

# Improving the Utilization of *Telfairia occidentalis* Leaf Meal with Cellulase-Glucanase-Xylanase Combination and Selected Probiotic in Broiler Diets

Ayodeji Fasuyi

## I. INTRODUCTION

**Abstract**—*Telfairia occidentalis* is a leafy vegetable commonly grown in the tropics for nutritional benefits. The use of enzymes and probiotics is becoming prominent due to the ban on antibiotics as growth promoters in many parts of the world. It is conceived that with enzymes and probiotics additives, fibrous leafy vegetables can be incorporated into poultry feeds as protein source. However, certain antinutrients were also found in the leaves of *Telfairia occidentalis*. Four broiler starter and finisher diets were formulated for the two phases of the broiler experiments. A mixture of fiber degrading enzymes, Roxazyme G2 (combination of cellulase, glucanase and xylanase) and probiotics (*Turbotox*), a growth promoter, were used in broiler diets at 1:1. The Roxazyme G2/*Turbotox* mixtures were used in diets containing four varying levels of *Telfairia occidentalis* leaf meal (TOLM) at 0, 10, 20 and 30%. Diets 1 were standard broiler diets without TOLM and Roxazyme G2 and *Turbotox* additives. Diets 2, 3 and 4 had enzymes and probiotics additives. Certain mineral elements such as Ca, P, K, Na, Mg, Fe, Mn, Cu and Zn were found in notable quantities viz. 2.6 g/100 g, 1.2 g/100 g, 6.2 g/100 g, 5.1 g/100 g, 4.7 g/100 g, 5875 ppm, 182 ppm, 136 ppm and 1036 ppm, respectively. Phytin, phytin-P, oxalate, tannin and HCN were also found in ample quantities viz. 189.2 mg/100 g, 120.1 mg/100 g, 80.7 mg/100 g, 43.1 mg/100 g and 61.2 mg/100 g, respectively. The average weight gain was highest at 46.3 g/bird/day for birds on 10% TOLM diet but similar ( $P > 0.05$ ) to 46.2 g/bird/day for birds on 20% TOLM. The feed conversion ratio (FCR) of 2.27 was the lowest and optimum for birds on 10% TOLM although similar ( $P > 0.05$ ) to 2.29 obtained for birds on 20% TOLM. FCR of 2.61 was the highest at 2.61 for birds on 30% TOLM diet. The lowest FCR of 2.27 was obtained for birds on 10% TOLM diet although similar ( $P > 0.05$ ) to 2.29 for birds on 20% TOLM diet. Most carcass characteristics and organ weights were similar ( $P > 0.05$ ) for the experimental birds on the different diets except for kidney, gizzard and intestinal length. The values for kidney, gizzard and intestinal length were significantly higher ( $P < 0.05$ ) for birds on the TOLM diets. The nitrogen retention had the highest value of  $72.37 \pm 0.10\%$  for birds on 10% TOLM diet although similar ( $P > 0.05$ ) to  $71.54 \pm 1.89$  obtained for birds on the control diet without TOLM and enzymes/probiotics mixture. There was evidence of a better utilization of TOLM as a plant protein source. The carcass characteristics and organ weights all showed evidence of uniform tissue buildup and muscles development particularly in diets containing 10% of TOLM level. There was also better nitrogen utilization in birds on the 10% TOLM diet. Considering the cheap cost of TOLM, it is envisaged that its introduction into poultry feeds as a plant protein source will ultimately reduce the cost of poultry feeds.

**Keyword**—*Telfairia occidentalis* leaf meal, enzymes, probiotics, additives.

Ayodeji Fasuyi is with the Ekiti State University, Nigeria (e-mail: ayodeji.fasuyi@eksu.edu.ng).

MANY countries all over the world have embraced the poultry industry and the production of poultry products (meat and egg) as important commercial economic activities which significantly reduce the challenges of animal protein intake and its acceptance. Plant leaves have been suggested as a source of abundant and naturally occurring protein for animal feed manufacturing. The resources needed for the synthesis and polymerization of amino acid monomers into plant proteins are naturally occurring [1]. The polymerization of the amino acids in plant leaves will also involve the build-up of unwanted anti-nutritional factors that render the polymerized amino acids less nutritionally valuable for man and animal. These anti-nutritional factors are identified as the high fibre content and other anti-nutrients [2], [3].

Poultry diets can be improved by monitored supplementation with exogenous enzymes like carbohydrases and phytases [4], and other cellulases and proteolytic enzymes [5]. Many benefits have been attributed to the use of exogenous enzymes in poultry diets such as improvement in nutrient digestibility and assimilation, increased breakdown of complex phytin-phosphorous and subsequent increased reduction of nitrogen and phosphorous excretion, increased use of non-conventional feed resources in feed manufacturing, stability and predictability of nutrient status and quality of feed ingredients and reduction in the cases of wet litter in poultry when fed diets that are abundant in viscous grains [6].

The concept of probiotics in recent years is no more confusing as was earlier thought [7]. The use of probiotics as organic additives for growth promotion and stabilization of gut microbiota has gained more prominence against the background of the evolution of antimicrobial resistance among pathogenic bacteria. Most applied biotechnological researches in animal nutrition have focused on the use of probiotics as opposed to antibiotics and chemotherapeutic agents for growth promotion in poultry [7], [8]. The possibility of antibiotics ceasing completely to be used as growth stimulants is rife as the use of antibiotics in poultry feeds have been banned in some countries. The ban on all growth-promoting antibiotics started in Sweden in 1986, then the ban on avoparcin and virginiamycin in Denmark in 1995 and 1998 followed by the European Union (EU) ban on the use of avoparcin in 1997 and the four remaining antibiotics used for growth promotion in 1999 on the basis of the 'Precautionary Principle' [8]. Probiotics are being considered to fill this gap and already some farmers are using them in preference to antibiotics [9]-

[11].

The present study aimed at using a combination of enzymes and probiotics in reducing the fiber level and other antinutritional factors inherent in TOLM used as alternative plant protein nonconventional feedstuff in broiler diets.

## II. MATERIALS AND METHODS

### Preparing *Telfairia occidentalis* Leaf Meal (TOLM)

Fresh leaves of *Telfairia occidentalis* were harvested at 20-30 days after *Telfairia occidentalis* plants were transplanted to the field from the nursery. These fresh leaves were sun dried in an open cleaned concrete floor space to obtain a constant moisture content between 12 and 13%. The sun dried *Telfairia occidentalis* leaves were milled using commercial feed milling machine (Artec, model 20) and thereafter referred to as TOLM.

### Determination of Proximate Analysis, Gross Energy, Amino Acids and Mineral Content

The proximate composition of the TOLM was determined [12] and the amino acid profiling was done using amino acid analyzer model 80-2107-07 Auto Loader [3]. Mineral elements were also determined [3]. Gross energy of the TOLM sample and the four formulated experimental diets were determined against thermocouple grade benzoic acid using a Gallenkamp ballistic bomb calorimeter (Model CBB-330-0104L). The results of the determined proximate analysis, gross energy, amino acids and mineral contents are presented in Tables I and II.

### Cellulase-Glucanase-Xylanase Combination (Roxazyme G2)

Roxazyme G2 was obtained from Nutrivitas Ltd., Plot 33 Mobolaji Johnson Road, Eleganza Industrial Building, Oregun, Lagos, Nigeria. The detailed composition of Roxazyme G2 by the manufacturer is as follows: minimum of 1,600 U/g of cellulase, 3,600 U/g of endo-1,3(4)- $\beta$ -glucanase and 5,200 U/g of endo-1,4- $\beta$ -xylanase. Single domain cellulase 5a (Cel5a) from *cellvibrio mixtus* and a truncated derivative of xylanase 11a (Xyn11a) from *Clostridium thermocellum* termed GH11-CBM6 were the recombinant enzymes used in this experiment [13]. Bacterial xylanase has been established as a modular enzyme containing a catalytic domain and a noncatalytic xylan-binding module separated by a short linker sequence [13]. Under the control of a T7 promoter, the plasmid proteins that contained the DNA encoding regions proteins in the prokaryotic expression vector pET21a (Novagen) were transformed in *Escherichia coli* BL21 cells. The growth of recombinant *E. coli* strains on Luria Bertani gene expression was facilitated by the addition of isopropyl  $\beta$ -D-thiogalactoside to 1 nM final concentration. The collection of cells was done after 5h induction at 37 °C, and protein extracts were prepared by ultrasonication as described [13]. Extracts were incubated at 50 °C for 20 min and centrifuged for 30 min at 10,000 X g to remove much of the *E. coli* proteins (both recombinant enzymes are thermostable at the referred temperature). Total enzyme used

in each treatment was commercial polysaccharidase mixture, 0.1 g/kg of Roxazyme G; recombinant xylanase, 4,000 U/kg of GH11-CBM6; and recombinant cellulase plus a xylanase, 4,000 U/kg of GH11-CBM6 plus 4,000 U/kg of Cel5a (1 U of enzyme activity released 1 mol of product/min at 37 °C).

### Probiotics (*Turbotox*) and Composition

TABLE I  
PROXIMATE COMPOSITION (G/100G DRY MATTER), GROSS ENERGY (KCAL/G) AND AMINO ACID PROFILE (G/16G N) OF TOLM (MEANS, N = 2)

Composition (g/100 g)	TOLM
Dry matter	90.96
Crude protein	35.14
Ether extracts	9.61
Crude fiber	12.68
Ash	10.87
Nitrogen free extract	31.72
Gross energy (kcalg <sup>-1</sup> )	3.21
Amino acids	
Alanine	406.9
Aspartic acid	388.1
Arginine	313.8
Glycine	381.3
Glutamic acid	688.1
Histidine	86.3
Isoleucine	318.8
Lysine	131.3
Methionine	155.0
Cystine	67.5
Meth. + Cys.	285.0
Leucine	473.8
Serine	244.4
Threonine	238.1
Phenylalanine	303.1
Valine	387.5
Tyrosine	351.3
Tryptophan	195.0

The probiotics, *Turbotox*, used in this present study, was obtained from Afrimash, a reputable agroallied company located in Akobo, Ibadan, Oyo State, Nigeria. *Turbotox* has been identified by the manufacturer as a multi-action probiotic feed supplement containing *Saccharomyces cerevisiae* extract conferring improved feed efficiency and optimal performance to ensure proper and maximum growth of many farm animals. *Turbotox* has been identified as a non-antibiotic growth enhancement. *Turbotox* is also especially reported to improve the integrity of the digestive system. It is carefully prepared in feed formulation proven to promote an ideal gut health and multiplication of beneficial flora. Its major features include inactive yeast, mannan oligosaccharides (MOS), diatomaceous earth and organic acid combination, non-antibiotic growth promoter, control of mycotoxins, bacteria, molds and vectors. The composition of *Turbotox* is mannan oligosaccharides which are known for adsorption of pathogenic bacteria, modulation of the immune system, improvement of intestinal function and binding of mycotoxins. Its composition of inactivated *Saccharomyces cerevisiae* extract is a natural source of vitamins, amino acids, minerals, and enzymes. The

presence of organic acids and their salts (propionic acid, formic acid, citric acid) is known for their ability in improving feed digestibility with acidifying effect while also stimulating the pancreatic enzymes and providing antifungal and antibacterial control in the feed and digestive tract; diatomaceous earth which is a mycotoxin adsorbent and mechanical insect repellent.

The experimentally established activities of *Turbotox* probiotics as performance enhancer include lowering pH in the stomach improving digestion, supporting normal gut flora, improving breakdown of feed and protection against pathogens. Other performance enhancing activities include extra supply of highly bioavailable amino acids, vitamins, and minerals. *Turbotox* is also a natural energy source. Absorption and binding of toxins have been detected in poultry diets where *Turbotox* were used as additives protecting against the negative effects on the hepatic and renal functions [14]. It is also known to prevent molds, and reduce bacterial colonization in the feed. There is evidence of improved intestinal humoral nonspecific immunity giving protection and saving energy. The overall result of *Turbotox* as additive in poultry rations is better FCR [14]. The recommended additive inclusion rate for performing enhancing for poultry is 1 kg/ton.

TABLE II  
MINERAL COMPOSITION OF TOLM (MEANS, N = 2) [3]

Ca	P	K	Na	Mg	Fe	Mn	Cu	Zn
g/100g				(ppm)				
2.6	1.2	6.2	5.1	4.7	5875	182	136	1036

TABLE III  
PHYTIC ACID, PHYTIN-P, OXALIC ACID, TANNIC ACID AND CYANIDE  
CONTENTS OF TOLM (MEANS, N = 2) [2], [3]

Phytic acid (mg/100g)	Phytin-P (mg/100g)	Phytin-P (% of total P)	Oxalate (mg/100g)	Tannin (mg/100g)	HCN (mg/100g)
189.2	120.1	8.05	80.7	43.0	61.2

#### Experimental Ration Formulation

All the ingredients used in ration formulation were locally purchased from a reputable commercial feed miller in Ado-Ekiti, Ekiti State, Nigeria. The TOLM was prepared as described above. Roxazyme (G2) and *Turbotox* probiotic (Turbotox) were sourced as discussed earlier. The results of the proximate analyses on TOLM were used as guides in the manual ration formulation of the experimental diets. The experimental diets were manually mixed on the floor of the Poultry Unit of the Teaching and Research Farm, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria. The four diets were compounded to contain identical crude protein content (isonitrogenous) and metabolisable energy (isocaloric) at both starter and finisher phases of the broiler experiments. Control diet contained neither the Roxazyme G2 nor *Turbotox* probiotic. The inclusion rates of TOLM varied in other experimental diets. Diet 2 had TOLM at 10%, diet 3 had 20% TOLM and diet 4 had TOLM at 30%. The Roxazyme G2 and *Turbotox* fortification addition were done by mixing the recommended manufacturers' specified addition levels to the

diets. Feed grade methionine, lysine and mineral/vitamin premix were used in all diets.

### III. RESULTS AND DISCUSSION

#### Proximate Gross Energy, Amino Acids, Mineral Content and Notable Antinutrients

Table I shows the proximate composition. Tables II and III present the mineral composition and some important detectable antinutrients. Crude protein at 35.14% was relatively high in TOLM with a potential of being considered as a researchable plant ingredient in animal feed. The fat content (ether extracts) which had a relatively high value of 9.61% was higher than mostly consumed leafy vegetables commonly eaten in Africa like bitter leaf at 3.4% ether extract, water leaf at 1.5% ether extract and *Amaranthus* at 2.8% ether extract [1]. Nitrogen free extracts (soluble sugars) value at 31.72% was also comparable with other plant protein sources [15], [1]. The values of mineral elements such as Ca, K, Na, Mg, Fe and Zn compared favorably with values reported for most plant proteins. Significant traces of phytic acid (189.2 mg/100 g), phytin phosphorous (120.1 mg/100 g), oxalate (80.7%), tannin (43 mg/100 g) and cyanide (61.2 mg/100 g) were detected.

The amino acid profile of TOLM indicated a rich source of some essential amino acids comparable to conventional plant protein sources already in use in animal feed manufacturing. The TOLM contents of lysine (2.10 g/16 g N) and methionine (2.48 g/16 g N) were remarkably higher than most plant protein sources. This agreed with previous works [15]-[17] that surmised that some plant leaves and grasses could contain amino acid patterns that are as rich as or even better than the best seed proteins such as soybeans. Leaf meals such as TOLM are generally inhibited as an ingredient in monogastric diet especially poultry because of their high fiber levels and some antinutritional factors present in most green leaves [2], [3]. It is conceivable therefore, that the supplementary addition of the combination of Roxazyme G2 enzyme and *Turbotox* probiotic would facilitate the breakdown of cellulose and other nonstarch polysaccharides which are mainly found in the cell wall of plant leaves and which are bound together in a complex matrix. The above process could have ensured the unlocking of the encapsulated starch molecules thereby increasing accessibility to digestive gastrointestinal digestive enzymes. The above process facilitated the encapsulation of the starch molecules which were unlocked by solubilization of the cell wall structure thereby increasing accessibility to digestive enzymes. The addition of *Turbotox* probiotic would probably enhance the utilization of TOLM by lowering pH in the stomach improving digestion, supporting normal gut flora, improving breakdown of feed and protection against pathogens. *Turbotox* probiotic has also been identified to enhance other activities during digestion such as extra supply of highly bioavailable amino acids, vitamins, and minerals [14]. All these attributes could have enhanced nutrient availability for growth in the broilers fed TOLM as a plant protein supplement. Reports from some previous studies

supported these recent findings as certain enzymes have been reported to significantly improve performances in poultry production [18], [19].

TABLE IV  
COMPOSITION OF EXPERIMENTAL DIETS FOR BROILER STARTER

	Diets			
	1	2	3	4
	Inclusion rate, %			
	0	10	20	30
Ingredients				
Maize (9.0 %CP)	50.1	47.6	44.6	34.6
Soybean (45.0 %CP)	33.5	26.0	19.0	11.0
PKC (18.8 %CP)	10.0	10.0	10.0	10.0
Fish meal (72.0 %CP)	2.0	2.0	2.0	2.0
TOLM (35.1 %CP)	-	10.0	20.0	30.0
Bone meal	2.5	2.5	2.5	2.5
Oyster shell	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
DL-Methionine	0.2	0.2	0.2	0.2
L-Lysine	0.2	0.2	0.2	0.2
Premix**	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0
Calculated composition				
Crude protein, %	22.9	23.0	22.9	23.1
Crude fiber, %	5.2	6.1	6.4	7.6
Ether extract, %	6.7	7.1	6.7	7.1
ME kcal/kg				
Analyzed composition				
Crude protein, %	23.1	22.9	23.1	23.0
Crude fiber, %	4.7	7.1	9.8	11.1
Ether extract, %	6.2	5.9	5.2	4.6

\*\* contained vitamin A (100,000,000 iu); D (2,000,000 iu); E [35,000 iu]; K (1900 mg); B12 (19 mg); Riboflavin (7,000 mg); Pyridoxine (3800 mg); Thiamine (2,200 mg); D pantothenic acid [11,000 mg]; Nicotinic acid [45,000 mg]; Folic acid [1,400 mg]; Biotin [113 mg]; and Trace element as CU [8000 mg]; Mn [64,000 mg]; Zn [40,000 mg]; Fe [32,000 mg]; Se [160 mg]; I<sub>2</sub> [800 mg] and other items as Co [400 mg]; choline (475,000 mg); Methionine (50,000 mg); BHT (5,000 mg); and Spiramycin (5,000 mg) per 2.5 kg.

The average weight gain was highest at 46.3 g/bird/day for birds on 10% TOLM diet with enzymes and probiotics inclusion but similar ( $P > 0.05$ ) to 46.2 g/bird/day for birds on 20% TOLM with enzymes and probiotic inclusion. The final body weight of 2642 g/bird obtained for broilers on 10% TOLM was the significantly highest value although similar ( $P > 0.05$ ) to 2638 g/bird obtained for broilers on 20% TOLM inclusion but significantly higher ( $P < 0.05$ ) than the other body weight values. The lowest final body weight of 2413 g/bird obtained for broilers on 30% TOLM diets was similar ( $P > 0.05$ ) to 2420 g/bird obtained for the broilers on the control diet which did not have TOLM or enzyme and probiotic supplementation. Many reports have corroborated the enhanced broiler growth performance when growth promoting enzymes [1], [20]-[22], and probiotics [23]-[27] were used as additives. This result agreed with previous work [28] who reported a significant final body weight values for birds after 5 weeks of growth. FCR of 2.27 was the lowest and optimum for birds on 10% TOLM with enzymes and probiotics inclusion although similar ( $P > 0.05$ ) to 2.29 obtained for birds on 20% TOLM with enzymes and

probiotics inclusion. FCR of 2.61 was the highest at 2.61 for birds on 30% TOLM diet with enzymes and probiotics additive. The lowest FCR of 2.27 was obtained for birds on 10% TOLM diet with enzymes and probiotics additives although similar ( $P > 0.05$ ) to 2.29 for birds on 20% TOLM diet with enzyme and probiotic additives.

Most carcass characteristics and organ weights were similar ( $P > 0.05$ ) for the experimental birds on the different diets except for kidney, gizzard and intestinal length. This result agreed with previous work [29] where the effects of feeding probiotic supplements were studied on morphometric traits such as the yield stress of the tibia. The tibiotarsi weight, length, and weight/length index, robusticity index, diaphysis diameter, modulus of elasticity, yield stress parameters, and percentage Ca content were not affected by the dietary supplementation of the probiotic. However, the findings from this study seemed to disagree with some previous studies [29]-[31] where occurrence of a significantly ( $P < 0.01$ ) higher carcass yield in broiler chicks fed with the probiotics on the 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> week of age were reported.

TABLE V  
COMPOSITION OF EXPERIMENTAL DIETS FOR BROILER FINISHER

	Diets			
	1	2	3	4
	Inclusion rate, %			
	0	10	20	30
Ingredients				
Maize (9.0 % CP)	50.1	47.6	44.6	34.6
Soybean (45.0 %CP)	26.5	20.0	15.0	9.0
PKC (18.8 %CP)	17.0	16.0	14.0	12.0
Fish meal (72.0 %CP)	2.0	2.0	2.0	2.0
TOLM* (35.1 %CP)	-	10.0	20.0	30.0
Bone meal	2.5	2.5	2.5	2.5
Oyster shell	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
DL-Methionine	0.2	0.2	0.2	0.2
L-Lysine	0.2	0.2	0.2	0.2
Premix**	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0
Calculated composition				
Crude protein, %	20.2	19.8	20.1	20.1
Crude fiber, %	5.2	6.1	6.4	7.6
Ether extract, %	6.7	7.1	6.7	7.1
ME kcal/kg				
Analyzed composition				
Crude protein, %	23.1	22.9	23.1	23.0
Crude fiber, %	4.7	7.1	9.8	11.1
Ether extract, %	6.2	5.9	5.2	4.6

\*\* contained vitamin A (100,000,000 iu); D (2,000,000 iu); E [35,000 iu]; K (1900 mg); B12 (19 mg); Riboflavin (7,000 mg); Pyridoxine (3800 mg); Thiamine (2,200 mg); D pantothenic acid [11,000 mg]; Nicotinic acid [45,000 mg]; Folic acid [1,400 mg]; Biotin [113 mg]; and Trace element as CU [8000 mg]; Mn [64,000 mg]; Zn [40,000 mg]; Fe [32,000 mg]; Se [160 mg]; I<sub>2</sub> [800 mg] and other items as Co [400 mg]; choline (475,000 mg); Methionine (50,000 mg); BHT (5,000 mg); and Spiramycin (5,000 mg) per 2.5 kg.

The carcass characteristics and organ weights all showed evidence of uniform tissue buildup and muscles development particularly in diets containing 10% of TOLM level. The values for kidney, gizzard and intestinal length were

significantly higher ( $P < 0.05$ ) for birds on the TOLM diets with enzymes and probiotics additives.

TABLE VI  
GROWTH PERFORMANCE OF CHICKENS ON EXPERIMENTAL STARTER DIETS

Diets	1	2	3	4	SEM	p
Inclusion of TOLM, %	0	10	20	30		
Initial weight, g/bird	51.2	51.4	51.2	51.5	0.12	0.20
Final live body weight, g/bird	2420 <sup>b</sup>	2642 <sup>a</sup>	2638 <sup>a</sup>	2413 <sup>b</sup>	67.1	0.002
Average weight gain, g/bird/day	42.3 <sup>b</sup>	46.3 <sup>a</sup>	46.2 <sup>a</sup>	42.2 <sup>b</sup>	2.03	0.002
Average feed intake, g/bird/day	98.9	105.1	105.6	110.1	2.10	0.31
FCR	2.34 <sup>b</sup>	2.27 <sup>c</sup>	2.29 <sup>bc</sup>	2.61 <sup>a</sup>	0.16	0.003

<sup>ab</sup> Means without common superscripts along the same row are different at  $P < 0.05$

The nitrogen retention (NR) had the highest value of  $72.37 \pm 0.10\%$  for birds on 10% TOLM diet with enzymes and probiotics although similar ( $P > 0.05$ ) to  $71.54 \pm 1.89$  obtained for birds on the control diet without TOLM and enzymes/probiotics mixture. There was evidence of a better utilization of TOLM as a plant protein source. There was also better nitrogen utilization in birds on the 10% TOLM diet that had the best NR value. It is obvious that enzyme and probiotic fortified diets facilitated the accessibility to intracellular entrapped nutrients [5].

The Roxazyme G2 and Turbotox probiotic supplemented diets in the present study clearly and consistently exhibited

better NR values than the unsupplemented control diet. Proteolytic activities as a result of the Roxazyme G2 supplementation seemed to have increased among the broilers on Roxazyme G2 and Turbotox probiotic supplemented diets. It was suggestive that the apparent breakdown of the nonstarch polysaccharides with a concomitant utilization of the initially bound amino acids was responsible for the improved protein retention and utilization in the experimental broilers birds on TOLM diets supplemented with Roxazyme G2 and Turbotox probiotic.

TABLE VII  
CARCASS EVALUATION (% BODY WEIGHT) AND RELATIVE ORGAN WEIGHTS (GKG<sup>-1</sup> BODY WEIGHT) OF BROILER CHICKENS FED ENZYME COMBINATION AND TURBOTOX PROBIOTIC ADDITIVES

Diets	1	2	3	4	SEM	p
Inclusion of TOLM, %	0	10	20	30		
Carcass characteristics, %						
LW	51.2	51.4	51.2	51.5	0.12	0.20
Relative organ weights, gkg <sup>-1</sup>						
LW						
Heart	4.29	4.32	4.40	4.23	0.43	0.12
Lung	5.60	5.62	5.56	5.52	0.13	0.11
Liver	21.3	21.4	21.5	21.8	0.57	0.003
Kidney	4.12 <sup>b</sup>	4.69 <sup>a</sup>	4.70 <sup>a</sup>	4.74 <sup>a</sup>	0.15	0.004
Gizzard	17.9 <sup>b</sup>	20.3 <sup>a</sup>	20.3 <sup>a</sup>	20.5 <sup>a</sup>	0.71	0.002
Spleen	1.12	1.10	1.07	1.08	0.07	0.12
Intestinal length, cm	240 <sup>b</sup>	262 <sup>ab</sup>	271 <sup>a</sup>	270 <sup>a</sup>	11.4	0.26

<sup>ab</sup> Means without common superscripts along the same row are different at  $P < 0.05$

TABLE VIII  
NITROGEN UTILIZATION OF BROILER BIRDS FED TOLM BASED DIETS

Diets	1	2	3	4	SEM	p
Inclusion of TOLM, %	0	10	20	30		
Nitrogen intake, g/chick	2.53 <sup>a</sup> ±0.09	2.57 <sup>a</sup> ±1.01	2.32 <sup>a</sup> ±0.62	2.41 <sup>a</sup> ±0.15	0.43	0.12
Nitrogen in droppings, g/chick	0.72±0.34	0.71±0.32	0.81±0.32	0.82±0.32	0.13	0.11
Nitrogen Retention, %	71.54 <sup>a</sup> ±1.89	72.37 <sup>a</sup> ±0.10	65.09 <sup>b</sup> ±1.87	65.98 <sup>b</sup> ±1.52	0.57	0.003

<sup>ab</sup> Means without common superscripts along the same row are different at  $P < 0.05$

#### IV. CONCLUSION

The present study revealed the optimum growth performance of broilers on 10% TOLM inclusion dietary levels with Roxazyme G2 and Turbotox probiotic supplementation. However, similarities in growth performance parameters detected for broilers on 30% TOLM inclusion levels (with Roxazyme G2 and Turbotox probiotic supplementation) and the control diet without TOLM and without the enzyme and probiotic suggested the possibility of increasing dietary inclusion levels of TOLM to 30% without significant reduction in the growth indices. Conducting the economic analyses (cost benefit analyses) on this experiment may be necessary to ascertain the commercial viability of the present findings in different parts of the world particularly regions where protein ingredients are very expensive.

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