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EFFECT OFENZYMESCOCKTAILAND YEASTCOMBINATION ON BLOOD HEMATOLOGY AND BIOCHEMISTRY OF JAPANESE QUAILS FED PROCESSED YAM PEEL MEAL BASED DIET

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ABSTRACT

There has been a global attempt to eradicate the use of antibiotic in poultry production due to increased antibiotic-resistant microbes and antibiotic residue in animal products. Therefore, the need for alternative feed additives is increasing and the beneficial contributions of enzyme complexes and probiotic may be considerable to improving productive performance. This study was carried out to evaluate the combined effect of dietary enzymes cocktail and yeast(ECY) supplementation on hematology and serum biochemistry of growing Japanese quails fed processed yam peel meal (PYPM) based diets. A total number of 240 healthy Japanese quail chicks of two weeks old were used for this study. Four experimental diets containing 0, 25, 50, and 75%ECY supplemented PYPM replacement levels for maize were formulated. The birds were randomly allotted to the four dietary treatments with four replicates of 15 birds each in a completely randomized design. Blood samples were collected from three birds in each replicate at the end of the study through the wing vein for blood hematological and biochemical analysis. Results revealed that ECY supplementation did not have any significant (P>0.05) effect on all blood indices evaluated except for haemoglobin, white blood cells, serum protein, serum glucose and serum cholesterol, which ranged from 6.95g/dl (25%PYPM) – 8.20g/dl (75%PYPM), 5.79 x10^9/l (control) – 6.87 x10^9/l (75% PYPM), 4.66g/dl (75%PYPM) – 5.83g/dl (50%PYPM), 117.67mg/dl (25%PYPM) – 139.04mg/dl (50%PYPM) and 228.09mg/dl (control) – 191.16mg/dl (50%PYPM), respectively. It is therefore, concluded that incorporating up to 75% PYPM with enzymes cocktail and yeast in diets growing Japanese quails will not adversely affect their blood characteristics.

Keywords: Japanese quails, yam peel, multi-enzymes, yeast, haematology, serum.

Introduction

In recent times, the use of untraditional feedstuffs in feed formulation is gaining more recognition in poultry production, mainly as a result of the high cost of traditional feeds such as maize and soybean, which has been a contributory factor resulting to exorbitant cost of poultry production in Nigeria (Thirumalaisamy *et al.*, 2016). The high cost of these conventional feeds arises from their seasonal production schedules, scarcity and high demand due to close competition for utilization among man, livestock and industries (Agbabiaka *et al.*, 2013; Abd El-Hack *et al.*, 2015; Sunmola *et al.*, 2019). This has stimulated research interests from poultry scientists to seek for the need to improve the utilization of untraditional feed ingredients which are seen as cheap and

readily available alternatives to reduce the dependency on use of conventional feed resources in poultry nutrition without any adverse effect on health status of the animals. Yam peel is a basic by-product when yam is peeled during processing for cooking and other purposes for human consumption in especially Nigeria (Akinmutimi *et al.*, 2006; Ezieshi *et al*., 2011).Yam peels are mostly discarded as waste and are majorly allowed to be degraded, hence resulting to waste disposal predicaments, which can potentially constitute a high risk of health and environmental hazards to humans (Enkenyem *et al*., 2006;Edache*et al*., 2012).Yam peel meal is considered to be a potential feed ingredient that could be incorporated in poultry diets if properly harnessed by detoxification process to reduce the level of inherent anti-nutritional factors (Akinmutimi and Onen, 2008; Ezieshi *et al*., 2011; Edache *et al*., 2012; Aguihe *et al.*, 2015). Previous studies with yam peel meal revealed that it can replace maize up to 50% in poultry diet without adverse effect on the performance (Ezieshi and Olomu, 2011).However, the higher crude fiber content in form of non-starch polysaccharides (NSPs) and low digestibility could be considered limiting factors for the greater inclusion level of yam peels in diet of poultry birds (Ezieshi *et al*., 2011; Alu *et al*., 2012).These limitations can be resolved by supplementation of feed additives such as enzymes and yeast which has the capacity to enhance the nutritional value of feedstuffs containing high contents of soluble NSPs (Khan *et al*., 2011;Ezema*et al*., 2012;Alagawany*et al*., 2018; Attia *et al*., 2020).Addition of multi-enzymes and yeast in poultry ration can permit the use of wide range of low quality ingredients without compromising bird's performance and also provide great flexibility in least cost formulation (El-Deek *et al*., 2008; Alu *et al*., 2012; Ogungbesan *et al*., 2014; Al-Harthi, 2016).An increased use of exogenous enzymes is expected not only from the nutritional and economic aspects but also from the health and environmental point of view (Moghaddam *et al.*, 2012; Berwanger *et al*., 2017). Until recently, information on combination of exogenous multi-enzymes and yeast supplementation in diet of growing quails on blood parameters has been inadequate, although its application into poultry production is on the increase. Therefore, the objectives of this study were to investigate the combined effects of supplementation of enzymes cocktail and yeast on the haemato-biochemical parameters of Japanese quails during their growing period.

Materials and Methods

Experimental site: The study was carried out at the Poultry Unit of Teaching and Research Farm of the Department of Animal Production Technology, Federal College of Wildlife Management, New Bussa, Niger State, Nigeria. New Bussa is located at longitude 9°81′ 95′′ N and 9°49′10′′N and latitude 4°58′05′′N and 4°34′49′′N in the Savanna Areas of Niger Basin, North Central Zone of Nigeria.

Preparation of experimental test ingredient: Yam peels were obtained from yam processing centers in New Bussa, Borgu Local Government Area, Niger state. The yam peels were soaked in fresh water daily for three days and thereafter sun-dried for 48 hours in order to decrease the levels of inherent anti-nutritional factors present. The processed peels were milled in a heavy-duty high rotation hammer mill passing through 1 mm mesh sieve, to produce processed yam peel meal (PYPM). The sample PYPM was subjected to proximate analysis according to AOAC(2010) procedure (Table I).

Table I: Proximate composition of processed yam peel and maize meals

Nutrients	[1]PYPM	[2]Maize
Dry matter	94.40	86.50
Ash	8.15	2.70
Crude protein	10.52	9.10
Crude fibre	11.25	1.30
Ether extract	1.11	4.00
Nitrogen free extract	68.97	83.00
Energy (Kcal/kg ME)	2927.70	3432.32

[1]PYPM-Processed yam peel meal; [2]Aduku (2005),

Experimental birds and management: A total of two hundred and eight (208),two-weeks old quails were used in this experiment. They were randomly distributed to four dietary treatment groups on weight basis and each group was replicated in four pens of 13 quails per replicate in a completely randomize design. The birds were raised and managed under a deep litter system with wood shavings serving as litter material and housed in a typical tropical open-sided and well-ventilated poultry facility. The trial period lasted for 28 days with the provision of experimental feed and clean water supplied *ad-libitum*. Standard procedures for vaccination and medication were strictly observed throughout the experimental period.

Experimental diets and treatment: Four isocaloric and isonitrogenous diets were formulated to meet or exceed all nutrient requirements for growing Japanese quails according to Musa *et al*. (2008). Dietary treatment 1 (T_1) contained no yam peel meal (0% PYPM) and supplemental ECY while T_2, T_3 and T_4 contained 25, 50 and 75%graded levels of PYPM substituted at the expense of corn meal with ECY supplementation at 1g per kg diet.

The ECY composition: The ECY supplement used in this study is a microbial preparation originated from the bacteria *Asperigillus oryzae* and each gram of ECY contained mixture of phytase (2500 FTU), cellulase (10,000 IU), ß-glucanase (200 IU), xylanase (10,000 IU) and yeast (*Saccharomyces cerevisiae*, $1x10^9$cfu).The inclusion rate of the ECY in the feed, were performed according to the recommendation of the manufacturer of the supplement product.

Table 2: Ingredient composition of experimental diet fed to growing Japanese quails.

Ingredients	T_1 (0% PYPM)	T_2 (25% PYPM)	T_3 (50% PYPM)	T_4 (75% PYPM)
Maize	46.00	34.50	23.00	11.50
Yam Peel Meal	-	11.50	23.00	34.50
Soy bean meal	36.00	36.00	36.00	36.00
Fish meal	3.00	3.00	3.00	3.00
Rice offal	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00

Lime stone	1.00	1.00	1.00	1.00
Premix	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
ECY	-	0.01	0.01	0.01
Total	100	100	100	100
Crude protein (CP%)	22.87	23.04	23.23	23.39
ME (Kcal/kg)	2923.4	2866.68	2809.93	2753.18

Haematological and serum biochemical indices analysis: On day 28 of the experiment, three birds per replicate of average mean weight were selected and fasted 2 hours for the evaluation of haematological and serum biochemical variables. Blood samples (2mls) were collected from the wing vein of the birds into bottles containing ethylene diamine tetra-acetic acid (EDTA) for haematological analysis. Haematological indices determined were packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC) and haemoglobin concentration (Hb). The improved Nuebaer Haemocytometer method described by Jain (1986) was used to estimate the RBC and WBC, haemoglobin were determined according to Jain (1986). PCV was determined using Wintrobe Micro haematocrit method (Dacie and Lewis, 1991).Red cell indices were calculated as described by Jain (1986) according to the following equations:

$$\text{Mean Corpuscular Volume (MCV)}(\mu m3) = PCVx\ 10/RBC$$

$$\text{Mean Corpuscular Hemoglobin (MCH)(Pg)} = Hbx\ 10/RBC$$

$$\text{Mean Corpuscular Hemoglobin Concentration (MCHC)(g/dl)} = Hbx\ 100/PCV$$

For preparation of serum samples, 2mls of blood was collected without anticoagulant in the sterile test tube. The tube containing blood was placed in slanting position at room temperature for clotting. Blood samples were centrifuged at 3000 rpm for 10 minutes, then collected and stored at -20. Serum total protein, albumin, cholesterol, Aspartate amino transferse (AST), glucose, uric acid, and creatinine were determined colorimetrically using available commercial kits.

Statistical analysis: The data generated were subjected to one way analysis of variance using the General Linear Model (GLM) procedure of the o SAS Institute (2012) for a completely randomized design. Significant differences among means were compared using Tukey test. In all analysis, the significance was declared at 5%.

Results and Discussion

The data presented in Table 3 shows the result of the effect ECY supplement in the diet of growing quails containing graded levels of PYPM on hematological parameters. The result showed that all hematological parameters evaluated in the present study with the exception of WBC and Hb, were not significantly ($p>0.05$) affected by dietary ECY supplementation to PYPM based diets. The non-significant effect recorded in PVC, RBC and differentials of RBC may indicate the evidence of the absence of macrocytic and hypochronic anaemia (Sunmola *et al.*,

2019). It also showed that the processing technique which gave rise to PYPM may have reduced the levels of anti-nutritional factors, thereby increasing its energy and protein release and utilization, due to impact of inclusion of exogenous enzymes cocktail and yeast supplement. The non-significant variation in these heamatological parameters due to enzyme complex and yeast supplementation supports the earlier reports (Al-Mansour *et al*., 2011; Shehab *et al*., 2012; Ezema *et al*., 2012).Supplementation of ECY significantly increased Hb concentration of the growing quails as the level of PYPM increases in their diets. Quails fed 75% PYPM diet had higher Hb while those on 25% PYPM diet gave the lowest Hb mean values compared to other dietary treatments. This also confirmed the fact that haematological traits, especially higher values of Hb were correlated with the nutritional status and blood quality of the broiler chicken (Iheukwmere, 2008). The WBC of groups fed PYPM diet supplemented with ECY were higher ($p<0.05$) compared with those fed control diet group. This is probably due to the fact that yeast stimulated immune system of birds by increasing the total WBC and lymphocyte proliferation (Aathouri *et al*., 2001; Khaksefidi and Ghoorchi, 2006); thus, an indication that the quails acquired good immunity to increase their resistance to infection and good oxygen carrying capacity of the blood that ensures vitality and general well-being (Ubua *et al*., 2018). And this is in accordance with the report by Naidu *et al*. (1999) who revealed that the inclusion of yeast increased the number of T-lymphocytes, antibody-secreting cells, natural killer cells activity and enhanced lymphocyte proliferation. Furthermore, the mean values recorded for MCH, MCV and MCHC across the dietary treatments were within the normal range for healthy poultry birds as stated by Talebi *et al*. (2005) and Akinola and Etuk (2015) who reported MCH values 24.4 pg– 57.2 pg, MCV value 110 fL – 144 fL, MCHC value 23.4 g/dL – 47.2 g/dL. This observation indicated that the nutrients were sufficiently utilized by the growing quails fed ECY supplemented PYPM; thus, explaining why the birds were healthy, not anaemic and capable of withstanding stress (Sunmola *et al*., 2019).

Table 3: Haematological indices of growing Japanese quails fed processed yam peel meal with supplemental ECY

Parameters	T_1 (0% YPM)	T_2 (25% YPM)	T_3 (50% YPM)	T_4 (75% YPM)	SEM
Hb (g/dl)	7.13^{bc}	6.95^{c}	7.88^{ab}	8.20^{a}	0.88
PCV (%)	36.01	35.60	36.30	33.44	1.31
RBC	3.17	3.55	3.11	3.20	0.24
($x10^9$/L)	5.79^{b}	6.11^{ab}	6.81^{a}	6.87^{b}	0.47
WBC ($x10^9$/L)					
MCV (fl)	113.59	110.28	116.72	114.50	8.03
MCH (Pg)	32.49	29.58	33.34	32.62	2.08
MCHC (g/dl)	29.80	29.52	31.71	34.52	2.22

$^{(a-c)}$ Means within a row lacking a common superscript are significantly different ($p<0.05$)

The results of the constituents of blood biochemistry of growing quails fed ECY supplemented PYPM based diet is shown in Table 4.The total protein, glucose and cholesterol were significantly affected by the dietary treatments, whereas serum albumin, aspartate amino transferase (AST), uric acid and creatinine were not

significantly (P>0.05) affected by the dietary multi-enzyme and yeast supplementation. The birds on T_3 diet had the highest (P<0.05) total protein, which is similar to those on T_2 and control diet but differed from those on T_4 diet group. The highest value of total protein was recorded in the bird fed diet T_3 (50% PYPM), suggesting good quality protein of this diet due to multi-enzyme and yeast supplementation, since the higher the value of the total protein, the better the quality of the test feedstuff (Ewuola *et al.*, 2004; Olorode *et. al.*, 2007). In line with this result, Shehab *et al*. (2012) and Attia *et al*. (2020) reported a significant increase on serum total protein with enzyme cocktail supplementation in the diet of growing Japanese quails and broilers, respectively. The glucose concentration of birds on T_3 was higher (p<0.05) than the rest of the treatment groups where birds on T_2 had the least concentration. This shows that multienzymes complexes might have engaged the degradation of fiber components in form non-starch polysaccharides (NSPs) which might have released reasonable amount of energy for utilization by the animals (Shirmohammad *et al*., 2011; Wealleans *et al*., 2017; Behera *et al*., 2019), thus leading to an increase in serum glucose. The concentration of serum cholesterol was reduced (p<0.05) in quails fed enzymes cocktail + yeast supplementation to PYPM based diet in the present experiment. This could be attributed to the possibility of the micro-organisms present in the yeast possessing the potential to assimilate the cholesterol present in the gastrointestinal tract for their own cellular metabolism, thus reducing the amount absorbed cholesterol, as suggested by Mohan *et al.* (1995) and Jin *et al*. (1998). According to Khan *et al*. (2011) and Al-Harthi (2016), this decrease could be attributed to the capability of bacteria/yeast to absorb or break down cholesterol and bile salts, followed by conjugation to prevent the re-synthesis of cholesterol. Previous studies have also found that additions of yeast and enzyme cocktail in poultry diets has led to a significant decrease in cholesterol in the blood serum (Abdel-Azeem *et al*., 2005; Shareef and Al-Dabbagh 2009; Behera *et al*., 2019; Attia *et al*., 2020). Nutritional factors such diet quality and composition as well as feed additive fortification also affect intermediary metabolism, resulting in the changes of serum metabolite levels in poultry (Buyse *et al*., 2002; Swennen *et al*., 2005).

Table 4: Serum biochemistry of Japanese quails fed processed yam peel meal with ECY supplement.

Parameters	T_1 (0% YPM)	T_2 (25% YPM)	T_3 (50% YPM)	T_4 (75% YPM)	SEM
Total protein(g/dl)	5.32^{ab}	5.12^{bc}	5.83^{a}	4.66^{c}	0.25
Albumin(g/dl)	3.15	2.78	3.62	2.86	1.68
AST(U/L)	221.83	241.08	238.33	236.68	18.23
Uric acid (mg/dl)	3.19	2.04	2.17	2.63	1.23
Creatinine(mg/dl)	5.66	5.20	5.56	5.50	0.87
Glucose(mg/dl)	134.13^{a}	117.67^{b}	139.04^{a}	128.86^{ab}	6.43
Cholesterol(mg/dl)	228.09^{a}	206.06^{b}	191.16^{b}	193.21^{b}	8.09

^{abc} Means with the same superscript in the same row are not significantly (p>0.05) different.

Conclusion and Recommendation

According to the findings in this study, incorporation of processed yam peel meal as a valuable ingredient in diets of growing Japanese quails can replace up to 75% maize with supplementation of ECY without any fear of blood dysfunction. Therefore, the results of the present study support the idea of using cheap and available agricultural by-products to reduce dependence of farmers in using costly conventional feedstuffs, which can increase economic returns without any detrimental impact on blood composition of birds while decreasing environmental pollution.

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