Nutrient Utilization in Yankasa Rams Fed Crop Residue Supplemented With Xylanase and Glucanase Combinations

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Abstract

Background: There is less information in Nigeria with regard the understanding of the use of exogenous enzymes widely used in monogastric diets in ruminant’s rations. The study evaluated the effects of supplementing xylanase and glucanase in combination in rations of Yankasa rams. Sixteen yearling Yankasa rams (average 21 kg) were used. Four treatments were evaluated: control (without enzyme combination), 50:50, 75:25 and 25:75 xylanase-glucanase combinations denoted as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> respectively. The basal roughage was cowpea husk and sorghum husk. The feeding trial was conducted using complete randomize design.

Results: There were differences (p<0.05) with regard to nutrient intake and total digestibility coefficient except crude protein and nitrogen free extract digestibility (p>0.05). The intake and digestibility increased with supplementation of xylanase and glucanase combination at 25:75 ratio respectively. It increased DM intake by 211.90 g/d, DM digestibility by 17.73%, ADF digestibility by 1.17% and NDF digestibility by 1.02%. The nitrogen balance in the body did not increase (p>0.05) with supplementation of 50:50 xylanase-glucanase combination. The efficiency of nitrogen utilization did not differ between the control and 50:50 xylanase-glucanase combination.

Conclusion: The combination of xylanase and glucanase at ratio 25:75 respectively improved nutrient intake and digestibility but did not influence nitrogen utilization.

Background

The scarcity of feeds is one of the major constraint to livestock production in the northern region of Nigeria (1). These are due to the shortage & cost of conventional feeds (consumed by humans) and the crop residues (consumed by a large number of animals) available are of poor nutritional status. These poor quality roughages comprise a huge part of the feed readily available and its consumption results to low plane of nutrition with attendant low productivity of the country's indigenous animals (2). Even though these crop residues are available to the ruminants and are produced in large quantity annually (most of them comes as threshing by-product), little quantity is used as feeds. The rest are usually left on farm to rot or thrown away as waste (3).

These problems has directed research efforts towards harnessing and enhancing the utilization of these abundant arable crop residues. Therefore, the concept of matching available/abundant feed resources with the production of ruminants has consequently intensified research into the evaluation of crop residues for feeding livestock rather than discarding it as waste (4). Similarly, since animal performance on these crop residues are poor, one of the ways to improve the quality of these crop residues is through enzyme supplementation (5). Commercial fibrolytic enzymes such as xylanase and glucanase have been used in monogastric rations over the years. Few trials have tested its efficacy in ruminates especially in Nigeria (6). Such exogenous enzymes have been tried in other regions to have recorded improved weight gain, feed efficiency, digestibility and rumen degradability (7–13). Therefore, the aim of this study was to
determine the effects of using xylanase and glucanase designed for use in monogastric rations, could be used as feed enzyme additive for Yankasa rams to enhance nutrient utilization.

**Methods**

This experiment was carried out at the Small Ruminant Unit of Adamawa State University Teaching and Research Farm, Mubi. All research protocols and use of animals were approved by Ahmadu Bello University Committee of Animal Use and Care. It certifies that the procedures adhere to the International standards on animal use and practice.

The feed ingredients [cowpea husk, sorghum husk, local brewer’s residue (burukutu waste), maize offal, bone meal and salt] for the experiment 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, Kaduna State. 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).

The study evaluated the following dietary treatments: T\(_1\), control (no supplementation); T\(_2\), with 50:50 ratio of xylanase-glucanase combination; T\(_3\), with 75:25 ratio of xylanase-glucanase combination; T\(_4\), with 25:75 ratio of xylanase-glucanase combination (Table 1). The difference in enzyme combination makes the difference in ration. The enzymes were incorporated into the rations at the rate of 100 g per tonne of feed (manufacturer’s recommendation). The ration was formulated using computer method (least cost ration formulation software).
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 (%)**

|                        | \( T_1 \) | \( T_2 \) | \( T_3 \) | \( T_4 \) |
|------------------------|------------|------------|------------|------------|
| Energy (kcal/kg)       | 2994.30    | 2994.30    | 2994.30    | 2994.30    |
| Dry matter             | 94.50      | 94.50      | 94.50      | 94.50      |
| Crude protein          | 18.56      | 18.56      | 18.56      | 18.56      |
| Ether extract          | 6.51       | 6.51       | 6.51       | 6.51       |
| Ash                    | 6.80       | 6.80       | 6.80       | 6.80       |
| 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      |

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 rations were mixed on a concrete floor by use of shovel, then bagged and kept safe for the experiment. A sample from the formulated ration was collected, and its proximate compositions was determined using the procedures described by (14). Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) was determined by the methods of (15).

Sixteen yearling Yankasa rams *Ovis aries* with an average initial weight of 21 kg were used in this experiment. The rams were purchased from *TIKE* cattle market. Prior to the experiment, the rams were adapted to the experimental conditions for 2 weeks. 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 adaptation period was over, the feeding trial commenced.

The rams were housed in a well ventilated, individual enclosures (2 × 2 m) with concrete floor and equipped with individual feeders and water troughs. These stalls were washed properly and disinfected a week before the commencement of the feeding trial.

The rations were assigned randomly to each group of rams using Completely Randomise Design with 4 rams per treatment. The rams were fed at 3% body weight and water was offered ad libitum. The total ration for a day was separated into two portions of equal weight and supplied to the animals at 8:00 h and 14:00 h. Feed offered and left over was measured daily using electronic kitchen scale (WH-B05). The left over is deduced from the feed offered to compute the feed intake daily until the end of the feeding trial. The feeding trial lasted for 12 weeks.

For the digestibility trial, the rams were housed in individual metabolic cages according to the procedures of (16). The metabolic cages was designed for separate collection of urine and faeces. Twenty one days was used as adjustment period on the crates, while seven days was for the collection of urine and faeces. Daily faeces and urine voided out by each ram was collected and measured. At the end of the collection period, the total faeces was then bulked and representative aliquot (10%) sample was collected for chemical analysis. Total urine collection was made over urine container acidified with sulphuric acid; 10 ml 0.1N H₂SO₄ (16). This was to avoid nitrogen loss. The urine was measured and 20 ml aliquot were collected for nitrogen analysis at the Lab. The nitrogen was analysed using the Kjeldahl method of nitrogen analysis (14).

Nutrient intake and digestibility coefficient of the experimental rations was calculated using the methods described by (17). Nitrogen retention was computed as the difference between nitrogen intake and nitrogen losses (urine and faeces) while nitrogen as percentage of intake was calculated from the nitrogen retention expressed as a percentage of nitrogen intake.

All statistical procedure were carried out and analysed according to PROC GLM procedures of (18). The effect of treatment means were tested at probability level of 95% (p < 0.05) and significant effects were compared using Duncan Multiple Range Test.

**Results And Discussion**

**Intake and digestibility**

There were differences (p < 0.05) among treatments with regards to all parameters measured (average daily feed intake, ADFI; dry matter intake, DMI; crude protein intake, CPI; nitrogen free extract intake, NFEI; acid detergent fibre intake, ADFI and neutral detergent fibre intake, NDFI) for nutrient intake (Table 2). T₄ (ADFI, 1239.70 g; DMI, 1171.50 g; CPI, 230.09 g; NFEI, 621.50 g; NDFI, 637.60 g, ADFI, 577.10 g) was observed to be higher statistically (p < 0.05) for all the parameters compared to other treatments.
Table 2
Nutrient intake in Yankasa rams fed experimental diets

| Parameters (g)               | \(T_1\)     | \(T_2\)     | \(T_3\)     | \(T_4\)     | SEM  |
|------------------------------|-------------|-------------|-------------|-------------|------|
| Average daily feed intake    | 1015.40\textsuperscript{b} | 976.80\textsuperscript{b} | 969.50\textsuperscript{b} | 1239.70\textsuperscript{a} | 94.99 |
| Dry matter intake            | 959.60\textsuperscript{b} | 923.00\textsuperscript{b} | 916.20\textsuperscript{b} | 1171.50\textsuperscript{a} | 89.77 |
| Crude protein intake         | 188.46\textsuperscript{b} | 181.29\textsuperscript{b} | 179.95\textsuperscript{b} | 230.09\textsuperscript{a} | 17.63 |
| NFE intake                   | 509.00\textsuperscript{b} | 489.60\textsuperscript{b} | 486.00\textsuperscript{b} | 621.50\textsuperscript{a} | 47.62 |
| Neutral detergent fibre intake| 522.20\textsuperscript{b} | 502.30\textsuperscript{b} | 498.60\textsuperscript{b} | 637.60\textsuperscript{a} | 48.85 |
| Acid detergent fibre intake  | 472.70\textsuperscript{b} | 454.70\textsuperscript{b} | 451.30\textsuperscript{b} | 577.10\textsuperscript{a} | 44.22 |

\textsuperscript{ab}: Means with different superscript are significantly different, NFE: nitrogen free intake, SEM: standard error of means

Increased nutrient intake is an important factor for improved productivity in and utilization of feed by ruminants (19) which may be associated with some factors such as the palatability of the feed, low fibre fraction etc. (20). This translates to the reason which may have brought about this results. High feed intake as observed with the rams receiving 25:75 xylanase-glucanase combination was an indicator that the rams ate the ration due to the taste or smell (21), and consequently influence the prehension and intake frequency of the rams (22). Similarly, the increased nutrient intake may be due to the ration having less lignified cell wall tissues as a result of enzyme supplementation. This decreases ration retention time in the rumen (23). The improvement may have been associated with the enzyme activity that was aimed at enhancing the fibre hydrolysis (5). The results obtained here differ from those reported by several authors who studied the use of various enzyme complex and discovered that its supplementation did not affect nutrient intake in sheep (9, 24, 25).

Statistical influence (\(p < 0.05\)) was observed in all parameters measured for digestibility coefficient (Table 3) except crude protein and nitrogen free extract (\(p > 0.05\)). \(T_4\) was observed to have improved dry matter (73.01%), acid detergent fibre, ADF (65.94%) and neutral detergent fibre, NDF (79.82%) compared to other treatments.
Table 3
Nutrient digestibility of experimental diets in Yankasa rams

| Parameters (%)            | T₁   | T₂   | T₃   | T₄   | SEM  |
|---------------------------|------|------|------|------|------|
| Dry matter                | 55.28ᵇ | 60.53ᵇ | 62.28ᵇ | 73.01ᵃ | 5.16 |
| Crude protein             | 94.04 | 94.85 | 95.75 | 95.80 | 2.15 |
| Ether extract             | 97.68ᵃ | 96.96ᵇ | 97.41ᵃᵇ | 97.95ᵃ | 0.25 |
| Ash                       | 66.59ᵃ | 56.83ᵇ | 61.31ᵃᵇ | 68.76ᵃ | 4.55 |
| Nitrogen free extract     | 75.85 | 76.06 | 75.75 | 76.65 | 0.86 |
| Acid detergent fibre      | 64.77ᶜ | 65.14ᵇ | 65.09ᵃᵇ | 65.94ᵃ | 0.16 |
| Neutral detergent fibre   | 78.80ᶜ | 79.39ᵇ | 78.63ᶜ | 79.82ᵃ | 0.20 |

ᵃᵇᶜ: Means of different superscript are significantly different, SEM: standard error of means

Improved DM, ADF and NDF observed in this study might be as a result of enzyme supplementation (5). These was an indication that the use of 25:75 xylanase-glucanase combination in the ration was successful in improving the digestibility of the crop residues within the gut of the rams. The results was consistent with the works of various authors who reported that exogenous enzymes supplementation in fibrous ration increased fibre digestibility in sheep (26–29). These positive improvements associated with enzyme combination may have resulted from enhanced colonization of feed by increasing the number of ruminal fibrolytic microbes (30), non-fibrolytic microbes (31), increased rate of fibre degradation in the rumen (28), increased rumen microbial protein synthesis (32) and total tract digestibility (33). However, the increased digestion of the fibre fraction (ADF and NDF) in the 25:75 xylanase-glucanase combination compared to the control may also be related to reduced digesta viscosity (34); altered ruminal fermentation (33) or reduction of rumen physical fill over time which will consequently increase nutrient intake (Table 2, Salem et al., 2011).

**Nitrogen utilization**

The results records no statistical (p < 0.05) difference among treatments for nitrogen intake and urine nitrogen (Table 4). Nitrogen losses was relatively higher in T₄ (0.61 g/d), it might be attributed to higher loss of nitrogen in the faeces.
Table 4
Nitrogen balance in Yankasa rams fed experimental diets

| Parameters (g/d)               | T₁   | T₂   | T₃   | T₄   | SEM  |
|--------------------------------|------|------|------|------|------|
| Nitrogen intake                | 2.97 | 2.97 | 2.97 | 2.97 | 0.00 |
| Faecal nitrogen                | 0.21ᵇ | 0.22ᵇ | 0.30ᵃ | 0.31ᵃ | 0.01 |
| Urine nitrogen                 | 0.29 | 0.30 | 0.30 | 0.30 | 0.01 |
| Nitrogen losses                | 0.50ᶜ | 0.51ᵇ | 0.60ᵃ | 0.61ᵃ | 0.01 |
| Nitrogen absorbed              | 2.76ᵃ | 2.76ᵃ | 2.67ᵇ | 2.66ᵇ | 0.01 |
| Nitrogen retained/balance      | 2.47ᵃ | 2.46ᵃ | 2.37ᵇ | 2.36ᵇ | 0.01 |
| Nitrogen balance as % of intake (%) | 83.27ᵃ | 82.92ᵃ | 79.66ᵇ | 79.44ᵇ | 0.41 |

ᵃᵇ: Means of difference superscript are significantly different, SEM: standard error of means.

Nitrogen obtained from the ration consumed by an animal is lost through urine (about 90%) and through faeces (about 10%). In a situation where the losses in faeces is high, it may lead to high nitrogen loss (36). Literatures point out that the major reason which affects the loss of nitrogen through faeces is the forage: concentrate ratio since a high level of concentrate in the diet results in an increased rate of passage which consequently increases nitrogen loss through the activities of the microbes (17, 37, 38). Hence, the absence of any significant effect for urine nitrogen may also be explained by forage: concentrate ratio of being same (60: 40) in the treatments. The absence of effects may also be due to the fact of the experimental ration been isonitrogenous. The nutritional demands of ruminants highlights the synchronization between protein and dietary carbohydrates in the rumen to maximize microbial synthesis, thereby reducing nitrogen loss through urine (39).

The higher nitrogen absorbed (2.76 g/d for both T₁ and T₂) and nitrogen retained (T₁, 2.47 g/d; T₂, 2.46 g/d) was not influenced (p < 0.05) by supplementing the ration with xylanase and glucanase at 50:50 combination because they are statistically same. Nitrogen retention is often referred as a good method of estimating the quantity of nitrogen available for body tissue deposition (39). This is an indication that the diets were able to present adequate levels of nitrogen (Table 1). Increased nitrogen retained/balance is associated with higher urea production in the liver and lower excretion in the urine (40).

The nitrogen balance as percentage of intake (T₁, 83.27%; T₂, 82.92%) was not influenced (p < 0.05) by xylanase and glucanase supplementation at 50:50 combination. Thus, the positive nitrogen balance as percentage of intake noted in all the treatments indicates that there was no excessive loss of nitrogenous compounds during the trial (39). This confirms that the fraction of protein in the ration was adsorbed
efficiently by the animals (41). Also, this positive balance is an indication that the protein and energy demands (Table 1) of the rams were satisfied most likely and there was protein retention in the body of the rams thereby avoiding weight loss (42).

**Conclusion**

The supplementation of Yankasa rams fed crop residues with xylanase and glucanase combinations can increase nutrient intake, nutrient digestibility, but may not influence increased nitrogen retention. The improvement for the nutrient intake and digestibility might be as a result of the combination of xylanase and glucanase at a ratio of 25:75 respectively.

**Abbreviations**

DM: dry matter; ADF: acid detergent fibre; NDF: neutral detergent fibre; DSM: Dutch State Mines; EC: enzyme commission number; PROC GLM: generalised linear model procedure; DMI: dry matter intake; CPI: crude protein intake; NFEI: nitrogen free extract intake; ADFI: acid detergent fibre intake; NDFI: neutral detergent fibre intake.

**Declarations**

**Ethics approval:**

All research protocols and use of animals were approved by Ahmadu Bello University Committee of Animal Use and Care. It certifies that the procedures adhere to the International standards on animal use and practice.

**Consent for publication:**

Not applicable

**Availability of data and materials:**

the authors don't really understand what is been referred here.

**Competing interests:**

The authors declare that they have no competing interests.

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**Author’s contributions:**
J JM, conceived the study, performed the statistical analysis, made major contributions to draft of manuscript, and coordinated the research group. AW and HP carried out the experimental trial, and helped draft the manuscript. All authors read and approved the final manuscript.

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