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 x 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 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.

| 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 40,000mg, 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:

\[
MCV \text{ (fl)} = 10 \times PCV \text{ (%)}/RBC \text{ counts (millions/µl)}
\]
MCH (pg) = [Haemoglobin (g/dl) x 10] / [RBCs count (million /µl)]
MCHC (g/dl) = haemoglobin (g/dl) x 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 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 cellulose composition of the faeces into the prediction equation of Moe and Tyrrell (1979) as follows:

\[
\text{Methane (CH}_4\text{)} = 3.406 + 0.510 \text{ (soluble residue)} + 1.736 \text{ (hemi-cellulose)} + 2.648 \text{ (cellulose)}
\]

Where;
\[
\text{CH}_4 \text{ is in MJ/d and soluble residue, hemicelluloses and cellulose in kg feed/d (R2 = 0.67).}
\]

**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 MCHC value 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 (x10^12/µl) | MCHC (g/dl) | HbC (g/dl) | MCV (fl) | MCH (pg) | WBC count (x10^9/µl) | Neutrophils (x10^3/µl) | Lymphocytes (x10^3/µl) | Monocytes (x10^3/µl) | Eosinophils (x10^3/µl) |
|-------|---------|-----------------|-------------|------------|---------|---------|--------------------|----------------------|----------------------|--------------------|----------------------|
| **Main effect of roughage: concentrate ratio** |
| HR | 35.79** | 11.26NS | 32.97** | 10.33* | 30.33** | 9.83 | 5.94 | 3.35** | 2.26NS | 0.60* | 0.07** |
| HC | 36.04** | 11.62NS | 33.18** | 11.18* | 31.24** | 10.70* | 7.30* | 3.59** | 2.24NS | 0.75* | 0.07** |
| **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 S. cerevisiae (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** |
| 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** |
| 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** |
| **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 S. cerevisiae supplementation)** |
| HR(SC-0g/kg) | 30.75** | 8.80** | 36.86** | 8.58** | 23.57** | 8.39* | 4.26* | 2.31** | 1.65WS | 0.26* | 0.10** |
| HR(SC-0.75g/kg) | 37.12** | 11.82** | 31.29** | 10.74** | 33.35** | 9.61* | 6.40* | 3.49** | 2.20** | 0.76* | 0.06** |
| HR(SC-1.5g/kg) | 39.50** | 13.16** | 30.76** | 11.69** | 34.05** | 11.48* | 7.16* | 4.26* | 2.93** | 0.79* | 0.06** |
| HC(SC-0g/kg) | 30.62** | 8.57** | 37.90** | 8.99** | 25.40** | 9.11* | 4.31* | 2.45** | 1.21** | 0.61* | 0.09** |
| HC(SC-0.75g/kg) | 38.75** | 13.31** | 30.54** | 12.72** | 35.84** | 11.71* | 8.88* | 4.43** | 3.01WS | 0.83* | 0.06** |
| **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** | 3.29NS | 10.06* | 5.93** | 142.88** | 4.86** |
| High Concentrate | 8.64* | 4.31** | 3.20NS | 9.73* | 6.01** | 142.65** | 4.71** |
| **Standard error of the mean** | 0.08 | 0.07 | 0.08 | 0.08 | 0.05 | 0.32 | 0.07 |
| **Main effect of S. cerevisiae supplementation** |
| 0g/kg | 7.06* | 4.30NS | 2.63* | 12.13* | 7.29* | 142.98** | 4.81NS |
| 0.75g/kg | 8.42* | 4.34** | 3.21* | 8.87* | 5.23* | 142.85** | 4.74** |
| 1.5g/kg | 8.50* | 4.15** | 3.91* | 8.70* | 5.38* | 142.47** | 4.81** |
| **Standard error of the mean** | 0.09 | 0.08 | 0.10 | 0.10 | 0.07 | 0.39 | 0.08 |
| **Interaction (roughage: concentrate ratio with S. cerevisiae supplementation)** |
| HR(SC-0g/kg) | 6.97* | 4.29** | 2.68NS | 12.04* | 7.25** | 142.91** | 4.94** |
| HR(SC-0.75g/kg) | 7.45* | 4.35** | 3.10** | 9.28* | 5.18** | 143.01** | 4.78** |
| HR(SC-1.5g/kg) | 7.63* | 4.03** | 4.09** | 8.88* | 5.35** | 142.71** | 4.88** |
| HC(SC-0g/kg) | 7.15* | 4.31** | 2.59NS | 12.21* | 7.33** | 143.05** | 4.68** |
| HC(SC-0.75g/kg) | 9.38* | 4.33** | 3.31** | 8.26* | 5.29** | 142.69** | 4.70** |
| HC(SC-1.5g/kg) | 9.37* | 4.28** | 3.72** | 8.33* | 5.41** | 142.22** | 4.75** |
| **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. CEREVISAIE 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.

### 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-Khalak 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 Omfade et al. (1999) who reported significant decreases of Ca2+ 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 Kingston-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 H₂ 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.

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