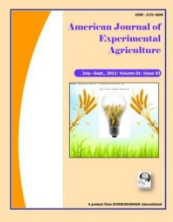

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Bioconversion and Enzymes Fortification of Palm Kernel Meal as Protein Supplement in Broiler Rations

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Authors' contributions

The studies were designed and supervised by the author AOF as an undergraduate research work for the co-authors.

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ABSTRACT

The study was designed to exploit the advantages in solid state fermentation (SSF) and the use of fibrolytic enzymes for proper degradation of the high crude fibre content of palm kernel meal (PKM). Complete randomized design was used in these studies. The research studies were carried out at the Teaching & Research Farm of Ekiti State University, Ado-Ekiti, Nigeria. Two sets of 336 broiler chicks were used in 2 independent phases (broiler starter and finisher phases) of the studies. Ensiled PKM (ePKM) and ensiled plus Roxazyme G2 (cellulase, glucanase and xylanase) fortified PKM (ePKMf) were used to replace significant quantities of energy and protein ingredients at 10%, 20% and 30% inclusion levels in broiler starter diets and 30%, 40% and 50% inclusion levels in broiler finisher diets. Growth performance parameters were generally similar (*P>.05*) and sometimes better for broiler starter and finisher chicks on ePKMf diets than for chicks on diets where PKM was neither ensiled nor fortified with enzymes. The feed conversion ratio (FCR) values of 2.29±0.21, 2.30±0.14 and 2.40±0.07 obtained from starter birds on 10%, 20% and 30% ePKMf diets, respectively were similar (*P>.05*) to the FCR value (2.30±0.12) obtained from the birds on the control diet and FCR values for finisher birds were either similar or better than the control values. Most carcass characteristics and organs weights had similar values (*P>.05*) and were consistently better for birds on ePKMf diets. Most haematological parameters and serum metabolites examined had similar (*P>.05*) values comparable with normal existing values in literature. The use of

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ensiled PKM and fibrolytic enzymes (Roxazyme G2) can be safely practiced to further improve the utilization of PKM in poultry diets. The optimum inclusion levels of 30% ePKMf and 50% ePKMf were tolerable and found to support investigated growth performance indices and nitrogen digestibility parameters in broiler starter (0-28days) and broiler finisher (29-57days), respectively.

Keywords: Processing technique; enzyme fortification; bio-fermentation; fibrolytic enzymes.

1. INTRODUCTION

Palm Kernel Meal (PKM) is the residue obtained after the extraction of palm kernel oil from the seed. Palm oil is widely used industrially and has huge export potentials facilitating the availability of its by-products including PKM in large quantities. PKM also known as palm kernel cake is aflatoxin free, palatable and has considerable potential as carbohydrate and protein sources [1]. PKM may contain from 12 to 23 % crude protein depending upon the efficiency of the process used to extract the oil [2].

The crude fibre content of PKM, ranging from 16%-18%, is acceptable to most ruminants, but is considered high for non-ruminants. It may not be appropriate if included at high levels in poultry or pig diets. PKM has been variously suggested as a filler to increase the bulkiness of the feed for non-ruminants, while providing some protein, energy, minerals and vitamins. Analysis revealed that more than 60% of PKM is cell wall components. The fibrous component is composed of mainly insoluble mannose-based polysaccharides (mannan). It has been shown that the cell wall consisted of 58% mannan, 12% cellulose and 4% xylan [3]. The amino acid content also revealed that lysine appears to be the first limiting amino acid, followed by the sulphur amino acids (methionine, cystein) and tryptophan. The average availability of the amino acids in PKM was 85% which was lower than that in most oil-seed meals [4,5]. It has also been reported that the amino acid availability for poultry ranged from 62%– 87% [6]. Another more recent study [7] showed that the overall true amino acid availability was 65%. Most studies carried out on the use of PKM in poultry diets indicated that birds could not perform optimally with high levels of PKM except when proper balancing of diets with synthetic amino acids or fish meal was applied. This suggests that PKM may not be particularly suitable for use as a protein source.

Bioconversion of low quality lignocellulose biomasses using beneficial microbes to animal feed has been applied to improve the quality of the biomasses. Solid-state fermentation (SSF) is one of the methods of choice for this purpose as it stimulates the natural environment of most microorganisms, especially fungi [8]. PKM can therefore be treated with microbes producing cellulase, xylanase and mannanase (termed fibrolytic microbes) to improve its value as animal feed. The advantages of treating PKM using microbes include the fact that the fibrolytic microbes use PKM as a growth substrate and degrade the fibrous materials and that the growth of fibrolytic microbes increases the protein content of PKM (single cell protein) and improves the overall nutritive value of PKM. This research paper is thus conceived to promote the use of PKM as a protein source in broiler diets by combining the inherent advantages in SSF and the use of fibrolytic enzymes.

2. MATERIALS AND METHODS

2.1 Pre-experimental Operations

The research study was carried out at the Teaching & Research Farm of Ekiti State University, Ado-Ekiti, a town in the Southwest Nigeria in the rain forest zone on latitude 7°40' North of the equator and longitude 5°15' East of the Greenwich Meridian with ambient temperature, 25-37°C; relative humidity, 70%; wind, SSW at 11mph (18km/h); barometric pressure, 29.68' Hg(F) during the summer of year 2012. Palm kernel meals (PKM) were obtained from local communities (especially Ogotun-Ekiti) around Ado-Ekiti where palm oil is produced majorly by solvent extraction method. Mixtures of the PKM and molasses were prepared using a ratio of 50litres of water to 50kg of PKM and 2.5litres of molasses and gently compressed into 120-L plastic containers [9,10]. The compressing of the materials into containers was done manually at about 1 foot height interval until the containers were about ¾ filled. The containers were carefully covered with thick nylon covering with sand used to fill the spaces left. There were further compressions and another thick nylon was spread across the rims of the containers before the containers were finally covered with their lids to ensure air–tightness.

Containers containing the ensiled PKM were opened on day 21 [10]. Samples were taken for laboratory analyses. The ensiled PKM was later sun-dried to achieve a moisture content of 12%. Dried samples of the ensiled PKMs were then analysed for proximate, mineral and amino acid composition before incorporation into feed formulation.

2.2 Laboratory Analyses (Proximate, Mineral and Amino Acid Determinations)

Proximate compositions of the fresh un-ensiled PKM and ensiled PKM were determined [11] while the amino acids were determined using amino acid analyzer model 80-2107-07 Auto Loader. The results showing the above determinations are presented in Tables 1 and 2.

Table 1. Proximate analysis (%) of ensiled palm kernel meal in contrast

Proximate Composition	*Existing Literature	Un-ensiled PKM	Ensiled PKM
Dry matter	88.0 – 94.5	85.6±2.3	92.0±1.1
Crude protein	14.5 – 19.6	18.9±3.1	22.3±2.3
Crude fibre	13.0 – 20.0	14.2±1.9	10.1±0.2
Ether extract	5.0 – 8.0	7.2±2.4	3.8±0.5
Ash	3.0 – 12.0	11.4±1.8	11.9±2.2
Nitrogen-free extract	46.7 – 58.8	49.2±2.0	57.6±1.2
Metabolisable energy (MJ Kg^{-1})	6.5 – 7.5	6.8±3.2	7.8±2.4

**Existing literature: Onwudike (1986); Alimon and Hair-Bejo (1995)*

2.3 Site preparation

Prior to the arrival of broiler chicks, the poultry house and metabolism cage were thoroughly washed and fumigated with diskol (a disinfectant containing 4% benzalkonium chloride, 3% glutaraldehyde, 14% formaldehyde, stabilizers, antioxidants and activators). The house was well covered to prevent heat loss and brooding equipment were put in place.

Table 2. Amino acid contents of ensiled palm kernel meal (g/16 g n) in contrast

Amino Acid	*Existing Literature	Un-ensiled PKM	Ensiled PKM
Alanine	3.83	3.10±2.3	4.12±0.1
Arginine	11.56	9.26±1.7	12.71±1.2
Aspartic acid	3.63	2.54±2.9	4.30±0.3
Cystine	1.13	0.68±3.1	0.71±1.3
Glycine	4.17	2.76±2.2	3.18±1.4
Glutamic acid	16.80	14.7±3.0	15.10±2.1
Histidine	1.91	1.07±2.4	1.21±1.1
Isoleucine	3.22	3.29±3.1	4.11±2.0
Leucine	6.07	6.54±2.5	7.01±2.1
Lysine	2.68	2.72±2.4	3.21±1.7
Methionine	1.75	2.43±2.8	3.50±2.1
Phenyalanine	3.96	3.78±2.1	4.02±1.2
Proline	3.31	3.43±2.7	3.92±2.5
Serine	4.11	3.59±3.2	4.15±2.9
Threonine	2.75	3.09±3.1	3.50±3.1
Tyrosine	2.60	2.14±2.7	2.73±1.71
Valine	5.05	4.82±3.5	5.10±1.3

**Existing literature: Onwudike (1986)*

2.4 Experimental diets

Tables 3 and 4 show the 7 experimental diets for the broiler chickens at both starter and finisher phases, respectively. The formulations were guided by the Feedstuff Analysis Table [12] and the proximate analysis of ensiled PKM in our laboratory.

Table 3 shows the experimental compositions of broiler starter phase in experiment 1:

I Diet 1 served as the control diet without the inclusion of PKM and Roxazyme.
ii Diet 2 contained 10% of ensiled PKM (10% ePKM) as partial replacement for protein and energy ingredients.
iii Diet 3 contained 10% of ensiled PKM fortified with Roxazyme G2 (ePKMf).
iv Diet 4 had 20% ensiled PKM without Roxazyme G2 (20% ePKM).
v Diet 5 had 20% ensiled PKM with Roxazyme G2 fortification (20% ePKMf).
vi Diet 6 had 30% ensiled PKM (ePKM).
vii Diet 7 had 30% ensiled PKM with Roxazyme G2 fortification (30% ePKMf).

Table 4 is the experimental composition of diets for broiler finisher phase:

1. Diet 1 is the control diet also without PKM and Roxazyme G2.
2. Diet 2 contained 30% of ensiled PKM (30% ePKM) as partial replacement for protein and energy ingredients.
3. Diet 3 contained 30% of ensiled PKM fortified with Roxazyme G2 (30% ePKMf).
4. Diet 4 had 40% ensiled PKM without Roxazyme G2 (40% ePKM).
5. Diet 5 had 40% ensiled PKM with Roxazyme G2 fortification (40% ePKMf).
6. Diet 6 had 50% ensiled PKM without Roxazyme G2 (50% ePKM).
7. Diet 7 had 50% ensiled PKM with Roxazyme fortification (50% ePKMf). Roxazyme G2 fortification was made at 1kg/t for all Roxazyme G2 fortified diets.

Table 3. Experimental diets for broilers (starting phase, 0-28day)

Ingredients	Diets						
	1	2	3	4	5	6	7
	% PKM inclusion level and enzyme supplementation						
	Control	10	10*	20	20*	30	30*
Maize	56.0	34.2	34.2	26.4	26.4	17.6	17.6
Soybean meal	37.6	30.4	30.4	27.2	27.2	25.0	25.0
PKM	-	30.0	30.0	40.0	40.0	50.0	50.0
Palm oil	-	1.0	1.0	2.0	2.0	3.0	3.0
Fish meal	2.0	-	-	-	-	-	-
Bone meal	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Oyster shell	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Premix**	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL–Met.	0.2	0.2	0.2	0.2	0.2	0.2	0.2
L-Lysine	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated							
CP %	22.6	22.5	22.5	22.7	22.7	22.6	22.6
ME (kcal/kg)	3115.4	2975.6	2975.6	3110.4	3110.4	3120.0	3120.0
Crude Fibre	4.7	5.5	4.8	6.8	5.2	9.7	7.2
Ether extract	5.4	4.3	4.3	5.4	5.4	6.26.2	

**Enzyme (Roxazyme G2) fortification, **contained vitamins A (4,000,000iu); D(800,000 iu); E (14,000 iu); K (760mg); B12 (7.6mg);Riboflavin (2,800mg); Pyridoxine (1,520mg); Thiamine (880mg); D Pantothenic acid (4,400mg);Nicotinic acid (18,000mg); Folic acid (560mg); Biotin (45.2mg); and Trace elements as Cu(3,200mg); Mn (25,600mg); Zn (16,000mg); Fe (12,800mg) Se (64mg); I2 (320mg) and other items asCo (160mg); Choline (190,000mg); Methionine (20,000mg); BHT (2,000mg) and Spiramycin (2,000mg) per kg.*

2.5 Fortification of Diets with Enzymes (Roxazyme G2)

Roxazyme G2 contains a minimum of 1600 Ug^{-1} of cellulase, 3600 Ug^{-1} of endo-1,3(4)-β-glucanase, and 5200 Ug^{-1} of endo-1,4-β-xylanase. The recombinant enzymes used in this experiment were the single domain cellulase 5a (Cel5a) from Cellvibrio mixtus [13] and a truncated derivative of xylanase 11a (Xyn11a) from *Clostridium thermocellum* termed GH11-CBM6 [14]. The bacterial xylanase is a modular enzyme containing a catalytic domain and a noncatalytic xylan-binding module separated by a short linker sequence [14]. Plasmids containing the DNA encoding regions of both proteins, under the control of a T7 promoter in the prokaryotic expression vector pET21a (Novagen, Merck KGaA, Darmstadt, Germany), were transformed in *Escherichia coli* BL21 cells. Recombinant *E. coli* strains were grown on Luria Bertani gene expression induced by adding isopropyl b-d-thiogalactoside to a final concentration of 1mm. Cells were collected after 5h induction at 37ºC and protein extracts prepared by ultrasonication [14]. Extracts were incubated at 50 °C for 20 min and centrifuged for 30 min at 10 000g 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^{-1} of Roxazyme G; recombinant xylanase, 4000 U kg^{-1} of GH11-CBM6; and recombinant cellulase plus a xylanase, 4000U kg^{-1} of GH11-CBM6 plus 4000U kg^{-1} of Cel5a (1U of enzyme activity released 1m min^{-1} at 37ºC). Enzymes were introduced into the diets by carefully mixing with small quantities (carriers) of

the diets before finally mixing with the larger volumes of the diets for thorough blending and kept as described above.

Table 4. Experimental diets for broilers (finisher phase, 29-57day)

Ingredients	Diets						
	1	2	3	4	5	6	7
	% PKM inclusion level and enzyme supplementation						
	Control	30	30*	40	40*	5	50*
Maize	55.0	52.0	52.0	48.5	48.5	44.0	44.0
Soybean meal	28.0	24.3	24.3	20.3	20.3	17.5	17.5
PKM	-	10.0	10.0	20.0	20.0	30.0	30.0
Wheat offal	8.3	6.0	6.0	4.5	4.5	2.3	2.3
Fish meal	4.0	3.0	3.0	2.0	2.0	1.0	1.0
Bone meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Limestone	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Premix**	0.3	0.3	0.3	0.3	0.3	0.3	0.3
DL–Met.	0.1	0.1	0.1	0.1	0.1	0.1	0.1
L-Lysine	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated							
CP %	20.1	19.7	19.7	19.9	19.9	20.1	20.1
ME (kcal/kg)	801.3	2800.6	2800.6	2850.4	2850.4	2801.0	2801.0
Crude Fibre	8.2	9.7	9.7	10.2	10.2	10.5	10.5
Ether extract	6.3	8.3	8.3	7.8	7.8	6.46.4	

**Enzyme (Roxazyme G2) fortification, **contained vitamins A (4,000,000iu); D(800,000 iu); E (14,000 iu); K (760mg); B12 (7.6mg);Riboflavin (2,800mg); Pyridoxine (1,520mg); Thiamine (880mg); D Pantothenic acid (4,400mg);Nicotinic acid (18,000mg); Folic acid (560mg); Biotin (45.2mg); and Trace elements as Cu(3,200mg); Mn (25,600mg); Zn (16,000mg); Fe (12,800mg) Se (64mg); I2 (320mg) and other items asCo (160mg); Choline (190,000mg); Methionine (20,000mg); BHT (2,000mg) and Spiramycin(2,000mg) per kg.*

2.6 Management of Experimental Birds

Two sets of three hundred and thirty six broiler chicks were used for the two phases (starter and finisher) of the experiment. They were purchased from a reputable hatchery in Ibadan, South Western part of Nigeria. The two sets of chicks were brooded in a brooder house using electricity supplied constantly by 1KVA stand-by power generating plant at the Ekiti State University Teaching and Research Farms. During the first week, the chicks were fed on commercial chicks mash containing 23% crude protein (CP). Chicks were sexed on the 3rd day of arrival [15]. The chicks were managed on the floor for the two phases of experiment.

The following veterinary routines were observed from day old:

i. Intraocular vaccination against Newcastle disease at day one.
ii. Neoceryl (Antibiotics) for a period of 4 days from 3 days of age.
iii. Coccidiostat for the treatment/control of coccidiosis and chronic respiratory diseases.
iv. Gumboro vaccine at 2 weeks of age
v. Lasota vaccine (New castle booster) administered in a day at the age of about 3 weeks.

A 5-day acclimatization period was observed before the commencement of the first phase (5-28days) of the experiment using a set of three hundred and thirty six birds. Another set of three hundred and thirty six birds were used for the second phase (29-57days) of the experiment. The experimental birds were reared on the floor within demarcated pens that were randomly allocated to the 7 experimental treatments as designed.

2.7 Experimental Design

At the beginning of the two experiments, 48 chicks were randomly placed on each of the 7 dietary treatments of each experiment. Each group contained 4 replicates comprising of 12 chicks in a completely randomized design. The average weight of birds in each replicate were taken and carefully balanced to ensure uniformity of weights in all treatments. This exercise was repeated for the experimental broiler birds used for the finisher phase of the experiment at the onset of the 2nd phase of the experiment.

2.8 Data Collection

Feeds were supplied ad libitum for the 21-day trial periods of the 1st and 2nd phases of the experiment with records of feed consumption and 3-day periodic weight gain recorded. Estimation of nitrogen intake, nitrogen retention and protein efficiency ratio were done in a nitrogen balance study. Total faeces voided during the last 5 days were collected for the two phases of the experiment, weighed, dried at 65-70ºC in an air circulating oven for 72 h and preserved while the corresponding feed consumed was also recorded for nitrogen studies. Nitrogen content of the samples was determined by the method of AOAC, 2010. Nitrogen retention of the chicks was calculated as the difference between nitrogen in the feed and nitrogen in droppings. Protein efficiency ratio was calculated as the ratio of weight gained to total protein consumed. Nitrogen digestibility was computed by expressing the nitrogen retained as a fraction of the nitrogen intake multiplied by 100.

2.9 Blood Collection for Analysis

At the end of the feeding trial all birds were starved overnight. One male broiler chick from each replicate was randomly picked and blood collected through the wing-web vein using a 2ml syringe and needle. Blood samples were put in labeled bijour bottles containing a speck of EDTA. The bottles were covered and content mixed by inversion. The blood samples collected were used for haematological studies. The packed cell volume (PCV %) was estimated in heparinized capillary tubes in an haematocrit micro centrifuge for 5minutes with 1400 RPM. Total red blood cell (RBC) count was determined using Drabkin solution to easily recognize red blood cells from other components of the blood under microscope. The haemoglobin concentration (HBC) was estimated, whereas Mean Corpuscular Heamoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) were calculated.

2.10 Carcass and Organ Characteristics

After slaughtering and bleeding the birds, the carcasses were scalded at 55-60ºC in water bath for 30 seconds before de-feathering. The dressed chicks were eviscerated. The data taken were fasted weight, dressed weight %, eviscerated weight, thigh, drumstick, shank, chest, back, neck, wing, fat and head weight. Also organs (heart, spleen, liver, lungs, kidney, gizzard, intestine and bursa of fabricius) were dissected out and weighed.

2.11 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) and means among treatments were separated [16] using Computer Minitab Statistical Package [17].

3. RESULTS AND DISCUSSION

3.1 Proximate and Amino Acid Compositions of Ensiled PKM

The proximate and amino acid compositions of PKM are presented in Tables 1 and 2. The proximate composition of ensiled PKM revealed higher values for crude protein and nitrogen free extracts and lower values for crude fibre and ether extracts compared with PKM samples that were not ensiled and also with other values obtained from existing literatures [5,18]. The amino acid composition of the ensiled PKM had appreciably higher values for all analysed amino acids compared with the un-ensiled PKM and almost higher than most values obtained from existing literature [5,18] except for cystine, glycine, glutamic acid and histidine. The ensiling process has been identified as a means of improving the nutritional value of food/feed resource while reducing the pH and fibre contents [19]. Ensiling also increases digestibility of crude protein by breaking linkages between protein and fibre [20]. Ensiling has been associated with improvement of palatability and reduction of toxic substances present in fresh leaves and plant by-products to safe level concentrations [21].

3.2 Growth Performance and Nitrogen Utilization of Broiler Birds Fed Varying Levels of Ensiled PKM with and Without Enzyme

The growth performance indicators and nitrogen utilization parameters for the two phases of experiment (0-28days and 29-57days) are presented in Tables 5, 6, 7 and 8. The average daily feed intakes (AFIs) for the broiler starter chicks (0-28days) were almost similar ($P > .05$) for all birds on all experimental diets except for birds on 30% ePKM that had the highest value of 92.10±3.32g/chick/day but still similar to AFI value for birds fed 30% ePKMf and 20% ePKMf at 89.20±0.14g/chick/day and 89.20±1.14 g/chick/day, respectively.

Average daily weight gains (AWGs) for broiler starter birds were generally similar ($P>.05$) among all experimental birds but highest for birds on 20% ePKMf at 38.80±1.41g/chick/day. However, the best feed conversion ratio value (FCR) of 2.29±0.21 was obtained for birds on 10% ePKMf diet with close similarity ($P > .05$) to FCR values obtained for birds on the control diet at 2.30±1.12, 10% ePKM diet at 2.37±0.14, 20% ePKMf diet at 2.30±0.14, 20% ePKM diet at 2.35±0.21 and 30% ePKMf diet at 2.40±0.07. Only birds on 30% ePKM diet had the poorest FCR of 3.00±0.21. Protein efficiency ratio was highest for the control diet at 1.94±0.78 and lowest for birds on 30% ePKM diet. Nitrogen retention was highest (2.75±0.65g/chick/day) for birds on the control diet but similar ($P>.05$) to 2.59±0.65g/chick/day obtained for birds on diet 7 (30% PKM enzyme fortified diet). The apparent nitrogen digestibility (AND) was highest at 77.8%±0.67 for birds on 30% ePKMf but also similar ($P >.05$) to AND value of 75.6%±0.54 obtained for the birds on the control diet.

The AFIs of all experimental broiler birds (29-57days) were similar ($P>.05$) ranging from 140.78±0.07g/chick/day for birds on the control diet to 141.96g/chick/day for birds on the 50% ePKMf diet. The AWG values were highest at an average of 56.31g/chick/day for birds on 40% ePKMf but similar ($P>.05$) to AWG values of 53.94±2.19g/chick/day, 53.82±0.30g/chick/day, 54.06±1.44g/chick/day and 55.89±0.86g/chick/day obtained for birds

on control diet 1, 30% ePKM diet, 30% ePKMf diet and 50% ePKMf diet, respectively. The FCR was optimum for birds fed 40% ePKMf diet at 2.52±0.06 although similar (*P>.05*) to the FCR values obtained from birds on the control diet at 2.61±0.10, 30% ePKM diet, 30% ePKMf and 50% ePKMf diet. All PER values were similar (*P>.05*) with a range of 1.98±0.01 for bird on the control diet to 2.15 for birds on 40% ePKMf. The nitrogen retention values for broilers finisher birds was highest at 2.93±0.40 for birds on control diet but similar (*P>.05*) to 2.61±0.45g/chick/day obtained for birds on 30% ePKMf diet. AND values also had the same trend with 66.74%±o.63 being the highest value for birds on the control diet but similar (*P>.05*) to 59.73%±0.64 and 58.51%±0.61 obtained for birds on 30% ePKMf and 40% ePKMf diets, respectively.

The poor utilization of PKM as an energy and protein supplement in monogastric livestock animal as against ruminant animals has been previously recognized [22]. The poor FCR values for birds on some PKM diets especially ePKM diets may have been caused by the presence of fibres such as mannan (hemicellulose) and cellulose which reduce the feed conversion efficiency in poultry [22] PKM also lacks certain essential amino acids such as lysine and methionine [18,22,23]. Various studies in which PKM was used as either protein or energy supplement [6,24-26] indicated that the birds could not perform optimally with high levels of PKM except with proper balancing of diets with synthetic amino acids or fish meal suggesting that PKM may not be suitable for use as a protein source.

The use of a combination of processing techniques in this present study such as ensiling of PKM and further addition of enzymes with cellulase, xylanase and phytase activities could be adduced to the improved utilization and subsequent growth performance recorded for birds on ePKMf diets. The advantages of treating PKC using microbes include the fact that the fibrolytic microbes use PKC as a growth substrate and degrade the fibrous materials and that the growth of fibrolytic microbes increases the protein content of PKC (single cell protein) and improves the overall nutritive value of PKC. It has been asserted that PKM can be treated with microbes producing cellulase, xylanase and mannanase (termed fibrolytic microbes) to improve its quality as animal feed [8]. Bioconversion of low quality lignocellulose biomasses using beneficial microbes to animal feed has been applied to improve the quality of the biomasses. Solid-state fermentation (SSF) is one of the methods of choice for this purpose as it stimulates the natural environment of most microorganisms, especially fungi [8]. A certain amount of molasses and duration of ensiling have been prescribed as optimal and most suitable for practical ensiling of different materials [10]. This is corroborated by previous study [27] which revealed on analysis of nutritional composition of solid substrate fermented PKC an increment in CP (24.7%) compared with untreated PKC (17.5%). Neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose and cellulose levels in fermented PKC also significantly decreased and the total ash content was higher in the fermented PKC [28]. Similar study using *Trichoderma viride* as fermenting agent for PKC and rice bran mixture had a crude protein increase of 29.58% and crude fibre decrease of 22.53% [27]. Enzymes (Mannanase, α-galactosidase and protease) supplementation of diets high in NSPs and other fibres have also been identified to significantly improve apparent metabolizable energy (AME) and true metabolizable energy ($TME_{n)}$ than PKC diets with no enzyme supplementation in laying hens [23]. An established fact in the addition of exogenous enzymes including Roxazyme G2 is that they function through enhancing the availability and retention of nutrients present in the diet Fig. 1 [29].

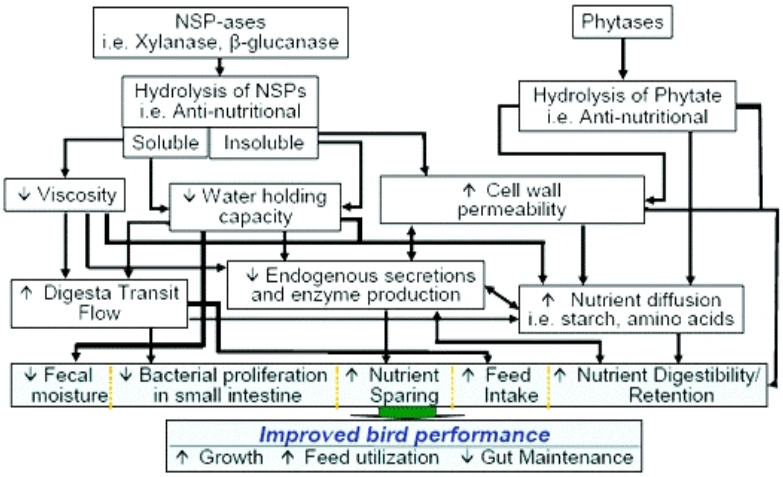

Fig. 1. Working path for mode(s) of action of feed enzyme
Source: Wyalt et al. [29] (Carolina Poultry Nutrition Conference)

3.3 Carcass Characteristics and Organs Weights of Broiler Birds on the Different Levels of Ensiled PKM with/without Enzyme Fortification

Birds on Roxazyme G2 fortified ePKM diets had better carcass characteristics and organs weights than the other birds raised on diets with equal ePKM inclusions but unfortified with Roxazyme G2. The live weights, carcass weights, dressing percentages of broilers (0-28 day and 29-57 day) were consistently higher for birds on ePKMf diets.

For instance, the live weights of broiler starter (0-28day) birds on 20% ePKMf diet were significantly higher ($P = .05$) at 1025.0±0.57g/chick/day than 857.0±0.62g obtained for birds on 20% ePKM but similar ($P > .05$) to the live weight of 1030.0±0.71g obtained for the birds on the control diet that were fed standard broiler starter diet without PKM or Roxazyme G2. This trend was common for most of the carcass characteristics such as carcass weight, dressing percentage, head, wings, neck, thigh, drumstick, breast and shank where the weights of the carcass parts were, in most cases consistently higher for birds on ePKMf diets than for birds on ePKM diets Table 9. The trend of results in Table 9 for broiler starter (0-28day) was virtually repeated in Table 10 for broiler finisher (29-57 day) birds where the live weights, dressed weights, eviscerated weight and dressing percentages of birds on ePKMf diets were consistently higher ($P = .05$) than for birds on equal inclusion levels of ePKM diets.

Expectedly, most other carcass characteristics and organs weights were either similar ($P >.05$) in values or had higher values ($P = .05$) recorded for birds on ePKMf Tables 11 and 12.

3.4 Haematological and Serum Biochemistry of Broilers fed Different Levels of Ensiled PKM with/without Roxazyme G2

The haemoglobin concentration (Hbc) had similar values ($P > .05$) for all mature sacrificed birds on all experimental diets Table 13). Most other haematological parameters examined such as PCV, ESR, MCV, MCH and MCHC had similar standard values ($P > .05$).

Table 5. Growth performance of broilers (0-28day) fed different levels of ensiled PKM with and without Roxazyme G2

Parameters	Diets						
	1	2	3	4	5	6	7
	% PKM inclusion level and enzyme supplementation						
	Control	10	10*	20	20*	30	30*
Ave. Daily Feed Intake (g/chick/day)	85.75±0.07^{b}	85.40±0.14^{b}	86.30±6.36^{b}	87.00±0.28^{b}	89.20±1.4^{ab}	92.10±3.32^{a}	89.20±0.14^{ab}
Ave. Daily weight gain (g/chick/day)	37.30±1.40^{a}	36.03±1.5^{b}	37.70±0.14^{ab}	37.02±2.12^{b}	38.80±1.41^{ab}	30.70±2.19^{b}	37.17±0.28^{a}
Feed Conversion Ratio (FCR)	2.30±0.12^{ab}	2.37±0.14^{b}	2.29±0.21^{b}	2.35±0.21^{b}	2.30±0.14^{ab}	3.00 ±0.21^{c}	2.40±0.07^{b}
Protein Efficiency Ratio (PER)	1.94±0.78^{b}	1.37±0.52^{a}	1.57±0.86^{c}	1.64±0.76^{bc}	1.72±0.71^{b}	1.27±0.64^{a}	1.54±0.56^{c}

** Enzyme (RoxazymeG2) fortification.*

$^{a, b, c..}$ Mean values within rows with different superscripts letters are significantly different (P = .05)

Table 6. Growth performance of broilers (29-57day) fed different levels of ensiled PKM with and without Roxazyme G2

Parameters	Diets						
	1	2	3	4	5	6	7
	% PKM inclusion level and enzyme supplementation						
	Control	30	30*	40	40*	50	50*
Ave. Daily Feed Intake (g/chick/day)	140.78±0.07	141.01±1.25	140.01±1.03	141.63±0.32	141.91±0.19	141.91±0.19	141.96±0.12
Ave. Daily weight gain (g/chick/day)	53.94±2.19^{ab}	53.82±0.30^{ab}	54.06±1.44^{ab}	52.85±1.05^{ac}	56.31±1.38^{b}	51.98±0.08^{c}	55.89±0.86^{b}
Feed Conversion Ratio (FCR)	2.61±0.10^{ab}	2.62±0.01^{a}	2.59±0.08^{a}	2.68±0.06^{b}	2.52±0.06^{a}	2.73±0.01^{b}	2.54±0.04^{a}
Protein Efficiency Ratio (PER)	1.98±0.01	2.06±0.07	2.07±0.08	2.08±0.04	2.15±0.05	2.00±0.01	2.04±0.09

** Enzyme (RoxazymeG2) fortification.*

$^{a, b, c..}$ Mean values within rows with different superscripts letters are significantly different (P = .05)

Table 7. Nitrogen Utilization of Broiler (0-28day) Chicks fed Different Levels of Ensiled PKM with and without Roxazyme G2

Parameters	Diets						
	1	2	3	4	5	6	7
	% PKM inclusion level and enzyme supplementation						
	Control	10	10*	20	20*	30	30*
Nitrogen Intake (gN/chick/day)	3.64±0.61	3.57±0.64	3.14±0.71	3.18±0.64	3.18±0.64	3.14±0.75	3.33±0.83
Nitrogen in droppings (FN+UN) (gN/chick/day)	0.89±0.73	1.15±0.65	0.92±0.64	0.88±0.51	0.88±0.51	0.83±0.65	0.74±0.74
Nitrogen Retention (gN/chick/day)	2.75±0.65^{a}	2.22±0.71^{b}	2.42±0.56^{bc}	2.30±0.52^{b}	2.30±0.52^{b}	2.31±0.76^{b}	2.59±0.65^{ac}
Apparent NDigestibility (%)	75.6±0.54^{a}	70.8±0.52^{b}	70.7±0.62^{b}	70.3±0.65^{b}	74.3±0.65^{b}	72.4±0.85^{b}	77.8±0.67^{a}

** Enzyme (Roxazyme G2) fortification; FN, Faecal Nitrogen; UN, urinary Nitrogen*

$^{a, b, c..}$ Mean values within rows with different superscripts letters are significantly different (P = .05)

Table 8. Nitrogen utilization of broiler (29-57day) chicks fed different levels of ensiled PKM with and without Roxazyme G2

Parameters	Diets						
	1	2	3	4	5	6	7
	% PKM inclusion level and enzyme supplementation						
	Control	30	30*	40	40*	50	50*
Nitrogen Intake (gN/chick/day)	4.39±0.60	4.40±0.63	4.37±0.43	4.07±0.55	4.23±0.58	4.18±0.45	4.22±0.58
Nitrogen in dropping (FN+UN) (gN/chick/day)	1.46±0.60	2.02±0.71	1.76±0.54	1.96±0.52	1.78±0.49	1.85±0.64	1.97±0.52
Nitrogen Retention (gN/chick/day)	2.93±0.40^{a}	2.38±0.40^{bc}	2.61±0.45^{ab}	2.35±0.44^{bc}	2.51±0.42^{b}	2.33±0.43^{bc}	2.35±0.46^{bc}
Apparent N Digestibility (%)	66.74±0.63^{a}	54.09±0.63^{b}	59.73±0.64^{ab}	54.52±0.62^{b}	58.51±0.61^{ab}	44.23±0.64^{c}	54.52±0.65^{b}

** Enzyme (Roxazyme G2) fortification; FN, Faecal Nitrogen; UN, urinary Nitrogen*

$^{a, b, c..}$ Mean values within rows with different superscripts letters are significantly different (P = .05)

Table 9. Carcass characteristics at slaughter of broilers (0-28day) fed different levels of ensiled PKM with and without Roxazyme G2

Parameters	Diets						
	1	2	3	4	5	6	7
	% PKM inclusion level and enzyme supplementation						
	Control	10	10*	20	20*	30	30*
Live weight (g)	1030.0±0.71^{a}	855.0±0.51^{b}	1044.0±0.71^{a}	857.0±0.62^{b}	1025.0±0.57^{a}	751.0±0.76^{c}	814.0±0.53^{b}
Carcass weight (g)	950.0±0.62^{a}	774.0±0.58^{b}	944.0±0.79^{a}	809.0±0.51^{b}	941.0±0.75^{a}	678.0±0.57^{c}	750.0±0.55^{b}
Dressing percentage (%)	89.9±0.51^{a}	90.5±0.76^{a}	90.4±0.66^{a}	94.4±0.86^{b}	91.8±0.83^{ab}	89.6±0.88^{a}	92.1±0.71^{ab}
Head (g)	39.2±0.84^{a}	28.0±0.75^{c}	39.0±0.59^{a}	35.0±0.83^{ab}	39.0±0.52^{a}	29.0±0.73^{c}	34.0±0.54^{ab}
Wings (g)	89.0±0.65^{a}	77.0±0.71^{b}	96.0±0.81^{a}	65.0±0.79^{c}	88.0±0.73^{a}	66.0±0.67^{c}	66.0±0.76^{c}
Neck (g)	50.60±0.57^{a}	43.0±0.53^{b}	50.0±0.52^{a}	46.0±0.61^{ab}	40.0±0.83^{b}	35.0±0.55^{c}	36.0±0.85^{bc}
Thigh (g)	107.0±0.76^{a}	83.0±0.76^{b}	103.0±0.55^{a}	82.0±0.56^{b}	109.0±0.75^{a}	71.0±0.60^{c}	78.0±0.71^{bc}
Drumstick (g)	98.0±0.86^{a}	70.0±0.86^{b}	93.0±0.61^{a}	75.0±0.76^{b}	96.0±0.59^{a}	66.0±0.70^{c}	73.0±0.53^{b}
Breast (g)	230.0±0.65^{a}	167.0±0.58^{b}	199.0±0.71^{ab}	181.0±0.64^{b}	229.9±0.8d^{a}	143.0±0.86^{c}	173.0±0.87^{b}
Backfat (g)	62.0±0.84^{a}	79.0±0.67^{b}	93.0±0.65^{c}	77.0±0.71^{b}	87.0±0.77^{bc}	66.0±0.81^{a}	70.0±0.59^{b}
Bursa of fabricius (g)	7.0±0.52	7.0±0.62	6.8±0.63	7.1±0.51	7.0±0.65	8.0±0.78	7.2±0.66
Shank (g)	59.0±0.72^{a}	41.0±0.51^{b}	58.0±0.59^{a}	41.0±0.72^{b}	45.0±0.64^{b}	39.0±0.56^{b}	45.0±0.70^{b}

** Enzyme (RoxazymeG2) fortification.* $^{a, b, c..}$ *Mean values within rows with different superscripts letters are significantly different (P = .05)*

Table 10. Carcass characteristics at slaughter of broilers (29-57day) fed different levels of ensiled PKM with and without Roxazyme G2

Parameters	Diets						
	1	2	3	4	5	6	7
	% PKM inclusion level and enzyme supplementation						
	Control	30	30*	40	40*	50	50*
Live weight(g)	2065±70.2^{a}	1832±70.6^{ab}	2055±70.4^{a}	1669±70.7^{b}	1829±70.3^{ab}	1655±70.4^{b}	1809±70.7^{ab}
Dressed weight (g)	1903±70.5^{a}	1642±73.7^{ab}	1894±83.4^{a}	1530±74.7^{b}	1631±72.7^{ab}	1507±71.7^{b}	1651±70.6^{ab}
Eviscerated weight (g)	1594±73.7^{a}	1386±72.3^{ab}	1590±74.7^{ab}	1331±79.1^{ab}	1411±74.6^{ab}	1268±76.0^{b}	1389±74.7^{ab}
Dressing percentage (%)	58.15±0.71^{a}	65.39±0.71^{b}	65.79±0.73^{b}	65.77±0.76^{b}	85.8±0.77^{c}	63.32±0.72^{b}	66.0±0.74^{b}
Head (g)	43±0.60^{ad}	45±0.78^{ac}	50±0.62^{e}	42±0.66^{d}	42±0.54^{d}	46±0.81^{c}	54±0.71^{b}
Wings (g)	165±0.76^{a}	168±0.71^{e}	176±0.81^{f}	137±0.84^{d}	154±0.89^{b}	129±0.74^{c}	155±0.72^{b}

Table 10 Continued…

Neck (g)	83±0.74^{a}	95±0.71^{d}	95±0.71^{d}	85±0.87^{a}	89±0.66^{c}	82±0.86^{a}	73±0.66^{b}
Thigh (g)	200±0.51^{a}	189±0.78^{e}	210±0.71^{f}	175±0.62^{d}	181±0.66^{b}	151±0.55^{c}	182±0.61^{b}
Drumstick (g)	223±0.90^{a}	207±0.88^{b}	215±0.70^{a}	170±0.89^{c}	200±0.83^{b}	165±0.80^{bc}	191±0.98^{b}
Breast (g)	530±0.88^{a}	405±0.87^{bc}	482±0.90^{abc}	374±0.91^{c}	490±0.81^{ab}	392±0.71^{bc}	409±0.8^{bc}
Backfat (g)	209±0.12^{b}	210±0.16^{b}	231±0.15^{a}	203±0.20^{b}	213±0.18^{d}	203±0.13^{b}	173±0.18^{c}
Shank (g)	84±0.82^{a}	72±0.86^{b}	76±0.77^{f}	60±0.71^{e}	65±0.91^{d}	57±0.81^{c}	70±0.90^{b}

** Enzyme (RoxazymeG2) fortification.*

$^{a, b, c..}$ Mean values within rows with different superscripts letters are significantly different (P = .05)

Table 11. Relative weights of organs at slaughter of broilers (0-28day) fed different levels of ensiled PKM with and without Roxazyme G2

Parameters	**Diets**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
	% PKM inclusion level and enzyme supplementation						
	Control	**10**	**10***	**20**	**20***	**30**	**30***
Liver	23.0±0.55^{a}	23.0±0.85^{a}	23.0±0.67^{a}	22.0±0.67^{a}	23.0±0.71^{a}	16.0±0.83^{b}	14.0±0.53^{b}
Kidney	7.0±0.81^{ab}	5.0±0.53^{a}	5.0±0.89^{a}	8.0±0.65^{ab}	8.0±0.85^{b}	5.0±0.56^{a}	6.0±0.58^{ab}
Heart	6.0±0.72^{a}	4.0±0.62^{c}	5.0±0.73^{ac}	5.0±0.54^{ac}	7.0±0.83^{a}	2.0±0.72^{b}	3.0±0.71^{bc}
Spleen	1.0±0.67	1.0±0.76	1.0±0.55	1.0±0.63	1.0±0.76	1.0±0.77	1.0±0.76
Gizzard	24.0±064^{a}	27.0±0.67^{ab}	27.0±0.54^{ab}	30.0±0.75^{c}	29.0±0.79^{bc}	29.0±0.78^{bc}	27.0±0.88^{ab}
Lungs	8.0±0.71^{b}	4.0±0.83^{a}	4.0±0.71^{a}	7.0±0.58^{b}	8.0±0.69^{b}	4.0±0.89^{a}	4.0±0.79^{a}
Intestine	76.0±0.62^{a}	86.0±0.57^{b}	86.0±0.66^{b}	99.0±0.78^{c}	79.0±0.71^{a}	77.0±0.67^{a}	72.0±0.77^{a}

** Enzyme (RoxazymeG2) fortification.*

$^{a, b, c.}$ Mean values within rows with different superscripts letters are significantly different (P = .05)

Table 12. Relative weights of organs at slaughter of broilers (29-57day) fed different levels of ensiled PKM with and without Roxazyme G2

Parameters	Diets						
	1	2	3	4	5	6	7
	% PKM inclusion level and enzyme supplementation						
	Control	30	30*	40	40*	50	50*
Liver	31.0±0.88[a]	32.0±0.90[a]	36.0±0.94[b]	30.0±0.94[ab]	27.0±0.85[b]	32.0±0.84[a]	30.0±0.90[ab]
Kidney	7.0±0.80[a]	5.0±0.80[b]	7.0±0.81[a]	8.0±0.86[a]	5.0±0.83[b]	10.0±0.84[c]	6.0±0.85[ab]
Heart	8.0±0.22	7.0±0.20	7.0±0.26	7.0±0.25	7.0±0.24	7.0±0.28	7.0±0.29
Spleen	2.0±0.40	2.0±0.58	2.0±0.59	2.0±0.50	2.0±0.53	2.0±0.47	2.0±0.49
Gizzard	45.0±0.22[a]	49.0±0.33[b]	55.0±0.36[b]	46.0±0.25[a]	52.0±0.30[bc]	49.0±0.26[b]	53.0±0.28[bc]
Lungs	11.0±0.54	10.0±0.73	12.0±0.71	11.0±0.69	12.0±0.70	11.0±0.66	12.0±0.61
Intestine	118.0±0.11[b]	98.0±0.15[a]	108.0±0.13[ab]	98.0±0.13[a]	103.0±0.17[ab]	103.0±0.17[ab]	113.0±0.19[b]
Proventriculus	9.0±0.55	8.0±0.60	10.0±0.59	9.0±0.61	10.0±0.68	8.0±0.66	8.0±0.63
Pancreas	6.0±0.70[a]	5.0±0.79[ab]	5.0±0.73[ab]	4.0±0.69[b]	4.0±0.74[b]	4.0±0.71[b]	4.0±0.73[b]
Bursarfabricius	2.0±0.10	2.0±0.07	2.0±0.11	2.0±0.03	2.0±0.01	2.0±0.09	2.0±0.08
Crop	23.0±0.55	22.0±0.68	22.0±0.70	23.0±0.63	23.0±0.61	22.0±0.59	22.0±0.60

** Enzyme (RoxazymeG2) fortification.*

[a, b, c..] Mean values within rows with different superscripts letters are significantly different (P = .05)

Table 13. Haematological and some serum biochemical indices of broilers at maturity fed different levels of ensiled PKM with and without Roxazyme G2

Haematological	Diets						
	1	2	3	4	5	6	7
	% PKM inclusion level & enzyme supplementation						
Parameters	**Control**	**30**	**30***	**40**	**40***	**50**	**50***
Hbc (g/dl)	9.86±0.61	10.68±0.65	8.21±0.62	9.86±0.63	8.21±0.67	9.04±0.66	9.04±0.64
PCV (%)	26.0±0.72^{a}	30.0±0.76^{ab}	27.0±0.75^{a}	29.0±0.73^{ab}	25.0±0.71^{a}	31.0±0.71^{b}	26.0±0.74^{a}
ESR (mm^3/l)	4.0±0.54^{ab}	2.0±0.51^{ab}	3.0±0.56^{b}	3.0±0.55^{b}	3.0±0.57^{b}	7.0±0.28^{ab}	5.0±0.29^{b}
MCVx10^{-6} (µl)	0.12±0.02	0.13±0.04	0.11±0.05	0.14±0.06	0.13±0.07	0.14±0.04	0.13±0.05
MCH x10^{-6} (µg)	4.93±0.20	4.77±0.22	4.41±0.21	4.61±0.23	4.32±0.25	4.19±0.22	4.52±0.24
MCHC (g/dl)	41.80±0.66^{a}	32.60±0.64^{bc}	51.31±0.63^{d}	34.0±0.69^{bc}	32.84±0.68^{c}	36.16±0.64^{b}	34.77±0.67^{bc}
Serum Biochemical Parameters (g/100ml)							
Total protein	31.53±0.44^{ab}	27.07±0.46^{b}	20.53±0.45^{d}	28.26±0.49^{ab}	30.94±0.47^{ac}	27.37±0.46^{b}	28.26±0.48^{bc}
Albumin	25.33±0.30^{a}	13.65±0.34^{d}	19.56±0.31^{b}	24.77±0.35^{ac}	22.51±0.35^{c}	17.73±0.34^{b}	26.03±0.32^{ab}
Globulin	6.2±0.19^{a}	3.42±0.17^{d}	0.97±0.15^{a}	4.09±0.16^{ac}	8.43±0.18^{ab}	9.64±0.14^{b}	2.23±0.18^{ce}
Alb/Glo ratio	4.09±0.21^{ab}	1.02±0.19^{d}	20.16±0.26^{e}	6.06±0.25^{c}	2.67±0.24^{ad}	1.84±0.23^{a}	11.67±0.22^{b}

***** *Enzyme (RoxazymeG2) fortification.*

$^{a, b, c..}$ *Mean values within rows with different superscripts letters are significantly different (P = .05) Packed cell volume, PCV; Red blood cell, RBC; Erythrocyte sedimentation rate, ESR; Heamoglobin concentration, HBC; Mean cell Heamoglobin Concentration, MCHC; Mean cell Heamoglobin, MCH; Mean cell volume, MCV*

4. CONCLUSION

The use of ensiled PKM and fibrolytic enzymes (Roxazyme G2) can be safely practiced to further improve the utilization of PKM in poultry diets. The maximum inclusion levels in this study of 30% ePKMf and 50% ePKMf in the starter and finisher phases of the 2 experiments, respectively were found to support investigated growth performance and nitrogen digestibility parameters. Although the nitrogen digestibility had lower values for all PKM diets, this was not enough to threaten or inhibit the growth of the experimental birds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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