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INVESTIGATING THE EFFECT OF PAWPAW (Carica papaya) ESSENTIAL OIL DIETARY SUPPLEMENTATION ON THE GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILERS

Daniel Nnadozie Anorue[1*], Friday Ubong[2], Alagbe Olujimi John[3]

[1]Department of Animal Science, University of Abuja, Nigeria
[2]Petsvets Essential Limited, Gwagwalada, Nigeria
[3]Sumitra Research Institute, Gujarat, India

Abstract. The aim of this study was to determine the effect of dietary supplementation of Carica papaya essential oil (CPEO) on the growth performance and carcass characteristics of broiler chickens. 200 1-day Cobb 500 chicks of mixed sex were used for the seven weeks trials. In a completely randomized design 100 birds were randomly distributed into 4 groups consisting of 10 birds. Each treatment group has 5 replicates, birds in group 1 (G_1) was fed basal diet with no Carica papaya essential oil, G_2, G_3 and G_4 were fed basal diet with 100 mg, 200 mg and 300 mg/kg respectively. Clean water was supplied ad libitum. Results on overall growth performance revealed that average daily weight gain, average daily feed intake were higher ($P<0.05$) in G_2, G_3 and G_4 relative to G_1. Mortality and feed conversion ratio were significantly ($P<0.05$) influenced by dietary supplementation of CPEO. Higher mortality was observed in G_1 while none was recorded in other treatments ($P<0.05$). Dressed weight, eviscerated weight and dressing percentage were higher ($P<0.05$) in G2, G3 and G4 than in other treatment group. Weight of legs, heart, spleen, gizzard, thigh, breast, back, drum stick and neck were significantly ($P<0.05$) different among the groups except for the weights of the head, spleen and livers ($P>0.05$). It was concluded that CPEO can be supplemented up to 300 mg/kg in the diets of broilers without negatively affecting their general performance and health status.

Keywords: *Phytochemicals, broilers, growth, performance, carcass, food safety*.

[*]***Corresponding Author:*** *Daniel Nnadozie Anorue, Department of Animal Science, University of Abuja, Nigeria, e-mail: anorued@gmail.com*

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1. Introduction

In order to lower the risk of antibiotic resistance, there is a strong demand for chicken producers to use fewer antibiotics. In order to prevent, treat, and control illnesses and infections, antibiotics are frequently utilized (Manu, 2020; Anne, 2022). Antimicrobial resistance, a serious hazard to the human population, can be brought on by the abuse of antibiotics in the chicken business, though (Albert, 2021; Liz, 2020). Phytogenics or plant extracts, have been suggested as a potential antibiotic replacement because they are efficient and environmentally friendly while leaving no hazardous residue in animal products (meat and eggs). Essential oils and spices are examples of phytogenic feed additives that fall into various categories and each have a unique impact on birds (Jenny, 2020; Jan & Ester, 2021). Hydro-distillation and steam distillation are two methods that can be used to extract essential oils from plant stems, bark, seeds, buds, flowers and seeds (Shittu & Alagbe, 2021).

According to the World Health Organization (2008), there are over 50,000 species of therapeutic plants, some of which still have unrealized promise. Papaya, also known as *Carica papaya* Linn, is a member of the Caricaceae family and one of the promising therapeutic plants (Yogiraj *et al*., 2014; Velluti *et al*., 2003). The papaya is a large, tree-like plant with a single stem that can reach heights of up to 10 meters (Van Zyl *et al*., 2006; Valko *et al*., 2004). The top of the trunk only has spirally arranged leaves. According to Braide *et al*. (2012); Yamaguchi *et al*. (1998), the fruit are high in sugars, fiber, minerals and vitamins (A, B and C). As opposed to mature leaves, the leaf and fruit were said to contain carotenoids, such as -carotene, lycopene and anthraquinones glycoside and as a result, they have medicinal qualities like anti-inflammatory, hypoglycaemic, anti-fertility, abortifacient, hepatoprotective and wound healing properties (Yadegarinia *et al*., 2006; Xu *et al*., 2011).

The seeds of the papaya plant are used as a carminative, emmenagogue, vermifuge, abortifacient, counterirritant, paste for the treatment of ringworm and psoriasis and antifertility medications for men. The black seeds have a pungent, spicy flavor and are edible. They are occasionally powdered and utilized as a black pepper alternative (Wood *et al*., 2006; Woldemichael *et al*., 2008). According to Khaled *et al*. (2013), papaya seeds include phytocomponents like flavonoids, tannins, alkaloids, carbohydrates, and triterpenes. According to Khaled *et al*. (2013), papaya oil had strong antibacterial action against *Staphylococcus spp*, *Streptococcus sp*, *Escherichia coli* and *Proteus mirabilis*. Studies have been done on a variety of papaya seed qualities, including their antioxidant and free radical scavenging activity, anticancer, anti-inflammatory, anti-dengue fever, anti-diabetic, wound healing and antifertility effects. Seeds have the nutraceutical feature because of all these potentials.

The secretion of digestive juices and nutrient absorption, as well as the activities of pathogenic organisms, may be positively impacted by experiments on various essential oils (such as garlic, turmeric, clove, ginger, rosemary, peppermint and thymol), according to several authors (Adebayo *et al*., 2018; Oloruntola *et al*., 2019). Quality, nutritional value and food safety are all related; ensuring public health has necessitated extensive study in the livestock sector. Additionally, there is a lack of knowledge regarding the usage of papaya oil as a dietary supplement for broiler chickens.

Therefore, the aim of this experiment is to investigate the effect of papaya oil on the growth performance and carcass characteristics of broiler chickens.

2. Materials and Methods

Site of the study, ethical consent/approval

The research investigation was conducted at the Sumitra Institute's Livestock Unit, which is located between 23º 13' N and 72º 41' E. In the months of September and October 2023, the study was carried out in accordance with the guidelines and requirements of the protocols approved by the research ethics committee of the Sumitra Research Institute in Gujarat, India (AS/092/2023).

Collection and preparation of Carica papaya essential oil

A qualified taxonomist (Mr. Xing Lin) collected fresh Carica papaya seeds from Punsari village and brought them to the institute's biological science section for thorough identification and certification. To preserve the seeds' active ingredients, the collected seeds were air-dried on a flat metallic tray for 14 days. The seeds were then pulverized to minimize their surface area. Carica papaya essential oil was extracted using the steam distillation method. An H-shaped clavenger apparatus, a heating mantle, a Graham condenser, a safety tube, a seperatory funnel, a round bottle flask, and a beaker are needed for the method. Papaya seeds that had been ground up weighing 200 grams were soaked in 500 mL of water with a heating mantle in a round bottom flask (RBF), and a delivery tube with a Graham's condenser was attached above the RBF. The sample was heated to 60ºC and kept at boiling point; the resultant water vapor then travels through a condenser to a separatory funnel, where it forms a layer with water and oil. The essential oil was slowly discharged from the tube and gathered in a beaker.

Design, experimental feeding and handling of animals

For the experiment, 200 mixed-sex Cobb 500 broiler chicks at one day old were used. The birds were obtained from a renowned hatchery in India and divided into 4 groups, each of which contained 50 birds (5 duplicates, each containing 10 birds). Chicks were kept in semi-closed pens in a metallic battery cage that measured (160 cm ×100 cm × 90 cm) (length breath height) and was 120 cm above the ground. It was furnished with aluminum feeders and nipple drinkers. Birds were given Vitamix® and Glucomol® at a ratio of 5 grams per 10 liters of water when they arrived. Diets were created based on NRC's (1994) nutritional advice for broilers. Throughout the duration of the experiment, additional standard management procedures and a vaccination campaign were implemented. These were the experimental groups:

An essential oil-free standard feed control group: Group 1 Carica papaya essential oil supplemented group, which included group 2, group 3 and group 4 (base feed with 100 mg, 200 mg and 300 mg/kg, respectively.

Carica papaya essential oil analysis using gas chromatography - mass spectrophotometry

Using an Agilent 7000B triple quadrupole GC/MS instrument, the bioactive components in *Carica papaya* essential oil were analyzed. It has the following technical specifications: mode (standard), EI (high sensitivity extraction source), ion source material (non-coated proprietary inert source), ion source temperature (106 to 350ºC), filaments (dual filaments for EI), electron energy (100 to 300 eV), mass range (10 to 1.050 m/z), dynamic range (106), scan rate (up to 6.250 u/s) and mass axis stabilization. Total gas flow (up to 80 mL/min GC carrier plus another 5 mL/min of methane for Cl operation plus an additional 1-2 mL/min of N2 and He for the collision cell gases), pumping system (dual stage turbomolecular pump), detector (triple axis HED-EM with extended life EM and dynamically ramped iris) and detector (Agilent mass hunter acquisit) are all dependent on the collision cell gas and detector configuration.

Traits associated with performance

The amount of feed consumed was calculated by deducting the amount of feed rejected from the quantity of feed provided. The ratio of average feed intake to average body weight growth was used to calculate the feed conversion ratio, or the amount of feed required to create one unit of gain. The difference between the subject's initial and final body weights was used to compute the weight increase. By dividing the weight gained for each treatment by the number of trial days, the average daily body weight was calculated.

Carcass and organ weight estimation

40 broiler chicken (2 per replicates) were chosen at random at the end of seven weeks and starved for ten hours to empty their crops for each treatments. They were

slaughtered via neck using a sharp knife and let to bleed, then scalded in warm water. They were then transferred to the lab, where further measurements were made within 30 minutes of the animal's death, including the dressed weight, the weight of the cut parts or sections and those of the organs. The weight of the cut-up parts and organs was reported as a percentage of live weight.

Dressing % = Eviscerated weight⁄ Live weight multiply by 100

Proximate analysis of experimental diet

Simultaneous determination of all the parameters of interest within 1.5 minutes was carried out using NIR Analyzers InfraLUM FT-12 new versatile instrument with the following technical specification; spectral range (13200–8700 cm-1 (760–1150 nm), resolution (8, 16, 32, 64 cm-1), power consumption (110 W) and power requirements (110/220 Vac, 60/50 Hz).

Statistical analysis

Data on performance and carcass weights were subjected to analysis of variance in a completely randomized design using the Statistical Package for Social Sciences (SPSS; 21.0). Duncan multiple range test of the same software was used to test the significant difference between the means at $P \leq 0.05$ level of significance.

Gas chromatography and mass spectrometry (GC/MS) analysis of Carica papaya oil

GC/MS analysis of oil from *Carica papaya* seed (Table 2) revealed the presence of 23 compounds representing 95.07 %. The oil has benzylisothiocyanate (44.50 %), phytol (10.80 %), farnesyl acetone (7.12 %), hepatodecanol (5.41 %), α-pinene (4.80 %) and α-terpineol (3.50 %) were predominant amongst the identified compounds in the oil. Infection prevention, wound healing, pain relief, nausea reduction, anti-inflammation and anti-anxiety are just a few of the pharmacological effects of essential oils (Halcon, 2002). According to geographical distribution, harvesting period, growing environment, and extraction technique, plants' essential oil components and quality vary (Alagbe, 2022; Oluwafemi *et al*., 2021). A number of these metabolites have medicinal characteristics and their concentration in plant tissues is thought to be the primary determinant of an herb's therapeutic usefulness and quality (Wills *et al*., 2000). Scientific research has demonstrated the efficacy of traditional medicines that contain essential oils in treating a variety of illnesses like malaria and other microbial origin diseases. According to recent research (Kabera *et al*., 2014), benzylisothiocyanate has chemopreventive, antibacterial and antioxidant activities. According to Kris-Etherton *et al*. (2002), limonene, linalool, myrcene-pinene and -terpineol are well-known antibacterial, antioxidant, immune-stimulating, hepatoprotective, antioxidant, chemopreventive and therapeutic agents against tumor cells. Inhibitory, anti-inflammatory anti-carcinogenic and other biological activities of farnesyl acetone, squalene, geraniol and geranyl acetate may prevent oxidative stress (Park *et al*., 2001).

Table 1. Ingredient and chemical composition of experimental diet (% DM)

Ingredients	Starters' mash (0- 21days)	Growers' mash (22 – 42 days)
Yellow maize	50.00	54.00
Wheat bran	2.00	5.00
Soy bean meal	31.05	29.00
Fish meal (72 %)	2.00	2.00
Groundnut meal	8.00	5.00
Oyster shell	2.00	3.00
Bone meal	4.00	6.00
Lysine	0.20	0.20
Methionine	0.20	0.20
Premix	0.25	0.25
Salt	0.35	0.40
Toxin binder	0.05	0.05
Total	100.00	100.00
Calculated analysis		
Crude protein	22.15	20.61
Crude fibre	4.50	5.13
Ether extract	3.80	4.75
Calcium	1.50	1.87
Phosphorus	0.63	0.91
Lysine	1.50	1.67
Meth +Cysteine	0.91	0.95
Energy (Kcal/kg)	2994.1	3168.6
Determined analysis		
Crude protein	23.41	20.95
Crude fibre	4.00	4.40
Ether extract	4.33	4.51
Calcium	1.65	1.81
Phosphorus	0.65	0.81
Lysine	1.96	1.98
Meth +Cysteine	1.10	1.32
Energy (Kcal/kg)	2998.5	3100.3

*Starters' premix: Min/vitamin premix supplied per kg diet: - vitamin A, 10,000 I.U; vitamin E, 28.0 mg; vitamin D 4,000I.U, vitamin K, 5.00mg; vitamin B2, 5.0mg; Niacin, 80 mg; vitamin B12, 25 mg; choline chloride, 100 mg; Manganese, 10.0 mg; Zinc, 40.1mg; Copper, 8.0g; folic acid, 4.5mg; Iron, 5.1g; pantothenic acid, 30mg; biotin, 31.5g; antioxidant, 70mg.

**Growers' premix: Min/vitamin premix supplied per kg diet: - vitamin A, 8,000 I.U; vitamin E, 35.0 mg; vitamin D 3,000I.U, vitamin K, 4.00mg; vitamin B2, 5.0mg; Niacin, 70 mg; vitamin B12, 20 mg; choline chloride, 80 mg; Manganese, 8.0 mg; Zinc, 35.1mg; Copper, 7.0g; folic acid, 4.0mg; Iron, 6.7g; pantothenic acid, 25mg; biotin, 25.5g; antioxidant, 60mg

Table 2. Gas chromatography and mass spectrometry analysis of *Carica papaya* oil

No.	Compounds	Reaction time (minutes)	Percentage area
1	Myrcene	5.002	0.77
2	α-pinene	5.332	4.80
3	α-terpineol	7.445	3.05
4	Limonene	8.031	1.26
5	Benzylisothiocyanate	8.631	44.50
6	Linalool	8.874	0.33
7	Carvone	9.338	2.11
8	Phytol	9.500	10.80
9	farnesyl acetone	9.871	7.12
10	Hepatacosane	9.990	2.69
11	Hepatodecanol	11.440	5.41
12	Geranyl acetate	11.591	1.42
13	9- hexadecen-1-ol	12.550	2.08
14	Squalene	12.671	1.66
15	α-terpinyl acetate	12.700	0.05
16	Benzyl acetate	12.906	1.21
17	2-methyl propyl acetate	13.111	0.11
18	Oxacyclohexadec-2-one	13.223	0.90
19	Cis-methyl dihydrojasmonate	13.601	0.26
20	n-Hexyl salicylate	15.667	0.04
21	Geraniol	17.008	1.35
22	γ-terpene	17.221	2.11
23	Veloutone	17.302	1.04
			95.07 %

Effect of Carica papaya essential oil on the performance of broilers

Effect of *Carica papaya* essential oil on the performance of broilers (Table 2). Average daily weight gain, feed intake and feed conversion ratio at the starter's and growers phase (0 - 21 d) were not significantly (P>0.05) affected by the treatments. At the growers phase (21 - 42 d), average daily weight gain and feed conversion ratio were influenced (P<0.05) by dietary supplementation of *Carica papaya* essential oil. Body weight gain in group 2, 3 and 4 were higher (*P*<0.05) than birds in group 1. Average daily feed intake in group 1 and 2 were similar (*P*>0.05) while mortality were higher in group 1 relative to other treatments (*P*<0.05). Overall production (0 - 42 d), average daily weight gain, feed intake and mortality were significantly influenced by the treatments (*P*<0.05). Lowest weight gain were recorded among birds in group 1 relative to the other treatment. Conversely, feed intake among birds in group 3 and 4 were higher (*P*<0.05) than those in other treatments. Higher mortality rate was recorded in group 1 (1.61 %) while none was recorded in the other treatments fed diets supplemented with CPEO. Higher feed conversion ratio were recorded in group 2, 3 and 4 relative to birds in group 1 (*P*<0.05). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the animal's body and these chemical substances are called phytochemicals (Singh *et al*., 2021; Adewale *et al*., 2021. These are non- nutritive chemicals that have disease preventive properties and translate to better feed conversion among birds (Shittu *et al*., 2021). The results obtained in this experiment suggests that CPEO effectively enhanced digestion and absorption of nutrients, host metabolism and maintaining a healthy gut to mount against diseases among birds in group 2, 3 and 4. This result corroborates with the findings of Osowe *et al*. (2023) when phytogenics (fig leaf powder) was fed to broiler chickens. Saponins have been reported to increase the absorption of amino acids, minerals and vitamins in the gastrointestinal tract of animals (Cheeke, 2000). No mortality was recorded among birds in fed CPEO which indicates that it is capable of preventing dysbacteriosis or dysbiosis in the gut, thus lowering the amount of pathogenic bacteria and increase the quantity of beneficial bacteria (symbionts) (Olafeadehan *et al*., 2021; Oloruntola *et al*., 2018). The presence of benzylisothiocyanate in CPEO suggests that it has antimicrobial and antibacterial properties, thus lowering the intestinal pH against pathogenic organisms (Singh *et al*., 2021). The outcome of this result is in consonance with the reports of (Olafadehan *et al*., 2021).

Table 3. Effect of *Carica papaya* essential oil on the performance of broilers

Parameters	Group 1	Group 2	Group 3	Group 4	SEM	*P*-value
Starters' phase (0-21 days)						
Initial body weight (g/b)	45.91	46.00	45.88	45.67	0.06	0.27
Final body weight (g/b)	977.10	977.61	980.12	979.10	16.73	0.05
Body weight gain (g/b)	931.19	931.61	934.24	933.43	15.08	0.48
Average daily body weight (g/b)	44.34	44.36	44.48	44.45	0.05	0.25
Total feed intake (g/b)	1234.1	1232.8	1233.1	1233.6	25.10	0.04
Average daily feed intake (g/b)	58.76	58.70	58.71	58.74	0.07	0.28
Feed conversion ratio	1.33	1.32	1.32	1.32	0.02	0.05
Mortality (%)	1.11	-	-	-		
Growers' phase (22 - 42 days)						
Body weight gain (g/b)	1300.1^{b}	1516.2^{a}	1520.7^{a}	1523.4^{a}	28.60	0.06
Average daily body weight (g/b)	30.95^{b}	36.10^{a}	36.21^{a}	36.27^{a}	0.04	0.19
Total feed intake (g/b)	2800.4^{b}	2800.9^{b}	2810.3^{a}	2813.4^{a}	33.95	0.21
Average daily feed intake (g/b)	66.67	66.68	66.91	66.98	0.06	0.25
Feed conversion ratio	2.15^{a}	1.85^{b}	1.85^{b}	1.85^{b}	0.02	0.03
Mortality (%)	0.50	-	-	-	0.01	0.01
Overall production (0 - 42 days)						
Final body weight (g/b)	2231.9^{b}	2510.6^{a}	2500.5^{a}	2500.4^{a}	31.58	0.25
Body weight gain (g/b)	2186.1^{b}	2464.4^{a}	2454.62^{a}	2454.73^{a}	27.47	0.05
Average daily body weight (g/b)	52.05^{b}	58.68^{a}	58.44^{a}	58.45^{a}	0.05	0.16
Total feed intake (g/b)	4034.5^{b}	4034.7^{b}	4043.4^{a}	4047.0^{a}	60.85	0.58
Average daily feed intake (g/b)	96.06^{b}	96.06^{b}	96.27^{a}	96.36^{a}	0.07	0.12
Feed conversion ratio	2.20^{a}	1.65^{b}	1.64^{b}	1.64^{b}	0.03	0.01
Mortality (%)	1.61	-	-	-		

Means on the same column with different superscripts are significant (P<0.05); G_1: basal diet with no *Carica papaya* oil (CPEO); G_2: basal diet plus 100 mg/kg CPEO; G_3: basal diet plus 200 mg/kg CPEO; G_4: basal diet plus 300 mg/kg CPEO; SEM: standard error of mean.

Carcass characteristics of broilers fed diet supplemented with Carica papaya essential oil

Carcass characteristics of broilers fed diet supplemented with *Carica papaya* essential oil (Table 4). Dressed weight, eviscerated weight and dressing percentage were higher (*P*<0.05) in group 2, 3 and 4 than birds in group 1. Weights of legs, heart, gizzard, thigh, back, breast, back, wings and neck) were higher (*P*<0.05) in group 2, 3 and 4 relative to those in group 1 except for head, kidneys and liver weights were not influenced (*P*>0.05) by dietary supplementation of CPEO. Higher dress weights among birds fed CPEO suggests that it is nutritionally non-toxic and safe for birds. It was also observed that the organs were not atrophied indicating that the absence of high concentrations of anti-nutrients in the test ingredients (CPEO). The result obtained is in consonance with the reports of Alagawany *et al*. (2015); Erener *et al*. (2011). A rapid secretion of hydrochloric acid and pepsinogen from the proventriculus, which in turn enhances feed degradation and reduces the entry of pathogenic bacteria into the digestive tract, is indicated by an increase in the relative weight of the gizzards of birds fed diets supplemented with CPEO (Attia *et al*., 2014). Similar to this, increased relative weights of the hearts and livers in groups 2, 3 and 4 demonstrate that the animals' bodies are sufficiently supplied with oxygen and are able to process nutrition.

Table 4. Carcass characteristics of broilers fed diet supplemented with *Carica papaya* essential oil

Parameters	Group 1	Group 2	Group 3	Group 4	SEM	*P*-value
Live weight (g)	2202.1^{b}	2578.9^{a}	2500.6^{a}	2534.8^{a}	28.82	0.06
Dressed weight (DW) (g)	19118.6^{b}	2105.5^{a}	2191.7^{a}	2111.9^{a}	26.07	0.05
Eviscerated weight (g)	1710.2^{b}	1960.6^{a}	1976.5^{a}	1980.1^{a}	21.28	0.03
Dressing percentage (%)	71.34^{b}	77.02^{a}	77.04^{a}	77.09^{a}	0.08	0.11
Organ weight (% of DW)						
Head	3.45	3.44	3.51	3.58	0.14	0.01
Legs	1.87^{b}	2.08^{a}	2.17^{a}	2.13^{a}	0.22	0.05
Spleen	0.18	0.17	0.18	0.18	0.01	0.001
Heart	0.37^{b}	0.39^{a}	0.41^{a}	0.40^{a}	0.01	0.001
Kidneys	0.26	0.27	0.28	0.27	0.11	0.02
Livers	2.16	2.13	2.18	2.11	0.10	0.02
Gizzard	3.72^{b}	4.69^{a}	4.77^{a}	4.71^{a}	0.01	0.001
Cut parts (% of DW)						
Thigh	9.29^{b}	11.88^{a}	11.56^{a}	11.30^{a}	0.21	0.02
Back	18.56^{b}	18.73^{a}	18.50^{a}	18.69^{a}	0.27	0.01
Breast	25.18^{b}	25.92^{a}	25.98^{a}	26.00^{a}	0.09	0.11
Drum stick	7.67^{b}	10.73^{a}	10.85^{a}	10.88^{a}	0.02	0.001
Wing	5.85^{b}	9.87^{a}	9.96^{a}	9.98^{a}	0.03	0.01
Neck	3.06^{b}	3.86^{a}	3.95^{a}	3.96^{a}	0.10	0.001

Means on the same column with different superscripts are significant (P<0.05); G_1: basal diet with no *Carica papaya* oil (CPEO); G_2: basal diet plus 100 mg/kg CPEO; G_3: basal diet plus 200 mg/kg CPEO; G_4: basal diet plus 300 mg/kg CPEO; SEM: standard error of mean.

3. Conclusion

In conclusion, *Carica papaya* essential oil contains several bioactive compounds which have several therapeutic properties. 300 mg/kg supplementation of *Carica papaya* essential oil is nutritionally non-toxic and safe without affecting their performance and health condition.

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