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Research Article

Effects of Alkali Treated Groundnut Shells Supplemented with Xylanase in Rations of Yankasa Rams on Haematological and Serum Biochemical Indices

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Abstract

Sixteen yearling *Yankasa* rams with a mean weight of 17 ± 0.68 kg were used to assess its haematology and serum biochemical indices fed rations containing treated groundnut shells supplemented with xylanase. The rations were compounded to contain the treated groundnut shells as UTGNS, UGNS, ULGNS and ULGNS with xylanase supplementation and fed to four rams per group in a completely random design. Feed and water were supplied without restriction. Blood was collected from the jugular vein for the determination of haematological and serum biochemistry parameters. The results observed showed that there were significant ($p < 0.05$) effects amongst the treatments involved. Most of the blood parameters measured were within normal reference range of values recommended for healthy sheep. The PCV, Hb, RBC and lymphocyte values were higher ($p < 0.05$) in LGNS (36.50%, 12.1g/dL, 12.27×10^{12}/L, and 85%, respectively). White blood cells and eosinophil values were higher ($p < 0.05$) in ULGNS (7.9×10^9/L and 2% respectively) while neutrophils and monocytes were higher ($p < 0.05$) in UTGNS (26%) and UGNS (5%) respectively. Alanine aminotransferase (18.5 U/L) and glucose (4 mmol/L) values were higher ($p < 0.05$) in UTGNS. Total protein (7.57 g/dL) and albumin (2.77g/dL) were higher ($p < 0.05$) in LGNS while BUN (5.8 mmol/L) and creatinine (135.07 µmol/L) values were higher ($p < 0.05$) in ULGNS. From the results of the present study, it could be concluded that alkali treated groundnut shells with xylanase supplementation in rations of *Yankasa* rams did not have disastrous effects on blood or organ health throughout the trial.

Keywords: Exogenous enzyme; Haematology; Lime; Serum biochemistry; Sheep; Urea

Abbreviations

ADSUIACEC: Adamawa State University, Institutional Animal Care and Ethics Committee; ALP: Alkaline Phosphatase; ALT: Alanine Transaminase; AST: Aspartate Transaminase; BUN: Blood Urea Nitrogen; EDTA: Ethylenediaminetetraacetic Acid; Hb: Haemoglobin; LGA: Local Government Area; LGNS: Lime Treated Groundnut Shell; PCV: Packed Cell Volume; PICS: Perdue Improved Cowpea Sack; PROC GLM: Procedure General Linear Model; RB: Red Blood Cells; TP: Total Protein; UGNS: Urea Treated Groundnut Shell; ULGNS: Urea-Lime Treated Groundnut Shell; UTGNS: Untreated Groundnut Shell; WBC: White Blood Cells

Introduction

Lack of feed availability is one of the most major hurdles to successful production of tropical small ruminant animals, especially when only low-nutrient roughages are available [1]. The cost of animal feeds and feedstuffs has risen dramatically in recent years. As a result, in many developing nations, such as Nigeria, the cost of feeding becomes a big issue for livestock producers. According to reports, feed costs account for roughly 70-80 percent of overall animal production costs [2]. This necessitates and piques interest in locating abundant and underutilized feed materials and crop residues, such as groundnut hulls, which are frequently obtained in groundnut threshing areas in northern groundnut-producing states [3]. These threshing cites can be found throughout those states and are typically a source of environmental pollution, particularly when left to rot in the field or when rain washes them away, clogging drainage systems and causing flooding; and air pollution when they are incinerated, causing greenhouse effects, which is a global concern [4].

Crop residue utilization as ruminant feed resources has piqued the interest of a number of animal nutritionists who want to learn more about it in order to combat the threat of groundnut shell problems, such as reducing their negative environmental impact, solving the problem of dry season feeding, and lowering production costs [5]. This will allow low-income farmers to switch between conventional and commercial feeds without affecting the animals' health or performance. Groundnut shells are easily available and inexpensive, particularly in threshing towns, and can be processed/treated with alkali chemicals to weaken their fibrous structure and enhance their protein value [6]. Treatment with alkali chemicals alone will not promote groundnut shell use; consequently, supplementation, particularly with exogenous fibrolytic enzymes, may be beneficial [7,8].

For a feedstuff to project as a better feed material and be used as an alternative to commercial/conventional feedstuff, the feedstuff's nutritional quality should be higher [9]. The nutritional value of feeds can be determined by their performance in animals and the use of animal blood profiling [10]. Dietary studies should not be restricted to performance alone but should also consider the effect on blood constituents as crucial instruments for diagnosing any deviation from normal in the animal's physical state [11]. Animals' physiological status can be measured using haematology and serum biochemistry measures. Changes in these characteristics can be utilized to find out how animals react to different physiological circumstances [12]. When additional tissue-related data are unavailable, haemato-biochemical indices have been found to be effective for assessing body condition, nutritional health, and immunological status in animals [1]. The purpose of this study was to see how alkali treated groundnut shells with xylanase supplementation affects the haematological and biochemical indices in *Yankasa* rams".

Materials and Methods

Adamawa State University's Institutional Animal Care and Ethics Committee (ADSUIACEC/2020/006) approved all research protocols and animal use. It attests to the procedures' adherence to international animal use and practice standards.

Description of study area

The research was carried out at Adamawa State University Teaching and Research Farm, Sahuda Road, Mubi North, Adamawa, Nigeria. With 560 meters above sea level, the University is located between latitude 10°16.6'6.9" north of the equator and longitude 13°16'1.2" east Greenwich Meridian [13].

Source and processing of groundnut shells

The groundnut shells for this study came from a local farmer in Adamawa State's Dirbishi Ward, Mubi South Local Government Area (LGA). The groundnut shells were milled to a size of 0.5 cm using a local grinding machine, then stored in bags until needed. The groundnut shells were treated with urea, lime, and urea-lime at a concentration of 5% each (i.e., 5 g urea dissolved in 1 litre of water to treat 1 kg of groundnut shells; 5 g of lime dissolved in 1 litre of water to treat 1 kg of groundnut shells; and 2.5 g of urea plus 2.5 g of lime mixed together dissolved in 1 litre of water to treat 1 kg of groundnut shells respectively). The urea, lime, or urea-lime solution was evenly sprayed on the milled groundnut shells and thoroughly mixed with a shovel on a clean concrete floor [14]. According to [15], the treated groundnut shells were ensiled in airtight Perdue Improved Cowpea Storage (PICS) bags for 21 days. Prior to the start of the experiment, the treated groundnut shells were spread on a polythene sheet to air dry, bagged, and stored. The other ingredients for the experimental rations came from *TIKE* livestock market in Mubi South LGA, Adamawa State. RONOZYME® Multi Grain (MG), DSM Nutritional Products Ltd, Switzerland provided the enzyme (xylanase): xylanase (Endo-1, 4-β-xylanase; EC 3.2.1.8).

Experimental rations and proximate compositions

Four rations containing the treated groundnut shells and enzyme supplementation were fashioned using least-cost ration formulation (Table 1). The recipe was formed to meet the needs of the rams. The enzyme was added directly to the rations as recommended by the manufacturer (100 g per tonne). Each ration was thoroughly mixed using a shovel on a clean concrete floor, then bagged and stored safely for the experiment. The experimental ration was compounded 1 week before the commencement of the trial. Each experimental ration had a sample taken from it, and the proximate compositions were determined using the procedures described in [16]. The proximate determinations was achieved at Adamawa State University's Mubi Nutrition and Biochemistry Laboratory, which is part of the Department of Animal Production.

Ingredients (kg)	UTGNS	UGNS	LGNS	ULGNS
Groundnut shells	40.00	40.00	40.00	40.00
Maize offal	32.50	48.00	46.10	55.00
Cotton seed cake	25.50	10.00	11.90	3.00
Bone meal	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50
Xylanase	0.10	0.10	0.10	0.10
Proximate compositions (%)				
Energy (kcal/kg)	1596.45	2177.10	2093.40	2446.65
Dry matter	93.0	93.5	93.0	91.5
Crude protein	9.1	11.3	11.3	13.5
Crude fibre	40.75	33.6	38.35	30.25
Ether extract	5.5	6.5	7.0	6.5
Ash	13.0	7.0	6.0	8.0
Nitrogen free extract	22.4	34.7	31.2	40.0
Feed cost (₦/kg) diet	62.22	53.11	71.44	57.38

Table 1: Gross compositions of the experimental rations.

UGNS: Untreated Groundnut Shell; UTGNS: Urea Treated Groundnut Shell; LTGNS: Lime Treated Groundnut Shell; ULGNS: Urea-Lime Treated Groundnut Shell.

Management of experimental animals

The study used sixteen yearling (12-15 months) *Yankasa* (*Ovis aries*) rams from the *TIKE* livestock market in Uba Town, Askira/Uba LGA, Borno State, with an average weight of 17 ± 68 kg. The animals were ear-tagged prior to the start of the experiment (for identification). Prophylactic treatments for the rams included intramuscular injections of long-acting antibiotics (Oxytetracycline LA®) and a multivitamin at a dose of 1 ml/10 kg body weight. They were given 1 ml/10 kg body weight of albendazole for endoparasites and 0.5 ml/10 kg body weight of ivermectin (Ivomec®) to treat ectoparasites. For four weeks, the rams were quarantined. The rams were given *ad libitum* access to adequate feed and clean, fresh water on a daily basis.

Experimental housing, design and data collection

The experimental animals were housed in a well-ventilated stall under a building with a corrugated iron roof and concrete floors. The stalls were fitted with individual feeding and water troughs (1.5 × 1.5 m in length). A week prior to the start of the feeding trial, these stalls were thoroughly cleaned and disinfected. In a completely randomised design, each ration was assigned to a group of four rams at random. Throughout the 90-day feeding trial, the rams were allowed access to the experimental rations and clean water without restriction on a daily basis. The daily ration was divided into two equal-weight portions and given to the animals at 8:00 a.m. and 2:00 p.m.

Blood samples were taken from the jugular veins of fasted (for 12 hours) rams using a 5 ml syringe over EDTA (Ethylenediaminetetraacetic acid) bottles for packed cell volume, haemoglobin count, red blood cells, and white blood cells determination; a centrifuge bottle for total protein, liver enzymes, creatinine, and blood urea determination; and a fluoride oxalate bottle for glucose analysis. The samples were taken to the Mubi General Hospital's Laboratory Service Department for blood analysis. Acid haematin method [17] and Wintrobe's tube [18] were used to determine haemoglobin and packed cell volume, respectively. The enzymes in the liver were measured using the methods described in [19]. The Glucose Oxidase Principle [20] was used to determine blood glucose levels, total protein was determined using the method of [21], creatinine was determined using the method of [22], and blood urea nitrogen was determined using the method of [23].

Statistical analysis

The Generalised Linear Model procedure (PROC GLM) of [24] was used to analyse the experimental data. The effects of dietary treatments were tested at a 95% confidence level ($p < 0.05$), and the Duncan Multiple Range Test was used to determine whether there was a significant difference within the treatment means.

Results and Discussion

Blood haematological profile

Table 2 depicts the results pertaining to blood profile of the experimental rations. A significant ($p < 0.05$) interaction was observed among all the rations fed except for basophiles. Addition of xylanase and alkali treatment had higher significant ($p < 0.05$) values in all parameters measured except neutrophils which was highest in the untreated group. The majority of the haematological values were well within normal physiological ranges advocated for healthy sheep [25], implying that the rams' overall health remained stable throughout the experiment. Lymphocytic counts, on the other hand, are above the reference range. The higher PCV, Hb, and RBC levels in the LGNS group could be attributed to the animals' improved nutrition as a result of the enzyme supplementation and lime treatment. It might have improved erythropoiesis or reduced the establishment of adult nematodes [26]. This is an indication that neither the total cell concentration of the blood was affected by the chemical treatment involved in the ration and the enzyme supplementation to cause anaemia, nor the oxygen carrying capacity of the blood was affected by chemical treatment involved and the enzyme supplementation while the rams were consuming the ration [1]. The findings of this study contradict those of [27], who found that adding exogenous enzymes to the rations of growing lambs had no effect on any of the haematological parameters measured. However, it is similar to the results reported by [1], who fed *Yankasa* rams a xylanase and glucanase combination in their rations. The increase in WBC counts ($p < 0.05$) in the ULGNS group could be due to the production of more antibodies, which play an important role in the biological system's defence against infection [28]. The results of WBC observed in this study are in agreement with those of [1] who also reported a higher value above normal ranges when *Yankasa* rams were fed with ration containing xylanase and glucanase combinations.

For the blood differential WBC counts, the neutrophils, monocytes and eosinophil values were within normal physiological range for healthy sheep [25] suggesting that the animals are not likely to be predispose to infection [29]; or there was no problem with the cell production nor likely to come down with leukaemia [30]. It may also mean that the rams were safe from parasitic infestation of the intestine and any form of allergy respectively [25]. The results were comparable with the findings of several authors who studied the haematology of *Yankasa* rams in the semi-arid areas of the Nigeria [31,32]. Obviously, the lymphocyte count was observed to be above normal range, it may be attributed to the animals having sufficient potential to ward off attacks that could cause health challenge [28,33]. The observed results are in tandem with the findings of some authors [32,34] who reported similar values for sheep in the Arid zone of Nigeria while others [35] recorded lower values.

Parameters	UTGNS	UGNS	LGNS	ULGNS	SEM	Ref. ranges
Packed cell volume (%)	30.50^{b}	31.50^{b}	36.50^{a}	28.50^{c}	0.86	27-45
Haemoglobin (g/dL)	10.48^{b}	10.16^{bc}	12.10^{a}	9.44^{c}	0.24	9-15
Red blood cell ($\times10^{12}$/L)	10.48^{b}	11.50^{a}	12.27^{a}	10.00^{b}	0.46	9-15
White blood cell ($\times10^{9}$/L)	5.60^{b}	7.67^{a}	7.87^{a}	7.90^{a}	0.52	4-8
Neutrophils (%)	26.00^{a}	20.00^{b}	12.50^{c}	25.00^{a}	1.48	10-50
Lymphocytes (%)	70.00^{c}	73.50^{b}	85.00^{a}	70.50^{bc}	1.50	40-55
Monocytes (%)	2.50^{b}	5.00^{a}	0.50^{c}	2.50^{b}	0.18	0-6
Eosinophils (%)	1.00^{c}	1.50^{b}	1.00^{c}	2.00^{a}	0.07	0-10
Basophiles (%)	0.00	0.00	0.00	0.00	0.00	0-3

Table 2: Blood haematology in *Yankasa* rams fed experimental rations.

^{abc} Mean values within same row with different superscript are significantly ($p < 0.05$) different, UTGNS: untreated groundnut shells, UGNS: urea treated groundnut shells, LGNS: lime treated groundnut shells, ULGNS: urea-lime groundnut shells, SEM: significant error of means (Source: [25]).

Serum biochemical indices

Blood metabolites concentrations are presented in table 3. The inclusion of xylanase and alkali treatment had no effect ($p > 0.05$) on AST and ALP while other parameters measured were significantly ($p < 0.05$) affected. However, higher ($p < 0.05$) values were recorded in both the untreated and treated groups. Most of the biochemical indices measured were within normal reference ranges recommended for healthy sheep [25] which indicates that the organs of the rams were functioning in a state of normal health condition during the study.

Parameters	UTGNS	UGNS	LGNS	ULGNS	SEM	Ref. ranges
Aspartate transaminase (IU/L)	58.50	64.50	65.00	65.00	5.83	60-280
Alanine transaminase (IU/L)	18.50^{a}	16.00^{b}	11.00^{c}	8.50^{d}	0.98	26-34
Alanine phosphatase (IU/L)	15.50	19.00	17.50	18.00	2.49	68-387
Total protein (g/dL)	5.20^{c}	7.37^{ab}	7.57^{a}	7.07^{b}	0.21	6.0-7.9
Albumin (g/dL)	2.80^{b}	2.50^{c}	2.77^{a}	2.60^{b}	0.04	2.4-3.0
Glucose (mmol/L)	4.00^{a}	2.97^{c}	3.87^{a}	3.40^{b}	0.09	2.78-4.44
Urea Nitrogen (mmol/L)	4.10^{d}	4.97^{c}	5.17^{b}	5.80^{a}	0.28	2.8-7.1
Creatinine (µmol/L)	131.20^{a}	127.87^{a}	106.52^{b}	135.07^{a}	8.66	106-168

Table 3: Serum biochemical indices in *Yankasa* rams fed experimental rations.

^{abc} Mean values within same row with different superscript are significantly ($p < 0.05$) different, UTGNS: untreated groundnut shells, UGNS: urea treated groundnut shells, LGNS: lime treated groundnut shells, ULGNS: urea-lime groundnut shells, SEM: significant error of means (Source: [25]).

Blood biochemical test determines the levels of certain parameters to assess the level of energy metabolism, liver damage and kidney malfunction in a body system. Furthermore, levels of some of these parameters that are above or below reference range are of great concern. For the liver function test profile of the rams, the values for liver enzymes (AST and ALP) are not significant ($p > 0.05$) while ALT ($p < 0.05$) values are below normal physiological range for healthy sheep [25], suggesting that ALT (been liver specific) are still been produced within the liver and are not spilled into the blood stream to show that there is no injury/damage to the liver [11,32-37]. The current study's findings ($p < 0.05$) contradict the findings of [27] and [38] in sheep and goats for non-significant differences in hepatic enzyme levels when fed rations supplemented with exogenous enzymes.

In the hepatic functioning, the mean values for TP and Albumin were within normal physiological range suggested for healthy sheep [25]. Higher values for TP and Albumin in the group of rams receiving LGNS could be an indication that the animals received an adequate dietary protein supply (Table 1; crude protein) that was sufficient for protein metabolism in the liver. The result observed for Albumin being within the normal reference range may also be due to isoalbuminemia as a result of acceptable protein supply in the ration [39].

The metabolizable energy in feed varied among rations as observed in table 1, nevertheless none of the supplemental rations affected plasma glucose because all values were within normal physiological range recommended for healthy sheep [25]. However, the concentration of plasma glucose showed higher ($p < 0.05$) values in the group of rams receiving UTGNS and LGNS. This may have stem from the enzyme supplementation which was able to hydrolyse the fibre fraction probably due to crude fibre digestibility of the ration which consequently increased its absorption along with the non-structural carbohydrates in the ration [40]. It might also be attributed to increased intake of carbohydrates, leading to increased level of blood glucose [10,11]. The findings back up the findings of some authors who found that blood glucose levels in sheep were related to the amount of energy in their rations [10,31,32,37].

Looking at the kidney function attributes of the *Yankasa* rams, values of measured parameters (blood urea nitrogen, BUN and creatinine) in all the group of animals were within normal physiological range for healthy sheep [25]. Increased BUN levels in the ULGNS group may be due to more efficient utilization of dietary proteins due to the addition of xylanase than in the other treatments [41]. It could also be attributed to the ULGNS groups' optimal rate of protein degradation, as well as better utilization of generated ammonia in the rumen, which was represented in terms of normal BUN [1]. The serum creatinine levels in the rams given experimental ration being within the normal reference range, indicates that the enzyme supplementation had no adverse effect on glomerular filtration and was thus safe for renal function. Also, it is an indication of normal wear and tear of the muscle in the system created during normal functioning of the muscle [39]. The findings of significant effect on serum creatinine in the present study are not similar to those described by [42] for feed additive supplementation in sheep. Creatinine levels in combination with BUN concentrations indicate that the kidneys are functioning normally.

Conclusions

From the results obtained in the present study, it was concluded that UGNS was cost effective and the combination of urea and lime treatment of groundnut shells with xylanase supplementation in rations of *Yankasa* rams did not pose any health implication when fed throughout the trial. This is because most of the indices measured were within normal reference range of values recommended for healthy sheep. Thus, urea or lime treated groundnut shells with xylanase supplementation can be incorporated in the rations of *Yankasa* without any health hazards on haem, liver and kidney.

Conflict of Interest

There was no conflict of interest among the authors for the present research.

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