they can best be used and developed (FAO, 2015).

The study areas are potential in sheep production, little works was done to characterize or to improve the indigenous sheep population of Tanqua-Abergelle, Kola-Tembien and Adwa districts by Gizaw (2008) under the name of Sokota sheep breed providing some information on some physical body measurements and characteristics.

Therefore this research was initiated to characterized phenotype of the existing sheep population in their production system. This will serve to develop genotypic characterization and develop improvement strategies and productivity schemes of the local sheep. Since genetic resources and production systems are not static and thus routine inventories and thus on-going monitoring is needed (Sölkner et al., 1998).

The objective of this study was to characterize sheep in Central Zone of Tigray in their production system based on their qualitative traits so that suitable selection criteria for sheep may be suggested.

MATERIALS AND METHODS

Description of study area

The study was conducted in three districts of central zone of Tigray, Ethiopia (Tanqua Abergell, Kola-Tembien and Adwa) (Figure 1).

The central zone of Tigray covers about 9741 km² with a total population of 1,132,229. The central zone is divided into nine districts and three major marketing towns-Axum, Adwa and Abyi Adi. The zone consists of about 406,018 sheep (CSA, 2015). The elevation of the area ranges from 1332 to 2921 m a.s.l. Annual rainfall is vary within a range of 466 to 758 mm. Temperature ranges from 14 to 22°C. Most of the lands are cultivated for crop purpose with some patchy grazing bottomlands and degraded hilly sites. Major soil types in the study areas include Eutric and Chromic Cambisols, Eutric and Lithic Leptosols, Eutric Cambisols and Lithic Leptosols (CSA, 2015).

Site selection and sampling technique

From among the nine districts of central zone of Tigray, three districts were selected using multi-stage purposive sampling techniques, based on the sheep population density and road accessibility. From each selected districts two rural kebeles (Felege-Hiwet and Gera from Tanqua Abergelle district, Werka-Emba and Debre-Tsehay from Kola-Tembien and Debre-Gent and Endamaryam-Shewito from Adwa) were selected purposively based on the sheep flock density and accessibility for transportation. Accordingly, a total of 450 healthy adult sheep (135 male and 315 non pregnant females in the proportion of 30 male: 70 female) were selected randomly (209 from Tanqua-Abergelle, 143, from Kola-Tembien and 98 from Adwa district). The sheep were identified by sex, districts and four age groups (1PPI-1 pair of permanent incisor), 2PPI (2pair of permanent incisor), 3PPI (3 pair of permanent incisor) and 4PPI (4 pair of permanent incisors) for the base line data collection (characterization) as per the method of Wilson and Durkin (1984).

Data type and methods data collection

Data on morphological variables on the selected animals for qualitative traits (morphological) were recorded as the following.

Qualitative traits (morphological variables)

The standard breed descriptor list for sheep developed by FAO (2012) was followed in selecting morphological variables. Data for qualitative traits like: Coat color pattern, coat color type, hair type, head profile, ears orientation, wattle, presence or absences of horn, ruff and tail type and shape were recorded using individual interviews, group discussion and observation on the animal.

Data management and analysis

Qualitative traits

Qualitative data from individual observation were analyzed

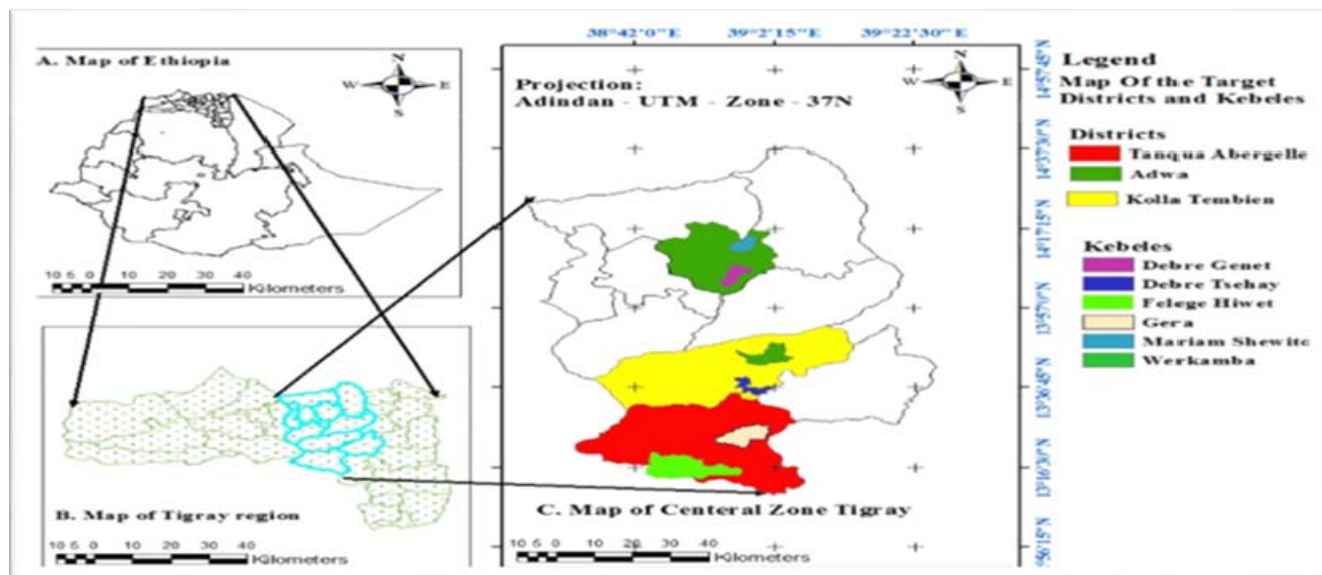


Figure 1. Map of the study area.

separately for male and female sheep using frequency procedure of Statistical Analysis System SAS version release 9.2 (2008) within population. Chi-square was employed when required to test the independence of categories or to assess the statistical significance ($p \leq 0.05$) within population. Chi-square was also calculated across/between population to test the existence of significance differences between populations. Multiple correspondence analysis was carried out to evaluate the typical features or associations of each district sampled sheep population morphologically.

RESULT AND DISCUSSION

Morphological traits

The number and percentage of each level of the 13 qualitative traits recorded in male and female for each districts are presented in Table 1. In Tanqua-Abergelle district the dominant coat color type were dark red, white and black with the proportions of 50.7, 12.4 and 11.5%, respectively. Grey mixture local name ('*Agua*'), black dominant, white dominant and dark red dominant were also observed with the proportions of 10.5, 9.1, 4.8 and 1.0%, respectively. This finding is in agreement with that of Tajebe et al. (2011) who reported the dominated coat color type of Abergelle sheep breed was dark red followed by white and black, whereas in Kola-Tembien and Adwa sampled sheep population grey ('*Agua*'), dark red and black coat color were observed dominantly with the proportion of 30.1, 25.2, 14.0% and 40.8, 24.4 and 9.2%, respectively. Nearly 54.5, 27.8 and 18.2% of the sampled sheep in Tanqua-Abergelle had plain coat color pattern, spotted pattern and patchy pattern, respectively.

Similarly the proportion of plain coat color pattern, spotted pattern and patchy pattern for the sampled sheep

of Kola-Tembien and Adwa districts were 59.4, 25.2, 15.4% and 67.3, 22.4 and 10.2%, respectively. The present finding is in line with the finding of Melesse et al. (2013) reported that coat color pattern of Kembata Tembaro-Hadiya, zone of southern Ethiopia of sampled sheep were dominantly by plain coat colour pattern with 75.5%.

Almost all the male and female sampled sheep population across the study districts were short fat tailed followed by short thin and medium tail fat type with curved tip, strait tip and docked tail with the proportion of 91.5, 7.2% 1, 81.3, 11.5 and 7.2% for Tanqua-Abergelle, 71.3, 12.6, 16.1, 80.4, 11.9 and 7.7% for Kola-Tembien and 63.2, 29.6 8.2, 77.6, 15.3 and 9.2% for Adwa sheep. Majority of the sampled sheep population in all the study districts had smooth hair type and concave head profile with the percentage of 63.2 and 52.2 for Tanqua-Abergelle, 60.1 and 55.2 for Kola-Tembien and 56.1 for Adwa sheep. This finding is comparable with the previous work done Hayelom et al. (2014) who reported that majority sampled sheep of Degua-Tembien had smooth hair type and concave face profile with proportion of 75 and 64%, respectively. In Tanqua-Abergelle district most of the sampled sheep population had rudimentary ear, 61.7, 20.6% erect, 12.4% semi pendulous, 4.8% pendulous and 0.5% horizontal, while in Kola-Tembien and Adwa the sampled sheep had dominantly semi pendulous and erect ear, 30.8, 23.8% and 38.8%, 17.3%, respectively. The remaining small proportion of sampled sheep had rudimentary, pendulous and carried horizontally in their order were 17.5, 18.2, 9.8% and 12.2, 15.3 and 16.3%, respectively.

Most of male and female sampled sheep populations across the study districts were polled or hornless 82.8%

Table 1. Description of qualitative traits of sheep populations in the study districts.

Traits	Attributes	Tanqua-Abergelle						Kolla Tembien						Adwa					
		M		F		Total		M		F		T	M		F		T		
		N	%	N	%	N	%	N	N%	N	%	N	%	N	N%	N	%	N	%
Coat colour type	Black	11	5.3	13	6.2	24	11.5	7	5.0	13	9.1	20	14.0	2	2.0	7	7.1	9	9.2
	White	6	2.9	20	9.6	26	12.4	8	5.6	10	7.0	18	12.6	2	2.0	9	9.2	11	11.2
	Dark red	38	18.2	68	33.0	106	50.7	16	11.2	20	14.0	36	25.2	11	11.2	9	9.2	20	20.4
	Gray	6	2.9	16	7.7	22	10.5	8	5.6	35	24.5	43	30.1	5	5.1	35	35.7	40	40.8
	Black dominant	5	2.4	14	6.7	19	9.1	3	2.1	10	7.0	13	9.1	1	1.0	12	12.2	13	13.3
	White dominant	3	1.5	7	3.3	10	4.8	2	1.4	5	3.5	7	5.0	-	-	4	4.1	4	4.1
	Dark red dominant	-	-	2	1.0	2	1.0	1	0.7	5	3.5	6	4.2	-	-	1	1.0	1	1.0
	χ^2 -w/n p	15.4 ^{ns}						5.1 ^{ns}						12.3 ^{ns}					
χ^2 -b/n p	35.9 ^{ns}																		
Coat colour pattern	Plain	46	22.0	68	32.5	114	54.5	25	17.5	60	42.0	85	59.4	16	16.3	50	51.0	66	67.3
	Patchy	9	4.3	29	13.9	34	18.2	5	3.5	17	11.9	22	15.4	3	3.1	7	7.1	10	10.2
	Spotted	14	6.7	44	21.1	53	27.8	15	10.5	21	14.7	36	25.2	2	2.0	20	20.4	22	22.4
	χ^2 - w/np	4.23 ^{ns}						2.49 ^{ns}						3.4 ^{ns}					
χ^2 - b/np	3.2 ^{ns}																		
Hair type	Smooth	38	18.2	94	45.0	123	63.2	27	18.9	59	41.3	86	60.1	12	12.2	45	45.9	57	58.2
	Course	31	14.8	47	22.5	78	37.3	18	12.6	39	27.3	57	39.9	9	9.2	32	32.7	41	41.8
	χ^2 -w/np.	1.7 ^{ns}						0.01 ^{ns}						0.00 ^{ns}					
χ^2 -b/np.	0.35 ^{ns}																		
Tail type	Short fat	67	29.2	125	59.8	192	91.9	34	23.8	68	47.6	102	71.3	16	16.3	45	45.9	61	62.2
	medium fat	1	0.5	1	0.5	2	1.0	6	4.2	17	11.9	23	16.1	3	3.1	26	26.5	29	29.6
	Short thin	1	0.5	14	6.7	15	7.2	5	3.5	13	9.1	18	12.6	2	2.0	6	2.0	8	8.2
	χ^2 b/np	5.7 ^{ns}						0.27 ^{ns}						2.57 ^{ns}					
χ^2 w/np	57.1 ^{**}																		
Tail form	Curved tip	61	30.4	109	52.2	170	81.3	36	25.2	79	55.2	115	80.4	15	15.3	61	62.2	76	77.6
	Strait tip	2	1.0	22	10.5	24	11.5	5	3.5	12	8.4	17	11.9	2	2.0	13	13.3	15	15.3
	Docked	6	2.9	9	4.3	15	7.2	4	2.8	7	4.9	11	7.7	4	4.1	5	5.1	9	9.2
	χ^2 w/n pon	8.2 ^{ns}						0.15 ^{ns}						4.2 ^{ns}					
χ^2 b/n pon.	0.32 ^{ns}																		
Head profile	Strait	24	11.5	42	20.1	66	31.6	11	7.7	29	20.3	40	28.0	4	4.1	20	36.4	24	24.5
	Convex	12	5.7	22	10.5	34	16.3	6	6.7	18	12.6	21	16.8	3	3.1	16	16.3	19	19.4
	Concave	33	15.8	76	36.4	109	52.2	28	19.6	51	35.7	79	55.2	14	14.3	41	41.8	55	56.1
	χ^2 w/n pon	2.5 ^{ns}						0.91 ^{ns}						2.3 ^{ns}					

Table 1. Contd.

	χ^2 b/n pon.																		
Horn	Present	32	15.3	4	1.9	36	17.2	17	11.9	3	2.1	20	14.0	10	10.2	2	2.0	12	
	Absent	37	17.7	136	65.1	173	82.8	28	19.6	95	66.4	123	86.0	11	11.2	75	76.5	97	
		57.9 ^{ns}					29.95					35.6 ^{ns}							
Horn orientation	Lateral	13	36.1	2	5.6	15	41.7	6	30.0	1	5	7	35.0	3	23.1	2	15.4	5	34.5
	Backward	19	52.8	2	5.6	21	58.3	11	55.0	2	10	13	65.0	7	53.9	1	7.7	8	61.5
	χ^2 w/n pon				0.13 ^{ns}						0.0004 ^{ns}						0.98 ^{ns}		
	χ^2 b/n pon.										0.24 ^{ns}								
Horn shape	Straight	10	27.8	-	-	10	27.8	4	20.0	-	-	4	20.0	3	23.1	1	7.7	4	30.8
	Curved	13	36.1	2	5.6	15	41.7	7	35.0	2	10.0	9	45.0	5	38.5	1	7.7	6	46.2
	Spiral	8	22.2	2	5.6	10	27.8	6	30.0	1	5.0	7	35.0	2	13.4	1	7.7	3	23.1
	Corkscrew	1	2.8	-	-	1	2.8	-	-	-	-	-	-	-	-	-	-	-	-
	χ^2 - w/np.	2.3 ^{ns}																	
	χ^2 -b/np.										7.9 ^{ns}								
Ear orientation	rudimentary	40	19.1	89	42.6	129	61.7	7	4.9	18	12.6	25	17.5	5	5.1	7	7.1	12	12.2
	Erect	13	6.2	30	14.4	43	20.6	10	7.0	24	16.8	34	23.8	3	3.1	14	14.3	17	17.3
	Semi-pendulous	10	4.8	16	7.7	26	12.4	16	11.2	28	19.6	44	30.8	8	8.2	30	30.6	38	38.8
	pendulous	5	2.4	5	2.4	10	4.8	9	6.3	17	11.9	26	18.2	-	-	15	15.3	15	15.3
	Carried horizontally	1	0.5	-	-	1	0.5	3	2.1	11	7.7	14	9.8	5	5.1	11	11.2	16	16.3
	χ^2 -w/np.	3.6 ^{ns}									2.0 ^{ns}						9.2 ^{ns}		
	χ^2 -b/np.										134.9 ^{**}								
Ruff	Present	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Absent	69	33	140	67.0	209	100	45	31.5	98	68.5	143	100	21	21.4	77	78.6	98	100
Wattle	Present	16	7.7	4	1.9	20	9.6	0	0	7	4.9	7	4.9	-	-	1	1.0	1	1.0
	Absent	53	25.4	136	65.1	181	90.4	45	31.5	91	63.6	133	95.1	21	21.4	76	77.6	108	99.0
	χ^2 -w/np.				20.55 ^{**}						3.5 ^{ns}						0.24 ^{ns}		
	χ^2 -b/np.										10.4 ^{**}								

N = Number of observations; % = percentage of observations; M = male; F = female; χ^2 -w/np = chi-square within population; χ^2 -b/np = chi-square between population; ** = significant at (p<0.05); ns = non-significant.

in Tanqua-Abergelle, 86% in Kola-Tembien and 87.8% in Adwa districts. The remaining small proportion of both sexes had horn 17.2% in

Tanqua-Abergelle, 14% in Kola-Tembien and 12.2% in Adwa district. The current result is in consistence with the previous work done Hayelom

et al. (2014) in Tanqua-Abergelle, 86% in Kola-Tembien and 87.8% in Adwa districts. The remaining small proportion of both sexes had horn

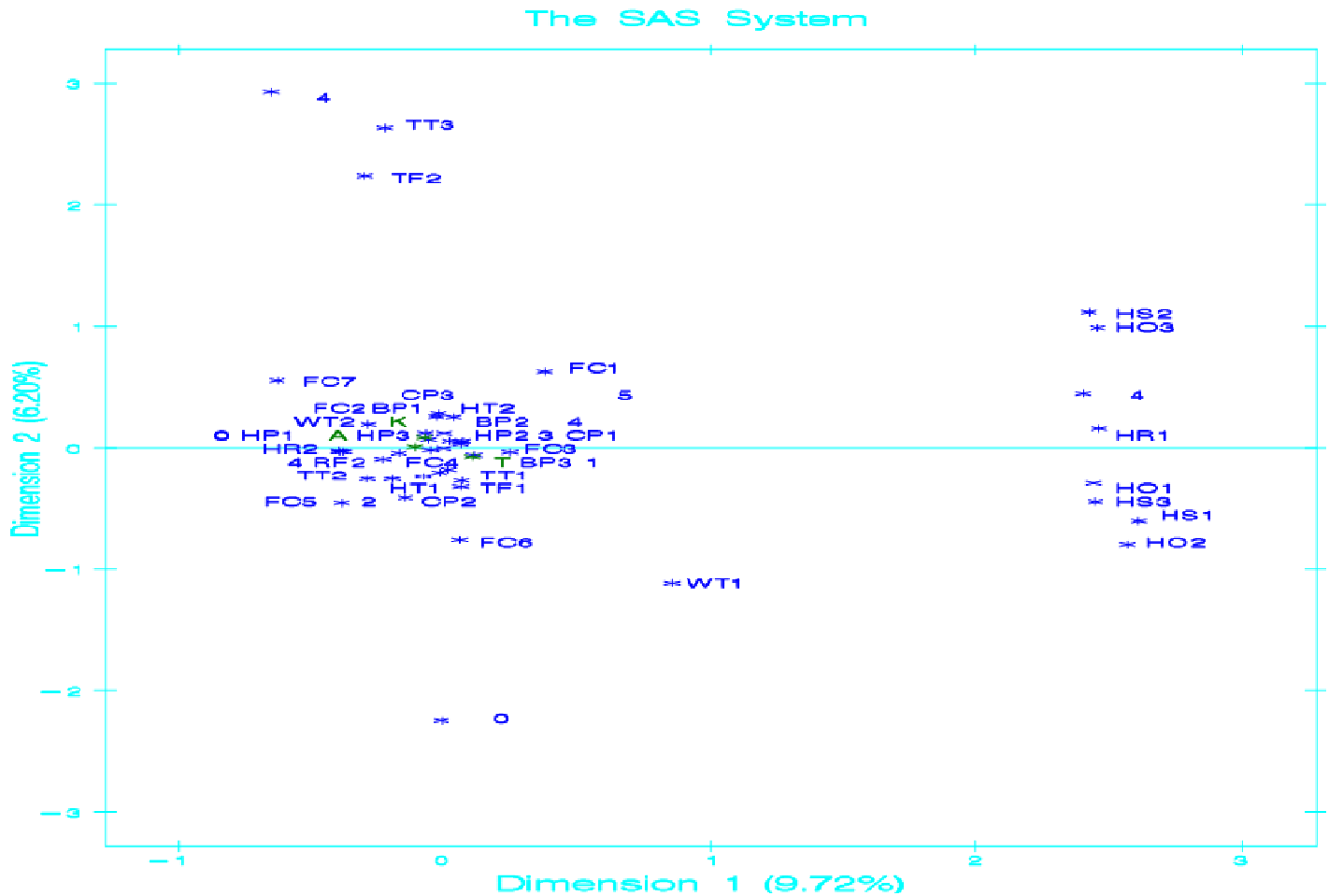


Figure 2. Association (relation) among different categories of qualitative traits using multiple correspondence analysis techniques.

17.2% in Tanqua-Abergelle, 14% in Kola-Tembien and 12.2% in Adwa district. The current result is in consistency with the previous work done Hayelom et al. (2014) reported that majority 80% of the sampled sheep of Tigray highland were polled but, different from the previous work done (Getachew, 2008; Edea, 2008) reported that both sexes of Afar and Horro sheep were polled or hornless.

In all the study districts all the sample sheep population 100% had no ruff and about 7.7% male and 1.9% female sampled sheep in Tanqua-Abergelle had wattle, whereas in Kola-Tembien and Adwa districts the male sampled sheep had no wattle rather female 4.9 and 1%, respectively had wattles. The present finding is in line with that of Mohamed et al. (2015) and Getachew (2008) who reported male sampled sheep in Habru and Afar sheep had no wattles.

Chi-square test for assumption of equal proportion of categorical variables in all the study districts (Tanqua-Abergelle, Kola-Tembien and Adwa) sample sheep population indicated that among the variables considered in this study coat colour type, tail type, ear orientation and

wattle presence were significant ($p < 0.05$) between districts. Among the three districts only within Tanqua-Abergelle district attributes of coat colour type, tail form, horn and wattle were found to be significant ($p < 0.05$). The uniformity in coat colour type in Kola-Tembien and Adwa sheep might be due to similar coat colour preference and selection practice.

Multiple correspondence

Multiple correspondence analyses was carried out to evaluate the typical features of each district sampled sheep population morphologically. Figure 2, shows a dimensional graph representing the association among the categories of the analyzed qualitative traits. The association is based on the point found in approximately the same direction from the origin in approximately in the same region of the space. From the figure shown that 15.92% of the total morphological variation in sheep population is explained by the first two dimensions 9.72% by the first and 6.20% by the second dimensions. From



Figure 3. Morphological views of the sampled sheep in the study districts.

Table 2. Key abbreviation.

Variable	Description
Districts	T= Tanqua-Abergelle, K= Kola-Tembien, A= Adwa
Coat colour type	C1=Black, C2=Gray, C3=Dark red, C4=Dark red dominant, C5= Black Dominant, C6= White dominant, C7= White
Coat colour pattern	CP1=Plain, CP2= Patchy, CP3= Spotted
Hair type	HT1= Short smooth, HT2= Course
Tail type	TT1=Short fat, TT2=Short thin, TT3= Medium fat tail
Tail form	TF1=Curved tip, TF2=Strait tip, TF3=Docked
Head profile	HP1=Strait, HP2=Convex, HP3=Concave
Horn	HR1=Present, HR2=Absent
Horn orientation	HO1=Upward, HO2=Lateral, HO3=Backward
Horn shape	HS1= Strait, HS2=Curved, HS3=Spiral
Back profile	BP1=Strait, BP2=Slops towards rump, BP3=Slops down from rump
Ear orientation	EO1=Rudimentary, EO2=Erect, EO3=Semi-pendulous, EO4= Pendulous, EO5=Carried horizontally
Ruff	RF1= Present, RF2=Absent
Wattle	WT1= Present, WT2= Absent

the identified dimensions the sample sheep population in Kola-Tembien and Adwa districts clustered together with characters grey and white coat colour type, spotted coat color pattern, concave head profile, strait back profile, medium tail fat with strait tip and with no wattle and ruffs, whereas the sampled sheep in Tanqua-Abergelle district the traits clustered together closely associated with dark red coat colour type, short fat tail type with curved tip, upward and later horn orientation with strait and spiral horn shape and slops down from rump back profile as it indicate in (Figure 3).

Conclusions

Sheep population across the three districts were short fat tailed followed by short thin and rumped fat tail type. Coat colour, tail type, ear orientation and presence of wattle were significantly different ($p < 0.05$) between districts studied. Multiple correspondence analysis indicated that

the sampled sheep population in the study districts were clustered into two groups based on their unique morphological characteristics; Kola-Tembien and Adwa sheep together as one group and Tanqua-Abergelle district sheep as the other group. Among the three districts only within Tanqua-Abergelle district, attributes of coat colour type, tail form, horn and wattle were found to be significant ($p < 0.05$).

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests

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Full Length Research Paper

Substitution of dried mulberry (*Morus indica* L.) leaf meal for concentrate mix on feed intake, digestibility, body weight gain and carcass characteristics of Abergelle sheep

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The study was conducted at Abergelle Agricultural Research Center, Ethiopia using 24 yearling intact male Abergelle sheep with initial body weight of 17.52 ± 1.13 kg. The objectives were to evaluate substitution of dried mulberry (*Morus indica* L.) leaf meal (DMLM) for concentrate mix (CM) on feed intake, digestibility, body weight gain and carcass characteristics, and to determine cost benefit analysis of the supplementary regimes. The study comprised 90 days of feeding trial, 7 days of digestibility trial and carcass evaluation at the end. Six sheep were randomly assigned to each treatment using randomized complete block design. The treatments included feeding of ad libitum natural grass hay and supplementation with 100% CM (T1); 75% CM + 25% DMLM (T2); 50% CM + 50% DMLM (T3) and 25% CM + 75% DMLM (T4) on DM basis. The supplements were prepared iso-nitrogenous and they were offered to meet daily CP requirements of 80.4, 95.7 and 108 g for rams with body weight of 15, 20 and 25 kg, respectively. At beginning of experiment, amount of supplements provided were 439.49, 440.51, 440.75 and 440.50 g on DM basis for T1, T2, T3 and T4, respectively. The CM contained noug seed cake (NSC) and wheat bran (WB) at ratio of 19.28: 80.72, 14.14: 60.86, 9.04: 40.96 and 3.92: 21.08 for T1, T2, T3 and T4, respectively. The CP content of grass hay, WB, NSC and DMLM were 7.20, 16.80, 33.40 and 20.20%, respectively. The OM, CP, NDF, ADF and ADL intakes and digestibilities were similar among treatments but, hay and total DM intakes as well as DM digestibility were significantly higher ($p < 0.05$) in T3 than T1. Calculated ME was greater ($p < 0.01$) in T3 and T2 than T4 but similar in T1, T2 and T3. There was no significant differences among treatments for feed conversion efficiency, final body weight, ADG and most carcass parameters measured. The partial budget analysis showed that T4 had highest economical gain. Therefore, T4 is recommended on both biologically and economically efficient supplementary regime for Abergelle sheep.

Key words: Economical gain, feed conversion efficiency, isonitrogenous, noug seed cake, wheat bran.

INTRODUCTION

There are about 1.82 and 29.33 million sheep in Tigray region and Ethiopia, respectively (CSA, 2015). Indigenous sheep in Ethiopia have a multipurpose role for

smallholder farmers like sources of income, meat, skin, manure and coarse wool (Markos, 2006; Mengesha and Tsega, 2012; Gizaw et al., 2013).

As reported by FAO (2013) sheep meat consumption trends in Ethiopia grow from 36000 ton in 2000 to 86000 ton in 2012. Sheep accounted 34% of the live animal exports (Gizaw et al., 2013). Moreover, sheep together with goats contributed 86% of the total value of meat exports (Legese and Fadiga, 2014). Even though the sheep population provided considerable roles to both smallholder farmers and the country's economy but their present contribution is far below their potential. This is because productivity of sheep is hampered by many factors. However, Yami (2008) indicated that the nutrition of sheep and goats is the most important factor affecting their performance. Similarly, Gizaw et al. (2010) and Tesfay et al. (2012) noted that small ruminants usually suffer from feed shortage and poor nutrition. The common feeds in Ethiopia such as crop residues and matured natural pasture are inherently low in crude protein (CP), minerals and digestibility (Tolera, 2008; Gizaw et al., 2010).

One way of improving the poor quality of the feed resources is by supplementation with other high quality feeds. Concentrate feeds (agro-industrial by products) have a good potential of supplementation value but in most developing countries like Ethiopia concentrates are expensive, are in short supply and may not be easily accessible to smallholder farmers (Tolera et al., 2000). Hence, there is a need to search for supplement sources that could be applied at smallholder farmer levels with affordable costs. The use of leaves' of some trees and shrubs like leaf of mulberry plant as substitution to concentrate supplementation may be one of the alternative solutions. It has been noted that the nutritive value of mulberry plants is one of the highest found in products of vegetable origin and of far superior to traditional forages like alfalfa (Benavides, 2000; Doran et al., 2007).

Though the availability has not been well quantified, large numbers of mulberry trees are present in different areas of Ethiopia particularly in Tigray region. However, in most of the areas, their leaves remained unused due to non-availability of silkworm rearing. Therefore, the objectives of the study were to evaluate substitution of dried mulberry (*Morus indica* L.) leaf meal (DMLM) for concentrate mix (CM) on feed intake, digestibility, body weight gain and carcass characteristics of Abergelle sheep as well as to determine cost benefit analysis of the supplementary regimes.

MATERIALS AND METHODS

Study site

The study was conducted at Abergelle Agricultural Research

Center in Tanqua Abergelle district, Ethiopia. It is located at 13°14'06"N latitude and 38°58'50"E longitude. The district is categorized as hot to warm sub-moist lowland (SM1-4) sub-agro ecological zone of Tigray region with an altitude of 1300 - 1800 masl. Its mean annual rainfall ranges from 400 - 650 mm and the mean annual temperature ranges from 21 to 41°C.

Feed preparation and feeding

Natural grass hay was harvested from Abyi-Adi skill development and training center in Kola Tembien district while the mulberry leaves were collected from both farmers and forage nursery site in Tanqua Abergelle district during September. The mulberry leaves were harvested manually with leaf picking method and immediately, they were spread thinly on plastic sheet under shed in well-ventilated room for drying with turning of 10 - 12 times/day. Finally, they were milled using mortar and pestle to make in form of meal. Wheat bran (WB) and noug seed cake (NSC) were purchased from market in Mekelle city. The natural grass hay was offered ad libitum (about 20% refusal on DM basis) and the supplements were given twice daily in equal quantities at 8.00 am and 4.00 pm to each experimental ram.

Experimental animals' management

Twenty four yearling intact Abergelle rams were selected randomly and purchased from market at Yechila town in Tanqua Abergelle district, Ethiopia. The rams were ear tagged for identification. They were treated against internal and external parasites with Albendazole and Vetazine 60% EC, respectively as per the recommended dosage. They were also vaccinated against ovine pasturelosis which is the most common disease in the area, and housed in individual pens equipped with feeding and watering troughs. The rams were adapted to experimental feeds for 15 days and then followed by 90 days of feeding trial. Besides, each experimental ram had free access to salt block and fresh water daily.

Experimental design and treatments

The experiment was conducted using a randomized complete block design (RCBD) with 4 treatments and 6 replications. Initial body weight of the rams was applied as block and experimental rams were randomly assigned to the dietary treatments within the block. The initial body weight of experimental rams was 17.52 ± 1.13 kg (mean ± SD) while the average initial body weights of the rams in each group were 17.50, 17.52, 17.53 and 17.52 kg for T1, T2, T3 and T4, respectively. The supplements were prepared isonitrogenous having almost the same 20% CP and they were offered according to recommendation of Ranjhan (2004) to meet daily CP requirements of 80.4 g, 95.7 g and 108 g for growing lambs with body weight of 15, 20 and 25 kg, respectively to get daily gain of 100 - 120 g. The dietary treatments included feeding of ad libitum natural grass hay and supplementation with 100% CM (T1); 75% CM + 25% DMLM (T2); 50% CM + 50% DMLM (T3) and 25% CM + 75% DMLM (T4) on DM basis. The CM was formulated using NSC and WB at ratio of 19.28: 80.72, 14.14: 60.86, 9.04: 40.96 and 3.92: 21.08 for T1, T2, T3 and T4, respectively. At the beginning of the study, CP requirements of the experimental

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rams were 87.90, 88.10, 88.15 and 88.10 g while the amounts of supplements provided to meet the aforementioned CP requirements were 439.49, 440.51, 440.75 and 440.50 g on DM basis for T1, T2, T3 and T4, respectively and they were changed fortnightly according to the change in body weight.

Feed intake and body weight measurements

The amounts of feed offers and refusals for each ram were recorded every day throughout the experimental period. The DM intake was calculated as difference between amounts of feed offered and amounts of feed refusals on DM basis. Similarly, nutrient intake was computed as difference between nutrient content of feed offered and nutrient content in feed refusals. The body weight measurement was taken fortnightly for each ram for the whole experimental period after overnight fasting. The initial body weight of experimental rams was measured at the end of adaptation period while final body weight was measured at the end of feeding trial (growth period). The average daily gain (ADG) was calculated by subtracting initial body weight from final body weight and then after dividing it to the number of feeding days. Feed conversion efficiency was determined as ratio of ADG to daily DM intake.

Digestibility trial

Digestibility trial was conducted following the feeding trial. The total wet feces for each ram were measured for 7 consecutive days after 3 days of adaptation of the rams for carrying of the fecal bags. The daily fecal excretion of each ram was mixed thoroughly and 10% was sampled and kept in airtight plastic bags and stored at -20 °C refrigerator. At the end of the fecal collection period the fecal samples were pooled per treatments and 20% were sub-sampled to determine chemical composition. The apparent digestibility coefficient of DM and nutrients were calculated as follows;

$$\text{Apparent DM digestibility coefficient} = \frac{\text{Total DM intake} - \text{DM in feces}}{\text{Total DM intake}}$$

$$\text{Apparent nutrient digestibility coefficient} = \frac{\text{Total nutrient intake} - \text{Nutrient in feces}}{\text{Total nutrient intake}}$$

The percent (%) total digestible nutrients (TDN) for each dietary treatment was computed by summing % digestible CP, % digestible crude fiber (CF), % digestible nitrogen free extract (NFE) and % digestible ether extract (EE) multiplied by a factor of 2.25. The TDN value of the dietary treatments was used to derive metabolizable energy (ME) using the relation of 1 kg TDN is equal to 3616 Kcal ME.

Carcass evaluation

At the end of digestibility trial, all experimental rams were taken to Abergelle International Export Abattoir and slaughtered after overnight fasting for evaluation of carcass parameters using a standard slaughtering method. Slaughter weight (SW) was taken immediately before slaughter. Hot carcass weight was measured after removal of blood, skin, head, feet (legs below the hock and knee joints), gastrointestinal tract and internal organs. Liver, bile without gallbladder, heart, heart fat, reticulo-rumen, abomasum-omasum, testicles, kidney, omental and kidney fats, tongue, tail, small and large intestines were weighed, and recorded as total edible offals while blood, head without tongue, skin with feet, lungs, trachea and esophagus, penis, gallbladder without bile, spleen,

pancreases and gut content were weighed, and recorded as total non-edible offals. Empty body weight was calculated as the difference between SW and gut content. Dressing percentage was determined as proportion of hot carcass weight to SW.

The ribeye area is determined by measuring area of the *Longissimus dorsi* muscle exposed by cutting or "ribbing" the carcass between the 12th and 13th ribs (O'Rourke et al., 2004). The backfat which is the most common measure of subcutaneous fat on a carcass is measured at a point ¾ of length of ribeye (*Longissimus dorsi*) muscle from the split chine bone, perpendicular to the surface fat, at the 12th rib (Williams, 2002).

Chemical analysis

Chemical analysis of representative samples of feed (hay and supplements) offer, hay refusals, and feces for dry matter (DM), Ash, crude fiber (CF), ether extract (EE) and nitrogen (N) content were analyzed using the procedures outlined by AOAC (2005). The N content was determined by Kjeldahl method and then the CP was calculated by N content multiplied by 6.25. The organic matter (OM) was calculated by subtracting total ash content from DM content of a feed sample while NFE was computed by subtracting sum of CP, CF, EE and total ash contents from DM content of a feed sample. Neutral detergent fiber (NDFom), acid detergent fiber (ADFom) and acid detergent lignin (ADL) of the samples were analyzed according to method of Van Soest et al. (1991). Sulfite and Alpha-amylase were not applied as reagents in the analysis of NDFom. Pre - experiment analysis for DM and CP contents of DMLM, WB and NSC were done at Mekelle soil laboratory research center and the remaining all other parameters were analyzed at Haramaya University Animal nutrition laboratory.

Partial budget analysis

The partial budget analysis was performed to determine cost benefit analysis of substitution of DMLM for CM as supplement to Abergelle sheep fed a basal diet grass hay. At the end of experiment, selling prices of each experimental ram were estimated. Variable costs included feed costs for both hay and supplement consumed. Total return (TR) was determined by subtracting purchasing prices of experimental rams from their estimated selling prices. Net return (NR) was calculated as the difference between TR and total variable costs (TVC) while change in net return (Δ NR) was calculated as difference between change in total return (Δ TR) and change in total variable costs (Δ TVC).

Statistical analysis

The data obtained from the experiment were subjected to analysis of variance (ANOVA) using the general linear model procedure of SAS version 9.2 (SAS, 2008). Significant treatment means were compared using Tukey's studentized range (HSD) test. The statistical model used for the data analysis was:

$$Y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij}$$

where Y_{ij} = response variable; μ = overall mean; τ_i = effect of treatment i ;

β_j = effect of block j and ϵ_{ij} = random error.

RESULTS AND DISCUSSION

Chemical composition of experimental feeds

The hay refusals had lower CP and relatively higher NDF,

Table 1. Chemical composition of experimental feeds and hay refusals.

Experimental feeds	DM (g/kg)	Chemical composition DM (g/kg)						
		CP	Ash	NDF	ADF	ADL	CF	EE
Dried mulberry leaf	943.0	202.0	120.6	329.7	230.6	44.0	118.6	40.2
Wheat bran	924.0	168.0	63.0	483.1	143.2	34.0	121.1	44.1
Noug seed cake	945.0	334.0	82.9	365.4	262.0	104.0	181.7	88.7
Grass hay offers	945.7	72.0	84.4	748.6	509.8	113.6	385.5	14.0
100% CM	928.0	200.3	66.8	410.4	176.0	47.5	132.8	52.7
75% CM + 25% DMLM	931.7	200.1	80.2	388.1	169.8	46.4	129.0	49.4
50% CM + 50% DMLM	935.4	200.4	93.6	365.7	170.1	44.8	125.3	46.2
25% CM + 75% DMLM	939.1	200.0	107.0	353.4	174.0	44.2	121.6	42.9
Grass hay refusals	946.1	49.7	63.2	767.7	536.3	128.9	435.0	12.2

CM = concentrate mix (the CM contained noug seed cake and wheat bran at ratio of 19.28: 80.72, 14.14: 60.86, 9.04: 40.96 and 3.92: 21.08 for 100% CM, 75% CM, 50% CM and 25% CM, respectively); DMLM = dried mulberry leaf meal; DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL= acid detergent lignin; CF = crude fiber and EE = ether extract.

ADF and ADL contents than hay offers (Table 1). The CP content of grass hay in the present study was similar to the CP value of 73 g/kg DM reported by Gebru (2015) for native hay. Moreover, it was very close to border-line level of 60- 70 g/kg DM CP required to create an appropriate rumen environment to promote DM digestibility and intake (Yami, 2008). The CP content of DMLM in this study was comparable with previously reported values of 201 g/kg DM (Kandylis et al., 2009) and 203 g/kg DM (Vu et al., 2011). The CP content of WB in the present study was similar with values of 168 g/kg DM (Tolera, 2008) and 168.2 g/kg DM (Hagos and Melaku, 2009). The CP content of NSC in the present study was comparable to value of 331 g/kg DM noted in earlier study (Abraham et al., 2015). Tolera (2008) indicated that CP and fat content of NSC varies depending upon the method and efficiency of oil extraction from the noug seeds.

The NDF and ADF contents of the grass hay in the present study were within the ranges of 731 – 772.5 and 429.9 – 537.6 g/kg DM, respectively reported by Tesfay et al. (2009) for different grass species when harvested at mid-September. The grass hay nutritive value declined with advanced stage of harvesting and in general grasses should be harvested for hay making immediately before or at the beginning of flowering (Tolera, 2008). The NDF content of DMLM was similar to value of 324 g/kg DM reported by Vu et al. (2011) while its ADF content was within the range of 186.7 – 246.7 g/kg DM reported by Kabi and Bareeba (2008) for mulberry leaves. The ADL of DMLM in the present study was nearly similar with previous findings of 41 g/kg DM (Kandylis et al., 2009) and 46.5 g/kg DM (Atiso et al., 2012). As reported by Sanchez (2000) the chemical composition of mulberry leaves varies depending on variety, harvesting stage and growing conditions. Comparable to the NDF content of WB in the present study, values of 484 g/kg DM by Gebeyew et al. (2015) and 480.1 g/kg DM by Gebru et al.

(2015) were documented. The ADF content of WB in this study was greater than the value of 105 g/kg DM reported in earlier study (Megersa et al., 2013). It has been noted that fiber content of WB may vary depending upon the quality of wheat being milled and the exact processing method used (Tolera, 2008). In consistent with the NDF content of NSC in this study, values of 365 g/kg DM by Kebede (2014), 363 g/kg DM by Girma et al. (2014) and 367 g/kg DM by Abraham et al. (2015) were reported. Comparable to ADF content of NSC in the present study values of 264 g/kg DM by Asmare et al. (2010) and 261 g/kg DM by Nurfetaa et al. (2013) have been reported.

Dry matter and nutrient intake

All the quantities of supplements offered to the experimental rams were completely consumed without any refusals. It is observed that offering of dried mulberry leaves as meal form avoided selection intakes of the leaves and also facilitated quick consumption. There was significance differences ($p < 0.05$) for hay and total DM intakes between T1 and T3 (Table 2). The reason for the difference in hay and total DM intakes between T1 and T3 might be due to the higher DM digestibility observed in T3 than T1 because in ruminants there is a positive relationship between the digestibility of feeds and their intake (McDonald et al., 2010). In consistence with the present study, Atiso et al. (2012) reported increasing total DM intake with substitution of 50% of mulberry leaves for concentrate compared to sole CM supplementation in dairy cows. The OM, CP, NDF, ADF and ADL intakes of experimental rams were similar among treatments.

The daily CP intake of Abergelle rams in the present experiment was higher than the CP intakes of sheep in many other studies (Gebru, 2012; Hadgu, 2014; Teklehaymanot, 2015). The higher CP intake of Abergelle rams observed in the present study might be due to the

Table 2. Daily dry matter and nutrient intakes of Abergelle rams supplemented with graded levels of dried mulberry leaf meal and concentrate mix.

Intake (g/day/head)	Treatments				SEM	SL
	T1	T2	T3	T4		
Hay DM	449.99 ^b	462.87 ^{ab}	482.21 ^a	465.90 ^{ab}	6.90	*
Supplement DM	469.96	476.32	479.25	478.12	4.39	ns
Total DM	919.95 ^b	939.19 ^{ab}	961.46 ^a	944.03 ^{ab}	9.23	*
OM	847.09	858.84	873.30	850.26	8.60	ns
CP	130.20	131.91	133.51	132.61	1.07	ns
NDF	526.59	528.59	533.90	514.78	6.06	ns
ADF	309.45	313.61	322.06	316.98	3.90	ns
ADL	69.21	70.96	73.14	70.11	0.97	ns

^{abc} = mean in the same row with different superscript differ significantly; * = p<0.05; ns= non-significant; T1 = ad libitum natural grass hay (NGH) + 100% CM; T2 = ad libitum NGH + 75% CM + 25% DMLM; T3 = ad libitum NGH + 50% CM + 50% DMLM; T4 = ad libitum NGH + 25% CM + 75% DMLM; DM = dry matter; OM = organic matter ; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; SEM = standard error of mean and SL = significance level.

Table 3. Apparent dry matter and nutrient digestibility coefficient of graded levels of dried mulberry leaf meal and concentrate mix supplemented for Abergelle rams.

Digestibility coefficient	Treatments				SEM	SL
	T1	T2	T3	T4		
DM	0.693 ^b	0.700 ^{ab}	0.702 ^a	0.700 ^{ab}	0.18	**
OM	0.70	0.70	0.71	0.71	0.18	ns
CP	0.76	0.76	0.76	0.76	0.14	ns
NDF	0.58	0.59	0.60	0.59	0.47	ns
ADF	0.56	0.56	0.57	0.57	0.65	ns
Calculated ME (MJ/day)	10.95 ^{ab}	11.00 ^a	11.01 ^a	10.87 ^b	5.35	**

^{abc} = mean in the same row with different superscript differ significantly; ** = p<0.01; ns= non-significant; T1 = ad libitum natural grass hay (NGH) + 100% CM (control); T2 = ad libitum NGH + 75% CM + 25% DMLM; T3 = ad libitum NGH + 50% CM + 50% DMLM; T4 = ad libitum NGH + 25% CM + 75% DMLM; DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ME= metabolizable energy; SEM = standard error of mean and SL = significance level.

amount of supplements were prepared according the daily CP requirement of each ram and they were also changed fortnightly based on the change of body weight, and the high digestibility of CP.

Apparent dry matter and nutrient digestibility

The DM digestibility coefficient was significantly different (p<0.01) between T1 and T3 with higher value for T3 but similar in T2, T3 and T4 (Table 3). The lower DM digestibility observed in T1 compared to T3 might be associated with the relatively higher NDF content of both WB and NSC, and greater ADL concentration of NSC existed in the supplement of T1 as the fibre fraction of a feed has the greatest influence on its digestibility (McDonald et al., 2010). Moreover, McDonald et al. (2010) also indicated that the digestibility of a feed is

influenced by the composition of other feeds consumed with it and the associative effects could be negative or positive. Comparable values to DM digestibility coefficient in the present study have been reported for dried mulberry leaves partially substituted lucerne hay and concentrates in Karagouniko sheep breed by Kandyliis et al. (2009) and for graded levels of dried mulberry leaves and concentrate mix in Arsi-Bale goats by Worku (2015).

The OM, CP, NDF and ADF digestibility coefficients were insignificant (p>0.05) among dietary treatments. Similar to OM digestibility coefficient in this study, value of 0.70 for mature indigenous Malin rams supplemented at 1.2% of body weight with mixture of mulberry foliage (50%) and urea-rice bran (50%) has been noted (Yulistiani et al., 2014). In agreement to the present study Worku (2015) reported CP digestibility coefficient of 0.75 – 0.77 for dried mulberry leaves included at different levels in concentrate for Arsi- Bale goats.

Table 4. Body weight gain and feed conversion efficiency of Abergelle rams supplemented with graded levels of dried mulberry leaf meal and concentrate mix.

Parameters	Treatments				SEM	SL
	T1	T2	T3	T4		
Initial body weight (kg)	17.50	17.52	17.53	17.52	0.171	ns
Final body weight (kg)	22.98	23.70	23.9	23.75	0.476	ns
ADG (g/day)	60.93	68.70	70.74	69.26	5.019	ns
FCE (g weight gain /g DM intake)	0.066	0.073	0.074	0.073	0.005	ns

T1 = ad libitum natural grass hay (NGH) + 100% CM; T2 = ad libitum NGH + 75% CM + 25% DMLM; T3 = ad libitum NGH + 50% CM + 50% DMLM; T4 = ad libitum NGH + 25% CM + 75% DMLM; ADG = average daily gain; FCE= Feed conversion efficiency; SEM = standard error of mean; SL = significance level and ns = non-significant.

Table 5. Carcass characteristics of Abergelle rams supplemented with graded levels of dried mulberry leaf meal and concentrate mix.

Parameters	Treatments				SEM	SL
	T1	T2	T3	T4		
SW (kg)	22.83	23.57	23.77	23.62	0.469	ns
Empty body weight (kg)	18.78	19.35	19.47	19.30	0.397	ns
Hot carcass (kg)	9.52	9.86	10.01	9.88	0.202	ns
Dressing percentage based on SW (%)	41.70 ^b	41.83 ^b	42.11 ^a	41.85 ^b	0.052	***
Ribeye area (cm ²)	8.43	8.92	8.98	8.93	0.206	ns
Back fat thickness (mm)	2.82	2.87	2.92	2.88	0.075	ns
TEOs (g)	3032.88	3096.68	3155.07	3100.38	45.488	ns
TNEOs (g)	10004.20	10321.83	10337.88	10333.28	224.151	ns

^{ab} = mean in the same row with different superscript differ significantly; *** = P<0.0001; ns = non significant; T1 = ad libitum natural grass hay (NGH) + 100% CM; T2 = ad libitum NGH + 75% CM + 25% DMLM; T3 = ad libitum NGH + 50% CM + 50% DMLM; T4 = ad libitum NGH + 25% CM + 75% DMLM; SW = Slaughter weight; TEOs = total edible offal's; TNEOs = total non-edible offal's; SEM = standard error of mean and SL = significance level.

The calculated ME were lower ($p < 0.01$) in T4 than T2 and T3 but similar in T1, T2 and T3. Ranjhan (2004) recommended that 0.35 kg TDN for daily maintenance requirement of Indian sheep of 30 kg body weight as well as 0.32, 0.40 and 0.51 kg TDN for daily growth requirement of Indian lambs of 15, 20 and 25 kg body weight, respectively. According to recommendation of the above author both maintenance and growth ME requirements for T1, T2, T3 and T4 are 9.10, 9.26, 9.31 and 9.29 MJ, respectively. However, the actually used ME of Abergelle rams in the present study exhibited an increment of 20.32, 18.79, 18.25 and 17.09% compared to the expected total ME requirements of maintenance and growth for T1, T2, T3 and T4, respectively and this difference might be due to variations in breed, environment and other factors.

Body weight gain and feed conversion efficiency

There was no significant difference among treatments for final body weight, ADG and FCE (Table 4). Similar to the present study, insignificant results for final body weight, ADG and FCE were reported by Worku (2015) for Arsi-bale goats when supplemented with inclusion of graded

levels of dried mulberry leaf in CM consisted of WB and NSC. The ADG of Abergelle rams in the present experiment was higher than ADG of 55 g reported by Liu et al. (2001) for growing sheep of the Huzhou breed supplemented with 240 g air dried mulberry leaves but lower than ADG of 76.2 g and 86.2 g (Gonzalez and Milera, 2000); 121 g (Miller et al., 2005) and between 80 g and 90 g (Martin et al., 2014) were reported in previous studies for goat breeds supplemented with mulberry leaves. The FCE of Abergelle rams obtained in the present study was similar to previously reported values for Tigray highland sheep with supplementation of 200 g WB and 400 g air dried *Acacia saligna* leaves (Gebru, 2012) and with supplementation of 306 g sole WB (Berihe et al., 2014).

Carcass characteristics

There was no significant difference ($p > 0.05$) among treatments for SW, empty body weight, hot carcass, ribeye area, backfat thickness, total edible offal's and total non-edible offal's but T3 had significantly higher ($p < 0.001$) dressing percentage based on SW than T1, T2 and T4 (Table 5). Except for the dressing percentage

Table 6. Partial budget analysis for Abergelle rams supplemented with graded levels of dried mulberry leaf meal and concentrate mix.

Parameters	Treatments			
	T1	T2	T3	T4
Number of rams	6	6	6	6
Purchasing price of ram (ETB/head)	430.00	430.00	430.00	430.00
Total hay consumed (kg/head)	42.83	44.05	45.90	44.35
Total wheat bran consumed (kg/head)	36.95	28.17	19.12	9.89
Total noug seed cake consumed (kg/head)	8.63	6.40	4.13	1.80
Total dried mulberry leaf meal consumed (kg/head)	0.00	12.16	22.87	34.13
Total feed consumed as fed-basis (kg/head)	88.41	90.78	92.02	90.17
Cost for hay (ETB/head)	53.54	55.06	57.37	55.44
Cost for wheat bran (ETB/head)	155.19	118.31	80.3	41.54
Cost for noug seed cake (ETB/head)	56.10	41.60	26.85	11.70
Cost for dried mulberry leaf meal (ETB/head)	0.00	30.40	57.18	85.33
Total cost of supplement (ETB/head)	211.29	190.31	164.33	138.57
Total feed Cost or TVC (ETB/head)	264.83	245.37	221.70	194.01
Selling price of ram /Gross return (ETB/head)	930.83	960.83	979.17	962.50
Total return (ETB/head)	500.83	530.83	549.17	532.50
Net return (ETB/head)	236.00	285.46	327.47	338.49
Δ TR	-	30	48.34	31.67
Δ TVC	-	-19.46	-43.13	-70.82
Δ NR	-	49.46	91.47	102.49

T1 = ad libitum natural grass hay (NGH) + 100% CM; T2 = ad libitum NGH + 75% CM + 25% DMLM; T3 = ad libitum NGH + 50% CM + 50% DMLM; T4 = ad libitum NGH + 25% CM + 75% DMLM; TVC = total variable costs; Δ TR = change in total return; Δ TVC change in total variable costs and Δ NR = change in net return.

based on SW, the present study result was consistent with the finding of Worku (2015) for SW, empty body weight, hot carcass, RER, total edible offal's and total non-edible offal's for Arsi-bale goats supplemented with graded levels of dried mulberry leaf substituted portions of CM. The difference for dressing percentage based on SW among T3 and T1, T2 and T4 might be due to the numerically higher hot carcass weight score for T3 compared to the rest treatments, and also influence of other factors such as gut content and weight of head. It has been noted that SW is positively correlated with hot carcass weight (Jaramillo et al., 2012).

The ribeye area in the present study was within the range of 8 - 9.4 cm² reported by Hagos and Melaku (2009) for Afar rams. Ribeye area is affected by the weight and muscularity of the live animal (O'Rourke et al., 2004) and it is increased with carcass weight (Park et al., 2002). The backfat thickness in the present study was slightly lower but within range of 2.8 - 7 mm reported by Degu et al. (2009) for Tigray highland sheep fed cactus (*Opuntia ficus-indica*)-teff straw and supplemented with isonitrogenous oil seed cakes. The USDA yield grades categorized lamb carcass into five groups with yield grade one having highest expected yield of trimmed retail cuts and backfat thickness \leq 0.15 inch (3.83 mm), and yield grade five with the lowest yield and \geq 0.46 inch (11.73 mm) backfat thickness (O'Rourke et al., 2004).

According to the USDA yield grades, the value of backfat thickness in the present study was classified under the corresponding backfat thickness for yield grade one.

Partial budget analysis

The result of partial budget analysis as shown in Table 6, revealed that total feed cost reduced with increased amounts of DMLM substitution for CM. Substitution of DMLM for CM minimized total feed cost by 7.35, 16.29 and 26.74% and CM cost by 9.93, 22.23 and 34.42% for T2, T3, and T4, respectively as compared to T1. The net return and change of net return (Δ NR) were linearly increased with increased levels of substitution of DMLM for CM. The Δ NR exhibited a profit of 20.96, 38.76 and 43.43% for T2, T3 and T4 compared to T1, respectively. The increment in net return and Δ NR as the levels of substitution of DMLM for CM increased could be partly attributed to the differences in selling price of the rams but mainly due to lower cost of DMLM as compared to CM.

Conclusion

All the dietary treatments provided comparable results in

terms of growth performance, most nutritional and most carcass parameters measured. However, the net return and Δ NR parameters in the partial budget analysis showed that T4 had highest economical gain. Therefore, substitution of 75% DMLM for CM contained WB and NSC is recommended on both biologically and economically efficient supplementary regime for Abergelle sheep reared by smallholder farmers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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