Effect of *Ficus sur* fruits supplementation on rumen ammonia nitrogen, pH concentration, and blood profile of Hararghe highland sheep fed natural pasture hay basal diets

Diriba Diba^{a*}, Yoseph Mekasha^{b#}, Mengistu Urge^b, Adugna Tolera^c and Tesfaheywet Zeryehun^d ^a Wollega University, College of Agriculture and Natural Resources, Department of Animal Sciences; P.O.Box 395, Nekemte, Ethiopia; ^bHaramaya University, School of Animal and Range Sciences; P.O.Box 138, Dire Dawa, Ethiopia; ^cHawassa University, College of Agriculture, School of Animal and Range Sciences; P.O.Box 05; Hawassa, Ethiopia; [#]International Livestock Research Institute, P.O.Box 5689, Addis Ababa, Ethiopia

^{*} Corresponding author: <u>diriba.diba@yahoo.com</u>, <u>dnazerawi2010@gmail.com</u>

Abstract

The experiment was conducted for 90 days in Haramaya University to evaluate the rumen ammonia nitrogen, rumen pH concentration, and blood profile of Hararghe highland sheep consumed natural pasture hay (NPH) basal diet supplemented with varied proportions of ground Ficus sur fruits (FSF) and ground oats grain (OG) diets at isonitrogenous level provision of noug seed cake (NSC) (Gizotia Abysinica). The treatment diets were ad libitum natural pasture hay (control); 100%FSF:0%OG [100FSF]; 67%FSF:33%OG [67FSF]; 33%FSF:67%OG [33FSF]; 0%FSF:100%OG [0FSF]. The experiment was laid out as a Randomized complete block design. Four animals from each treatment were used to collect about 30-40ml of rumen fluid using stomach tube at 4 hours post feeding for ammonia nitrogen (NH3-N) and pH profile determination. Additional rumen fluid samples were taken at 0, 2, 4, 8, 12, and 24 hours post feeding for rumen fluid pH dynamics determination. About 10 ml of blood sample was taken from jugular vein of the sheep and RBC, WBC and hemoglobin concentration were determined by employing the Neubauer counting chamber. The packed cell volume (PCV) was determined by spinning blood field capillary tubes in a microhematocrit centrifuge and reading the value on heamatocrit reader (model AIC 1490). Sera components were determined using spectrophotometer and refractometer. The mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) were calculated from the PCV, Hb, and RBC count. The study indicated that feeding Ficus sur fruits (FSF) at varied proportion with ground oats grain (OG) resulted in nearly neutral rumen liquor pH (7.12-7.24), optimum NH₃-N concentration (84.7-85.7mg/l), and normal count of WBC ($\hat{1}1.5-12.7l^3/\mu l$), $\hat{RBC}(11.0-11.5l^6/\mu l)$, optimum levels of PCV (36.7-40.8), Hgb (11.8-13.3 g/dl), and sera metabolites, which all indicated healthy rumen and cardiovascular physiology. Hence, FSF can be used as a source of energy ingredient, in replacement for OG, as the present study proved that it has positive effect on rumen environment and blood profile of lambs.

Keywords: Ammonia nitrogen, blood profile, pH, rumen fluid, Hararghe highland sheep

Introduction

In multi-cellular organisms, different biological systems jointly function to help the organism perform normal physiological activities. For example, the digestive system provides soluble nutrients which are absorbed from small intestine into the hepatic portal vein of the cardiovascular systems (Breves and Wolffram, 2008; Barej et al., 1980) so that every cell at the periphery can get nutrients through the blood transport system. One of such nutrients is ammonia nitrogen (NH3-N). Consumption of diets containing excess nitrogen concentration, upon degradation, results in release of higher level of ammonia (McDonald et al., 2002). If too much ammonia is released within a short period, the ammonia level in the blood rise and become toxic and the pH in the rumen simultaneously rises to the extent that rumen ceases to function normally (Nauhaus, 2008).

Among many other roles, the blood functions in nutrient transport, protection against pathogens, and regulation of optimum conditions (temperature, pH and body water balance). The excess or deficiency of nutrients in the blood above or below the normal concentration level could be an indication of some nutritional disorders or abnormal hematological physiology (Zvonko Antunović et al., 2011; Ogbuewu et al., 2010; Van Saun, 2000).

In feed quality evaluation, some of the most common practices include laboratory chemical analysis (Cherney, 2000); intake (Romney and Gill, 2000); digestibility (Jones and Theodorou, 2000; Rymer, 2000; Tilley and Terry, 1963) and growth (Tamminga and Chen, 2000); rumen fermentation (Niwińska, 2009; Ørskov, 2000; Ørskov et al., 1980), and hematological profile of the animals (Harvey, 2006). When the feed is a non-conventional, which is not common in the diets of livestock, examination of its effect on rumen pH and level of blood constituents is important.

Ficus sur fruits (FSF) belongs to the genus *Ficus* that comprises about 750 species, with about 100 species in Africa, 500 species in tropical Asia and Australia, and 150 species in tropical America (Lumbile & Mogotsi, 2008). The species in Ethiopia is named as *Ficus sur* (Cv. Forssk.) or commonly named as fig. It is widely distributed in almost all National Regional States of the country. In Ethiopia, the FSFs have been traditionally used as food and feed for centuries by man and livestock, respectively, in different parts of the country. During dry seasons of severe feed shortage where crop residues and aftermath are the main feed sources for livestock, the ripen fruits drop from the trees by wind and all classes of livestock freely access with no restriction. During this time, FSF become important natural concentrate supplements for livestock and many lambs and kids grow fast and reach market weight in few weeks.

Though small ruminants like sheep usually consume FSF in many parts of Ethiopia, its impact on their rumen ammonia, pH concentration, and blood profile was not yet studied and no scientific information was available. Therefore, this study was conducted to evaluate the effect of ground

FSF mixed with ground oats grain (OG) diet on the rumen ammonia nitrogen, rumen pH concentration, and blood profile of Hararghe highland sheep.

Materials and Methods

Study site

The feeding trial on the sheep was conducted in Haramaya University sheep farm, which is located at 9° 26'N latitude and $42^{\circ}3$ 'E longitude in eastern Ethiopia. The altitude of the area is about 1980 meters above sea levels and the mean annual rainfall is about 910 mm with a range of 560-1260 mm. The mean maximum and minimum temperatures are 23.4°C and 8.25°C, respectively (summarized report from Haramaya University Meteorological Station, 2012).

Management of the experimental animals

Intact male yearling lambs with average initial live weight of 13.62 kg were purchased from Kulubi local market. The animals were carefully transported on lorry to Haramaya University and quarantined for 3-weeks. During quarantine period, they were sprayed with accaricides (*diazinone*) to control ticks; treated with *ivermectin* injection against internal and some external parasites, and penistrep against Pneumonia disease. The animals were ear-tagged for ease of identification. Then they were assigned to individual pen arranged in a block based on the animals' initial live weight. The animals were handled with great care that assured their welfare until the end of the experiment.

Experimental diets and feeding management

Naturally ripen and dry FSF were collected from fig trees in Horro district, packed in clean sacks and taken to Haramaya University sheep farm. The fruits were further sun dried for three to four days depending on the weather condition (sunny or cloudy) to ensure grinding of the feed in conventional flour mill. The OG was purchased from Sheno town of North Shoa zone of Oromia National Regional State, ground in similar mill with the same particle size and taken to the sheep farm where FSF were stored. These two feeds (FSF and OG) were used as energy supplement being mixed at different proportions while noug seed cake (NSC) was given to all experimental animals as isonitrogenous protein supplement. Medium quality natural pasture hay, basal diet, was offered to the animals *ad libitum* at a refusal rate of 20%. Sheep were adapted to the treatment diets for two weeks. After completion of adaptation period, they were offered measured quantity of dietary treatments twice a day at 8:00pm and 16:00am in equal proportion. Lambs in each block were randomly assigned to one of the five diets. The animals were housed in individual pens furnished with feeder and watering bucket. Clean tap water was provided to the animals and it was changed whenever it is contaminated with feeces or other material not

appealing to the animal. The amount of supplement and basal diet offered and refused was measured and recorded every day before the next day's feed. The basal diet, natural pasture hay, was adjusted every 3-days to ensure 20% refusal. The live weight change of the animals was recorded every fortnight.

Dietary treatments and experimental design

The dietary treatments used in the experiments are *ad libitum* natural pasture hay (control); 100% *Ficus sur* fruits (FSF) and 0% oat grain (OG), which was represented as [100FSF]; 67%FSF:33%OG [67FSF]; 33%FSF:67%OG [33FSF]; 0%FSF:0FSF [0FSF]. Noug seed cake (NSC) was given to all animals with the purpose to fulfill at least the protein maintenance requirement of the control animal (at isonitrogenous level). The experiment was laid out in a randomized complete block design (RCBD) with five treatments and 6 replications each. The animals were grouped into six blocks based on their initial body weight and the five treatment diets were randomly distributed to the animals within each block.

^	Dietary proportions (DM basis)					
Ingredients (g)	Control	100FSF	67FSF	33FSF	0FSF	
Ficus sur fruits	0	300	201	99	0	
Oats grain	0	0	99	201	300	
Noug seed cake	225	210	190	170	150	
Nutrient composition of d	iets (%)					
Dry matter	91.8	91.4	91.4	91.4	91.4	
Ash	9.3	8.1	7.3	6.9	5.6	
Crude protein	15.4	15.4	15.4	15.4	15.4	
Neutral detergent fiber	58.3	33.7	35.2	36.8	38.6	
Acid detergent fiber	41.1	22.6	23.1	23.7	24.3	
Hemicelluloses	17.1	11.1	12.0	13.1	14.3	
Cellulose	33.6	17.2	17.7	18.3	18.8	
Acid detergent lignin	7.6	5.4	5.4	5.5	5.5	
ME calculated (MJ/kg	8.6	10.4	10.2	9.9	9.6	
DM)						

FSF= *Ficus sur* fruits; ME= metabolizable energy; DM= dry matter *Natural pasture hay was offered *ad libitum* whereas noug seed cake was given to make the diets of all animals isonitrogenous. Control= *ad libitum* natural pasture hay supplemented with noug cake; 100FSF = 100% FSF with 0% oats grain; 67FSF= 67% FSF with 33% oats grain; 33FSF= 33% FSF with 67% oats grain; 0FSF= 0% FSF with 100% oats grain

Laboratory analysis of experimental diets

The laboratory analysis of the experimental diets was performed in Haramaya University Animal Nutrition laboratory. The feed samples were taken each day and pooled over the experimental period in separate bag. At the end of the feeding trial, the bulked feed was thoroughly mixed and ample sample was taken for dry matter determination and chemical analysis.

After the samples were partially dried in forced draft oven at 65°C for 48 hours, it was ground to pass 1mm Wiley mill sieve size put in crucible and labeled. The chemical analysis for each sample was run in duplicates. When the results of the two replicates were not similar, the mean result was taken provided that the result of the two replicates did not vary by more than 5%. The DM and ash contents of the feed samples were determined following the procedure of AOAC (1995). The NDF, ADF, and ADL were determined based on the method described by Vansoest and Robertson (1985). Hemicelluloses and cellulose were calculated as NDF-ADF and ADF-(ADL+ADF ash), respectively. The ME (MJ/kg) of the diets was estimated according to Moran (2005). The N content of the samples was determined by the micro-Kjeldahl method and CP was calculated as N X 6.25.

Rumen fluid collection and analysis

The sample for rumen fluid was taken after the animals consumed the treatment diets for 90 days/period. Four animals from each treatment were randomly taken for collection of rumen liquor. About 30-40ml of rumen fluid was collected using stomach tube at 4 hours post feeding. The pH reading at 0, 2, 4, 8, 12, and 24 hours was taken immediately after the fluid was withdrawn from the rumen using Demetra model PM 53D portable pH meter. The rumen fluid was strained through double layers of cheesecloth and transferred into clean plastic bottles containing 10 ml sulfuric acid and stored in deep freeze at -20°C until used for rumen ammonia nitrogen analysis following the Kjeldahl procedure (ILRI, 1997).

Blood count, PCV determination, and sera biochemical analysis

Before taking about 10 ml of blood sample from jugular vein of the sheep, the animals were fasted for eight hours (Theml et al., 2004). Heparinized vacutainer tube was used to collect blood to prevent coagulation during sample collection. Half of the blood was used for the determination of RBC and WBC according to the Neubauer counting chamber (Jain, 1986; Bassert and McCurnin, 1985) and hemoglobin concentration according to Harvey (2001). Smears for differential leucocyte counts were stained by the Leishman technique, and the different cells of leucocyte series were enumerated by the longitudinal counting method. The other half of the blood was used to determine packed cell volume (PCV) and serum nutrient compositions. For PCV determination, four disposable capillary tubes of about 75mm length and

1.07-1.24 mm diameter was used to fill with about 50µm blood samples (WHO, 2000). After filling the samples a pliable sealing compound was used to close one end of the tube to prevent blood flow. A microhaematocrit centrifuge with 8cm radius was used to agitate the specimen in capillary tubes at 10,000 rpm for 5 minute. Then the PCV was read against PCV tube reader, model AIC 1490 and recorded. After blood for PCV determination was taken, the remaining blood was centrifuged in RCF K40R centrifuge to separate the plasma, which was then collected into separate plain tube and sealed and stored in deep freeze at a temperature of -20°C until it was used for analysis of urea by the urease–Berthelot method (Coles, 1986), glucose by Folin and Wu approach (Ullman et al., 1992), creatinine according to Henry (1974), globulin and cholesterol by spectrophotometer (Braham and Trinder, 1972; Merck, 1974; Doumas et al., 1981) and total protein concentrations according to Merck (1974) and Doumas et al. (1981).

Statistical analysis

The experiment was laid out in a randomized complete block design (RCBD) with 5 treatments and 6 replications each. Initial body weight of the animals was used for blocking and animals with similar body weight were grouped together. The animals were randomly allocated to treatments independent of the block. The data collected on rumen ammonia, rumen liquor pH, blood counts, and serum metabolites were analyzed by General Linear Model (GLM) procedure of Statistical Analysis System, SAS (2008). When F-test declared the existence of significance, means were compared using Tukey honestly significant difference test at P<0.05. The model, yijk= $\mu +\tau i +\beta j + \epsilon ijk$, where, μ =overall mean of the population, τi = The ith treatment effect, β = The jth block effect, and ϵijk =random error associated with yij, was used for data analysis.

Results

Chemical composition of experimental diets

The chemical constituents of the experimental diets are presented in Table 1. The DM content of the experimental diets was similar. The 100FSF diet had higher Ash and calculated ME compared to 0FSF. The fiber constituents were higher in 100FSF than 0FSF.

Rumen ammonia nitrogen and pH concentration

Table 2 shows rumen liquor pH concentration and ammonia nitrogen (NH₃-N) profile. The animals fed with the control diet (diet with no FSF or OF) had highest (P<0.001) pH concentration compared to the rest of the treatment diets followed by the group supplemented with sole OG, which is higher than those supplemented with sole FSF. The pH observed for all treatment is a little above neutral, tending to be slightly alkaline (7.34). The NH₃-N content of the rumen fluid was not significantly differed (P>0.05) between the treatments and it is higher in magnitude in treatments supplemented with sole OG or combination of OG and FSF as

compared to the control and Sole FSF fed group. The pH slightly increased during the first two hours and declined thereafter, until it stabilizes at about eight hours in all treatments (Fig 1).

Table 2: Rumen pH concentration and NH3-N profile of sheep supplemented with mixture ofFSF and OG at different proportions (samples collected at 4 hours post feeding)

		1	± ,	1	-		0,
Rumen							
Parameters	control	100FSF	67FSF	33FSF	0FSF	SEM	SL
Rumen pH	7.34 ^a	7.12 ^c	7.14 ^{bc}	7.19 ^{bc}	7.24 ^b	0.022	***
NH3-N (mg/l)	84.6	84.7	85.3	85.5	85.7	0.441	Ns
_~				100505 100			~ -

FSF= Ficus sur fruits; NH₃N =Ammonia Nitrogen; 100FSF =100% FSF: 0%OG, 67FSF =67%FSF:33%OG; 33FSF =33%FSF:67%OG; 0FSF = 0%FSF:100%OG; SEM= Standard error of the mean; SL= significance level; ns= non-significant.

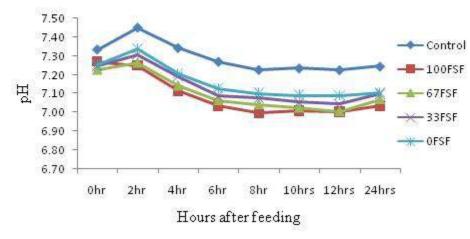


Fig 1: Effect of dietary treatments on rumen pH concentration dynamics of the sheep

Blood count

The analysis of variance indicated that white blood cell (WBC) did not statistically differ between treatments (P>0.05) (Table 3). Generally there was no clear trend in WBC count, being slightly higher in magnitude for the group fed the control diet. Red blood cell count is different among the treatments (P<0.001) and it is higher in 33FSF:67OG ratio than the group supplemented with either sole FSF or OG and 67FSF:33OG. Values for Hgb, MCV, and MCHC did not differ significantly (P>0.05) among treatments and there was no any trend in magnitude of the values. However, MCH is significantly higher in sole oat grain supplemented group than 33OG:67FSF and the control diet groups.

	-	Dietary treatments					
Blood parameters	control	100FSF	67FSF	33FSF	0FSF	SEM	SL
WBC (10 ³ /µl) RBC (10 ⁶ /µl)	12.8 13.2 ^{ab}	11.5 11.5 ^{bc}	11.7 12.0 ^{bc}	12.7 14.7 ^a	12.7 11.0 ^c	0.542 0.402	ns ***
Hgb (g/dl)	12.2	13.3	12.5	12.2	11.8	0.745	ns
PCV (%)	32.3	38.5	40.8	38.2	36.7	2.045	ns
MCV (fL)	24.5	33.5	34.0	26.0	33.4	2.708	ns
MCH (pg)	9.26 ^{bc}	11.7^{ab}	10.4^{abc}	8.33 [°]	12.6 ^a	0.069	**
MCHC (g/dl)	38.3	35.1	31.1	32.3	38.4	2.888	ns

Table 3: Blood counts of sheep fed different proportions of ground Ficus sur fruit and oat gra	ain
mixture	

FSF= Ficus sur fruits; SEM =Standard error of the mean; SL= significance level; ns= nonsignificant; WBC =white blood cells; RBC=red blood cells; Hgb= Hemoglobin; PCV= packed cell volume; MCV= mean corpuscular hemoglobin; MCHC =mean corpuscular hemoglobin concentration; 100FSF =100% FSF: 0%OG; 67FSF =67%FSF:33%OG, 33FSF = 33%FSF:67%OG; 0FSF = 0%FSF:100%OG;

Sera metabolites

The blood serum metabolites for the experimental animals as affected by varied proportions of ground Fig and MG mixed diets was presented in Table 4. There was no significant difference (P>0.05) between the treatments in sera metabolites.

Table 4: Blood serum metabolites of sheep fed different proportions of ground *Ficus sur* fruit and oat grain mixture

	Dietary treatments						
Serum metabolites	control	100FSF	67FSF	33FSF	0FSF	SEM	SL
Urea (mg/dl)	27.8	28.9	28.8	29.4	33.2	1.621	Ns
Creatinine (mg/dl)	1.29	1.33	1.37	1.37	1.38	0.032	Ns
Cholesterol (mg/dl)	58.3	55.2	59.8	62.7	59.8	2.915	Ns
Glucose (mg/dl)	59.0	61.0	63.5	59.7	61.3	2.419	Ns
Total protein (g/dl)	6.68	7.22	7.12	7.18	7.33	0.234	Ns
Albumin (g/dl)	3.65	3.95	3.77	3.83	3.77	0.111	Ns
Globulin (g/dl)	3.03	3.27	3.34	3.35	3.57	0.164	Ns
Albumin:Globulin	1.22	1.24	1.13	1.15	1.06	0.057	Ns

FSF= Ficus sur fruits; *SEM* =Standard error of the mean; *ns*= non-significant; *SL*= significance level; 100FSF =100% FSF: 0%OG; 67FSF =67% FSF:33%OG; 33FSF= 33% FSF:67%OG; 0FSF=0% FSF:100%OG.

Discussion

Chemical composition of experimental diets

All the four types of diets had almost similar values for DM. The OM content of the feeds was more influenced by its ash content. The higher ash content (7.15%) of FSF resulted in its ' relatively less OM content as compared to OG (3.7%). Nevertheless, the higher OM contained in OG does not necessarily mean higher availability of the nutrients to the animals. This is because the higher ADF composition in OG than in FSF can presumably reduce its digestibility (McDonald et al., 2002). Moreover, the higher NDF value in OG may influence voluntary feed intake and provide less nutrients for the proliferation of rumen microbes compared to FSF. This is because FSF has higher neutral detergent soluble fiber than OG that can support rumen microbes as sources of soluble carbon. In this regard, FSF could be important energy supplement than OG. This in turn influences the digestibility of the fibrous basal diet utilization. Moreover, the variations in nutrient composition and types of the diets may influence hematological profile of sheep (Abdollahzadeh and Abdulkarimi, 2012; Schaefer et al., 2009).

Rumen pH and ammonia nitrogen concentration

The highest pH concentration in control diets compared to the rest treatment diets may suggest that animals fed the control diet did not receive sufficient energy concentrate as readily soluble carbon supplements, since this group received only noug seed cake, which served as nitrogen source and is slightly alkaline in nature. This was not preferable for better microbial ecology (Owen and Goetsch, 1988). As a result, the rumen microbes may be less active and small proportion of the basal diet (natural pasture hay) might have been degraded to yield volatile fatty acids (VFA), which could have diluted the alkaline nature of ammonia released from protein supplement in the control diets (Bodine et al., 2000; Carey et al., 1993). The relatively lower pH values of rumen liquor in sheep consumed 100FSF was due to higher proportion of energy concentrate supplement, which contained less fiber portion compared to OG in 0FSF diet. The pH concentration in rumen of the sheep consumed 100FSF was nearly neutral, which is considered as important since it is conducive especially for fiber fermenting (cellulolytic) microorganisms (Owen and Goetsch, 1988). Relatively lower, but nearly neutral values (6.9-7.1) pH were reported by other researchers (Yoseph et al., 2003) for Menz sheep fed with natural pasture hay basal diet supplemented with different conventional protein sources, such as T. atella (traditional brewery residue), lentil hulls (L. culinaris), K. atella (traditional liquor residue), rough pea hulls (L. sativa) and field pea hulls (P. sativum). Baraka and Abdl-Rahman (2012) reported lower figures (6.4-6.6) than the current finding for sheep fed barley grain energy supplement to hay basal diet.

The nearly similar values of NH₃-N were probably due to the isonitrogenous levels of treatment diets fed to all sheep. The level of ammonia for all treatments in the present study was above the minimum level (50 mg/l) required to maximize microbial growth (Krebs et al., 2007) but lower than the amount (86.7-117.3 mg/l) reported by Baraka and Abdl-Rahman (2012) under in vitro evaluation of sheep rumen fermentation pattern. No sheep in the present study showed symptom of ammonia toxicity such as difficult breathing, rapid pulse, salvation, bloat, tremors, in coordination, staggering, and tetany (Nauhaus, 2008; Robson, 2007) indicating healthy conditions of the experimental diet. This might be due to reduction of the rumen fluid pH concentration from 2 hours to about 8hours post feeding (Nikkhah, 2011) after which it attained more or less stable trend for all treatments until 24 hours (Fig. 1) at nearly neutral reactions. The rise in pH concentration at early phase of degradation (from 0 to 2 hours), except in group supplemented with sole FSF, may be attributed to faster degradation of the protein supplement than the relatively longer lag phase (Ørskov et al., 1980) for fibrous natural pasture hay basal diet, which resulted in volatile fatty acids that might have reduced the pH during that phase. Rapid reduction in pH in group supplemented with sole FSF is attributed to the availability of soluble carbon to the microbes from the diet for ease of fiber fermentation.

Blood count

The values for WBC were all in the normal range $(11-22*10^3/\mu l)$ documented in literature (Radostits et al., 2006; Jackson and Cockcroft, 2002; Scott et al., 2006). White blood cell count above $22*.10^3/\mu l$ is known to be an indication of infection in sheep (Radostits et al., 2006). This indicates that the immunity of the animal is not affected by any of the supplement used in the present experiment.

The RBC counts in the present study was higher than the values $6.35-7.65*10^6/\mu$ l reported by Abdel et al. (2011) for sheep consumed Ca-Saponified Lemuru Oil Coated by Herbs and the 9.1- $10.7*10^6/\mu$ l reported by Astuti and Sudarman (2009) for urea treated or untreated groundnut hull supplemented with different protein source diets. Nevertheless, it was still in the normal range of RBC counts in healthy sheep $9-15*10^6/\mu$ l as documented by Jackson and Cockcroft (2007) indicating absence of any infection in the animal.

The overall Hgb concentration in this study (12.2-13.8 g/dl) was within the normal range (9-15 g/dl) described by Radostits et al. (2006) and Jackson and Cockcroft (2007), but less than the range (41.02-45.43g/dl) obtained by Addass et al. (2010) for different sheep breeds from different husbandry background. In contrary to the former report, Islam et al. (2005) obtained lower Hgb values (8.36-10.0 g/dl) for yearling sheep fed with diets containing different types of hematinics which improved the health of the animals. Hence, none of the experimental units fed with different experimental diets were suspected for anemia or parasitic case (Teleb et al., 2007) in the present study.

Compared to the present finding, relatively lower PCV values of 26.4 - 35.2% were obtained by Al-Saad et al. (2007) from their study on natural zinc deficiency in sheep with different age and sex. The authors reported that those sheep with PCV values of 26.4-28.4% were considered diseased. There was a controversy in literature in the value of PCV. Radostits et al. (2006) noted that PCV of 27% is the normal minimum value for sheep. The PCV value of an animal is affected by different factors, and lower PCV indicates that body has fewer red blood cells than normal. These include chronic kidney diseases, blood loss from hemodialysis, abnormally low levels of iron, vitamin B12, folic acid, species and age of animals (Egbe -Nwiyi et al., 2000). According to this, the PCV value obtained in the present experiment may be an optimum value and indicates no loss of blood and anemic case symptoms is occurred to the animals.

The mean cell volume (MCV) obtained for some of the treatments in the current study is somewhat lower compared to other findings (Radostits et al., 2006; Jackson and Cockcroft, 2007) who suggested 28-45 fl levels as normal value for sheep. Rise in MCV above 45 fl indicates liver and thyroid problems and deficiency of vitamin B12 and folate, and anaemia (Cox, 2009). Values lower than 28 fl shows existence of parasites, iron anemia, vitamin C deficiency, cobalt deficiency, low hydrochloric acid in their abomasums, rheumatoid arthritis, or lead toxicity (Hala et al., 2008; Saad and El_Sayed, 2014).

In both cases, the MCV value in the present study was free of hematological disorders, which were not observed in any of the experimental units in the present study. The MCH and MCHC are all in the optimum sera metabolites range (Radostits et al., 2006; Jackson and Cockcroft, 2007) recommended for different breeds of sheep at various husbandry conditions and no chronic hematological cases was observed.

Sera metabolites

Serum urea level is an indication of kidney function in lambs. The absence of significant variation in serum urea concentration among treatments was mainly due to equal level of dietary protein supplements. The least serum urea concentration in magnitude was recorded for sheep consumed control diets (natural pasture hay basal diet with noug seed cake protein supplement) while the highest was for those fed with 0FSF (100% OG). The reason for this was not clear yet. Similar range of results (19.5 mg/dl – 31.8 mg/dl) with the present study was reported by AL-Zghoul et al. (2008) for Awasi lambs kept in a closed farm and fed a regular fattening ration. The sera urea level in the present finding was in the range for normal renal physiology (17.12 – 42.8mg/dl) as documented by other researchers (Radostits et al., 2006). It can be considered that the kidney of all the experimental sheep is normal as far as the impact of sera urea concentration was concerned.

Creatinine level is an indicator of renal health status in animals. Some researchers (Zamiri and Rezaei-Roodbari, 2004) found a slightly lower creatinine concentration (1.02mg/dl to 1.13 mg/dl) for sheep consumed 50% alfalfa hay with 50% barley grain diets compared to the present study and no kidney problem was reported. In contrary to this, previous studies indicated that dehydration for two to six days in sheep kept on normal diets increased serum creatinine from 1.6 mg/dl to 4.9 mg/dl indicating some degree of muscle damage (Kataria and Kataria, 2007). Since increased level of creatinine is indicator of chronic renal dysfunction (Fartashvand et al., 2012), the concentration level found for sheep supplemented with the new feed resource, 100% FSF (1.33mg/dl) can be considered as the safest value.

The normal blood cholesterol concentration range 43-103 mg/dl in sheep of different age and breeds (Radostits et al., 2006). Different earlier reports confirmed the present finding (52-76 mg/dl, Landgraf et al., 1984); (53.7 – 64.1 mg/dl, Hatfield et al., 1998); (34.65-38.1 mg/dl, Khadem et al., 2007). However, Zamir and Rezaei-Roodbari, (2004) reported the lowest result (33.1- 40.63 mg/dl) for rams consumed alfalfa hay supplemented with barley grains. The Hararghe highland sheep used in the present study, therefore, had normal serum cholesterol level and one may not suspect any cardiac infarction or heart attack diseases (McDonald et al., 2002) that usually occurs in cases when the blood cholesterol rises beyond the normal level as a result of inclusion of mainly excess saturated fatty acids in their diets.

The sera total protein concentration for all treatments in the present study was similar, except for the control group. This could be an attribute of lack of soluble carbon as energy supplement in the diets of sheep received control diets. Soluble carbon supports optimum proliferation of rumen microbes, which ultimately contribute to microbial protein, which could be digested in the subsequent gastro intestinal tract and absorbed into blood system and increase blood protein concentration. The albumin, globulin, and their ratios (Albumin: Globulin) were all in the normal ranges (Jackson and Cockcroft, 2007) that indicated absence of parasitism, hepatic diseases, protein deficiency, starvation and malignancy. The result obtained rather indicates optimum proteinacious feed intake (Keser and Bilal T., 2008) and secured immune status.

Conclusion

The present study indicated that feeding *Ficus sur* fruits to the lambs could result in nearly neutral rumen liquor pH, optimum rumen NH₃-N concentration and normal count of WBC, RBC with optimum levels of PCV, Hgb and sera metabolites, which all indicated healthy rumen and cardiovascular physiology. Hence, the FSF can be used as sole supplement or as a replacement for OG as energy source where accessibility and cost of OG is a limiting factor, particularly in small holder sheep production system.

Acknowledgement

The authors are indebted to Dr Henock Ayalew and Dr Abdissa for their assistance in blood sample collection. Ms Jember and Meseret in facilitating and determination of some of the blood parameters are highly acknowledged. We acknowledge Haramaya University for allowing use of research facilities, and SIDA (Swedish International Development Agency) for providing the research fund.

Reference

- Abdel, A.A., Hameed, A.M., Salih, A.M. Fadel Elseed, E., Amasab, O., 2011. Effect of Feeding Untreated or Urea Treated Groundnut Hull Supplemented with Different Protein Sources on Blood Parameters of Sudan Desert Lambs; Online Journal of Animal and Feed Research 3, 40-46.
- Abdollahzadeh, F., Abdulkarimi R., 2012. The effects of some agricultural By-products on blood metabolites, chewing behavior and physical characteristics of dairy cow diets; Life Science Journal; 9, 270-274
- <u>Al-Saad</u>, K.M., <u>Al-Sadi</u> H.I., <u>Abdul-Majeed</u>, M.O., 2010. Clinical, Hematological, Biochemical and Pathological Studies on Zinc Deficiency (Hypozincemia) in Sheep; Veterinary Research 3, 14-20
- AL-Zghoul, M. B.F., AL-Rukibat, R. K., Talafha, A. Q., 2008. Cellular and Some Biochemical Changes in Blood and Peritoneal Fluid Constituents in Awassi Lambs Following Elective Castration; American Journal of Animal and Veterinary Sciences 3, 23-27
- AOAC., 1995. Official Methods of Analysis of AOAC International, 16th edition. Standard compendium of laboratory methods for analyzing foods and related substances; Gaithersburg, MD.
- Astuti, D.A., Sudarman, A., 2009. Physiological Status, Blood Profile and Body Composition of Sheep Fed with Ca-Saponified Lemuru Oil Coated by Herbs; Feed and Nutrition; the 1st International Seminar on Animal Industry; Bogor, Indonesia
- Baraka, T. A. M., Abdl-Rahman, M.A., 2012. *In vitro* Evaluation of Sheep Rumen Fermentation Pattern After Adding Different Levels of Eugenol – Fumaric acid Combinations; Vet. World; 5, 110-117

- Barej, W., Barbara, S., Jacek, G., Skolasinska K., 1980. The Depressive Action of Ammonium Chloride on the Hepatic Blood Flow in Sheep; Quarterly Journal of Experimental Physiology, 65, 99-104
- Bassert, J. M., McCurnin, D. M., 1985. Clinical Text Book for Veterinary Technicians, 7th eds., Jenkintown, PA; pp: 1456
- Bodine, T.N., Purvis, H.T., Cox, D.A., 2000. Effects of supplemental energy and Degradable Intake Protein on grazing behavior, forage intake, digestion and performance of steers grazing winter range; Animal Science Research Report, PP: 33-39
- Braham, D., Trinder, P.,1972. Methods for determination of blood glucose level by spectrophotometer, 97, 141-142
- Breves, G., Wolffram, S., 2008. Transport systems in the epithelia of the small and large intestines; In: Sejrsen K., T. Hvelplund and M.O. Nielsen (eds.); Ruminant physiology: Digestion, metabolism and impact of nutrition on gene expression, immunology and stress; Wageningen Academic Publishers, www.WageningenAcademic.com; Wageningen, the Netherlands; pp:139-189
- Brian, S. B., John, M.D., Koepke, A., Elkin, M.D., Simson, M.B., Ch.B., Med., M., Onno W. V., Assendelft, M.D., 2000. Procedure for Determining Packed Cell Volume by the Microhematocrit Method; Approved Standard-Third Edition; USA; Volume 20 Number 18
- Carey, D. A., Caton, J. S., Biondini, M., 1993 Influence of Energy Source on Forage Intake, Digestibility, In Situ Forage Degradation, and Ruminal Fermentation in Beef Steers Fed Medium-Quality Brome Hay; J. Anim. Sci. 71, 2260-2269
- Cherney, D.J.R., 2000. Characterization of Forages by Chemical Analysis. In: Givens D.I, E. Owen, R.F.E. Axford and H.A. Omed (eds.), Forage evaluation in ruminants nutrition. Wallingford, Oxon OX10 8DE; UK, pp: 281-300
- Cox, M., 2009. Interpreting Blood Tests and Investigations; Royal College of Nursing Conference PowerPoint presentations; pp: 1-44
- Coles, E. H., 1986. Veterinary Clinical Pathology. (4th eds.) Published, W.B. Saunders co. London, pp: 457.

- Doumas, B., Bayse, D.D., Carter, R.J., Peters, T., Schaffer, R.,1981. A candidate reference method for determination of total protein in serum. Clin. Chem., 27: 1642. 8th Ed. Edited by W.R. Faulkner and S.M. Mietes. Washington D.C., USA
- Egbe-Nwiyi T.N, Nwaosu, S.C. And Salami, H.A., 2000. Haematological Values of Appararently Healthy Sheep and Goats as Influenced by Age And Sex in Arid Zone Of Nigeria. Afr. J. Biomed. Res. 3, 109 115
- Fartashvand, M., Ghafour, M., Yaghoub, H., 2012. Gentamicin-Induced Nephrotoxicity in Adult Sheep; Advances in Bioresearch; 3, 116-120
- Hala A., Abou-Zeina A., Zaghawa A.A., Nasr S. M. and Keshta H.G.E., 2008. Effects of Dietary Cobalt Deficiency on Performance, Blood and Rumen Metabolites and Liver Pathology in Sheep. Global Veterinaria 2, 182-191
- Harvey, J. W., 2006. Erythrocyte Biochemistry; In: Douglas J. Weiss and K.Jane Wardrop (eds.), Schalm's Veterinary Hematology, (Six editions), pp: 131-135
- Harvey, J.W., 2001. Examination of blood samples; In: Atlas of veterinary hematology-blood and bone marrow of domestic animals; Philadelphia, PA, USA, pp: 3-41
- Hatfield, P. G., Hopkins, J. A., Ramsey, W. S., Gilmore, A., 1998. Effects of level of protein and type of molasses on digesta kinetics and blood metabolites in sheep, Small Ruminant Research 28, 161–170
- Henry, R. J., 1974. Clinical Chemistry: Principles and Technique (second editions.) Harper and Row,pp: 525
- International Livestock Research Institute (ILRI), 1997. Analytical methods for feeds, animal excrements and animal tissues. ILRI, Addis Ababa, Ethiopia. pp. 38-39.
- Islam, R. S., Rashid, M.H., Hossain, M. K, Rahman, M., 2005. Effects of Hematinics on Body Weight and Some Hematological Values in Sheep and Goats; International Journal of Agriculture & Biology; 7, 582–584
- Jackson, P.G.G., Cockcroft, P. D., 2007. Laboratory Reference Values: Haematology; Clinical Examination of Farm Animals; pp: 302, Oxford, UK
- Jain, N. C., 1986. Schalm's Veterinary Hematology. 4th ed. Lea and Febiger, Washington square, Philadelphia, USA.

- Jones, D.I.H., Theodorou, M.K., 2000. Enzyme Techniques for Estimating Digestibility; In: Givens D.I, E. Owen, R.F.E. Axford and H.A. Omed (eds.), Forage evaluation in ruminants nutrition. Wallingford, Oxon OX10 8DE; UK, PP: 155-173
- Kataria, N., Kataria A. K., 2007. Compartmental water management of Marwari sheep during dehydration and rehydration; Veterinarski Arhiv 77, 551-559
- Khadem, A.A., Soofizadeh, M., Afzalzadeh, A., 2007. Productivity, Blood Metabolites and Carcass Characteristics of Fattening Zandi Lambs Fed Sodium Bentonite Supplemented Total Mixed Rations; Pakistan Jownal of Biological Sciences 10, 3613-3619
- Krebs G. L., Howard, D. M., Dods, K., 2007. Feeding Acacia saligna to Sheep and Goats with or without the Addition of Urea or Polyethylene Glycol; Asian-Aust. J. Anim. Sci. 20, 1551-1556
- Landgraf, B. K., Peter K. F., Havstad, K. M., 1984. Utilization of leafy spurge (*Euphorbia esula*) by sheep, Weed Science. 32, 348-352
- Lumbile, A.U. & Mogotsi, K.K., 2008. *Ficus sur* Forssk. [Internet] Record from Protabase. In: Louppe, D., Oteng-Amoako, A.A. & Brink, M. (eds.). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands.
- Malone K. M., Hinman A. R. 2003. Vaccination Mandates: The public health imperative and individual rights; center for disease control and prevention; pp262-284
- McDonald I. W., 1958. The Utilization of Ammonia-Nitrogen by the Sheep; Sheep Biology Laboratory, Prospect, New South Wales. PP 46-51
- McDonald, P., Edwards, R. A, Greenhalgh, J. F. D., Morgan, C.A., 2002 Animal Nutrition: Microbial digestion in ruminants and other herbivores; 6th eds. Prentice Hall, London. PP: 179-195
- Merck, E., 1974. Clinical Laboratory Techniques. 11th Ed., 379 Dermasted, Federal Republic of Germany.
- Nauhaus, W.K., 2008. Urea poisoning, spotlight on Agriculture. Ministry of Agriculture, Water and Forestry, directorate of research and training, agricultural laboratory, PP: 1-4, Windhoek, Namibia.

- Nikkhah A., 2011. Eating Timing Regulates Post -Feeding Patterns of Rumen Ammonia and Blood Urea: A Dairy Cow Model; Veterinary Science Research; 2, 17-20
- Niwińska, B., 2009. Effect of carbohydrates in grass silage-based diets on *in sacco* ruminal degradability of barley (*Hordeum vulgare* L. *cv.* Lomerit) grain ground to different particle sizes; Czech J. Anim. Sci., 54, 260–269
- Ogbuewu, I.P., Uchegbu, M.C., Okoli, I.C., Iloeje, M.U., 2010. Assessment of Blood Chemistry, Weight Gain and Linear Body Measurements of Pre-Puberal Buck Rabbits fed Different Levels of Neem (*Azadirachta Indica* A. Juss.) Leaf Meals; Chilean Journal of Agricultural Research 70, 515-520
- Ørskov, E.R., 2000. The *In Situ* Technique for the Estimation of Forage Degradability in Ruminants; In: Givens D.I, E. Owen, R.F.E. Axford and H.A. Omed (eds.), Forage evaluation in ruminants nutrition. Wallingford, Oxon OX10 8DE; UK, PP: 175-189
- Ørskov, E R, DeB Hovell, F. D. Mould, F., 1980. The Use of the Nylon Bag Technique for the Evaluation of Feedstuffs; Paper first presented at the Third Annual Conference on Tropical Animal Production, Merida, Mexico; Trop Anim Prod. 5, 195-213
- Owen, F.N. and A.L. Goetsch. 1988. Ruminal Fermentation. In: The ruminant animal digestive physiology and nutrition; editions of D.C. Church. pp-172. Waveland press, USA.
- Radostits, O., M., Clive, G., Kenneth, W. H., Constable, P. D., 2006. Veterinary medicine. A textbook of the disease of cattle, sheep, goats, pigs and horses, 10th edition, ELSEVIER, PP: 2047-2050
- Robson, S., 2007. Nitrate and nitrite poisoning in livestock; Profitable and sustainable Primary Industries; www.dpi.nsw.gov.au; New South Wales, PP: 1-4; Australia
- Romney, D.L., Gill, M., 2000. Intake of Forages; In: Givens D.I, E. Owen, R.F.E. Axford and H.A. Omed (eds.), Forage evaluation in ruminants nutrition; UK, PP: 43-62
- Rymer C., 2000. The Measurement of Forage Digestib17ility *In Vivo*; In: Givens D.I, E. Owen, R.F.E. Axford and H.A. Omed (eds.), Forage evaluation in ruminants nutrition; UK, PP: 113-134
- Saad R.A., and El_Sayed M.H.,2014. Hemodynamic and cardiac functions in rats exposed to lead toxicity, the possible effect of vitamin C (ascorbic acid). Life Science Journal, 11, 167-179

- Schaefer, E., Joi, J., Gleason, A., Dansinger, M. L., 2009. Dietary Fructose and Glucose Differentially Affect Lipid and Glucose Homeostasis; J. Nutr., 139, 1257–1265.
- Scott, J.L., Ketheesan N., Summers P.M., 2006. Leucocyte population changes in the reproductive tract of the ewe in response to insemination. Reproduction, Fertility and Development, 18, 627–634
- Statistical Analysis System (SAS), 2008. The Little SAS® Book: A Primer, Fourth Edition, version 9.1.3. Statistical analysis system institute Inc., NC. USA.
- Tamminga, S., and Chen, X.B., 2000. Animal-based techniques for the estimation of protein value of Forages; In: Givens D.I, E. Owen, R.F.E. Axford and H.A. Omed (eds.), Forage evaluation in ruminants nutrition. Wallingford, Oxon OX10 8DE; UK, PP: 225-232
- Teleb D. F., Soliman, E. K. A., El-khalek, T.M.M, 2007. Effect of fascioliasis on hematological, serum biochemical and histopathological changes in sheep; Egyptian J. of Sheep and Goats Science, 2, 15 - 33
- Theml, H., Diem, H., Haferlach, T., 2004. Color Atlas of hematology. Second revised edition, PP: 208.
- Tilley, J. M. A., Terry, R. A., 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Brit. Grassland Soc. 18, 104-111.
- Van Soest, P.J., Robertson, J.B., 1985. Analysis of forages and fibrous feeds. Laboratory Manual for Animal Science 613. Cornell University Press, Ithaca, NY, pp: 202
- World Health Organization, WHO., 2000. Recommended method for the determination of packed cell volume by centrifugation; WHO/DIL/OO.2, pp: 1-7
- Ullman, J. E., Hjelmquist, H., Lundberg, J. M., 1992. Tolerance at haemorrhage during vasopressin antagonism and/or captopril treatment in conscious Sheep. Acta physiol Scand; 146, 457 465
- VanSaun, R. J., 2000. Blood Profiles as Indicators of Nutritional Status; Advances in Dairy Technology; (12) 401
- Van Soest, P.J., Robertson, J.B., 1985. Analysis of forages and fibrous feeds. Laboratory Manual for Animal Science 613, pp: 202 Cornell University Press, Ithaca, NY

- Zvonko, A., Ivica, M., Zdenko, S., Mensur, Novoselec, V. J., 2011 . Blood Metabolic Profile of The Dubrovnik Sheep: Croatian Endangered Breed; Macedonian Journal of Animal Science, 1(1) pp. 35–38
- Yoseph, M., Azage, T., Alemu, Y., Umunna, N. N., Nsahlai, I.V., 2003. Effect of supplementation of grass hay with non-conventional agro-industrial by-products on rumen fermentation characteristics and microbial nitrogen supply in rams. Small Ruminant Research 50, 141-151.
- Zamiri, M. J., Rezaei-Roodbari, A., 2004. Relationship between blood physiological attributes and carcass characteristics in iranian fat-tailed sheep; Iranian Journal of Science & Technology, Transaction A, 28 (A1).