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Xylanase and glucanase supplementation on growth performance and blood profile of *Yankasa* rams fed crop residues

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ABSTRACT: The study was carried out to evaluate the supplementation of xylanase and glucanase on growth performance and blood profile of *Yankasa* yearling rams fed crop residue. The study was designed in a 4×4 Latin square design with 4 yearling rams to measure feed intake, weight gain, feed conversion ratio, and some blood parameters. Four dietary treatments were fed, with T_1 as the control ration without enzyme combination. T_2, T_3, and T_4 contained 50:50, 75:25, and 25:75 xylanase: glucanase combination, respectively. The result of the study showed that no significant ($p>0.05$) effect was observed across treatments for all growth parameters while for blood profile, a significant ($p<0.05$) effect exists. Values for haematological characteristics show significant ($p<0.05$) differences across treatments for all parameters measured except for haemoglobin. Packed cell volume value was higher for T_2 (33.00%), red blood cells values were higher for both T_2 (5.63×10^{12}/L) and T_3 (5.63×10^{12}/L) and white blood cells values were higher for both T_3 (25.35×10^{9}/L) and T_1 (24.00×10^{9}/L). Significant differences ($p<0.05$) were observed for blood urea (3.58, 3.45 and 3.03 mmol/L) to be higher for T_3, T_1, and T_2 respectively while creatinine (110.28 and 107.80 µmol/L) were observed to be higher for T_4 and T_3 respectively. It was therefore concluded that supplementation with or without enzyme combination may not affect growth performance without any health hazard on serology.

Keywords: Enzymes, feed intake, haematology, serology, weight gain.

INTRODUCTION

The population of sheep in Nigeria stands at 22.1 million (Abdu et al., 2012), majority of which are found in the Northern Guinea Savanna zone. This zone coincidently is the major grain producing belt in the country. With these sheep population, its production contributes immensely to the Gross Domestic Product of the country and potentially, the supply of animal products such meat etc (FAO, 2012).

The demand of increased and subsequent high cost of usual feedstuffs have rekindled interest in the use of crop residue of cereal and legumes origins as feed for sheep in Nigeria (Fetuga, 2015). Increasing population pressure (over 182 million) and other developments in the country has led to the most cropped area being extended to land hitherto considered unsuitable for this purpose (Anya et al., 2018). According to Nayawo et al. (2017), ram potential (mutton) has been found to be relatively poor, however the poor yield in meat is not only as a result of the direct genetic and environmental effect, but also and more importantly is caused by poor roughage quality, low supplement inputs and high incidence of disease and parasites. These factors affect sheep productivity greatly, but the most important environmental factor that determines progressive livestock productivity is feed.

For a feed material to stand out as a better feedstuff to be used as an alternative to the conventional feedstuffs, it has to have a better nutritional quality. The nutritive value of feeds can be known from the performance of the animals as well as through blood profiling of the animals (Nayawo et al., 2017). Wada et al. (2014) observed that nutritional studies should not be limited to performance alone, but the effect on the blood constituents are also vital tools that help to dictate any derivation from normal in the animal's body. Growth performance and blood profile are good indices of measuring the physiological status of animals, and changes in the values of these parameters can be used to assess the response of animals to various physiological situations (Silanikove, 2010). Growth performance and blood parameters have been considered useful for the evaluation of body condition, nutritional and immune status in animals where other tissue related measurements are not available. When blood is examined, it provides a good opportunity to clinically investigate the presence of several metabolites and other constituents in the body of an animal (Millam et al., 2020). This is in line with the recommendation of the World Health Organisation on the use of blood parameters in medical nutritional assessment (BAPEN, 2016; Nayawo et al. 2017). A blood examination is also a good way of assessing the health status of an animal as it plays a vital role in the pathological status of an animal (Mohammed et al., 2016).

Since the production cost of raising rams can be reduced by making use of alternative fed to replace the conventional feeds (such as grains), along with animating the performance of the animals, one of such scenarios is by the use of enzymes (Donohue and Cunningham, 2009). It promotes growth parameters such as feed efficiency and body weight gain (Horvatovic et al., 2015). Exogenous enzymes such as xylanase (known to hydrolyse hemicellulose) and glucanase (known to hydrolyse cellulose) have been found to increase the hydrolysis of high amount of cell wall constituents. Higher fibre content that is tough for degradation are found to be reason for the low quality ascribed to crop residues which can be used as an alternative to the much common conventional feeds used in feeding rams (Saleh et al., 2019). Recent studies have been demonstrated to present the beneficial effects of supplementing fibrolytic enzymes in ruminant ration to improve fibre digestibility *in vivo* (Arriola et al., 2011; Gomaa et al., 2012; Bhasker et al., 2013; Hussain et al., 2014; Thammiah et al., 2017). Exogenous fibrolytic enzymes were evaluated on rams and the resulting effect on weight gain and feed intake was unchanged (Bueno et al., 2013; Torres et al., 2013). In another study (Arce-Cervantes et al., 2013), supplementing lignocellulolytic enzymes improved average daily gain and digestibility.

Even though the use of exogenous enzymes in ruminant rations in this country is at its preliminary stages, the focus of this research does not put into consideration the cost implication of the rations fed since most of the ingredients used were not purchased. Therefore, the objective of this study is to determine the efficacy of using commercial enzyme product (xylanase and glucanase) designed for use in non-ruminant rations, could be used as a feed enzyme additive for yearling *Yankasa* rams to enhance growth performance and some blood parameters.

MATERIALS AND METHODS

Description of the study area

The study was conducted at the Small Ruminant Unit of the Adamawa State University Teaching and Research Farm, Mubi. The area lies within the Northern-Guinea Savannah zone of Nigeria. It is located between latitude 10°16.6'6.9" north of the equator and longitude 13°16'1.2" east Greenwich Meridian with 560 meters above sea level. The dry season of the area commences in November and ends in March, while the wet season begins from April and end in late October. The mean annual rainfall is about 1050 mm. The relative humidity is extremely low (20-30%) between January and March but reaches a peak of about 80% in August and September. The maximum temperature can reach 40°C particularly in April while the minimum temperature is about 12°C between December and January (ADSU Metrological Station, 2019).

Source, preparation, and chemical composition of experimental ration

The ingredients [sorghum husk, cowpea husk, local brewer's residue (*burukutu* waste), maize offal, bone meal, and salt] for the experimental rations were obtained from *TIKE* cattle market, Mubi South Local Government Area, Adamawa State. The enzymes (Xylanase and Glucanase) were purchased from a local vendor in Kaduna State, Nigeria. The vendor obtained it from RONOZYME® MultiGrain (MG), DSM Nutritional Products Ltd, Switzerland; xylanase (endo-1,4-β-xylanase; EC 3.2.1.8) and glucanase (endo-1,3 (4)-β-glucanase; EC 3.2.1.6. and endo-1,4-β-glucanase: EC 3.2.1.4).

Four iso-nitrogenous and iso-caloric dietary treatments were formulated using computer method (least-cost ration formulation) to include the cowpea husk, sorghum husk, maize offal, local brewer's residue, bone meal, salt and the enzymes combinations (Table 1). The formulation was made to meet the requirement of the rams.

The enzymes were incorporated in the rations in different combinations or ratios. The difference in enzyme combination makes the difference in the rations. The experimental rations/treatments were denoted as T_1 (0, without enzyme combination or control), T_2 (50:50), T_3 (75:25), and T_4 (25:75) of the xylanase and glucanase combinations respectively to make 100 g per tonne of the ration (manufacturers recommendation). The rations were mixed on a clean concrete floor by use of a shovel, then bagged and kept safe for the experiment. A sample from

Table 1. Dietary composition of the experimental rations.

Parameters (%)	T_1	T_2	T_3	T_4
Cowpea husk	30.00	30.00	30.00	30.00
Sorghum husk	30.00	30.00	30.00	30.00
Maize offal	25.40	25.40	25.40	25.40
Local brewers' residue	12.60	12.60	12.60	12.60
Bone meal	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50
Enzyme combination	0.00	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00
Chemical analysis (%)				
Energy, kcal/kg	2994.3	2994.3	2994.3	2994.3
Crude protein	18.56	18.56	18.56	18.56
Ether extract	6.51	6.51	6.51	6.51
Ash	6.8	6.8	6.8	6.8
Nitrogen free extract	50.13	50.13	50.13	50.13
Acid detergent fibre	51.43	51.43	51.43	51.43
Neutral detergent fibre	46.55	46.55	46.55	46.55

T_1, T_2, T_3, and T_4 denotes 0, 50:50, 75:25, and 25:75 ratio of xylanase and glucanase combinations, Enzyme combination: the combination of both xylanase and glucanase at 50:50, 75:25 and 25:75 levels of inclusion to makeup 10 g.

the formulated experimental ration was collected, and its proximate compositions was determined using the procedures described by AOAC (2011). Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) was determined by the methods of Van Soest and Wine (1991).

Feeding and management of experimental animals

Four yearlings (1 year, using dentition method and confirmation from the merchant) *Yankasa* (*Ovis aries*) rams with an average weight of 21 kg used for the research were purchased from the *TIKE* cattle market. The experimental pens were properly washed and disinfected a week before the arrival of the rams. On arrival, the rams were kept for an adaptation period of 2 weeks while acclimatizing to the new environment before the commencement of the trial. While adjusting to the environment, the rams were fed voluntarily and water *ad lib*, given long-lasting antibiotics (Oxytetracycline LA®) and ivermectin (Ivomec®) against bacterial infection, ecto- and endo-parasites respectively. After the adjustment period, the trial commenced.

Experimental design and data collection

Each ram was housed individually in a well-ventilated pen with a concrete floor equipped with water and feed troughs. The dietary treatments were assigned randomly to each ram in a 4×4 Latin square design (Table 2). The experiment lasted for 60 days in period of 4, such that each period lasted for 15 days. The rams were fed an experimental ration at 3% body weight, twice a day at 08:00 and 14:00 hours; water was offered *ad libitum*. The initial weights (mean 22.7 kg) of the rams were taken at the beginning of the trial using the WeiHeng (WH-A series) potable electronic scale (WH-A08). The subsequent weight of the rams was recorded at the end of each period. Feed intakes were measured daily (using kitchen electronic scale; WH-B05) while the feed/gain ratio were computed weekly until the end of the trial.

Table 2. Experimental layout.

Period/Ram	1	2	3	4
1	T_1	T_4	T_3	T_2
2	T_3	T_2	T_1	T_4
3	T_4	T_1	T_2	T_3
4	T_2	T_3	T_4	T_1

At the end of each period, the rams were bled through the jugular vein as 5 ml of the blood samples were collected over centrifuge bottle for total protein, creatinine, and blood urea determination; fluoride oxalate bottle for total glucose determination; and EDTA (ethylenediamine-tetracetic acid) bottles for haematological parameters. The samples were taken to the Laboratory Service Department in General hospital, Mubi. Haemoglobin and packed cell volume were determined by the acid haematin method (Benjamin, 1985) and Wintrobe's tube (Hawk, 1965), respectively. Determination of the blood glucose levels was done by the Glucose Oxidase Principle (Beach and Turner, 1975); total protein by the method of Henry and Stobel (1957); Creatinine by the method described by Lamb (1991) and blood urea nitrogen by the method described by Tannins and Maylor (1968).

Statistical analysis

The experimental data were analyzed using the Generalised Linear Models Procedure (PROC GLM) of SAS (2002). The effects of dietary treatment were tested at a probability level of 95% ($p<0.05$) and significant differences among the treatment means were separated by Duncan's Multiple Range Test.

Ethical approval

The research protocols and the use of animals used for this study were approved and obtained from Ahmadu Bello University Committee of Animal Use and Care. It certifies that the procedures adhere to the international standards on animal use and practice.

RESULTS AND DISCUSSION

Growth performance of *Yankasa* rams fed a ration supplemented with xylanase and glucanase combinations

It was observed that there was no statistical variation for growth parameters among the experimental treatments (Table 3: $p>0.05$). Relatively higher average daily weight gain (155.14 g) was observed for T_1 compared to the other treatments that may be attributed to the higher feed intake as it is an important factor for accessing the quality and probability of feeds by ruminants (Jiwuba et al., 2016). However, Salem et al. (2011) and Norovsambuu et al. (2017) recorded significant improvement of average body weight gain of sheep fed wheat straw supplemented with enzyme over the control group.

Relatively higher average daily feed intake (1044.7 g) was observed for T_4 compared to other treatments that may be attributed to the enzyme activity (25:75 xylanase: glucanase combination) which was said to improve the palatability of the ration (Beauchemin et al., 2003). It may also be as a result of acceptably due to animal difference (Millam, 2016). The animal difference which refers to some animals accepting to eat particularly more than the other animals. The enzyme activity is usually a function of the rations post-ingestive feedback manifested after consumption (Menezes-Blackburn and Greiner, 2015) in which digestibility is increased to reduced gut fill and trigger the consumption of more feed. This result was similar with those reported by Salem et al. (2011) who fed wheat straw supplemented with an enzyme to sheep. Also, Almaraz et al. (2016) reported a non-significant effect in feed intake when lambs ration was supplemented with exogenous enzymes.

Relatively lower feed conversion ratio (7.53) was observed for T_3 compared to other treatments may be attributed to an efficient hydrolysis by xylanase-glucanase combination at ratio 75:25 in the gut (Menezes-Blackburn and Greiner, 2015). It indicates that the rams were able to convert relatively small amount of ration to a relatively appreciable body mass. The finding was consistence with the work of Arce-Cervantes et al. (2013) and Mendoza et al. (2013) who documented improved feed efficiency when lambs were fed with exogenous fibre enzymes in their rations.

Effects of rations containing xylanase and glucanase on blood parameters of *Yankasa* rams

It was observed that there was statistical variation for haematological parameters among the experimental treatments (Table 4: $p<0.05$) except for haemoglobin. Packed cell volume (PCV) appears to be highest in T_2 (33.00%) significantly compared to other treatments. The PCV values indicates that they were within the normal range (27-45%) recommended for sheep as reported by Weiss and Wardrop (2010) and Latimer (2011). The observed increased PCV may be associated with improved nutritional components (Table 1) of the rations as reported by Umar et al. (2010). This could also mean that *Yankasa* rams react to different rations differently (Gandi et al., 2020). Red blood cell concentration (RBC) was highest (5.63×10^{12}/L) significantly in both T_2 and T_3 compared to other treatments, even though the RBC values appeared to be below the normal range (9-15 $\times 10^{12}$/L) recommended for sheep (Weiss and Wardrop 2010; Latimer, 2011). Values below normal range for RBC points out that the experimental rations are more likely to cause anaemia to the experimental rams (Aruwayo et al., 2011). It does seem that supplementation of the rations with or without enzymes did not enhance absorption of iron and salt from the intestine (Osita et al., 2019). Shittu et al. (2020) reported that the quality and amount of red blood cells in the animal's body depend on nutrient availability, state of health and physiological status of the animal. It was reported that ruminants with the good haematological composition are likely to exhibit better performance and productivity (Isaac et al., 2013). Result of white blood cells (WBC) for T_3 (25.35×10^9/L) was higher significantly compared to other treatments. These values (WBC) indicate that they were above the normal range (4-8 $\times 10^9$/L) recommended for sheep as reported in the literature (Weiss and Wardrop 2010; Latimer, 2011). Increased WBC counts might be related to the production

Table 3. Growth performance of *Yankasa* rams supplemented with xylanase and glucanase

Parameters	T_1	T_2	T_3	T_4	SEM
Initial weight (kg)	23.09	22.90	22.57	22.22	0.59
Final weight (kg)	24.87	22.44	24.37	24.02	0.55
Weight gain (kg)	1.78	1.53	1.80	1.81	0.21
Average daily weight gain (g/d)	155.14	140.69	154.85	151.27	18.47
Feed intake (kg)	11.70	11.06	11.22	12.19	0.96
Average daily feed intake (g/d)	1026.80	976.80	969.50	1044.70	72.46
Feed conversion ratio	7.87	9.70	7.53	8.44	1.50

T_1, T_2, T_3, and T_4 denotes 0, 50:50, 75:25, and 25:75 ratio of xylanase and glucanase combinations, SEM: standard error of means.

Table 4. Blood haematological indices of *Yankasa* ram supplemented with xylanase and glucanase

Parameters	T_1	T_2	T_3	T_4	SEM	Normal range
PVC (%)	31.50^{ab}	33.00^{a}	30.00^{b}	29.00^{b}	0.95	27-45
Hb (g/L)	66.25	65.00	62.75	63.75	4.25	90-150
RBC ($\times 10^{12}$/L)	5.38^{ab}	5.63^{a}	5.63^{a}	4.75^{b}	0.19	9-15
WBC ($\times 10^{9}$/L)	24.00^{a}	19.80^{ab}	25.35^{a}	15.65^{b}	3.14	4-8

^{ab}Means of different superscript are significantly different ($P<0.05$), T_1, T_2, T_3, and T_4 denotes 0, 50:50, 75:25, and 25:75 ratio of xylanase and glucanase combinations, PCV: packed cell volume, Hb: haemoglobin, RBC: red blood cells, WBC: white blood cells. SEM: standard error of means (Source: Weiss and Wardrop, 2010: Latimer, 2011).

Table 5. Serum biochemical profile of *Yankasa* rams supplemented with xylanase and glucanase

Parameters	T_1	T_2	T_3	T_4	SEM	Normal range
Total protein (g/dL)	6.95	6.58	6.98	6.55	0.24	6.0-7.9
Glucose (mmol/L)	5.30	5.25	5.58	5.00	0.60	2.78-4.44
Urea (mmol/L)	3.45^{a}	3.03^{a}	3.58^{a}	1.83^{b}	0.36	2.8-7.1
Creatinine (µmol/L)	95.38^{b}	89.95^{b}	107.80^{a}	110.28^{a}	5.25	106-168

^{ab}Means of different superscript are significantly different ($P<0.05$), T_1, T_2, T_3, and T_4 denotes 0, 50:50, 75:25, and 25:75 ratio of xylanase and glucanase combinations, SEM: standard error of means (Source: Kaneko et al., 2008; Latimer, 2011).

of more immune cells (and thus antibodies) that play an important role in defending the biological system against diseases (Osita et al., 2019).

It was observed that there was statistical variation for serum biochemical indices among the experimental treatments (Table 5: $p<0.05$) except for total protein (TP) and glucose. Blood urea was observed to be highest significantly for T_3 (3.58 mmol/L) compared to other treatments. Blood urea measurement is used especially to indicate renal disease and to a lesser extent liver dysfunction. Higher values obtained for blood urea were within the normal range (2.8-7.1mmol/L) recommended for sheep as reported by Kaneko et al. (2008) and Latimer (2011). Higher values of blood urea and it being within normal range indicates that the dietary protein was well utilized since blood urea was used as an index of renal function. This result was in contrast with the reports of Rivero and Salem (2015), who documented a non-significant effect for blood urea when sheep were fed with mixed *Salix babylonica* extract and exogenous enzyme as feed additives. Creatinine was observed to be significantly highest for T_4 (110.28 µmol/L) compared to other treatments. Higher creatinine values were within the normal range (106-168 µmol/L) recommended for sheep (Kaneko et al., 2008; Latimer, 2011). The higher value and it being within normal range were an indication that the ration along with the enzyme (25:75 xylanase-glucanase) combination supports normal kidney functioning.

Conclusions

The outcome of this study from the chemical composition point of view shows that the rations met the requirements of the rams. Enzyme combinations may interfere with haematological activities. An enzyme combination may affect the activities of the pancreas. Therefore, it can be concluded that supplementation with or without enzyme combination may not affect growth performance without any health hazard on the liver, kidney, and a little on the haem of *Yankasa* rams.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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