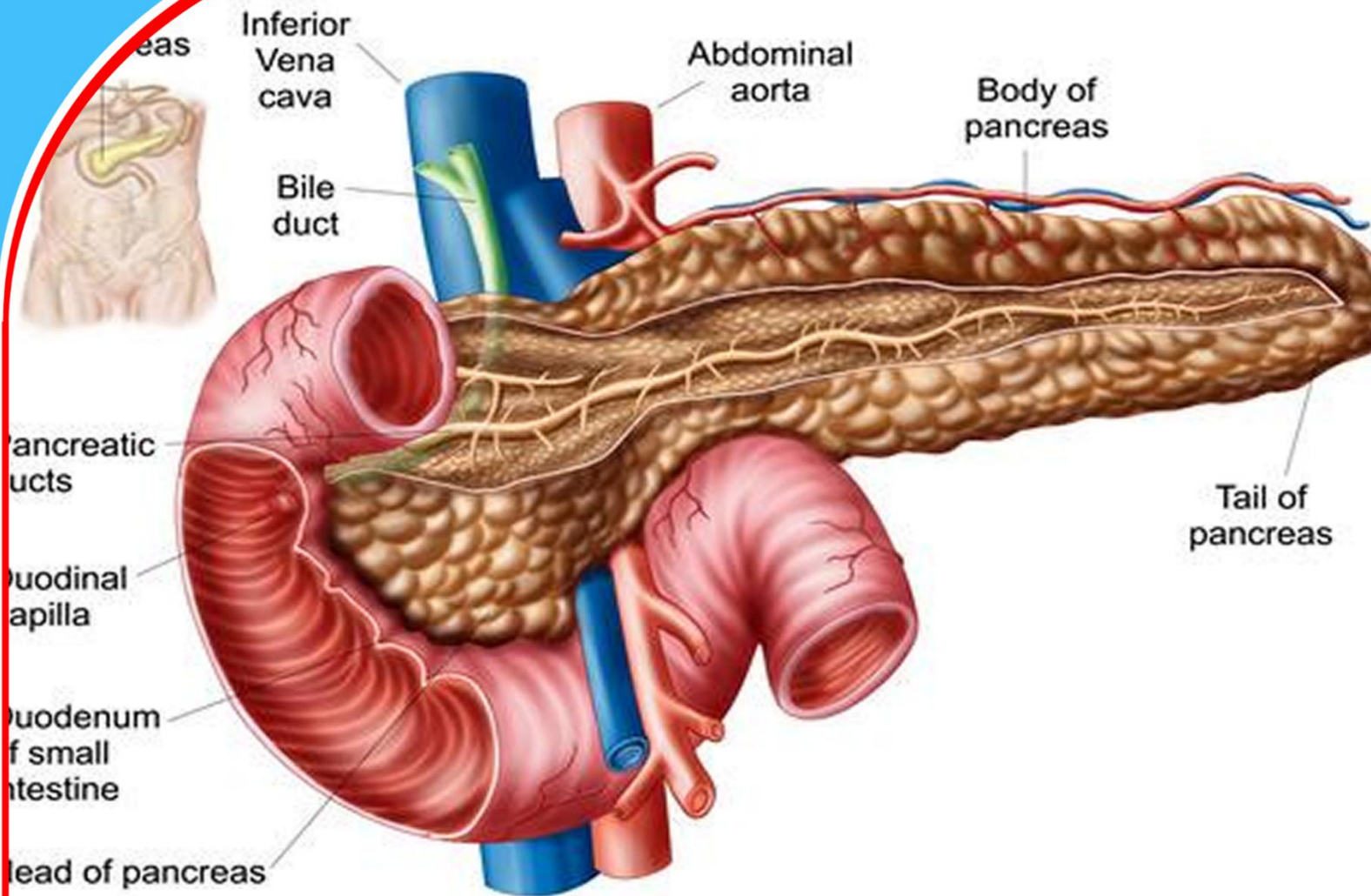


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Abstract

Purpose: Plant secondary metabolites available in *Tetrapleura tetraptera* fruit meal (TTFM) could be exploited as natural safe additive to enhance the physiological health status of rams. Thus, this study investigated the haematological and serum biochemical indices of West African Dwarf (WAD) rams fed diets containing varying levels of TTFM.

Methodology: Twenty five (25) WAD rams with an average live weight of 13.20 ± 0.20 kg were used in a completely randomized design for 140 days. Five concentrate diets containing varying levels (0, 0.5, 1.0, 1.5 and 2.0%) of TTFM were formulated while *Panicum maximum* was fed as a basal diet. Blood samples were collected at the onset and the end of the experiment for haematological and serum biochemical indices. Data collected were subjected to one way Analysis of Variance (ANOVA).

Findings: Results showed that haematological parameters were not significantly ($p > 0.05$) different in all dietary treatments except for eosinophil obtained at 20th week of the study with the lowest ($p > 0.05$) value at 1.5% TTFM. Total serum protein and glucose values were positively influenced as addition of TTFM increased. It can be concluded that TTFM could be utilized for ram productivity without any adverse effect on the health status of experimental rams.

Recommendation: It can therefore be recommended that TTFM could be included into the diets of WAD rams up to 2.0% level to improve the positive blood profile responses.

Keywords: *Tetrapleura*, rams, eosinophil, glucose

INTRODUCTION

The potency of *Tetrapleura tetraptera* in controlling schistosomiasis and trematode infections has been established (Aladesanmi, 2007). The documented biological and or pharmacological activities of *Tetrapleura tetraptera* are found to be effective in cardio-vascular, anti-ulcerative, neuromuscular, hypotensive, anti-convulsant, trypanocidal, entotoxicity, anti-microbial and the control of intestinal parasites (Adewumi *et al.*, 1990; Noamesi *et al.*, 1994; Amoako-Atta *et al.*, 2003; Aderibigbe *et al.*, 2007). Different solvent extracts of the fruit have been proved to have hypolipidaemic and hypokalaemic effects (Nwaichi & Igbino, 2012). Blood parameters have been shown to be major indices of physiological, pathological and nutritional status of an animal and change in the constituent compounds of blood when compared to normal values could be used to interpret the metabolic state, health status of an animal as well as quality of feed (Prvulovic *et al.*, 2012). Njidda *et al.* (2014) concluded that blood parameters are important in assessing the suitability and quality of feed ingredients and feeds in farm animals. This study was therefore designed to determine the effects of *Tetrapleura tetraptera* on the haematological and serum biochemical indices of West African dwarf rams.

MATERIALS AND METHODS

The study was conducted at the small ruminant unit of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan. The unit is located in the south western part of Nigeria. The area lies within the rain forest ecological zone and fall within longitude and latitude 7⁰-27⁰N and 3⁰-25⁰E respectively and altitude of 220-300m above sea level with the average rainfall of about 1250mm. The temperature and relative humidity ranges from 30-35⁰C and 76-84% respectively. Twenty five (25) West African dwarf rams randomly allotted to five dietary treatments in a completely randomized design with 5 replicates were chosen from each treatment. Rams between 6 and 8 months of age and average weight 13.00 ±0.02kg were used for the experiment. The fresh *Tetrapleura tetraptera* fruits were purchased from a reputable market in Ibadan, Oyo State Nigeria. This was identified and authenticated at the Herbarium unit of the Forest Research Institute of Nigeria (FRIN) Ibadan, Oyo State, Nigeria. The authenticated fruits were rinsed in sterile water and air-dried for two (2) consecutive weeks at room temperature and later milled into powdery form before compounding with other feedstuffs as fruit meal at 0%, 0.5%, 1.0%, 1.5% and 2.0% inclusion levels for treatments 1, 2, 3, 4 and 5 respectively. Each animal was served with *Panicum maximum* grass *ad libitum* and concentrate diets at 3% body weight twice daily.

Table 1. Gross compositions of concentrate diets containing varying levels of *Tetrapleura tetraptera* fruit meal for West African dwarf rams

Ingredients	Inclusion levels of TTFM (%)				
	0	0.5	1.0	1.5	2.0
Corn bran	30.00	30.00	30.00	30.00	30.00
Palm kernel cake	25.00	25.00	25.00	25.00	25.00
Rice bran	20.00	20.00	20.00	20.00	20.00
Wheat offal	15.00	15.00	15.00	15.00	15.00

Groundnut cake	5.00	5.00	5.00	5.00	5.00
TTFM	-	+	++	+++	++++
Dicalcium phosphate	3.00	3.00	3.00	3.00	3.00
□ Premix	1.00	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00

TTFM: *Tetrapleura tetraptera* fruit meal

+ (0.5kg TTFM), ++ (1.00kg TTFM), +++ (1.50kg TTFM), ++++ (2.00kg)

□ Contains Vitamin A (I.U.) 10,000,000; Vitamin D₂ (I.U.) 2,000,000; Vitamin E (I.U) 20,000; Vitamin K (mg) 2,250; Riboflavin (mg) 5000; Pyridoxine (mg) 275; Biotin (mg) 50; Pantothenic acid (mg) 7500; Vitamin B₁ (mg) 175; Vitamin B₁₂ (mg) 15.0; Niacin (mg) 27,500; Folic acid (mg) 7500. Choline Chloride (mg) 400; Antioxidant (mg) 125; Fe (g) 20.0; Zn (g) 50.0; Mn (g) 80.0; Cu (g) 5.0g; I (g) 12.0; Co (mg) 200; Se (mg) 200

Table 2: Chemical compositions of experimental diet containing varying levels of *Tetrapleura tetraptera* fruit meal

Parameters	Inclusion levels of TTFM (%)				
	0	0.5	1.0	1.5	2.0
Dry matter	81.50	81.40	81.10	80.90	81.55
Crude protein	15.20	15.28	15.34	15.38	15.43
Ether extract	8.40	8.70	8.95	8.96	9.02
Ash	11.00	10.95	10.75	11.02	10.93
Crude fibre	15.89	15.91	16.05	16.10	16.23
Nitrogen free extract	49.51	49.16	48.71	48.54	48.39
Neutral detergent fibre	48.64	52.62	54.69	58.19	60.38
Acid detergent fibre	34.64	36.84	38.93	43.64	47.19
Acid detergent lignin	9.87	11.64	14.62	16.32	17.11
Hemicelluloses	14.00	15.78	15.76	14.55	13.19
Cellulose	24.77	25.20	24.31	27.32	30.08
Tannin	0.32	0.38	0.45	0.56	0.74
Saponin	0.71	0.73	0.78	0.84	0.95
Flavonoid	2.32	2.44	2.67	2.82	3.54
Alkaloid	1.87	1.86	1.90	2.01	2.23

Hydrogen cyanide	0.12	0.15	0.22	0.25	0.26
Sterol	0.76	0.96	1.11	1.36	1.45
Macro minerals (g/kg)					
Calcium	0.84	0.92	1.24	1.68	2.31
Phosphorus	1.12	1.32	1.65	1.97	2.22
Magnesium	2.47	2.54	2.95	3.54	4.01
Potassium	0.74	0.56	0.98	0.79	0.98
Sodium	0.24	0.28	0.28	0.31	0.34
Micro minerals(mg/kg)					
Manganese	234.12	242.23	251.23	264.33	267.67
Iron	184.60	177.80	173.30	195.45	205.54
Copper	11.34	8.79	10.33	11.65	10.98
Zinc	55.32	44.76	45.65	51.21	48.87

Blood Sample Collection

About 10ml of blood sample was collected directly from jugular vein of each animal using hypodermic needle and syringe at the beginning and at end of the experiment (20th week). Five (5ml) of the sample was released into the sample bottles containing Lithium oxalate and ethylene tetra acetic acid as anti-coagulant and the bottles were turned gently to ensure proper mixing of the blood with the anti-coagulant to prevent coagulation, the remaining 5ml of blood samples were released into plain bottle to harvest serum. Packed cell volume, haemoglobin, red blood cell, white and red blood cell counts, serum glucose, total serum protein, albumin, cholesterol, neutrophil, lymphocyte, eosinophil, basophil and monocyte and uric acid were determined from the blood samples.

Statistical analysis and experimental design

Data obtained were subjected to analysis of variance using SAS (2013) in a completely randomized design. One-way analysis of variance (ANOVA) was used to determine the means and standard error. Treatment means were compared using Duncan's new multiple range test in the same package.

RESULTS AND DISCUSSION

Haematological Parameters of West African Dwarf Rams Fed Diets Containing *Tetrapleura tetraptera* Fruit Meal

Table 3 shows the haematological parameters of West African dwarf rams fed diets containing *Tetrapleura tetratepra* fruit meal. There were no significant differences ($P>0.05$) in all the haematological parameters of interest except in eosinophil at the end of the experiment. The values of packed cell volume ranged from 27.33- 33.67%. The haemoglobin value recorded in the experiment ranged from 8.70- 10.90 x 10³ µ/l. Red blood cells values ranged from 4.31- 5.51 which

were not significantly different ($P>0.05$). The white blood cells ranged from 42.17- 49.67% though not significantly different ($P>0.05$). The highest values for eosinophil obtained in this study at 2.00% and 0.5% TTFM which was significantly different from other treatments. The lowest value for eosinophil recorded at treatment four with 1.50% TTFM. The lymphocyte values ranged from 60.40- 66.40%.

Table 3: Haematological parameters of West African Dwarf rams fed diets containing varying levels of *Tetrapleura tetraptera*

Parameters	Inclusion levels of TTFM					SEM	Range
	0	0.5	1.0	1.5	2.0		
Packed cell volume (%)							21 - 35 ¹
Initial	30.40	31.60	31.60	30.40	29.20	0.36	
Final	33.00	33.20	34.20	32.40	33.00	0.44	
Variation	2.60	1.60	2.60	2.00	3.80	0.18	
Haemoglobin (g/dL)							7 - 15 ¹
Initial	9.42	9.62	10.66	9.70	10.56	0.37	
Final	10.36	10.66	12.70	11.44	11.96	0.14	
Variation	0.94	1.04	2.04	1.74	1.40	0.23	
Red blood cell ($\times 10^6$)							4 - 8 ²
Initial	4.62	4.03	4.37	4.13	4.07	0.22	
Final	5.49	4.70	5.17	5.32	4.91	0.13	
Variation	0.87	0.68	0.79	1.19	0.84	0.20	
White blood cell ($\times 10^3$)							4 - 13 ³
Initial	4.89	4.37	4.73	5.02	4.64	0.21	
Final	5.04	4.43	4.92	4.96	4.96	0.13	
Variation	0.15	0.06	0.19	0.06	0.32	0.14	
Platelets ($\times 10^4$)							3 - 8 ²
Initial	7.96	7.17	7.23	6.27	7.45	0.17	
Final	7.66	7.75	7.90	6.57	7.91	0.36	
Variation	-0.30	0.58	0.67	0.50	0.46	0.08	
Lymphocyte (%)							50 - 70 ³
Initial	66.00	60.40	62.20	60.40	64.20	0.40	
Final	66.40	64.40	63.00	61.60	64.20	0.51	
Variation	-0.40	4.00	0.80	1.20	0.00	0.32	

Monocyte (%)							0 -4 ³
Initial	1.40	1.80	1.60	1.40	1.60	0.37	
Final	1.80	1.80	2.00	1.60	2.00	0.40	
Variation	0.40	0.00	0.40	0.20	0.40	0.56	
Eosinophil (%)							1 -7 ⁴
Initial	1.60	2.00	1.20	1.40	1.60	0.25	
Final	1.60 ^{ab}	2.00 ^a	1.80 ^a	0.80 ^b	1.40 ^{ab}	0.18	
Variation	0.00	0.00	0.60	-0.60	-0.20	0.10	
Neutrophil (%)							30 - 48 ³
Initial	36.78	35.20	34.46	34.12	32.25	1.74	
Final	32.23	34.12	30.00	37.80	35.66	1.24	
Variation	4.55	-1.08	-4.46	3.68	3.41	0.29	
MCV (fL)							16-25 ¹
Initial	58.04	59.87	63.44	62.77	65.52	1.38	
Final	66.44	60.46	58.98	64.87	70.21	2.01	
Variation	8.40	0.59	-4.46	2.10	4.69	2.54	

Superscripts along the same row are significantly different ($p > 0.05$) TTFM-*Tetrapleura tetraptera* fruit meal, SEM- Standard Error of Means, MCV: Mean Corpuscular Volume, ¹Daramola et al. (2005), ²Latimer et al. (2003) ³Feldman et al. (2002), ⁴Fielder et al. (2016)

Serum Biochemical Parameters of West African Dwarf Sheep Diets Containing *Tetrapleura tetraptera* Fruit Meal

Table 4 shows the serum biochemical parameters of West African dwarf sheep fed diets containing *Tetrapleura tetraptera*. There were no significant differences ($P > 0.05$) in all the parameters assessed at the beginning of the experiment except in the glucose and alanine triphosphatase. All parameters of interest were not significant ($p > 0.05$) affected by the inclusion levels of TTFM except total protein and glucose. The initial and final values for glucose ranged from 23.31-36.23g/dl and 26.78-42.48g/dl respectively. The highest value was recorded with animals offered 2.0% TTFM (42.48g/dl) Positive variations were observed in all parameters determined except on low density lipoprotein. Animals fed diets containing 2.0% TTFM recorded the highest total protein (8.29g/dl) and the least value of (6.54g/dl) at the end of the experiment. The albumin values ranged from 2.89-3.20g/dl which was not significantly ($p > 0.05$) influenced by the TTFM inclusion. Inclusion of TTFM cause a slight decrease in the values of high density lipoprotein observed. Negative variations observed ranged from (-0.69 and -0.012). The initial and final HDL values ranged from 47.75-57.36mg/dl and 45.32-55.91mg/dl respectively. Increase in the values of cholesterol observed as the levels of inclusion of TTFM increases.

Table 4: Serum biochemical indices of West African Dwarf rams fed diets containing *Tetrapleura tetraptera* fruit meal

Parameters	Inclusion levels of TTFM (%)					SEM	Range
	0	0.5	1.0	1.5	2.0		
Total Protein (g/dL)							6.3-8.5 ¹
Initial	6.43	7.53	7.72	8.03	8.02	0.25	
Final	6.54 ^b	7.84 ^a	8.10 ^a	8.68 ^a	8.29 ^a	0.22	
Variation	0.13	0.31	0.38	0.65	0.27	0.09	
Albumin (g/dL)							2.7-3.8 ²
Initial	3.01	3.10	2.92	3.20	2.97	0.07	
Final	2.89	3.15	2.97	3.26	2.89	0.09	
Variation	-0.12	0.05	0.05	0.06	-0.08	0.43	
Glucose (mg/dL)							48-76 ²
Initial	23.31 ^b	28.86 ^{ab}	34.67 ^a	32.99 ^a	36.27 ^a	1.57	
Final	26.78 ^b	32.67 ^{ab}	37.06 ^{ab}	36.58 ^{ab}	42.48 ^a	1.82	
Variation	3.47	3.81	2.39	3.59	6.21	1.94	
Cholesterol (mg/dL)							65-136 ¹
Initial	70.39	80.07	72.30	80.93	76.36	2.20	
Final	74.09	87.01	76.99	83.53	84.83	2.36	
Variation	3.70	6.94	4.69	2.60	8.47	0.33	
ALT(I. µ/L)							15-52 ³
Initial	12.21	9.73	12.47	10.64	11.28	0.51	
Final	10.99	9.88	12.81	10.82	11.26	0.52	
Variation	-1.22	0.15	0.34	0.18	-0.02	0.19	
ALP (I. µ/L)							1.8-3.4 ⁵
Initial	2.88 ^b	3.46 ^{ab}	3.17 ^{ab}	3.54 ^{ab}	3.87 ^a	0.11	
Final	2.97	3.39	3.20	3.67	3.74	0.12	
Variation	0.09	-0.07	0.03	0.14	-0.12	0.52	
AST(I. µ/L)							12-38 ⁴
Initial	25.78	29.76	32.72	26.66	28.72	1.27	
Final	28.54	35.70	31.73	28.24	31.44	1.15	
Variation	2.74	5.94	-0.99	1.58	2.72	0.07	

LDL(mg/dL)							0.77-20.98 ¹
Initial	6.75	5.52	6.47	6.62	6.96	0.65	
Final	6.51	5.50	6.25	5.93	6.71	0.59	
Variation	-0.24	-0.01	-0.22	-0.69	-0.25	0.51	
HDL(I. µ/L)							25.14-114.56 ¹
Initial	57.38	47.75	53.11	49.80	55.21	2.71	
Final	55.45	45.28	52.24	45.33	55.92	2.29	
Variation	-1.93	-2.47	-0.87	-4.47	0.71	0.55	
Triglycerides (mg/dL)							6.19-61.87 ¹
Initial	35.98	34.93	48.33	48.49	45.62	2.60	
Final	38.15	34.07	49.09	48.65	49.68	2.59	
Variation	2.17	-0.86	0.76	0.16	4.06	0.10	

^{a,b,c} Means with different superscripts along the same row are significantly different ($p>0.05$).
 TTFM: *Tetrapleura tetraptera* fruit meal, ASP-Aspartate aminotransferase, TG- Triglycerides, ALT-Alanine aminotransferase, SEM- Standard Error of Means, ¹Daramola *et al.* (2005), ²Feldman *et al.* (2002), ³Opara *et al.* (2010), ⁴Babeker and Elmansoury, (2013), ⁵Latimer *et al.* (2003)

The haematological parameters obtained in this study were within the normal range reported for healthy sheep by Latimer *et al.*, (2003). The packed cell volume (PCV) obtained in the study (27.33-33.67%) were within the range reported by Alabi and Ososanya, 2017 fed rams with silage combinations of maize forage and *Muncunapruriens*. The values obtained for PCV in this study fall below the range of 24-75% reported by Latimer *et al.*, (2003). The result showed that the experimental rams were not anaemic. This revealed that experimental rams used in this study were not susceptible to anaemia related diseases. As a normal rule in animals, the PCV is directly related to the Red blood cell (RBC) and Haemoglobin (Hb) concentration (Wang *et al.*, 2019). The haemoglobin values obtained in this study was within the normal range of 7-15g/dL reported by Daramola *et al.*, (2005) for goats and sheep but below the normal range reported by Latimer *et al.*, (2003). The haemoglobin was closer to the value ranged of 9.0-12.4g/dL reported by Sowande *et al.*, (2008) for clinically healthy sheep. This is an indication that *Tetrapleura tetraptera* is capable of supporting high oxygen capacity in the experimental rams.

The values of White blood cell (WBC), lymphocytes and monocytes obtained fell within the range of $4-12 \times 10^3$ /g/d reported by Charles and Margi (2007) for sheep and goats and also within the normal range of $4-12 \times 10^3$ /g/d reported by Latimer *et al.* (2003) for clinical healthy sheep. The WBCs in animals possesses phagocytic function. The function of WBC is to respond to antigens or foreign substance by forming antibodies that circulates in the blood or in the development of cellular immunity as reported by An *et al.* (2020) and Daramola *et al.* (2005). But higher neutrophil levels were recorded as a component of white blood cell in this study indicated that the WAD rams seem to possess protective system, thereby providing a rapid and potent defense against any infection. The highest red blood cell of 5.51×10^6 /µL was obtained from Treatment 5 with 2%

Tetrapleura tetraptera fruit meal inclusion in the diets. This suggested that *Tetrapleura tetraptera* promoted higher red blood cell counts in the WAD sheep. The values obtained for RBC of $4.35\text{-}5.51 \times 10^6/\mu\text{L}$ were considerably within the range reported by Daramola *et al.* (2005) of $9.2\text{-}13.5 \times 10^6/\mu\text{L}$.

Serum parameters have been reported to be important in the proper maintenance of the osmotic pressure between the circulating fluid and the fluid in the tissue space so that the exchange of materials between the blood and cell could be facilitated (Rapeal *et al.*, 2017). Blood parameters are used to assess the physiological, nutritional and pathological status of farm animals. Serum concentrations of total protein and albumin provide indicators of protein intake in ruminants and inadequate dietary protein intake leads to decrease in total protein and albumin (Khajehdizaj *et al.*, 2014). The total protein values obtained in this study ranges from 6.58 to 8.80g/dL. It is within the range reported of 4.5-6.7g/dL reported by Alabi and Ososanya (2017) for healthy domestic rams fed silage combination maize offal and *Mucunapruriens* foliage. Serum Albumin did not differ ($p>0.05$) significantly among treatment groups. The increase in total protein and albumin probably reflects greater microbial protein synthesis and improved antioxidant status, which is supported by the reduced ruminal ammonia nitrogen and the higher antioxidant enzyme activity. Rams fed with 2% inclusion of *Tetrapleura tetraptera* have the highest range of (3.63g/DL), followed those fed diets 1, 2, 3 and 4. The range obtained for serum albumin within the normal range reported by Daramola *et al.*, (2005) of 2.5-3.7g/dL.

These results indicate that WAD rams fed diets mixed with *Tetrapleura tetraptera* will not be prone to excessive haemorrhage as diets promoted adequate serum albumin which is important in blood clotting (Feldman *et al.*, 2012). The globulin observed in this study indicated that there were no traces of anti-nutritional factors that could diminish nutrient permeability in the gut wall. The glucose is the chief source of energy of all living cells. The glucose levels obtained in this study were within the normal range of 55-131mg/dL reported for healthy sheep by Mitraka and Rawnsley (1977) but higher than the value 59-65mg/dL reported for serum glucose level by Elkholy *et al.*, (2009) and also higher than the range of 44-81mg/dL reported by Latimer *et al.*, (2003). The values reported across the treatment shows that the diets mixed with *Tetrapleura tetraptera* were adequate in supplying energy to the rams. The serum cholesterol levels obtained in the present study were below the normal range of 44-90mg/dL reported for normal healthy sheep by Taiwo and Ogunsanmi (2003).

The ranges of 16.56-29.67 mg/dL were also below the range reported by Elkholy *et al.* (2009) of 96-117mg/dL. Cholesterol is a group of fats vital to cell membranes, nerve fibres and bile salts, and a necessary precursor for the sex hormone. High levels indicate diet high in carbohydrates while low indicate low in fat, mal-absorption, or carbohydrates sensitivity. Mc Donald *et al.* (2011) established that cholesterol level of 180mg/dL and below is safe and may not result in arteriosclerosis in ruminants. Aspartate transaminase (AST), Alanine amino transaminase (ALT) values were also within the normal range which is an indication of normal function of liver. Therefore, the values obtained for AST, which is an indication of normal functioning of the liver were not significantly ($P>0.05$) different among the groups of animals fed the experimental diets. The concentrates of Alanine aminotransferase varied significantly from 4.80-10.00uL with treatment 4 having the highest concentrations, nonetheless, the values were within the range of normal animal blood indices (Latimer *et al.*, 2003).

CONCLUSION

It can be concluded from this study that inclusion of *Tetrapleura tetraptera* into diets up to 2% level did not have any adverse effect on the health status of the West African dwarf rams.

RECOMMENDATIONS

The study recommend that *Tetrapleura tetraptera* fruit meal could be added up to 2% level to improve the haematological parameters like packed cell volume of WAD rams. The inclusion level could also be recommended to reduce the health challenges and levels of toxicity in the diet of WAD rams. It could be suggested further studies to be carried on oxidative status and urinalysis test

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