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Phyto-additive effects of baobab (*Adansonia digitata* l.) fruit pulp on physiological, reproductive and antioxidant indices of male Japanese quail (*Coturnix coturnix japonica*)

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Abstract

A 12-week experiment was carried out to investigate the effect of varying levels of Baobab Fruit Pulp (BFP) on the haematological and serum biochemical parameters, testicular morphometrics, reproductive hormones and antioxidant indices of male Japanese quails (Coturnix coturnix japonica). Eighty (80) male Japanese quails (4 weeks old) were used for the experiment. The quails were randomly allotted to five dietary treatments T1, T2, T3, T4 and T5 of 16 birds each with two replicates in a Completely Randomized Design (CRD). The control diet was tagged T1 and did not contain BFP while T2, T3, T4 and T5 contained 0.2, 0.4, 0.6 and 0.8% BFP respectively. At the end of the 12th week, blood samples were collected for haematological, serum biochemical, reproductive hormones and oxidative stress indices assays. The birds were sacrificed and testes were carefully dissected for testicular morphometrics (testes weight, length and volume). Data were analyzed using ANOVA at $p<0.05$. The quails' packed cell volume, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration were all significantly ($p<0.05$) higher at 0.8% BFP supplementation level than other levels. Aspartate aminotransferase, Alanine aminotransferase, Low density lipoprotein, High density lipoprotein and urea were also significantly ($p<0.05$) higher at 0.8% BFP than other levels. Quails fed 0.2% BFP had significantly ($p<0.05$) larger mean testes volume than other treatments. Follicle stimulating hormone was significantly higher at 0.2 and 0.4% BFP than other treatments while glutathione was highest at 2% BFP. It was concluded that up to 0.8% BFP supplementation supported blood formation but could lead to liver and kidney damage. However, 0.2% supplementation showed the potential to enhance reproductive parameters and antioxidant indices of the male Japanese quail without adverse effect.

***Key Words:** Baobab Fruit Pulp, Quails, Blood, Testes, Hormones, Antioxidant*

Introduction

Japanese quail (*Coturnix japonica*) has been actively involved with humanity since the 12th century, and continues to play important roles in scientific research and industry. Quail has however since attained economic importance as an agricultural species producing eggs and meat that are enjoyed for their unique flavor. They have low maintenance cost because of their small body size (80- 300 g), short generation interval having three to four generations in a year (Zakari *et al*., 2020). Quail birds are more resistance to disease and they are an excellent laboratory animal because of their short generation interval (Sittie Omayyah, 2020).

Bioactive compounds are components of plants that can influence physiological or cellular activities resulting in beneficial health effects (Kris-Etherton *et al*., 2004). Baobab fruit pulp contains very high levels of minerals (calcium, iron, magnesium, and sodium), organic acids, essential amino acids, sugars, sterols, saponins, triterpenes, flavonoids (high levels of procyanidin B2 and (-)-epicatechin), cellulose, fibres and tannins which may be responsible for its antioxidant, anti-inflammatory and hepatoprotective properties (Kaboré *et al*., 2011).

Biochemical studies have shown that Baobab (*Adansonia digitata*) Fruit Pulp (BFP) is rich in vitamin C (De Caluwé*et al*., 2010). Vitamin C (ascorbic acid) is a naturally occurring organic compound with anti-oxidant properties, found in both animals and plants (Pehlivan, 2017). It functions as a redox buffer which can reduce, and thereby neutralize, reactive oxygen species. It is a cofactor for enzymes involved in hormone biosynthesis, and regenerating other antioxidants; which also regulates cell division and growth. It also plays several roles in physiological processes, such as immune stimulation, synthesis of collagen, hormones, neurotransmitters, and iron absorption (Pehlivan, 2017).

In an effort to achieve one of the United Nations Sustainable Development Goals (SDG), which is improvement of human nutrition, this research aimed at exploiting the potential of organic nutrients in BFP for the enhancement of animal protein supply via quail production and reproduction.

Materials and Method

Experimental site

The experiment was carried out at the Poultry Production and Research unit, Teaching and Research Farm of the Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The climatic condition of Ogbomoso has been described by Ayinla *et al*. (2015).

Animals and management

Eighty (80) male Japanese quails (4 weeks old) were used for the experiment. The quails were subjected to one week of acclimatization for physiological adjustment. They were maintained on a standard grower mash for 5 weeks. At the 10^{th} week, the quails were randomly allotted to five dietary treatments of 16 birds per treatment with two replicates in a Completely Randomized Design (CRD). The quails were fed diet containing 24% crude protein and about 2500 Kcal/kg ME. They were fed and provided with water *ad-libitum*. Litters were changed once at 6th week of the experimental period. Feeding trial lasted for twelve weeks.

Processing of Baobab Fruit Pulp (BFP)

Baobab fruits used for the experiment were obtained from the Ladoke Akintola University of Technology campus in Ogbomoso, Oyo state, Nigeria. Baobab fruits were dipped in water, scraped and cracked open manually using a wooden pestle. The pulp was separated from the fibers and seed by gently pounding in mortar with pestle and then sieving with a 2mm sieve to obtain a powdery material known as Baobab Fruit Pulp (BFP).

Preparation of experimental diets

Five treatment diets tagged T1, T2, T3, T4 and T5 were formulated to contain 24% crude protein and about 2500 Kcal/kg Metabolizable energy. The control diet was tagged T1 and did not contain BFP while T2, T3, T4 and T5 contained 0.2, 0.4, 0.6 and 0.8% BFP, respectively. The gross composition of the experimental diets is shown in Table 1.

Table 1: Gross composition of the experimental diets

Ingredients	T1 (0% BFP)	T2(0.2% BFP)	T3(0.4% BFP)	T4(0.6% BFP)	T5(0.8% BFP)
Maize	11.06	10.80	10.57	10.31	10.04
Soy Bean Meal	24.94	25.00	25.03	25.09	25.16
Baobab Fruit Pulp	0.00	0.20	0.40	0.60	0.80
Corn bran	20.00	20.00	20.00	20.00	20.00
Palm Kernel Cake	17.30	17.30	17.30	17.30	17.30
Wheat bran	9.00	9.00	9.00	9.00	9.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Groundnut cake	6.00	6.00	6.00	6.00	6.00
Oyster shell	4.00	4.00	4.00	4.00	4.00
Fish meal	4.00	4.00	4.00	4.00	4.00
Salt	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Calculated Nutrients					
Crude protein (%)	24.00	24.00	24.00	24.00	24.00
Metabolizable Energy (Kcal/Kg)	2451.04	2443.69	2436.60	2429.30	2421.91
Crude fibre (%)	8.23	8.23	8.22	8.22	8.22

***Vitamin premix**: Supply per kg diet: 2 000 000 iuvit. A; 400 000 iu D_3; 8.0 g vit. E; 4 g vit. b_1; 1.0 g vit. B_2; 0.6 g vit.; 0.4 mg vit. B_{12}; 24.0 g Niacin; 0.2 g Folic acid; 8.0 g Biotin; 48.0 g Choline; 320.0 g BHT; 16.0 g Manganese; 8.0 g iron; 7.2 g] Zinc; 0.32 copper; 0.25 iodine; 36. 0 mg cobalt; 16.0 mg selenium. ** Metabolizable Energy calculated using Pauzenga, (1985)

Data collection

At the end of the 12 –weeks feeding trial, blood was collected via the wing veins of the quails (all the 16 quails/ treatment). Blood meant for haematological assessment was collected into sample bottles containing Ethylene Diamine Tetra acetic Acid (EDTA) while blood meant for serum biochemical parameters, hormonal assay (luteinizing hormone, follicle stimulating hormone and testosterone) and antioxidant indices (catalase, malondialdehyde and glutathione peroxidase) were collected into the plain bottles that did not contain anticoagulant. Haematological assay was done using auto analyzer (SYSMEX, Model KX-21N, Japan). The protocol for the assays was according to the method described for the kits (Elabscience® ELISA Assay, USA). The 16 quails per treatment were humanely sacrificed in accordance with the ethics and regulation guiding the use of research animals as approved by the Faculty of Agricultural Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. Testes were carefully dissected from the sacrificed animals and trimmed off adhering tissues. Testis weight, testis length and testis volume were measured. The testis length was measured with the aid of a pair of vernier calipers, while the testis volume was measured by water displacement according to Archimedes principle (Adu and Egbunike, 2010).

Data analysis

Data obtained were analyzed by One-way analysis of variance using SAS (2000) and significant means were separated by Duncan's Multiple Range Test of the same statistical software.

Results and Discussion

The haematological parameters of

male Japanese quail fed varied BFP supplementation is shown in Table 2. Packed cell volume (PCV), Haemoglobin concentration (Hb), Red blood cell (RBC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) were all significantly ($p<0.05$) affected by the treatments. The PCV, RBC, MCV, MCH and MCHC were all significantly ($p<0.05$) increased at 0.8% BFP level. The Hb was significantly ($p<0.05$) lower at the control than other treatments. The rest of the haematological parameters were however not significantly ($p>0.05$) affected by the BFP. The results suggest that BFP supplementation influenced various hematological parameters in male Japanese quails. These effects may be indicative of changes in blood composition and oxygen-carrying capacity. The potential health and performance implications of this result on Japanese quails are improved oxygen transport. The increase in PCV, Hb and RBC suggests an enhancement in the blood formation and oxygen-carrying capacity of the blood. This could potentially lead to improved oxygen delivery to tissues and organs, supporting overall physiological functions and performance in quails (Evang *et al*., 2021)

Table 2: Effect of Baobab fruit pulp level on haematological parameters of male Japanese quails

Haematological parameters	T1 (0% BFP)	T2(0.2% BFP)	T3(0.4% BFP)	T4(0.6% BFP)	T5(0.8% BFP)	SEM
Packed Cell Volume (%)	50.65^{b}	52.75^{ab}	54.65^{ab}	57.93^{a}	57.07^{a}	0.96
Haemoglobin (g/dl)	14.73^{b}	16.69^{ab}	16.93^{ab}	18.34^{a}	17.07^{a}	0.35
Red blood cell ($\times10^{6}$/µl)	3.55^{b}	3.87^{b}	3.90^{b}	3.90^{b}	4.40^{a}	0.71
White blood cell ($\times10^{6}$/µl)	272.42	264.56	268.57	283.15	273.30	3.55
Platelet ($\times10^{6}$/µl)	34.50	23.67	29.17	49.00	23.33	4.57
Lymphocyte (%)	67.67	74.00	72.33	69.17	66.00	1.20
Heterophils (%)	24.00	22.00	25.50	28.33	30.00	1.47
Monocytes (%)	0.50	2.17	0.33	2.00	2.33	0.37
Eosinophils (%)	2.83	2.50	1.83	0.50	1.33	0.99
Mean Corpuscular Volume (fL)	142.95^{ab}	135.62^{b}	140.32^{ab}	137.95^{b}	149.32^{a}	1.70
Mean Corpuscular Haemoglobin (pg)	41.50^{b}	42.92^{ab}	43.42^{ab}	43.58^{ab}	44.53^{a}	0.39
Mean Corpuscular Haemoglobin Concentration (g/dl)	29.10^{b}	29.90^{ab}	30.97^{ab}	31.58^{a}	31.63^{a}	0.30

ab: Means on same row with different superscripts differ significantly ($P<0.05$). SEM: Standard Error of Mean

The White blood cell (WBC) count was not significantly ($p>0.05$) affected by the treatment. This indicates stability in the ability of the quails to resist disease invasion. A balanced haematological profile is crucial for maintaining immune function, and any alterations could influence the quails' ability to combat infections and diseases (Asogwa *et al*., 2021)

Similar to the report of Evang *et al.* (2021), the changes in haematological parameters, such as increased packed cell volume and haemoglobin levels, may have positive effects on cardiovascular health in quails. Improved blood viscosity and oxygen delivery could contribute to better cardiovascular function and overall wellbeing.

The changes in MCV and MCHC indicate potential alterations in the size and hemoglobin content of red blood cells. This could affect the efficiency of nutrient transport throughout the quail's body to ensure that essential nutrients reach cells and tissues more effectively (Silva *et al*., 2023). Although platelet count was not significantly affected by treatment, the variation across the groups suggests potential effects on blood clotting mechanisms.

Proper regulation of platelet function is essential for maintaining homeostasis and preventing excessive bleeding or clot formation (Evang *et al.*, 2021).

The combined effects of these haematological changes may contribute to improved health and performance outcomes in male Japanese quails. Enhanced oxygen transport, immune function, cardiovascular health, and nutrient delivery could collectively support better growth, reproductive efficiency, and overall vitality in quails.

Effect of BFP supplementation on the serum biochemical indices of male Japanese quails is presented in Table 3. Aspartate aminotransferase, Alanine aminotransferase, High density lipoprotein and urea were all significantly ($p<0.05$) increased with increasing level of BFP. The results suggest that Baobab fruit pulp supplementation may have various effects on serum biochemical indices in male Japanese quails. The significant increase in the levels of aspartate aminotransferase and alanine aminotransferase with increasing level of BFP is an indication of liver and kidney damage. Possibly the duration of feeding impacted negatively on these vital organs in the quails. The implication of this is that the use of BFP for any potential benefit in quail diets must be with caution. Also, the observed significant elevation in low density lipoprotein with increasing BFP level signals a potential danger since high LDL in the body causes health risk.

The findings from this study highlighted the potential influence of Baobab fruit pulp on metabolic and organ function in quails. Baobab fruit pulp supplementation may have potential benefits for quail nutrition and health, but may also require moderation in terms of dosage and duration of feeding.

The observed effects on some serum biochemical indices suggests the importance of carefully optimizing dosage levels to avoid adverse impacts on liver (Kaboré *et al*., 2011) and kidney function (Asogwa *et al*., 2021; Mohammed *et al.,* 2021; Wasihun *et al*., 2023).

Effect of varied Baobab fruit pulp on the testicular morphometrics of male Japanese quails is presented on Table 4. The BFP levels did not significantly ($p>0.05$) affect all the testicular parameters except mean testes volume. There was a significant ($p<0.05$) decrease in mean testes volume at 0.8% BFP level compared to the rest of the treatments. Probably this level was too high and the duration was too long for the quails.

Table 3: Effect of Baobab fruit pulp level on serum biochemical parameters of male Japanese quails

Parameters	T1 (0% BFP)	T2(0.2% BFP)	T3(0.4% BFP)	T4(0.6% BFP)	T5(0.8% BFP)	SEM
Albumin (g/dL)	2.17	2.46	2.55	2.12	2.37	0.06
Globulin (g/dL)	0.87	0.90	0.86	0.55	0.93	0.08
Total protein (g/dL)	3.04	3.36	3.11	2.67	3.30	0.11
Alkaline Phosphatase (U/L)	17.95	16.46	15.75	16.13	16.25	0.39
Aspartate aminotransferase (U/L)	41.84^{c}	52.90^{b}	58.95^{b}	60.00^{b}	78.16^{a}	3.54
Alanine aminotransferase (U/L)	49.73^{c}	57.31^{c}	68.25^{b}	67.98^{b}	77.97^{a}	3.32
Low Density Lipoprotein (mg/dl)	55.85^{c}	83.48^{b}	80.37^{b}	87.94^{b}	128.96^{a}	9.87
High Density Lipoprotein (mg/dl)	20.28^{c}	16.23^{c}	18.38^{c}	46.97^{b}	63.10^{a}	5.40
Creatinine (mg/dl)	0.92	0.95	1.03	0.67	1.03	0.07
Urea (mmol/l)	3.37^{ab}	3.06^{b}	3.81^{ab}	2.64^{b}	4.33^{a}	0.20
Triglyceride (mg/dl)	83.11	89.41	102.02	89.41	84.07	5.73
Total Cholesterol (mg/dl)	119.44	164.46	121.05	112.58	164.13	10.24

abc: Means on same row with different superscripts differ significantly (P<0.05)
SEM: Standard Error of Mean

Table 4: Effect of Baobab fruit pulp level on testicular characteristics of male Japanese quail

Testicular Parameters	T1 (0% BFP)	T2(0.2% BFP)	T3(0.4% BFP)	T4(0.6% BFP)	T5(0.8% BFP)	SEM
Mean Testis Length (cm)	2.28	2.24	2.80	2.70	2.30	0.07
Mean Testis Weight (g)	1.50	1.53	1.50	1.60	1.38	0.03
Mean Testis Volume (ml)	2.97^{a}	3.22^{a}	2.92^{a}	3.00^{a}	1.92^{b}	0.18

abc: Means on same row with different superscripts differ significantly (P<0.05)
SEM: Standard Error of Mean

Table 5 shows the effect of varied Baobab fruit pulp on reproductive hormones of male Japanese quails. Luteinizing hormone and testosterone were not significantly (p>0.05) influenced by varied BFP. However, follicle stimulating hormone increased significantly (p<0.05) up to 0.4% BFP level, beyond which it tended to decline. This result suggests that moderate concentrations of Baobab fruit pulp may have a stimulatory effect on FSH production in male Japanese quails. At higher concentrations of Baobab fruit pulp (0.6% and 0.8% - T4 and T5), the FSH levels did not differ from the control group (T1). This indicates that beyond a certain concentration, the stimulatory effect on FSH may diminish. This effect could be attributed to the nutritional composition of Baobab fruit pulp, including its rich content of vitamins, minerals, and antioxidants, which may support hormonal balance and reproductive function. The result however suggests a dose-dependent response, where intermediate concentrations of Baobab fruit pulp resulted in higher FSH level compared to both lower and higher concentrations. This indicates that there might be an optimal dosage for Baobab fruit pulp to exert its stimulatory effect on FSH level. Further research may be needed to elucidate the mechanisms underlying the observed effects and to determine the optimal dosage and duration of BFP supplementation for modulating FSH levels in quails. Additionally, studies investigating the long-term effects and potential interactions with other dietary components would be valuable for a comprehensive understanding of BFP's impact on reproductive hormones.

Table 5: Effect of Baobab fruit pulp level on reproductive hormones of male Japanese quails

Reproductive Hormones	T1 (0% BFP)	T2(0.2% BFP)	T3(0.4% BFP)	T4(0.6% BFP)	T5(0.8% BFP)	SEM
Luteinizing hormone (µmol/ml)	3.57	2.80	2.57	2.60	2.40	0.17
Follicle stimulating hormone (µmol/ml)	3.17^{b}	4.50^{a}	4.77^{a}	3.50^{b}	3.57^{b}	0.18
Testosterone (µmol/ml)	0.90	0.87	0.80	0.85	1.17	0.11

ab: Means on same row with different superscripts differ significantly (P<0.05)
SEM: Standard Error of Mean

Table 6 showed the effect of varied Baobab fruit pulp on antioxidant indices of male Japanese quails. The treatments did not have a significant (p>0.05) effect on both catalase and malondialdehyde while there was a significant (p<0.05) elevation in glutathione peroxidase at T2 (0.2% BFP) compared to the rest of the supplementation level. The decline in the glutathione peroxidase as BFP supplementation increased beyond 0.2% is an indication that an optimum level of supplementation might have been reached for activation of glutathione peroxidase. The BFP contains a lot of nutrients that work together in different proportions. These nutrients play essential roles in the overall health and function of the body, including reproductive systems. Baobab fruit pulp also contains antioxidants such as vitamin C and various polyphenols. Recent works (Ndiaye *et al.,* 2021; Vinha *et al*., 2024), have confirmed that antioxidants help in neutralizing harmful molecules called free radicals, which can cause oxidative stress and damage to cells, including those in the reproductive organs.

Table 6: Effect of Baobab fruit pulp on oxidative stress indices of male Japanese quails

Oxidative stress markers	T1 (0% BFP)	T2(0.2% BFP)	T3(0.4% BFP)	T4(0.6% BFP)	T5(0.8% BFP)	SEM
Catalase (µmol/ml)	1.52	1.01	1.81	1.52	1.18	0.43
Malondialdehyde (µmol/ml)	24.68	21.94	24.01	24.37	24.77	1.81
Glutathione peroxidase (µmol/ml)	3.10^{ab}	4.92^{a}	2.41^{b}	2.18^{b}	2.56^{b}	0.46

ab: Means on same row with different superscripts differ significantly (P<0.05)
SEM: Standard Error of Mean

Conclusion

The results from this experiment showed that 0.8% BFP supplementation level supported blood formation but could lead to liver and kidney damage. However, 0.2% supplementation showed the potential to improve the reproductive characteristics and antioxidant property of male Japanese quail without adverse health consequences. Further studies are needed to evaluate the overall reproductive and health implications of Baobab fruit pulp supplementation in quails under different conditions and different durations of feeding.

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