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Growth Performance and Carcass Quality of Broiler Chickens Fed Dried Pawpaw (*Carica Papaya Linn*) Latex

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ABSTRACT

This study is aimed to evaluate the effect of latex of pawpaw (*Carica papaya Linn*) as a feed additive and crude enzyme complex on growth performance, cell-mediated immunity, carcass and organ measurements of broiler chicken. Four experimental diets each were formulated during the starter (1-28d) and finisher (29-49d) phases such that the basal diets were supplemented with 0, 0.1, 0.15 and 0.2% Pawpaw Latex (PL). A total of 120 day-old Arbor Acre chicks were randomly allocated to the four dietary treatments comprising of three replicates each in a completely randomized design. Performance parameters measured include Daily Feed Intake (DFI) and Daily Weight Gain (DWG) while Feed to Gain Ratio (FGR) and Protein Efficiency Ratio (PER) were estimated. At day 49, twelve birds per treatment were randomly selected for slaughter in order to measure carcass cut-up parts (thigh, breast, neck, wing, back, drumstick and abdominal fat), and selected organs were weighed and expressed relative to live weight. The immunity index was also evaluated. Data collected were subjected to one-way analysis of variance and treatment means separated using Duncan Multiple Range Test. PL contained 54.9% crude protein, 6.28% ether extract, 4.65% crude fibre, 5.5% ash and 18.79% nitrogen free extracts. At the starter phase, there was a general decline in DFI, DWG and FGR as levels of PL increased. Between days 28-49, broilers on 0.1%PL had comparable DWG, FGR, PER and carcass yield with those fed PL-free diet. Hypertrophy of gizzard, liver and intestines were recorded with increase in level of PL. Generally, dietary inclusion of pawpaw latex decreased growth performance but maintained carcass yield, improved immune response and survivability of broiler chickens.

Keywords: Carcass, Carica papaya, Immunity, Papain, Performance, Poultry

INTRODUCTION

Carica papaya Linn (Pawpaw) is an invaluable plant that is prevalent throughout tropical Africa. It belongs to the family Caricaceae. Nigeria is the sixth largest producer of pawpaw globally, and the level of production has been estimated to be 836, 702 metric tonnes, after India, Brazil, Mexico, Indonesia, Dominican Republic (FAO, 2016; Pariona, 2017). The different parts of the pawpaw plant including leaves, seeds, and fruit have been shown to have excellent nutritional and medicinal values (Krishna et al., 2008; Afolabi et al 2011; Pradeep et al., 2014). Pawpaw latex that is obtained from the skin of unripe pawpaw fruitcontains proteolytic enzymes, papain and chymopapain; mixture of cysteine endopeptidases, glutamine cyclotransferase, chitinases, peptidase A and B, lysozymes and an inhibitor of serine protease (Pendzhiew, 2002; Dominguez et al., 2006; Arvind et al 2013). The latex is sometimes referred to as crude papain because papain production relies solely on it (Dubey et al, 2007; Macalood et al, 2013). Crude papain is of crucial importance in many vital biological processes in living organisms (Tsuge et al., 1999) and it is an enzyme of industrial use and of high research interest (Brocklehurst et al., 1981; Mellor et al, 1993; Thomas, 1994). Among the major applications of crude papain or latex are its use in the food industry (Neidlema, 1991), beer clarification (Caygill, 1979), meat tenderizing and preparation of protein hydrolysates (Dupaigne, 1973).

Nutritionally, pawpaw latex (papain) was found to increase body weight and egg production in layer chickens (Lien and Wu, 2012; Battaa et al., 2015); reduce the effect

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of heat stress in rabbits (El-Kholly, 2008), expand the use of soybean meal and acted as a growth promoter in the diets of weaned piglets (Singh et al, 2011; Baoming et al, 2012). This preliminary study therefore evaluates the inclusion levels of crude pawpaw latex as a feed additive and an enzyme supplement in the diets of broiler chickens. Growth performance, cell-mediated immunity and carcass characteristics were the measures of response.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Poultry Unit of Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Ogbomoso is located in the derived savannah that lies on longitude 4^0 10^1 East of Greenwich meridian and Latitude 8^0 10^1 North of the equator. The altitude is between 300m and 600m above sea level while the mean temperature and annual rainfalls are 27^0C and 1247mm, respectively as cited by Ayinla (2012).

Collection of pawpaw latex

Pawpaw latex (PL) also referred to as crude papain (Macalood et al., 2013) was obtained from fruits of pawpaw plants growing on the LAUTECH Teaching and Research farms plots. Three to four incisions (about 2 mm deep) were made on the epidermal layer (testa) of the unripe fruits during the early morning hours using clean razor blades. The fruits were tapped at intervals of 4-7 days into plastic containers. Daily collections of latex were subjected to sun drying within the temperature range of 30-40°C for 6-8 hours to obtain bristled particle matter. After drying, they were grounded in a Q link blender into powdered form. Samples of the dried pawpaw latex were analysed for proximate contents (AOAC, 2007) and remainder stored in airtight and light proof containers and kept in the refrigerator prior to use.

Experimental diets, birds and management

Four diets were formulated such that the control was maize – soybean meal basal diet without PL. Three other diets based on the control were thereafter formulated to contain 0.1%PL, 0.15%PL and 0.2%PL at the starter and finisher phases as shown in Tables 1 and 2 respectively. The diets were analysed for proximate composition (AOAC, 2007). The analysed crude protein and calculated metabolizable energy of the diets ranged from (22.68 – 22.93%) and (3054.87 – 3102.21 kcal/kg) for starter diets and (19.20 – 20.42%), (3061.69 – 3215.19 kcal/kg) for finisher diets, respectively. One hundred and twenty day-old Arbor acre broiler chicks were randomly allotted into the four dietary treatments of 3 replicates each. Each replicate had 10 birds to make a total of 30 birds per treatment in a Completely Randomized Design (CRD). On arrival, the birds were offered anti-stress and brooded for 2 weeks. Feed and fresh water were offered *ad-libitum* on a daily basis throughout the experiment, which lasted forty-nine days. Broiler starter diets were offered from 1-28 days while broiler finisher diets were fed from 29–49 days. Routine medications and vaccinations were strictly adhered to.

Data measurements

Growth performance. Data were collected daily on feed intake (DFI) and body weight gain (BWG) while feed to gain ratio (FGR) and protein efficiency ratio (PER) were computed using an appropriate formula.

Cell mediated immunity. The cell-mediated immunity was determined according to the formula of Fu-Chang et al. (2004) as follows:

$$\text{Spleen Index} = \frac{\text{Spleen weight}}{\text{Body weight}}$$

$$\text{Bursa index} = \frac{\text{Bursa weight}}{\text{Body weight}}$$

Carcass and organ evaluation

At day 49, 4 birds per replicate of similar body weights close to the average body weight of each replicate were slaughtered, properly bled by hanging them by their legs and scalded in water at temperature of 60°C. After de-feathering, they were eviscerated and dressed to get the dressed carcass weight. The weights of the cut-up parts (thigh, breast, neck, wing, back and drumstick), organs (heart, kidney, lungs, liver, spleen and gizzard), intestines and the abdominal fat pads were recorded and expressed as a percentage of live weight.

Ethical approval

This study was carried out in strict accordance with the recommendations of institutional guidelines for the care and use of laboratory animals. Chickens were humanely handled in respect of the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Statistical analysis

The data collected were subjected to one-way analysis of variance using the General Linear Model procedure of SAS (2012) to determine treatment effects. Significant mean differences were determined using Duncan Multiple Range Test of the same package. The experimental model was:

$$Yij = \mu + Ti + \in ij$$

Where:

Yij = Observed value of the dependent variable
μ = Population mean
Ti = Effect of treatment
$\in$ij = Experimental error assumed to be evenly distributed.

Table 1. Gross composition of starter diets (day 1- 28) (dry matter %)

Ingredients (%)	Control	0.1%PL	0.15%PL	0.2%PL
Maize	52.1	52.1	52.1	52.1
Soya bean meal	27.0	27.0	27.0	27.0
Groundnut cake	8.00	8.00	8.00	8.00
Wheat offal	5.00	4.90	4.85	4.80
Fishmeal (72%)	3.00	3.00	3.00	3.00
Dicalcium phosphate	3.00	3.00	3.00	3.00
Oyster shell	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25
Broiler premix*	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20
Pawpaw latex	-	0.10	0.15	0.20
Total	100	100	100	100
Analyzed composition (%)				
Crude protein	22.7	22.9	22.8	22.7
Ether extract	3.25	3.41	3.28	3.28
Crude fibre	3.00	3.40	4.11	3.32
Ash	8.00	7.10	7.20	7.34
Dry matter	92.5	91.6	92.1	92.8
Nitrogen free extract	55.5	54.8	54.7	56.2
ME (kcal/kg)**	3077.8	3071.7	3054.9	3102.2

[1] Premix supplied/kg diet: Vitamin A (15,000 I.U.), Vitamin D3 (3,000 I.U), Vitamin E (20 I.U.), Vitamin K (25 mg), Thiamin (2 mg), Riboflavin (6 mg), Pyridoxine (4 mg), Niacin (40 mg), Cobalamin (0.02 mg), Pantothenic acid (910 mg), Folic acid (1.0 mg), Biotin (0.08 mg), Choline Chloride (0.05 g), Manganese (0.096 g), Zinc (0.06 g), Iron (0.024 g), Copper (0.006 g), Iodine (0.004 g), Selenium (0.024 g), Cobalt (0.02 mg), Antioxidant (0.125 g), PL = Pawpaw Latex,
[2]Calculated Metabolizable energy

Table 2. Gross composition of finisher diets (day 29 – 49) (dry matter %)

Ingredients (%)	Control	0.1%PL	0.15%PL	0.2%PL
Maize	57.0	57.0	57.0	57.0
Soya bean meal	23.0	23.0	23.0	23.0
Groundnut cake	4.00	4.00	4.00	4.00
Wheat offal	8.60	8.50	8.45	8.40
Fishmeal (72%)	2.50	2.50	2.50	2.50
Dicalcium Phosphate	3.00	3.00	3.00	3.00
Oyster shell	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25
Broiler Premix*	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20
Pawpaw Latex	-	0.10	0.15	0.20
Total	100	100	100	100
Analyzed compositions (%)				
Crude protein	20.4	19.9	20.3	19.2
Ether extract	4.51	6.60	6.80	6.73
Crude fibre	5.00	4.60	4.60	5.00
Ash	8.00	6.30	7.00	5.00
Dry matter	92.5	92.0	91.6	90.9
Nitrogen free extract	54.6	54.6	52.9	55.0
Metabolizable energy (kcal/kg)**	3061.7	3215.2	3184.9	3213.4

[1]Premix supplied/kg diet: Vitamin A (15,000 I.U.), Vitamin D3 (3,000 I.U), Vitamin E (20 I.U.), Vitamin K (25 mg), Thiamin (2 mg), Riboflavin (6 mg), Pyridoxine (4 mg), Niacin (40 mg), Cobalamin (0.02 mg), Pantothenic acid (910 mg), Folic acid (1.0 mg), Biotin (0.08 mg), Choline Chloride (0.05 g), Manganese (0.096 g), Zinc (0.06 g), Iron (0.024 g), Copper (0.006 g), Iodine (0.004 g), Selenium (0.024 g), Cobalt (0.02 mg), Antioxidant (0.125 g), PL = Pawpaw Latex.
[2]Calculated Metabolizable Energy

RESULTS AND DISCUSSION

Proximate composition of pawpaw latex

The proximate content of PL has been presented in Table 3. The determined crude protein, ash and moisture contents were lower than the values obtained by Macalood et al. (2013) and Battaa et al. (2015) while crude fat and crude fibre were higher. Macalood et al. (2013) and Battaa et al. (2015) recorded crude protein values of 57.24% or 62.0%respectively compared to 54.9% recorded in our laboratory. The variations might be due to pawpaw varietal effects, fruit age and/or processing conditions of the latex. The proximate values showed a higher protein content of the latex compared to other nutrient components as previously observed (Macalood et al., 2013 and Battaa et al., 2015). The enzyme protease activity in the dried pawpaw latex was put at 2655 units/g and 285 units /g for pH 5.5 and pH 9.0 respectively (Macalood et al., 2013).

Table 3. Proximate composition of crude pawpaw latex

Parameters	Values (%)
Crude Protein	54.91+/-0.61
Ether Extract	6.28+/-0.18
Crude Fibre	4.65+/- 0.19
Ash	5.50+/-0.02
Moisture	9.87+/- 0.11
Nitrogen Free Extract	18.79+/- 0.16

Growth performance

The effects of PL on growth performance during starter (1-28d), finisher (29-49d) and combined starter/ finisher phases (1-49d) are presented in table 4. Between 1-28d, there was a linear decrease ($P<0.05$) in BWG, DFI and PER while the FGR worsened ($P<0.05$) as the level of PL increases in the diet between the period of 29-49days, birds fed the control diet had consumed ($P<0.05$) more feed however, there were no significant differences in BWG and PER between birds on the control diet and those on 0.1%PL. Considering the entire feeding period (1-49d), growth performance was significantly ($P<0.05$) influenced by the experimental diets (Table 4). There was a linear reduction in BWG and DFI as the level of PL increased. FGR and PER were similar between broilers on PL–free diet and 0.1%PL diet but better ($P<0.05$) than those on 0.15%PL and 0.2%PL based diets. Survivability of broiler chickens fed 0.2%PL was 100% compared to 90% for those on PL-free control group. The results in the present study have suggested that pawpaw latex impacted negatively on performance response, which does not follow the trends of previous reports in other animal models. El-Kholy et al. (2008) and Zeedan et al. (2009) observed that 0.7% papaya latex enhanced growth performance and immune response in heat stressed growing rabbits and concluded that the latex can be used as an alternative growth promoter. Battaa et al. (2015) fed 0.01, 0.03 and 0.05% natural enzyme (plant papain) to Egyptian local layers. They reported improved egg production, feed conversion ratio, nutrients digestibility, immunity and economic efficiency while feed intake was reduced at 0.05% addition of the natural enzyme. Baoming et al. (2012) fed soybean meal diets containing 0, 50 and 75mg/kg papain to weaned piglets in comparison to a diet containing high-grade animal protein ingredient. They observed that the addition of papain at 75 mg /kg level significantly increased *in-vitro* digestibility and effectively expanded the inclusion limit of soybean meal in piglet diets. The nature of protease enzyme in pawpaw latex is to digest protein, clean up the gastrointestinal tract wall and enable efficient nutrient absorption (Onyimonye and Onu, 2009). Judging by the positive role of pawpaw latex in studies involving layers, piglets and rabbits, it becomes somewhat difficult to explain the poor performance outcome with broiler chickens in this study. At the finisher phase it appears that age of the birds made it easier to deal with the presumed cause of poor performance during the starter phase. The poor response may be due to creation of nutrient imbalances and overlap of reactions rather than to a true failure of the enzyme in the pawpaw latex. Activity of crude pawpaw latex enzymes might have been influenced by the plant source, methods of extraction, purification and processing conditions (Rao et al., 1998) because Baoming et al. (2012) reported the good stability of papain at pH ranges of 5-9 or temperature ranges between 4-65^{o}C. Pawpaw latex with its protease content (expected to elicit nutritional improvement) may have different inherent characteristics causing divergent responses *in vivo* as observed in this study. Different performance responses of protease fed to non-ruminant animals might also be related to compatibility with endogenous proteases (Adeola and Cowieson, 2011). Some reports (Kotaro et al., 2004; Harrison and Bonning 2010) have suggested that cysteine protease to be the toxic component in pawpaw leaves that interact with the cellular aspect of insects resulting in growth inhibition, physiological damages and mortality. The exact mechanism by which this operates is still under investigation (Manjunath et al., 2014). Kotaro et al (2004) also noted that pawpaw is among the few latex-bearing plants whose noxious chemical contents have not been fully reported. Various parts of pawpaw contain carpaine, an alkaloid that is more prevalent in its leaves (Krishna et al., 2008) while other natural toxicants like benzyl glycosinate (BG) and benzyl isothiocyanate are parts of the plant's natural defence mechanism.

Table 4. Inclusion levels of pawpaw latex on growth performance of broiler chickens during starter, finisher and combined starter/ finisher phases

Diets / Parameters	Control	0.1%PL	0.15%PL	0.2%PL	SEM
Starter phase (1-28d)					
Daily weight gain, g	33.0^{a}	26.4^{b}	17.9^{c}	14.1^{d}	2.24
Daily feed intake, g	72.0^{a}	69.4^{a}	52.1^{b}	44.6^{b}	3.62
Feed: Gain	2.18^{c}	2.63^{bc}	2.93^{ab}	3.19^{a}	0.13
Protein Efficiency ratio	2.02^{a}	1.66^{b}	1.50^{bc}	1.39^{bc}	0.08
Finisher phase (29-49d)					
Daily weight gain, g	70.7^{a}	67.2^{ab}	61.6^{ab}	51.4^{c}	3.02
Daily feed intake, g	175.9^{a}	142.9^{b}	93.7^{c}	77.4^{d}	12.2
Feed: Gain	2.57^{a}	2.13^{b}	1.52^{d}	1.51^{c}	0.25
Protein Efficiency ratio	2.00^{c}	2.37^{c}	2.87^{b}	3.97^{a}	0.25
Combined phases (1-49d)					
Daily weight gain, g	49.2^{a}	43.8^{b}	36.6^{c}	30.1^{d}	1.32
Daily feed intake, g	116.5^{a}	100.9^{b}	93.6^{c}	65.6^{d}	3.93
Feed: Gain	2.39^{a}	2.30^{a}	1.83^{b}	1.86^{b}	0.15
Protein Efficiency ratio	2.07^{c}	2.19^{c}	2.40^{b}	2.86^{a}	0.06

SEM: ^{a,b,c,d} Treatments on the same row with different superscripts are significantly different ($P<0.05$)

Cell-Mediated immunity

The spleen and bursa are the immune organs (Table 5) of interest here and both were significantly ($P<0.05$) influenced. The immune response increased with increase in dietary levels of crude PL. This is in agreement with the observations of El-Kholly et al (2008) and Battaa et al (2015). Fu Chang et al. (2004) also reported that the index of both the spleen and the thymus determines the immunity strength. The bigger the immunity index the stronger the immune response. El-Kholly et al (2008) postulated that pawpaw latex improves lymphocytes production and thymus index, which lead to the production of T-cells. T-cells undergo maturation in the thymus gland and play a major role in cell-mediated immunity (Stephen, 2007). Pawpaw latex also has antibacterial and anti-fungal properties (Afolabi et al 2011), which may further enhance the activity of the immune system.

Table 5. Effect of inclusion level of pawpaw latex on cell-mediated immunity of broiler chickens

Diets / Parameters (%)	Control	0.1% PL	0.15% PL	0.2% PL	SEM
Spleen	0.10^{b}	0.11^{ab}	0.11^{ab}	0.12^{a}	0.003
Spleen Index ($\times10^{-5}$)	2.26^{c}	2.37^{bc}	2.43^{b}	5.54^{a}	2.2×10^{-6}
Bursa	0.05^{ab}	0.05^{b}	0.05^{b}	0.07^{a}	0.001
Bursa Index ($\times10^{-5}$)	4.33^{b}	4.96^{b}	5.74^{b}	8.77^{a}	0.000

$^{a,\ b\ and\ c}$ Treatments on the same row with different superscripts are significantly different ($P<0.05$)

Carcass and organ measurements

Table 6 has focused on the carcass, organs and intestinal measurements of broiler chickens. There was no significant difference in dressing percentage and percentage back of the broilers however; there were significant variations in the other cut up parts without any specific trend established. Interestingly, abdominal fat was significantly higher in broilers fed PL-based diets compared to those on PL-free diet. The gizzard, liver and intestinal weights were generally heavier in broiler chickens fed PL based diets whereas heart weights exhibited no significant variations. The highest values were demonstrated in broilers fed 0.2%PL. Hepatic hypertrophy, which was observed in the liver, gizzard and intestines of birds fed with PL, might have been provoked by the toxic effect of the cysteine protease in crude papain (Poulter and Caygall, 1985). Lien and Wu (2012) also observed increased liver weight as the inclusion level of crude papain had increased. The comparable values in the dressing percentage, breast, wing, back and thigh of birds fed diets containing 0 and 0.1%PL agreed with the findings of Esonu et al (2008), Bharathidhasan et al. (2009), Hana et al. (2010), Davood et al. (2012), Azarfar (2013) and Dalolio et al. (2015) that in a balanced and quality diet, enzyme might have no significant effect on carcass values.

Table 6. Carcass, organ and intestinal weights of broiler chickens fed supplemental levels of pawpaw latex (expressed as a percentage of live weight)

Parameters	Control	0.1%PL	0.15%PL	0.2%PL	SEM
Live Weight, kg	2266.7^{a}	2250.0^{a}	1866.7^{b}	1300.0^{c}	53.71
Dressed %	69.2	70.4	69.7	69.9	0.29
Cut-up parts (%)					
Neck	4.53^{c}	5.56^{b}	6.01^{a}	5.65^{ab}	0.87
Breast	23.6^{a}	22.2^{ab}	20.8^{b}	19.8^{c}	0.30
Wing	7.82^{b}	7.71^{b}	7.82^{b}	8.72^{a}	0.08
Thigh	10.6^{a}	10.6^{a}	9.93^{b}	11.1^{a}	0.11
Drum Stick	9.92^{c}	10.81^{b}	11.6^{a}	10.5^{b}	0.11
Back	12.1	12.3	12.5	12.4	0.13
Abdominal Fat	0.62^{b}	1.26^{a}	1.03^{a}	1.26^{a}	0.74
Organs (%)					
Kidney	0.53^{b}	0.59^{a}	0.59^{a}	0.49^{b}	0.01
Lung	0.61^{a}	0.54^{ab}	0.50^{b}	0.56^{ab}	0.01
Heart	0.45	0.44	0.45	0.41	0.01
Gizzard	2.19^{c}	2.25^{c}	3.03^{b}	3.23^{a}	0.06
Liver	1.69^{c}	1.71^{c}	1.90^{b}	2.09^{a}	0.05
Offals (%)					
Small Intestine	2.93^{b}	3.10^{b}	3.66^{a}	3.86^{a}	0.08
Large Intestine	0.17^{bc}	0.16^{c}	0.20^{ab}	0.21^{a}	0.01
Caecum	0.48	0.52	0.56	0.74	0.02

$^{a,\ b\ \text{and}\ c}$: Treatments on the same row with different superscripts are significantly different ($P<0.05$)

CONCLUSION

The crude pawpaw latex used in this study negatively affected the performance characteristics of broiler chickens at the starter phase, which however, was slightly ameliorated during the finishing stage. Improved immune response, sustained carcass yield and better survivability of broiler chickens were however, observed with pawpaw latex at 0.1%. Further research should investigate lower dosage and examine the processing conditions for the pawpaw latex.

DECLARATIONS

Competing interests

The authors declare that they have no competing interests.

Author's contributions

HarunaA. Moshood collected the samples; carried out the field work and wrote the first draft. Odunsi A. Adeyinka supervised the overall research and revised the draft and final script approved by both authors.

Consent to publish

Both authors gave their informed consent prior to their inclusion in the study.

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