



Effect of Levels of Feeding and *Saccharomyces Cerevisiae* on Some Haematological and Biochemical Indices in West African Dwarf Sheep

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Abstract | The objective of this study was to investigate the effects of dietary inclusion of yeast (*Saccharomyces cerevisiae*) on haematology, biochemical indices and methane emission by West African dwarf sheep. Forty eight lambs (24 males and 24 females) were distributed in a complete randomized 2 × 3 factorial design with high roughage and high concentrate diets supplemented with three levels of yeast (0, 0.75 and 1.5 g of *Saccharomyces cerevisiae* per kg of the basal diets). Sheep fed the high concentrate and high roughage diets supplemented with 0.75 g and 1.5 g of *Saccharomyces cerevisiae* per kg of the basal diet had higher ($p < 0.05$) monocyte value than sheep fed the other diets. The white blood cell count and total protein values for sheep fed high concentrate diets supplemented with 0.75 g and 1.5 g of *Saccharomyces cerevisiae* per kg of the basal diet were higher ($p < 0.05$) than those of sheep fed the other treatment diets. The group of sheep fed the high concentrate and high roughage diets without supplementary *S. cerevisiae* had higher ($p < 0.05$) calcium value than sheep fed the other diets. Methane emission values for sheep fed high roughage diet without *S. cerevisiae* and high roughage diet with 0.75 g of *S. cerevisiae* per kg of diet were higher ($p < 0.05$) than those of sheep fed other diets. Results suggested that *S. cerevisiae* can be added into high concentrate diet at 0.75 g per kg of the diet for optimum performance of West African dwarf sheep.

Keywords | Haematology, Biochemical, Sheep, Yeast, Methane

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INTRODUCTION

The interest in the use of probiotics to improve the productive performance and the general health status of livestock has been rekindled by legislations to curtail the use of sub therapeutic doses of antibiotics in animal diets (Plail, 2006). Fungal probiotics such as yeast (*Saccharomyces cerevisiae*) has yielded better results in adult ruminants. Yeasts have positive effects on blood haematology resulting in the improvement in the health status of animals (Agazzi et al., 2014). The addition of yeast cultures to feed has many positive effects on the absorption of some minerals and improves the metabolic health of the animals (Dolezal et al., 2012). Live yeasts, were reported to

influence blood constituents through the remodelling of the ruminal microbial populations. *Saccharomyces cerevisiae* supplementations have been shown to have significant ($p < 0.05$) effects on the hematological parameters such as haemoglobin concentration, packed cell volume and red blood cell counts in weaned Najdi ram lambs (Hussein, 2014). Milewski and Sobiech (2009) reported that feeding lambs with diets containing *Saccharomyces cerevisiae* had a significant ($p < 0.05$) effect on the blood's white blood cell count. Abdel-Rahman et al. (2012) reported that the concentration of albumin was significantly increased by *S. cerevisiae* supplementation in the diets of growing lambs. Galip (2006) had also shown that total protein was increased in the serum of rams that received dietary

supplemental yeast ($p < 0.01$) in comparison to control animals.

Fermentation of feeds in the rumen is the largest source of methane emission from enteric fermentation. It has been reported that the supplementation of ruminant feeds with probiotics such as *Saccharomyces cerevisiae* significantly reduced the level of methane emission. There were variable results obtained from researches on feeding sheep with *S. cerevisiae*. Several reasons for these variations include forage to concentrate ratios (Ali and Göksu, 2013), quality of the forage and nutrient compositions of the diet (Hassan et al., 2009). Mwenya et al. (2005) reported that sheep fed 70:30, forage: concentrate ratio diet emitted 10% less methane when their diet was supplemented with 4 g of a yeast culture daily. There was the need to identify the dietary situations in which probiotics can yield better results. Therefore, the objective of this study was to determine the effects of the dietary inclusion of yeast (*Saccharomyces cerevisiae*) on haematological and biochemical indices, and methane emission by West African dwarf (WAD) sheep.

MATERIALS AND METHODS

The experimental procedures complied with the provisions of the University of Nigeria, Nsukka Ethical committee on the use of animals for biometric research (2005).

EXPERIMENTAL ANIMALS AND MANAGEMENT

The study was carried out at the Sheep and Goat unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka, Enugu State, Nigeria. The study lasted 12 weeks.

Forty eight lambs (24 males and 24 females) of average weight of 9.80 ± 0.57 kg were assigned to six treatments in a completely randomized 2×3 factorial arrangement with high roughage (HR) and high concentrate (HC) diets supplemented with three levels of yeast (0, 0.75 and 1.5 g of *Saccharomyces cerevisiae* per kg of the basal diets). The animals were randomly divided into six treatment groups of eight sheep each. The HR diet was composed of forage: concentrate ratio of 60:40 while the forage: concentrate ratio of HC diet was 40:60. Table 1 shows the composition of the experimental diets. The six dietary treatments were as follows: Treatment 1 was a high roughage diet with no inclusion of *S. cerevisiae*; treatment 2 was a high roughage diet with 0.75 g of *S. cerevisiae* per kg of diet; treatment 3 was a high roughage diet with 1.5 g of *S. cerevisiae* per kg of diet; treatment 4 was a high concentrate diet with no supplemental *S. cerevisiae*; treatment 5 was a high concentrate diet with 0.75g of *S. cerevisiae* per kg of diet, and treatment 6 was a high concentrate diet with 1.5 g of *S. cerevisiae* per kg of diet. Each group was made

up of four replicates with two sheep serving as a single replicate. Diets were formulated to meet the recommended requirement for crude protein. Water was provided to the animals *ad libitum*. The animals were housed individually in pens. Twenty one days prior to the start of the experiment, the animals were allowed to acclimate and the experimental diets were gradually introduced. The animals were vaccinated with the PPR vaccine and dewormed with Albendazole.

Table 1: Composition of the experimental diets.

Ingredients (%)	High roughage diet	High concentrate diet
Panicum maximum hay	60	40
Palm kernel cake	5	24
Bambara nut waste	5	30
Brewer's spent grain	29	5
Salt	0.5	0.5
Vitamins and minerals	0.5	0.5

Vitamin/ mineral premix (Animal Care.OptimixR) Each 1.25kg supplied: Vit A 12,000,000 i.u., D3 3000,000 i.u., Vit. k2500mg, B1, 200mg, B2 500mg, B6 3,500mg, Niacin 40,00mg, B12 20mg, Pantothenic acid 10mg. Folic acid 1,000mg. Biotin 80mg, Choline chloride 200,000gm, Anti-oxidant 125,000mg, Manganese 70,000gm, Iron 40,000gm., Copper8000mg, Iodine1,200mg, Selenium 250mg, and Cobalt250mg.

BLOOD COLLECTION

At the 8th and 12th weeks of the experimental period, blood was collected in the morning from each sheep. Ten millilitres of blood was collected from the jugular vein of each animal using a sterile disposable syringe. Five mL were emptied into sterile sample bottles containing the anti-coagulant, Ethylene Diamine tetra acetic acid (EDTA) for laboratory analysis to determine haematological indices. The remaining 5ml of blood were emptied into sample bottles without EDTA for serum extraction and biochemical analysis.

The packed cell volume (PCV) was determined by the microhaematocrit method (Thrall and Weiser, 2002). The haemoglobin concentration (HbC) was determined by the cyanomethaemoglobin method (Higgins et al., 2008). The red blood cell (RBC) count and the total white blood cell (WBC) counts were determined by the haemocytometer method (Thrall and Weiser, 2002). The Differential White Blood Cell (Leukocyte) Count was determined by the Leishman Technique (Thrall and Weiser, 2002). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Schalm et al., 1975). The formulae used were:

$$MCV (\text{fl}) = 10 \times PCV (\%) / RBC \text{ counts (millions}/\mu\text{l}).$$

$MCH (pg) = \{Haemoglobin (g/dl) \times 10\} / \{RBCs \text{ count (million } /\mu l)\}$

$MCHC (g/dl) = haemoglobin (g/dl) \times 100/PCV (\%)$

The serum total protein (TPP) concentration was determined using the Tietz (1995) method while the serum albumin (ALB) concentration was determined using the method of Grant et al. (1987). The determination of the plasma globulin level was by the following formula:

Plasma globulin = Total protein (TP) - Plasma albumin (ALB)

The phosphorus (P) concentration was determined using the phosphomolybdate method as described by Pearson (1976). The sodium (Na), potassium (K) and calcium (Ca) concentrations were determined by the Flame photometric method as described by Pearson (1976).

ESTIMATION OF METHANE EMISSION

At the 8th and 12th weeks of the experimental period faecal matters were collected from each sheep and analysed to determine the soluble residue, hemicelluloses and cellulose composition in the faeces. The methane emissions were determined by fitting soluble residues, hemicelluloses and celluloses composition of the faeces into the prediction equation of Moe and Tyrrell (1979) as follows:

$Methane (CH_4) = 3.406 + 0.510 (\text{soluble residue}) + 1.736 (\text{hemi-cellulose}) + 2.648 (\text{cellulose})$

Where;

CH_4 is in MJ/d and soluble residue, hemicelluloses and cellulose in kg feed/d ($R^2 = 0.67$).

STATISTICAL ANALYSIS

The collected data were subjected to two way analysis of variance (ANOVA) for factorial arrangement in a completely randomized design (CRD) as described by Steel and Torrie (1980) using Statistical Package for the Social Sciences (SPSS, 2003). The model included the effects of feed types and *S. cerevisiae* supplementation. Significantly different means were separated using Duncan's New Multiple Range Test (Duncan, 1955). The treatment effects were considered significant at $p < 0.05$.

RESULTS

THE EFFECTS OF DIET TYPES, WITH OR WITHOUT *S. CEREVISIAE* SUPPLEMENTATION, ON THE HAEMATOLOGY OF WEST AFRICAN DWARF SHEEP

The main effects of diet types (DT) and *S. cerevisiae* supplementation levels (CL) and the DT X CL interactions on the haematology of WAD sheep are presented in Table

2. There were no significant effects ($p > 0.05$) due to the DT on PCV, RBC, MCHC, MCV, neutrophil, lymphocytes and eosinophil counts of sheep fed high roughage or high concentrate diets. The HbC value was significantly ($p < 0.05$) affected by the DT. The HbC, value of sheep fed the HC diet was higher ($p < 0.05$) than the HbC value of sheep fed the HR diet. There were significant effects ($p < 0.05$) due to CL on PCV, RBC, MCHC, HbC, MCV, neutrophil and lymphocytes counts of sheep fed the diets with or without *S. cerevisiae* supplementation.

The PCV, RBC, neutrophil and lymphocytes counts values of sheep fed the diets supplemented with 1.5 g of *S. cerevisiae* per kg of diet were the highest ($p < 0.05$) while the PCV, RBC, neutrophil and lymphocytes counts of sheep fed the diets without *S. cerevisiae* supplementation were the lowest ($p < 0.05$). The MCHC values of sheep fed the diets supplemented with *S. cerevisiae* were significantly lower ($p < 0.05$) than the MCHC value of sheep fed the diets without *S. cerevisiae* supplementation. The HbC, and MCV values of sheep fed the diets supplemented with *S. cerevisiae* were significantly higher ($p < 0.05$) than the HbC, and MCV values of sheep fed the diets without *S. cerevisiae* supplementation.

Significant effects ($p < 0.05$) due to DT X CL on MCH, WBC and monocytes values existed. However, there were no significant effect ($p > 0.05$) due to DT X CL on PCV, RBC, MCHC, HbC, MCV, neutrophil, lymphocytes and eosinophil counts values. The MCH values for sheep fed the high concentrate diets supplemented with *S. cerevisiae* and the high roughage diets supplemented with 1.5 g of *S. cerevisiae* per kg of diet were similar ($p > 0.05$) but were significantly higher ($p < 0.05$) than the MCH values of sheep fed other treatment diets. Sheep fed the high roughage diets without *S. cerevisiae* supplementation had the lowest ($p < 0.05$) MCH value. The WBC count values for sheep fed the high concentrate diets supplemented with *S. cerevisiae* were significantly higher ($p < 0.05$) than the WBC count values of sheep fed other treatment diets. The WBC count values for sheep fed the diets without *S. cerevisiae* supplementation were the lowest ($p < 0.05$). The groups of sheep fed the HR and HC diets supplemented with *S. cerevisiae* were significantly higher ($p < 0.05$) than those of sheep fed the HR and HC diets without *S. cerevisiae* supplementation. Sheep fed the high roughage diets without *S. cerevisiae* supplementation had the lowest ($p < 0.05$) monocyte count value.

THE EFFECTS OF DIET TYPES WITH OR WITHOUT *S. CEREVISIAE* SUPPLEMENTATION, ON THE BLOOD BIOCHEMICAL INDICES OF WEST AFRICAN DWARF SHEEP

The main effect of DT and CL and the DT X CL interactions on the blood biochemistry of WAD sheep are presented

Table 2: Effects of diet types with or without *Saccharomyces cerevisiae* supplementation on the haematology of West African dwarf sheep.

Items	PCV (%)	RBC (x10 ⁶ /μl)	MCHC (g/dl)	HbC (g/dl)	MCV (fl)	MCH (pg)	WBC count (x10 ³ /μl)	Neutrophil (x10 ³ /μl)	Lym-phocytes (x10 ³ /μl)	Mono-cytes (x10 ³ /μl)	Eosin-ophil (x10 ³ /μl)
Main effect of roughage: concentrate ratio											
HR	35.79 ^{NS}	11.26 ^{NS}	32.97 ^{NS}	10.33*	30.33 ^{NS}	9.83*	5.94*	3.35 ^{NS}	2.26 ^{NS}	0.60*	0.07 ^{NS}
HC	36.04 ^{NS}	11.62 ^{NS}	33.18 ^{NS}	11.18*	31.24 ^{NS}	10.70*	7.30*	3.59 ^{NS}	2.24 ^{NS}	0.75*	0.07 ^{NS}
Standard error of the mean	0.28	0.19	0.20	0.28	0.94	0.13	0.22	0.12	0.11	0.03	0.01
Main effect of <i>S. cerevisiae</i> (SC) supplementation											
0g/kg	30.69*	8.69*	37.38*	8.78*	24.49*	8.75*	4.29*	2.38*	1.43*	0.44*	0.09 ^{NS}
0.75g/kg	37.94*	12.40*	31.19*	11.28*	32.92*	10.45*	7.55*	3.69*	2.35*	0.78*	0.06 ^{NS}
1.5g/kg	39.12*	13.24*	30.65*	12.21*	34.94*	11.59*	8.02*	4.34*	2.97*	0.81*	0.06 ^{NS}
Standard error of the mean	0.34	0.24	0.25	0.35	1.15	0.16	0.27	0.15	0.13	0.03	0.02
Interaction (roughage: concentrate ratio with <i>S. cerevisiae</i> supplementation)											
HR (SC-0g/kg)	30.75 ^{NS}	8.80 ^{NS}	36.86 ^{NS}	8.58 ^{NS}	23.57 ^{NS}	8.39*	4.26 ^c	2.31 ^{NS}	1.65 ^{NS}	0.26*	0.10 ^{NS}
HR(SC-0.75g/kg)	37.12 ^{NS}	11.82 ^{NS}	31.29 ^{NS}	10.74 ^{NS}	33.35 ^{NS}	9.61*	6.40 ^b	3.49 ^{NS}	2.20 ^{NS}	0.76*	0.06 ^{NS}
HR(SC-1.5g/kg)	39.50 ^{NS}	13.16 ^{NS}	30.76 ^{NS}	11.69 ^{NS}	34.05 ^{NS}	11.48*	7.16 ^b	4.26 ^{NS}	2.93 ^{NS}	0.79*	0.06 ^{NS}
HC(SC-0g/kg)	30.62 ^{NS}	8.57 ^{NS}	37.90 ^{NS}	8.99 ^{NS}	25.40 ^{NS}	9.11*	4.31 ^c	2.45 ^{NS}	1.21 ^{NS}	0.61*	0.09 ^{NS}
HC(SC-0.75g/kg)	38.75 ^{NS}	12.98 ^{NS}	31.10 ^{NS}	11.81 ^{NS}	32.49 ^{NS}	11.29*	8.70 ^a	3.89 ^{NS}	2.50 ^{NS}	0.80*	0.06 ^{NS}
HC(SC-1.5g/kg)	38.75 ^{NS}	13.31 ^{NS}	30.54 ^{NS}	12.72 ^{NS}	35.84 ^{NS}	11.71*	8.88 ^a	4.43 ^{NS}	3.01 ^{NS}	0.83*	0.06 ^{NS}
Standard error of the mean	0.48	0.34	0.35	0.49	1.63	0.23	0.40	0.22	0.19	0.05	0.02

* - Values are significantly over control at P<0.05, NS – Not significant over control; HR(SC-0g/kg), HR(SC-0.75g/kg), and HR(SC-1.5g/kg): High roughage diet with *S. cerevisiae* supplementation at 0g, 0.75g and 1.5g / kg feed, respectively. HC (SC-0g/kg), HC (SC-0.75g/kg), and HC(SC-1.5g/kg): High concentrate diet with *S. cerevisiae* supplementation at 0g, 0.75g and 1.5g / kg feed, respectively.

Table 3: Effects of diet types with or without *Saccharomyces. cerevisiae* supplementation on the blood biochemistry of West African dwarf sheep.

Items	TPP (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Ca (mg/dl)	P (mg/dl)	Na (mmol/l)	K (mmol/l)
Main effect of roughage and concentrate ratio							
High Roughage	7.35*	4.22 ^{NS}	3.29 ^{NS}	10.06*	5.93 ^{NS}	142.88 ^{NS}	4.86 ^{NS}
High Concentrate	8.64*	4.31 ^{NS}	3.20 ^{NS}	9.73*	6.01 ^{NS}	142.65 ^{NS}	4.71 ^{NS}
Standard error of the mean	0.08	0.07	0.08	0.08	0.05	0.32	0.07
Main effect of <i>S. cerevisiae</i> supplementation							
0g/kg	7.06*	4.30 ^{NS}	2.63*	12.13*	7.29*	142.98 ^{NS}	4.81 ^{NS}
0.75g/kg	8.42*	4.34 ^{NS}	3.21*	8.87*	5.23*	142.85 ^{NS}	4.74 ^{NS}
1.5g/kg	8.50*	4.15 ^{NS}	3.91*	8.70*	5.38*	142.47 ^{NS}	4.81 ^{NS}
Standard error of the mean	0.09	0.08	0.10	0.10	0.07	0.39	0.08
Interaction (roughage: concentrate ratio with <i>S. cerevisiae</i> supplementation)							
HR (SC-0g/kg)	6.97*	4.29 ^{NS}	2.68 ^{NS}	12.04*	7.25 ^{NS}	142.91 ^{NS}	4.94 ^{NS}
HR(SC-0.75g/kg)	7.45*	4.35 ^{NS}	3.10 ^{NS}	9.28*	5.18 ^{NS}	143.01 ^{NS}	4.78 ^{NS}
HR(SC-1.5g/kg)	7.63*	4.03 ^{NS}	4.09 ^{NS}	8.88*	5.35 ^{NS}	142.71 ^{NS}	4.88 ^{NS}
HC(SC-0g/kg)	7.15*	4.31 ^{NS}	2.59 ^{NS}	12.21*	7.33 ^{NS}	143.05 ^{NS}	4.68 ^{NS}
HC(SC-0.75g/kg)	9.38*	4.33 ^{NS}	3.31 ^{NS}	8.26*	5.29 ^{NS}	142.69 ^{NS}	4.70 ^{NS}
HC(SC-1.5g/kg)	9.37*	4.28 ^{NS}	3.72 ^{NS}	8.33*	5.41 ^{NS}	142.22 ^{NS}	4.75 ^{NS}
Standard error of the mean	0.13	0.12	0.14	0.14	0.09	0.56	0.12

* -Values are significantly over control at P<0.05, NS – Not significant over control, HR(SC-0g/kg), HR(SC-0.75g/kg), and HR(SC-1.5g/kg): High roughage diet with *S. cerevisiae* supplementation at 0g, 0.75g and 1.5g / kg feed, respectively. HC (SC-0g/kg), HC (SC-0.75g/kg), and HC(SC-1.5g/kg): High concentrate diet with *S. cerevisiae* supplementation at 0g, 0.75g and 1.5g / kg feed, respectively. TPP: Total plasma proteins (mg/dl).

in Table 3. There were no significant effects ($p > 0.05$) due to DT on the albumin, globulin, Na, P and K values of sheep fed the high roughage or the high concentrate diets. However, there were significant effects ($p < 0.05$) due to DT on TPP and Ca levels of sheep fed the high roughage or the high concentrate diets. The TPP value of sheep fed HC diet was higher ($p < 0.05$) than the TPP value of sheep fed HR diet. The Ca level of sheep fed the HC diet was lower ($p < 0.05$) than the Ca level of sheep fed the HR diet. Results show that, although, there were no significant effects ($p > 0.05$) of CL on albumin, Na and K levels of sheep fed the diets with or without *S. cerevisiae* supplementation, TPP, globulin, Ca and P levels were significantly ($p < 0.05$) affected by CL. The TPP, and globulin values of sheep fed the diets supplemented with 1.5g of *S. cerevisiae* per kg of diet were the highest while the TPP, and globulin values of sheep fed the diets without *S. cerevisiae* supplementation were the lowest ($p < 0.05$). The Ca, and P levels of sheep fed diets supplemented with *S. cerevisiae* were significantly lower ($p < 0.05$) than the Ca, and P levels of sheep fed the diets without *S. cerevisiae* supplementation.

Significant effects ($p < 0.05$) due to DT X CL on TPP and Ca values existed. However, there were no significant effects ($p > 0.05$) due to DT X CL on albumin, globulin, P, Na and K levels. The TPP values for sheep fed the high concentrate diets supplemented with *S. cerevisiae* were significantly higher ($p < 0.05$) than the TPP values for sheep fed other treatment diets. The Ca values for sheep fed the diets without *S. cerevisiae* supplementation, were significantly higher ($p < 0.05$) than the Ca values for sheep fed the other treatment diets. The Ca values for sheep fed the high roughage diets supplemented with *S. cerevisiae* were significantly higher ($p < 0.05$) than the Ca values for sheep fed the high concentrate diets supplemented with *S. cerevisiae*.

THE EFFECTS OF DIET TYPES WITH OR WITHOUT S. CEREVISIAE SUPPLEMENTATION ON THE METHANE EMISSION BY WEST AFRICAN DWARF SHEEP

The main effects of DT and CL and the DT X CL interactions on the methane emission by WAD sheep are presented in Table 4. There were significant effects ($p < 0.05$) due to DT on methane emission values of sheep fed the high roughage or the high concentrate diets. The methane emission value for sheep the HR diet was higher ($p < 0.05$) than that of sheep fed the HC diet. There were significant effects ($p < 0.05$) of CL on methane emission by sheep fed the diets with or without *S. cerevisiae* supplementation. The methane emission value of sheep fed diets supplemented with *S. cerevisiae* were significantly lower ($p < 0.05$) than the methane emission value of sheep fed diets without *S. cerevisiae* supplementation.

Table 4: Effects of diet types with or without *Saccharomyces cerevisiae* supplementation on the methane emission by West African dwarf sheep.

Items	Methane (MJ/d)
Main effect of roughage: concentrate ratio	
High Roughage	3.68*
High Concentrate	3.08*
Standard error of the mean	0.02
Main effect of S. cerevisiae supplementation	
0g/kg	3.74*
0.75g/kg	3.11*
1.5g/kg	3.28*
Standard error of the mean	0.02
Interaction (roughage: concentrate ratio with S. cerevisiae supplementation)	
HR (SC-0g/kg)	3.80*
HR(SC-0.75g/kg)	3.64*
HR(SC-1.5g/kg)	3.59*
HC(SC-0g/kg)	3.68*
HC(SC-0.75g/kg)	2.57*
HC(SC-1.5g/kg)	2.98*
Standard error of the mean	0.03

* -Values are significantly over control at $P < 0.05$; HR(SC-0g/kg), HR(SC-0.75g/kg), and HR(SC-1.5g/kg): High roughage diet with *S. cerevisiae* supplementation at 0g, 0.75g and 1.5g / kg feed, respectively. HC (SC-0g/kg), HC (SC-0.75g/kg), and HC(SC-1.5g/kg): High concentrate diet with *S. cerevisiae* supplementation at 0g, 0.75g and 1.5g / kg feed, respectively.

Significant effects ($p < 0.05$) due to DT X CL on methane emission values existed. The methane emission values for sheep fed the high roughage diets without *S. cerevisiae* supplementation, and that of sheep fed the high roughage diets supplemented with 0.75 g of *S. cerevisiae* per kg of diet were similar ($p > 0.05$) but were significantly higher ($p < 0.05$) than the methane emission values of sheep fed the other treatment diets. The methane emission values for sheep fed the high roughage diet supplemented with 1.5 g of *S. cerevisiae* per kg of diet, and the high concentrate diets without *S. cerevisiae* supplementation were significantly higher ($p < 0.05$) than the methane emission values of sheep fed the high concentrate diets supplemented with *S. cerevisiae*.

DISCUSSION

The results of present study are consistent with Hussein (2014) who found that Hb, PCV and RBC's counts were higher ($P < 0.05$) in weaned Najdi ram lambs fed diets containing probiotics than those without it. These results would be explained as the supplementation of probiotics resulted in better iron salt absorption from the small intestine.

Also, probiotics were found to produce vitamins B, which affect positively the blood- cell forming processes (Kander, 2004). The present result is in agreement with Milewski and Sobiech (2009) who reported that feeding lambs with diets containing *Saccharomyces cerevisiae* had a significant ($p < 0.05$) effect on blood WBC's count. Increased WBC counts might be related to the production of more immune cells (and thus antibodies) (LaFleur, 2008) that plays an important role in defending the biological system against different diseases. According to Milewski et al. (2007), the immunostimulatory effect of *Saccharomyces cerevisiae* can be ascribed to the activity of β -1,3/1,6-D-glucans and mannan-oligosaccharides present in the yeast cell walls. This mechanism involves the stimulation of immunocompetent cells, mainly by β -1, 3/1,6-D-glucans. Our result supports the findings of Milewski and Sobiech (2009), who reported that dietary supplementation with yeast, caused an increase in the counts of erythrocytes and leukocytes, and in the levels of haemoglobin and haematocrit in ewes, which resulted in a significantly lower mean corpuscular haemoglobin concentration (MCHC). Milewski and Sobiech (2009), reported that feeding lambs with diets containing *Saccharomyces cerevisiae* had a significant ($p < 0.05$) effect on the blood's WBC count and contributed to higher lymphocyte percentages in the leukogram. In this study, the higher percentage of lymphocytes in probiotic groups could indicate improved immune system in lambs fed probiotic compared with control group lambs. Similar to our findings, Milewski and Sobiech (2009), reported that dietary supplementation with yeast significantly increased the values of the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin (MCH). The observed changes in the blood haematological indices suggest an improvement in their body condition.

The result of the present study is in agreement with Galip (2006) who had shown that albumin, serum Na^+ and K^+ concentrations were not significantly ($p > 0.05$) affected in serum of rams that received dietary supplemental yeast. Therefore, dietary *Saccharomyces cerevisiae* may be able to enhance the activities of hormones, involved in the maintenance of normal mineral balance. The biochemical result of the present work disagree with Abdel-Rahman et al. (2012) who reported that blood total protein or globulins were not significantly ($p > 0.05$) affected by *Saccharomyces cerevisiae* supplementation. Similar to our findings, El-Ashry et al. (2003) reported that yeast supplementation significantly increased plasma globulin values and this may be related to immunity in these animals. The result of the present study is in line with Galip (2006) who reported that total protein was increased in serum of rams that received dietary supplemental yeast in comparison to control animals. This result may be related to the beneficial effect of *Saccharomyces cerevisiae* supplementation on increasing protein digestibility (Abdel-Khalek et al., 2000). Yeast

culture has been found to stimulate microbial activity and increase the incorporation of nitrogen into microbial protein. Our result support the findings of Onifade et al. (1999) who reported significant decreases of Ca^{2+} and phosphorus concentrations in rabbits supplemented with *Saccharomyces cerevisiae* and they suggested that these variations would be related to enhancement of bone mineralization.

The result of the present study is in line with Yang et al. (2000) who reported that the proportion of concentrate within a diet has been reported to be negatively correlated with methane emissions. The result of the present study is in agreement with Kingeston-Smith et al. (2010) who had shown that forage rich diets result in acetic type fermentation, with an increase of methane emission compared to propionic type fermentation which, on the other hand, is stimulated by concentrates, with a decrease in methane emission.

Similar result with the present study were observed by Chung et al. (2011) and Mwenya et al. (2005) who reported that dietary supplementation of *S. cerevisiae* decreased methane emission. Yeast cultures of *Saccharomyces cerevisiae* potentially stimulate acetogenic microbes in the rumen, consuming H_2 to form acetate and thus potentially reducing methane production (Mwenya et al., 2005).

CONCLUSION

Results showed that the WBC count for sheep fed the HC diet supplemented with *S. cerevisiae* was improved. The TPP value was improved by the addition of *S. cerevisiae* to the high concentrate diet. The methane emission was reduced by the addition of *S. cerevisiae* to the HC diet. Based on the results obtained, the addition of 0.75g of *S. cerevisiae* per kg of the high concentrate diet was recommended.

AUTHORS CONTRIBUTION

Charles Onochie Osita and Augustine Ogbonna Ani conceived, designed and performed the experiment. Chika Ethelbert Oyeagu and Nnanna Ephraim Ikeh helped in data analysis. Ifeyinwa Eunice Ezemagu and Eunice Amaka Akuru read and approved the final manuscript. Valentine Chidozie Udeh assisted in writing part of the work and in data analysis.

CONFLICTS OF INTEREST

Authors declare that there are no conflicts of interest.

REFERENCES

- Abdel-Rahman H, Baraghit GA, Abu-El-Ella AA, Omar SS, Abo-Ammono FF, Kommona OF (2012). Physiological

- responses of sheep to diet supplementation with yeast culture. Egypt. J. Sheep Goat Sci. 7(1): 27-38. <https://doi.org/10.12816/0005005>
- Abdel-Khalek AE, Mehrez AF, Omar EA (2000). Effect of yeast culture (Lacto-Sacc) on rumen activity, blood constituents and growth of suckling Friesian calves. Proc. Conf. Anim. Prod. 21st Century, Sakha, 2000: 201-210.
 - Agazzi A, Tirloni EE, Stella S, Marocco S, Ripamonti B, Bersani C, Caputo JM, Dellorto V, Rota N, Savoini G (2014). Effects of species-specific probiotic addition to milk replacer on calf health and performance during the first month of life. Ann. Anim. Sci. 14(1): 101-115. <https://doi.org/10.2478/aoas-2013-0089>
 - Ali BM, Göksu S (2013). Effects of live yeast supplementation on ruminal parameters and lactation performance of dairy cows fed medium or high levels of dietary concentrate. Kafkas Univ. Vet. Fak. Derg. 19(1): 57-62.
 - Chung YH, Walker ND, McGinn SM, Beauchemin KA (2011). Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in non-lactating dairy cows. J. Dairy Sci. 94(5): 2431-2439. <https://doi.org/10.3168/jds.2010-3277>
 - Dolezal P, Dolezal J, Szwedziak K, Dvoracek J, Zeman L, Tukiendorf M, Havlicek Z (2012). Use of Yeast Culture in the TMR of Dairy Holstein Cows. Iran J. Appl. Anim. Sci. 2(1): 51-56.
 - Duncan DB (1955). Duncan new multiple range test. Biometrics. 1: 1-42. <https://doi.org/10.2307/3001478>
 - El-Ashry MA, Afaf MF, Youssef KM, Salem FA, Aziz HA (2003). Effect of feeding Flavomycin or yeast as feed supplement on lamb performance in Sina. Egypt. J. Nutr. Feeds. 6(1): 1009 – 1022.
 - Galip N (2006). Effect of supplemental yeast culture and sodium bicarbonate on ruminal fermentation and blood variables in rams. J. Anim. Physiol. Anim. Nutr. 90(11-12): 445-452. <https://doi.org/10.1111/j.1439-0396.2006.00625.x>
 - Grant GH, Silverman LM, Christenson RH (1987). Amino acids and proteins. In: N.W. Tietz (eds.), Fundamentals of clinical chemistry. WB Saunders Company, London, pp. 291-345.
 - Hassan SA, El-Saady YMA, Tawffek JA (2009). Effect substitution gradually percentages of reed silage with alfalfa hay fed with probiotic to Awassi lamb. 1- on daily feed intake, live weight gain and feed conversion ratio. Iraqi J. Agric. Sci. 40(1): 107-114.
 - Higgins T, Beutler E, Doumas BT (2008). Measurement of haemoglobin in blood. In: C.A. Burtis, E.R. Ashwood and D. E. Bruns (eds.). Tietz Fundam. Clin. Chem. Saunders Elsevier, Missouri. pp. 514 – 515.
 - Hussein AF (2014). Effect of biological additives on growth indices and physiological responses of weaned Najdi ram lambs. J. Exp. Biol. Agric. Sci. 2(6): 597-607.
 - Kander M (2004). Effect of *Bifidobacterium sp.* on the health state of piglets, determined on the basis of hematological and biochemical indices. Electron. J. Pol. Agric. Univ. 10(2): 443-455.
 - Kingeston-Smith AH, Edwards JE, Huws SA, Kim EJ, Abberton M (2010). Plant-based strategies towards minimizing 'livestock's long shadow'. Proc. Nutr. Soc. Ceredigion, UK. 210: 613-620. <https://doi.org/10.1017/S0029665110001953>
 - LaFleur-Brooks M, LaFleur-Brooks D (2008). Exploring medical language: A student-directed approach, seventh ed. Elsevier, Missouri, USA.
 - Milewski S, Wójcik R, Małaczewska J, Trapkowska S, Siwicki AK (2007). Effect of β -1,3/1,6-D-glucan on meat performance and non-specific humoral defense in lambs. Medycyna Wet. 63(3): 360-363.
 - Milewski S, Sobiech P (2009). Effect of dietary supplementation with *Saccharomyces cerevisiae* dried yeast on milk yield, blood biochemical and haematological indices in ewes. Bull. Vet. Inst. Pulawy. 53(4): 753-758.
 - Moe PW, Tyrrell HF (1979). Methane production in dairy cows. J. Dairy Sci. 62(10): 1583-1586. [https://doi.org/10.3168/jds.S0022-0302\(79\)83465-7](https://doi.org/10.3168/jds.S0022-0302(79)83465-7)
 - Mwenya B, Santoso B, Sar, C, Pen B, Morikawa R, Takaura K, Umetsu K, Kimura K, Takahashi J (2005). Effects of yeast culture and galactooligosaccharides on ruminal fermentation. J. Dairy Sci. 88(4): 1404-1412. [https://doi.org/10.3168/jds.S0022-0302\(05\)72808-3](https://doi.org/10.3168/jds.S0022-0302(05)72808-3)
 - Onifade AA, Obiyan RI, Onipede E, Adejumo DO, Abu OA, Babatunde GM (1999). Assessment of the effects of supplementing rabbit diets with a culture of *Saccharomyces cerevisiae* using growth performance, blood composition and clinical enzyme activities. Anim. Feed Sci. Technol. 77(1-2): 25-32. [https://doi.org/10.1016/S0377-8401\(98\)00244-2](https://doi.org/10.1016/S0377-8401(98)00244-2)
 - Pearson DA (1976). The Chemical Analysis of Foods, seventh ed, Churchill living stone, Edinburgh, New York.
 - Plail R (2006). The innovative power of probiotics. Poult. Int. 45(6): 34-36.
 - Schalm OW, Jain NC, Carol EJ (1975). Veterinary Haematology, third ed. Lea and Febinger, Philadelphia, USA.
 - SPSS (2003). Statistical Package for Social Sciences. Windows Version 8. SPSS Inc., USA.
 - Steel RGD, Torrie JH (1980). Principles and procedures of statistics. A biometric approach, third ed. McGraw-Hill Publishers, New York.
 - Thrall MA, Weiser MG (2000). Haematology. In: C.M. Hendrix (eds.), Laboratory procedures for veterinary technicians. Mosby Inc, Missouri. pp. 29-74. <https://doi.org/10.1111/j.1939-165X.2000.tb00404.x>
 - Tietz NW (1995). Clinical guide to laboratory tests, third ed. WB Saunders Company, Philadelphia.
 - Yan T, Agnew RE, Gordon FJ, Porter MG (2000). Prediction of methane energy output in dairy and beef cattle offered grass silage-based diets. Livest. Prod. Sci. 64(2-3): 253-263. [https://doi.org/10.1016/S0301-6226\(99\)00145-1](https://doi.org/10.1016/S0301-6226(99)00145-1)