HAEMATOLOGY AND SERUM ANTIOXIDANT STATUS IN LAYING BIRDS AS INFLUENCED BY PHYTOBIOTIC OF BLACK PEPPER (*Piper nigrum*) POWDER

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ABSTRACT
This study was done to evaluate the effect of Black pepper (BP) supplementation on health status of laying birds. One hundred and sixty-eight ISA Brown layers (25 weeks old) was used which was randomly allotted in a completely randomized design to four treatments; T1 (control, 0.0%), T2 (0.3% BP), T3 (0.6% BP), T4 (0.9% BP), with six replicates and seven birds per replicate. Haematology and serum antioxidant activities were assessed using a standard procedure. Antioxidant activity in the serum showed that ferric-reducing antioxidant power was elevated with BP at 0.6 and 0.9% (2.287 ug AAE/g and (2.176 ug AAE/g) compared with control (P<0.05). Black pepper supplementation did not have any significant effect on the haematological parameters assessed. The result obtained shows that Black pepper inclusion in the diets of laying birds increased antioxidant activities. Black pepper can be added to diets up to 0.9% without any negative effect on the health of laying birds.
Keywords: Phytobiotic, Black Pepper (*Piper Nigrum*), Productivity, Haematology, Serum, Antioxidant and Laying Birds

INTRODUCTION
Antibiotics growth promoters (AGPs) have shown encouraging effects among the phytobiotics feed additives (PFA) on poultry production output (Amad *et al.,* 2011; Gheisar *et al.,* 2015). Natural antioxidants from plant or phytogenic materials are becoming of increasing interest nowadays due to their roles as AGPs. In particular, plant-based materials that contain phenolic compounds of high antioxidant activities with the potential to alleviate the adverse effect of oxidative stress (Saracila *et al*., 2021). Phytobiotics, also known as phytogenics or phytochemicals, are an extensive subset of plant-based bioactive compounds. Over 5000 individuals dietary phytobiotics have been recognized to date in fruits, whole grains, vegetables, legumes, herbs, nuts, and essential oils (Liu, 2004; Landy *et al*., 2011). The concept of "phytogenic feed additives" thus refers to natural medicinal products derived from herbs used in livestock nutrition to enhance performance (Pliego *et al*., 2020). Spices and herbs are the main source of natural antioxidants (Xu *et al*., 2017; Alqethami and Aldhebiani, 2021) which are increasingly attractive due to safety concerns and are also cheaper compared to synthetic antioxidants (Stankovic *et al*., 2016). Black pepper (*Piper nigrum* L.*;* Piperaceae family) a flowering vine native to South Asia, renowned as the "King of Spices" due to its significant place culinarily for over 2000 years (Aggarwal, and Kunnumakkara, 2009). Traditionally it is used in the treatment of colds, neuropathic pain, gastric discomfort, respiratory diseases, etc. (Gülçin, 2005) and it has been reported to have numerous pharmacological actions, viz., antioxidant, anticancer, anti-asthmatic, antihypertensive, anti-inflammatory, anti-obesity, analgesic, CNS stimulant, hepatoprotective, immuno-modulatory, and antimicrobial properties (Gülçín, 2005; Agbor, *et al*., 2006; Zaveri *et al*., 2010; Saleem *et al*., 2022). The study aims at determining the influence of black pepper on the haematology and serum antioxidant status of laying birds.

MATERIALS AND METHODS
Experimental Site: The experiment was conducted on a commercial layer farm in Ilora Farm Settlement, Ilora, Oyo State, Nigeria. The general coordinates for Ilora are approximately 7.800° N latitude and 3.900° E longitude. Ilora Farm Settlement, located in the Southern Guinea Savanna of Oyo State, Nigeria, features a tropical savanna climate with distinct wet and dry seasons, annual rainfall of 1,000–1,500 mm, and temperatures ranging from 25°C to 35°C; it has gently undulating terrain (200–400m above sea level), well-drained sandy-loamy soil, mixed vegetation of grasses and scattered trees, seasonal water sources like boreholes and streams, and is primarily used for commercial poultry and crop farming under Oyo State's agricultural development program.
Experimental Material Sourcing and Processing: *Piper nigrum* (black pepper) seeds were sourced from Bodija market, Ibadan, ground into powder with a coffee blender and stored in a hermetic container until its use.

Experimental Design: The experimental design was a Completely Randomized Design of four (4) treatments with six (6) replicates each.

Experimental Birds and Management

One hundred and sixty-eight (168) ISA brown layers (25 weeks old) were used for the experiment. The birds were randomly divided into four (4) experimental treatments of seven (7) birds per replicate and six (6) replicates per treatment. Each experimental treatment has a total of forty-two birds.

Experimental Diets

The gross composition of the basal diet is presented in Table 1. Four dietary treatments containing graded levels of black pepper (*Piper nigrum*) at 0, 0.30%, 0.60%, 0.90% inclusion rates were formulated and designated as T_1, T_2, T_3, and T_4.

Diet 1= basal diet without BP inclusion (control)
Diet 2 = basal diet plus 0.30% of black pepper (300gm/100kg of feed)
Diet 3= basal diet plus 0.60% of black pepper (600gm/100kg of feed)
Diet 4= basal diet plus 0.90% 0f black pepper (900gm/100kg of feed)

Experimental Design and Duration of Experiment

Birds used for the study were weighed and randomly assigned to 4 dietary treatments with 6 replicates of 7 birds per replicate in a completely randomized design (CRD). The birds were acclimatized to the feed for 10 weeks after which data and sample collection was carried out for 6 weeks, covering the first phase of laying period from 25-40 weeks.

Parameters Measured

packed cell volume PCV (%), Haemoglobin (g/dl), red blood cells (RBC) count (μ/l), and white blood cell (WBC) count (μ/l). Mean corpuscular height and volume were also determined.

Table 1: Composition of Basal Diet

Ingredients	Quantity
Maize	51.80
Soybean meal	9.30
Groundnut cake	18.50
Wheat bran	6.30
Limestone	10.00
Di-Calcium phosphate	2.00
Vegetable Oil	0.85
Salt	0.31
L-Lysine	0.25
DL-Methionine	0.20
Toxin Binder	0.10
Choline-chloride	0.05
Layer premix	0.30
Enzyme	0.05
Calculated analysis	
Metabolizable energy (kcal/kg)	2590
Crude protein (%)	19.21
Crude fat (%)	3.60
Linoleic acid (%)	1.38
Crude fibre (%)	2.64
Calcium (%)	3.87
Available phosphorus (%)	0.58
Lysine (%)	0.96
Methionine (%)	0.43

Haematology

The blood samples were placed in specimen bottles with EDTA (Ethylene Diamine Tetra Acetate) for haematological studies. Blood was analysed for packed cell volume PCV (%), Haemoglobin (g/dl), red blood cells

(RBC) count (µ/l), and white blood cell (WBC) count (µ/l). Mean corpuscular height and volume were also determined.

Determination of Antioxidant Content and Activities: This was carried out according to (Manzocco *et al*., 2008) and Oyaizu (1986).

RESULTS

Mean values of polyphenol content and antioxidant activity detected in eggs are presented in Table 2. From the results it was observed that total flavonoid content (ug QE/g) was significantly higher at 0.9% compared with other treatments (P<0.05). There was however no significant effect of black pepper powder on total phenolic content (ug GAE/g), the highest value was at 0.3% BP inclusion level, while control had the lowest value. Ferrous reducing antioxidant power (FRAP ug AAE/g) was significantly higher at 0.6 and 0.9% than the control group (P<0.05). Diphenyl-2-picryl-hydrazyl radical scavenging activity (%) did not exhibit any significant effect of black pepper powder in the blood.

Table 2: Polyphenol Content and Antioxidant Activity Detected in Plasma of Laying Birds Fed Graded Level of Black Pepper Powder in Feed

	TFC	TPC	FRAP	DPPH
Control	2.552^{b}	1.295	2.010^{b}	31.67
0.3%	2.558^{b}	1.342	2.156^{ab}	32.05
0.6%	2.633^{b}	1.323	2.287^{a}	30.71
0.9%	3.247^{a}	1.339	2.176^{a}	34.03
SEM	0.092	0.011	0.027	0.772
P -value	0.041	0.418	0.014	0.500

^{a,b} means that a column with different superscripts are significantly different (P<0.05).

TFC: Total flavonoid content[1] (ug QE/g), TPC: Total phenolic content[1] (ug GAE/g), FRAP: Ferric reducing antioxidant power (ug AAE/g), DPPH: Diphenyl-2-picryl-hydrazyl radical scavenging activity (%), SEM: standard error of mean.

The results for haematological assessment carried out on laying birds fed graded levels of black pepper powder in diets are presented in Table 3. There was no significant effect of Black pepper on haematological indices assessed (P>0.05).

Table 3: Haematological Assessment of Layers Supplemented Black Pepper Powder in Diets

	PCV (%)	Hb (gm %)	RBC $*10^{10/L}$	WBC$*10^{8/L}$	MCV (FL)	MCH (Pg)
Control	18.7	61.7	369.7	61.3	54.7	17.7
0.3%	15.2	48.3	306.3	57.3	54.0	17.0
0.6%	19.7	64.2	336.5	58.0	59.2	19.2
0.9%	19.0	62.2	324.3	60.7	60.0	19.5
SEM	0.667	2.216	20.607	3.017	3.398	1.110
P -value	0.110	0.075	0.741	0.956	0.893	0.833

PCV: Packed cell volume, Hb: haemoglobin, RBC: red blood cell, WBC: white blood cell, MCV: mean corpuscular volume, MCH: mean corpuscular height.

DISCUSSION

Presently, this study highlighted elevated content of total flavonoids (27%) in serum of laying birds, alongside increased ferric reducing antioxidant power by up to 14% (0.9%), with Black pepper supplementation. However, total phenolic content and DPPH radical scavenging ability were not significantly influenced. The antioxidant activity of piperine, in vitro and in vivo is well-established (Mittal and Gupta, 2000; Abdel-Daim *et al*., 2019). The protective ability of piperine has been demonstrated against oxidative damage, via inhibition or elimination of free radicals and reactive oxygen species. Black pepper or piperine treatment has also exhibited ability to lower lipid peroxidation in vivo and beneficially influence cellular thiol status, antioxidant molecules and antioxidant enzymes during oxidative stress (Srinivasan, 2007). Piperine has also been reported to have anti-acetylcholinesterase activity (Manap *et al*., 2019; Aluko, 2021) and can restore the levels of antioxidant enzymes (Hritcu *et al*., 2015).

Phenols and flavonoids are secondary metabolites which play significant roles in the development and defence of plants (Sharma *et al*., 2023). According to González-Palma *et al*. (2016), a positive correlation exists between polyphenol content and antioxidant potential. Sharma *et al*. (2023), demonstrated that the antioxidative nature of the extracts of black pepper against reactive oxygen species was however dose-dependent. Irrespective, extracts of BP maintained a high level of cellular communication (Ghaffari *et al*., 2014) and still exerted a neuroprotective effect. In the same vein, Liu *et al*. (2021) observed that capsaicin supplementation, significantly increased the plasma glutathione peroxidase activity and reduced the plasma MDA content in laying duck, indicating improved redox status with phyto-additive in laying ducks.

The present study did not reflect any additive benefit of black pepper powder on the health status of the birds using haematological assessments as indices. Al-Kassi *et al* (2011) however reported that black pepper supplementation at up to 1% of the diet of broiler chicken resulted in a significant reduction in haemoglobin, packed cell volume and red blood cell count. They however observed no effect on white blood cell count. Abou-Elkhair *et al*. (2014) also reported that BP alone or in combination with other spices could enhance the health status of broiler chicken.

CONCLUSION

Black pepper can be added in diets up to 0.9% without any negative effect on the health of laying birds.

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