Full Length Research

Haematology and serum biochemistry of Uda rams fattened with diets containing graded levels of groundnut oil

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ABSTRACT: The study was conducted in Usmanu Danfodiyo University to determine the effects of groundnut oil on Haematology and serum biochemistry of fattened Uda rams. Four (4) dietary treatments were prepared; Treatment 1 which is the control, had no groundnut oil, while 2, 3 and 4 contained 15, 30 and 45 ml/kg groundnut oil. The results showed that except for packed cell volume, haemoglobin, MCV and MCH, which were significantly ($p<0.05$) higher on the control diet, no haematological index was influenced by dietary groundnut oil supplementation. Similarly, most of the serum biochemical parameters studied did not reveal any significant ($p>0.05$) effect of diet. However, total cholesterol was higher on T_1 (1.74 mmol/l), T_2 (1.72 mmol/l) and T_3 (1.68 mmol/l), which were the same, than on T_4 (1.41 mmol/l). High density lipoproteins for rams on T_2 (0.85 mmol/l) and T_3 (0.98 mmol/l) compared favourably with the control (0.60 mmol) which was lower than 1.25 mmol/l obtained on T_4. It was therefore concluded that the supplementation of groundnut oil in the feed of fattening Uda rams at 45 ml/kg feed improves serum concentration of high-density lipoproteins (HDL).

Keywords: Groundnut oil, haematology, serum biochemistry, Uda rams.

INTRODUCTION

Ruminants are one of the most valuable and renewable resources for humankind. Their unique ability to utilize non-competitive foods such as grass, crop residue and agro-industrial wastes make them indispensable to human beings as they are capable of meeting meat and milk demands. But a rapid increase in ruminant animal populations is also mentioned as a major challenge, as ruminants are responsible for up to one-third of methane emissions worldwide (Storm *et al.* 2012).

The use of concentrates has been recommended as a strategy for reducing methane production from ruminants (Holter and Young, 1992; Duan *et al.,* 2006). Lovett *et al.* (2003) demonstrated that increased concentrate use, compared with pasture, reduced the enteric methane per kg of animal product. Generally, a reduction in methane production is expected when the residence time of feed (concentrates and forage) in the rumen is reduced, since ruminal degradation decreases, and methanogenic bacteria are less able to compete in such conditions.

Supplementation of lipid rich feeds, either in the form of extracted lipids or whole oil seeds, is another strategy for modulating ruminal bio-hydrogenation as well as for increasing the energy contents of the diets. Hart *et al.* (2008) suggested two modes of action of essential oils (EO): one is EO affect the pattern of bacterial colonization of substrates, particularly starch rich substrates, as they enter the rumen. The other mode was the inhibition of hyper ammonia producing bacteria involved in deamination. Therefore, the objectives of the study is to determine the haematology and serum biochemistry of Uda rams fattened with diets containing graded levels of groundnut oil.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Livestock Teaching and

Research Farm, Department of Animal Science, Main Campus, Usmanu Danfodiyo University, Sokoto. Sokoto State is located in North-western Nigeria between latitudes 11°30' and 14°00'N and longitudes 4°00' and 6°40'E. The state covers a total land area of 32,000 km^2 (Mamman *et al.*, 2000) and at an altitude of 350 m above sea level (Mamman *et al.*, 2000). The state falls within the Sahel savannah vegetation zone. The hot dry spell extends from March to May and sometime to June in the extreme northern part. A short, cool, dry period (harmattan) occurs between October and February (Malami *et al.*, 2001). The annual rainfall is about 700 mm. The rainy season starts from June to early October with a peak in August, potential evapotranspiration has been reported to be 162 mm. Maximum temperature of 41°C has been reported in April and minimum of 13.2°C in January (SEPP, 1996). The state is one of the largest livestock producing area in Nigeria. Among the livestock produced in the area are cattle, sheep, goats, donkeys, poultry and camels.

Sources of the oils

The groundnut oil used in this experiment was bought from Gerewa oil mills in Kano State.

Experimental animal and their management

Sixteen (16) Uda rams were used for this study. The animals were allowed to rest for 3 to 4 weeks before commencement of the trials. The animals received prophylactic treatment against internal and external parasites.

Experimental diets

The composition of experimental diets is presented in Table 1. Graded levels of groundnut oil at 0, 15, 30 and 45 ml/kg were used in the diets designated as T_1, T_2, T_3, and T_4 respectively.

Experimental design and feeding

The rams were allotted to four treatments in replicates of four (4) using a completely randomized design. Each ram was housed in individual pen with adequate ventilation. They were balanced for weight before commencement of the experiment and weighed weekly thereafter throughout the period of the experiment. The experimental diets were offered at 2% of body weight while sorghum straw was fed *ad libitum* as basal diet for a period of 84 days (12 weeks). Clean drinking water was served *ad libitum*.

Collection of blood samples

At the end of the experiment, three (3) animals from each treatment were randomly selected and fasted overnight (12 hours) and bled the following morning (7:00 – 8:00am). Fasting was done to avoid the temporary elevation of blood metabolites following feeding (Jain, 1986) while morning bleeding was to prevent excessive bleeding (Kolo *et al.*, 2017). Blood samples were collected from jugular veins using sterilized disposable (5 ml) syringe. Two separates, properly-labelled sample bottles, were used for each ram. One of each set of bottles contained an anti-coagulant, ethylene diamine tetra – acetic acid (EDTA) while the other one was without EDTA. Samples collected in EDTA bottles were used for haematological studies while those in plain bottles were used for serum biochemistry analyses. For biochemical analysis, the samples were centrifuged for five minutes at 1400/rpm so as to separate the serum from blood cellular elements.

Analysis of haematological parameters

Packed cell volume (PCV), red blood cells (RBC) count, white blood cells (WBC) counts, Leucocytes differential counts and haemoglobin concentration (Hb) were determined in accordance with the methods outlined by Bush (1991).

Erythrocyte indices which include the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed in accordance with the standard formulae of Schalm *et al.* (1975) and Jain (1986) as shown below:

$$MCV = \frac{PCV}{RBC \text{ Count in } 10^6 \text{ /mm3}} \times \frac{10}{1}$$

$$MCH = \frac{Hb(g/dl}{RBC \text{ in } 10^6/mm3} \times \frac{10}{1}$$

$$MCHC = \frac{Hb(g/dl)}{PCV \text{ (\%)}} \times \frac{10}{1}$$

Serum biochemical analysis

The blood urea concentrations were estimated by Nessler's reaction (Tanis and Naylor, 1968). Serum total proteins were estimated by the biuret method as described by Kohn and Allen (1995). Albumins were determined by Bromo Cresol Green (BCG) method (Peter *et al.*, 1982), while globulin concentration was determined by difference between total protein and albumin. Albumin/globulin ratio was calculated by dividing albumin value by the calculated globulin value. Aspartate amino transferase (AST or SGOT), Alanine Amino transferase (ALT or SGPT), Alkaline phosphate (ALP) activities were determined using spectrophotometric method, as described by Rej and Hoder (1983). Total bilirubin was determined using orbital techniques as described by Stone (1954).

Table 1. Composition of experimental diets supplemented with graded levels of groundnut oil.

Ingredients (%)	Experimental diets			
	T1 (Control)	T2 (15 ml/kg)	T3 (30 ml/kg)	T4 (45 ml/kg)
Maize	20	20	20	20
Soybean	10	10	10	10
Cowpea husk	28	28	28	28
Wheat offal	25	25	25	25
Rice milling waste	16	16	16	16
Salt	1.0	1.0	1.0	1.0
Total	100	100	100	100
Calculated values				
ME Mcal/kg	2234	2234	2234	2234
Crude protein %	14.2	14.2	14.2	14.2
Crude fibre %	20	20	20	20
Ether extract (%)	4.1	4.1	4.1	4.1

ME = Metabolizable energy.

Statistical analysis

Data obtained from the experiment were subjected to Analysis of Variance using Completely Randomized Design (CRD) and significant difference between treatment means were separated using the Duncan`s Multiple Range Test at 5% probability level.

RESULTS

Haematological parameters of Uda rams fed diets containing graded levels of groundnut oil

The haematological parameters of fattened Uda rams supplemented with different levels of groundnut oils are shown in Table 2. There was significant (p<0.05) difference among rams fed graded levels of groundnut oil in Packed Cell Volume (PCV) values, Haemoglobin (Hb) values, Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH). Red Blood Cell (RBC), White Blood Cell (WBC), Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cell differential counts were not significantly influenced (p>0.05). However, all parameters measured are within the reference values for normal and healthy rams. The PCV of Uda rams supplemented with different levels of groundnut oil ranged from 31.16 to 35.50% for T_4 and T_1, respectively.

Values for Hb of Uda rams supplemented with different levels of groundnut oil ranged between 10.50 and 11.83 g/dl. The highest value was recorded on rams fed the control diet. Haemoglobin values differed significantly (p<0.05) among treatments. However, rams on treatment 3 (10.50g/dl) and those on treatment 4 did not differ (p>0.05). The Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) values ranged from

29.87 to 38.13 (fl) and 9.95 to 12.71 (pg), respectively.

Serum biochemistry of fattened Uda rams fed diets containing graded levels of groundnut oil

The result for serum biochemical analysis of fattened Uda rams supplemented with different levels of groundnut oil is shown in Table 3. The results indicated that high density lipoprotein (HDL), total cholesterol and triglyceride were significantly (p<0.05) different among rams in different treatment groups. Other parameters, which include total bilirubin, direct bilirubin, total protein, albumin, blood urea, creatinine, glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and LDL, were not statistically (p>0.05) different among the rams on treatment groups. With the exception of total bilirubin, TC and TG which are slightly lower than reference values, all values obtained in this study are within the standard reference values for normal and healthy rams. Total cholesterol value of fattened Uda rams fed diets supplemented with graded levels of groundnut oil ranged from 1.41 to 1.74 mmol/l. The lowest (p<0.05) mean value of total cholesterol was obtained on treatment 4 while all other treatments did not differ (p>0,05).

Mean values for triglycerides of fattened Uda rams fed diets supplemented with different levels of groundnut oil ranged from 0.06 to 0.14 mmol/l. The values were higher for treatments 4 (0.14 mmol/l), 3 (01.3 mmol/l) and 2 (0.11 mmol/l). However, the lower value of 0.06 mmol/l obtained on the control was comparable with treatment 2. High density lipoproteins (HDL) which was also significantly (p<0.05) affected, gave a mean value that ranged between 0.60 and 1.25 mmol/l. The higher values obtained on treatment 1 (1.25 mmol/l) did not differ from those on T_3 (0.98 mmol/l) and T_2 (0.85 mmol/l. The control group which

Table 2. Influence of graded levels of groundnut oil supplementation on hematological indices of fattened Uda rams.

Parameters	Experimental diets				SEM	Reference values**
	T₁ (Control)	T₂ (15 ml)	T₃ (30 ml)	T₄ (45 ml)		
Packed cell volume (%)	35.50[a]	33.00[b]	31.16[c]	31.50[c]	0.53	27-45
Haemoglobin g/dl	11.83[a]	11.00[b]	10.50[c]	10.50[c]	0.17	9-15
Red blood cell g/dl	9.33	9.50	10.55	10.50	0.21	9-15
White blood cells x 10⁹/L	9.00	9.33	9.66	9.10	0.35	4-12
MCV (fl)	38.13[a]	33.26[b]	29.87[b]	30.03[b]	1.17	28-40
MCH (pg)	12.71[a]	11.61[a]	9.95[b]	10.01[b]	0.38	8-12
MCHC (%)	33.33	33.33	33.33	32.22	0.27	31-34
WBC Differentials						
Lymphocytes (%)	34.33	39.00	44.66	46.66	2.46	2-90
Neutrophils (%)	63.33	58.66	53.00	51.33	2.23	
Monocytes (%)	0.66	0.66	0.00	0.00	0.26	0-0.8
Eosinophils (%)	0.00	0.00	0.00	0.33	0.08	0-1.0
Basophils (%)	0.33	0.66	1.00	0.00	0.14	0-3

SEM = Standard error of means, a, b = Means in the same row bearing different superscripts differ significantly (p<0.05); MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Haemoglobin; MCHC=Mean Corpuscular Haemoglobin Concentration; * *source = (Elmhurst *et al.*, 2002)

Table 3. Influence of graded levels of groundnut oil supplementation on serum biochemical parameters of fattened Uda rams.

Parameters	Experimental diets				SEM	Reference values**
	T₁ (Control)	T₂ (15 ml)	T₃ (30 ml)	T₄ (45 ml)		
Total Bilirubin (mg/100ml)	0.69	0.71	0.60	0.71	0.04	1.71-8.55
Conjugated Bilirubin (mg/100ml)	0.07	0.06	0.05	0.06	0.01	0 - 4.61
Total protein (g/dl)	5.86	5.90	6.23	6.93	0.23	6 - 7.9
Albumin (g/dl)	2.63	2.70	2.86	3.13	0.12	2.4-3
Glucose (mmol/l)	2.86	3.06	3.36	3.50	0.12	1.7-3.6
UREA (mmol/l)	8.23	7.50	7.33	6.10	0.34	3-10
Creatinine (mg/dl)	1.16	1.03	0.96	0.86	0.05	0.79-1.19
AST (iu/l)	145.66	145.66	169.00	184.33	7.44	60-280
ALT (iu/l)	41.00	43.00	43.00	44.66	2.97	22-38
ALP (iu/l)	64.33	70.33	75.66	79.33	3.14	70-390
TC mmol/l	1.74[a]	1.72[a]	1.68[a]	1.41[b]	0.06	1.05-1.5
TG mmol/l	0.06[b]	0.11[ab]	0.13[a]	0.14[a]	0.01	0.5-2.8
HDL mmol/l	0.60[b]	0.85[ab]	0.98[ab]	1.25[a]	0.13	0.8-2.6
LDL mmol/l	0.69	0.62	0.61	0.57	0.03	0.5-4.3

SEM = Standard error of means, a, b = Means in the same row bearing different superscripts differ significantly (p<0.05); AST – Aspartate Aminotransferase, ALT – Alanine Aminotransferase. ALP- Alkaline phosphatase, TC= Total cholesterol TG= Trigleceride HDL-high density lipoprotein, LDL- low density lipoprotein **source = (Elmhurst *et al.*, 2002).

had the lower mean value was however similar to T₂ and T₃.

DISCUSSION

Heamatological parameters of Uda rams fed diets containing graded levels of groundnut oil

The mean values of PCV of Udah rams obtained in this study were lower than 43.80.6% reported by Egbe-Nwiyi

et al. (2000) and 38-45% (Swenson 1990). The values were also within 27– 45% reported by Jain (1993) for normal and healthy sheep. Also, haemoglobin concentration decreased with increased levels of groundnut oil supplementation. However, all values obtained were within the normal range (8 – 16g/d*l*) for healthy sheep (Greenwood, 1977). Increase in the Hb concentration usually signifies better ability to fight infection while a lower level is an indication of poor nutrition and ability to stand against infectious micro-organisms (Tambuwal *et al.*, 2002).

The RBC values in this trial were slightly higher than 6.49 – 9.25 g/dl (Njidda *et al.*, 2014). Similarly, the values are similar to those reported by Frandson (1981) and Heath and Olusanya (1988) in sheep. The main function of the RBC is to carry oxygen from the lungs to other body tissues and carbon dioxide from tissues to the lungs. A higher RBC value may be related with conditions that cause the body to build too numerous red blood cells or impaired pulmonary function, while low RBC counts may be associated with iron deficiency, internal bleeding, some types of anemia or some vitamin deficiencies (Njidda *et al.*, 2014). The white blood cells (WBC) count obtained in the present study is within the range of 6.93 – 12.66 x 10^9/l reported by Fadiyimu *et al.* (2010) and 5.2 to 27.70 x 10^9/l (Njidda *et al.*, 2014) in similar experiments.

The values for percent lymphocytes obtained in this study are comparable with 45 – 76 % (Njidda *et al.*, 2014) and 40-75% (Coles 1986). The variations in values for WBC Differentials obtained in this study compared well with the report of Bush (1991) and may be due to differences in diets. Therefore, the differential counts value obtained in this study showed that the animals were in good health. This indicates that groundnut oil has no toxicity effects in animal.

The MCV, MCH and MCHC values reported for rams in this study were within the normal range of 33.12 – 54.09 fl, 10.46-17.89 pg and 15.40 – 33.90%, respectively as reported by Njidda *et al.* (2014). These parameters were used to measure the size and hemoglobin content of erythrocytes and the values are useful in diagnosing various forms of anemia. The higher MCH and MCV values may be attributed to age of the rams (Egbe-Nwiyi, 2000). Values of MCV and MCH are very important in the diagnosis of anemia and also serve as useful indices of bone marrow activity (Awodi *et al.*, 2005). Except for those on the control diet, MCV values obtained for the experimental rams were slightly lower than 35.3 – 43.7 fl reported by Borjesson *et al.* (2000). However, values of mean corpuscular haemoglobin were within the normal range (10.46 -17.89 pg) for normal and healthy sheep as reported by Njidda *et al.* (2014).

Serum biochemical parameters of Uda sheep fed diets containing graded levels of groundnut oil

The total and conjugated bilirubin values obtained in this study were in conformity with the findings of Coles (1986) who reported the total and conjugated bilirubin values of sheep as 0.082 - 0.185 and 0.066 -1.28 mg/dl, respectively. Slightly higher values 0.2750 and 1.7900 (mg/100ml) have also been reported by Kolo *et al.* (2017) in West African Dwarf goats. Bilirubin test measures is considered the true test for liver function (Frandson, 1981; Singh, 2004).

The values for total protein and albumin in this study were within the normal range of 5.5 - 9.4 and 2.3 - 3.3 g/dl,

respectively reported for normal and healthy rams (Njidda *et al.,* 2014). The total protein and albumin values were similar to the range of 6.0 – 9.3 g/dl and 30 – 38 g/dl, respectively reported for sheep (Borjesson *et al.,* 2000; Milne and Scott 2006). Kolo *et al.* (2017) reported that serum total protein and albumin of animals are indirect indices of assessing the nutritional protein adequacy in farm animals. Dairo (2005) reported that total protein and albumin are important blood clotting factors due to their ability to prevent haemorrhage, Therefore, the higher the values the better for the animals.

The values obtained for serum glucose were within the normal range (2.1-3.34 mmol/L) as reported by Njidda *et al.* (2014) for adult sheep and 2.5 - 3.87 mmol/L reported by Abbator *et al.* (2017) in a similar study. Serum glucose level is an indicator of metabolic efficiency (Coles, 1986). Lower values than the normal range is a sign of hypoglycemia while higher levels are indication of hyperglycemia (Olorunnisomo, 2012). Values for urea concentration obtained in this study were close to the average values (5.28 mg/dl) reported by Baneejee (2007) in adult sheep, within the reference range of 8-26 mg/dl reported by Babeker and Elmansoury (2013) but lower than 4.30–5.60 mg/dl (Antunovic, 2011). Kolo *et al.* (2017) asserted that when blood urea levels fall within the normal range, it is an indication that the kidneys and liver of the animals are normal.

The creatinine levels obtained in this study fell within the normal range (1.21 to 1.44 mg/dl) reported by Boyd (1984). Higher values of serum creatinine indicate poor protein and amino acid metabolism which may lead to impaired renal function and cardiac infarction (Gray and Howarth, 1980). The serum aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) values obtained in the present study were within the normal range reported by Mitruka and Rawnsley (1977) for healthy sheep. AST level is helpful for the diagnosis and of myocardial infarction, hepatocellular disease and skeletal muscle disorders (Alex and Laverne, 1983). ALT is a liver-specific hepatocellular enzyme that is used to assess liver damage (Mahgoub *et al.*, 2008).

Serum cholesterol values obtained in this study fell within the normal range reported for healthy ewes (Mitruka amd Rawnsley, 1977). Cholesterol values of 0.78 - 1.30 have been reported by Amin *et al.* (2014) which agrees with this study. The values of cholesterol obtained in the study are also in line with the normal range (1.33 – 1.95 mmol/*l*) reported for healthy sheep (Cox-Ganser *et al.*, 1994). The increased in serum cholesterol in this study is an indication of oil supplementation in the diets, but are lower than 1.9–3.5 mmol/l (Sirois, 1995). Consumption of saturated fatty acids has been associated with increased serum cholesterol concentrations which is a risk factor for coronary heart disease (Keys, 1970). The level of plasma lipids and lipoproteins determines the extent of adipose tissue deposition which affects fat composition of meat (Lewington *et al.,* 2007). Higher than normal level of blood

cholesterol may result in its deposition on the walls of blood vessels and these deposits may eventually harden to atheroschlerotic plaque, which may subsequently block important blood vessels and result in a myocardial infarction. The TG levels obtained in the study fell within the normal range as reported by Kaneko *et al.* (1997) but lower than 0.16 – 1.6 mmol/l (Daramola *et al.*, 2005). The HDL and LDL concentration in the current study fell within normal range of 1.47 – 2.80 mmol/l and 0.10 – 0.56 mmol/l, respectively (Khan *et al.*, 2016).

Conclusion

The study showed that the supplementation of Uda ram diets with groundnut oil at 45 ml/kg improves serum high density lipoproteins (HDL) levels thus reducing the risk of heart diseases in humans and animals.

CONFLICTS OF INTEREST

The author declares that there is no conflict of interest.

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