Some blood indices in finisher broiler chickens fed cocoa pod husk (*Theobroma cacao* L.) fermented with *Pleurotus ostreatus* or treated with enzymes as ingredients in their diets

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ABSTRACT

The dietary effects of pre-treated cocoa pod husk (CPH) on some blood indices of 21-day old COBB-500 finisher broiler chicks were evaluated in a 35-day experiment with a completely randomized design. The birds were allotted to seven treatments (diets) having 0-g kg^{-1}, 100-g kg^{-1}, 200-g kg^{-1} and 300-g kg^{-1} of either *Pleurotus ostreatus* fermented CPH (PF-CPH) or enzyme (Viscoyme®L+Pectinex®5XL) supplemented CPH (E-CPH). Each treatment was replicated three times with 12 chicks per replicate. The haemoglobin concentration (Hb), packed cell volume (PCV), activities of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), serum levels of cholesterol (CH), uric acid, total protein, albumin and globulin were determined. Across dietary treatments Hb, PCV, CH, uric acid and AST activity level in blood of birds varied significantly ($P < 0.05$). However, there was insignificant influence ($P > 0.05$) of pre-treated CPH on all the other evaluated parameters among treatment groups. All blood indices measured were within acceptable or reference ranges. The study has revealed that incorporating either E-CPH or PF-CPH up to 30 per cent of broiler finisher diet had no adverse effect on the blood values of the broiler chickens. However, up to 20 per cent PF-CPH and 10 per cent E-CPH incorporation rates for broiler finisher diets, based on best results observed for growth and feed conversion, is recommended.

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Introduction

As the price of conventional feed ingredients like soyabean and maize continue to increase, the need to explore locally produced agricultural solid wastes as unconventional feedstuffs has become increasingly impor-

tant. These unconventional feed resources, however, are of low nutritive value on account of their high levels of fibre and other anti-nutritional factors. The high fibre content in the unconventional feed could be improved by pre-treatment through the application of biotechnological methods such as fermentation or enzyme technology. These treatments could help break down cellulolytic bonds making such feeds available for digestion and absorption by mono-gastric animals and, therefore, good substitutes for their conventional feedstuffs (Iyayi & Aderolu, 2004; Iyayi & Davies, 2005; Nnenna, Emeka & Okpoko, 2006).

Cocoa (*Theobroma cacao* L.) pod husk (CPH) is a major under-utilized by-product from the cocoa industry in Ghana, currently the world's second highest producer of cocoa (ICCO, 2007). In a broiler chicken feeding trial, Alemawor *et al*. (2010) evaluated the feed value of CPH pre-treated with *Pleurotus ostreatus* (oyster mushroom) or enzyme supplement. The results indicated a 10-20 per cent optimum dietary inclusion level of the improved CPH to replace various proportions of maize, wheat bran and soyabean in broiler finisher rations. Though the growth and carcass performances were not adversely affected, information about the effect of the pre-treated CPH on the blood chemistry of the birds was not reported.

Blood chemistry constituents reflect the physiological responsiveness of the animal to its internal and external environments, which include feed and feeding (Esonu *et al*., 2001; Iheukwumere & Okoli, 2002). The determination of blood component values in laboratory examination is an important procedure to establish diagnostic baselines of blood characteristics to assist in routine management practices of farm animals (Bounous *et al*., 2000; Tambuwal, Agaie & Bangana, 2002).

The objective of the study, therefore, was to determine the effects of feeding CPH (either *P. ostreatus* fermented or enzyme-supplemented) on levels of some blood indices in finisher broiler chickens. This will be helpful to further consolidate the adoption of the novel treated CPH for poultry use.

Materials and methods

Processing of cocoa pod husk (CPH)

CPHs were treated by fermenting with grain spawn of *P. ostreatu*s as described by Alemawor *et al*. (2009), in which cocoa pods were subjected to a solid-state treatment. A second treatment, involved a combined supplementation of two commercial multi-enzymes (Novozymes North America Inc. USA), namely Viscozyme®L (beta endo -1,3(4) -glucanase activity of specific activity of 100 betaglucanase units g^{-1}) and Pectinex®5XL (polygalacturonase activity with specific activity of 4500 pectinase units ml^{-1}). Viscozyme®L and Pectinex®5XL were applied at a rate of 6 g kg^{-1} CPH and 8 g kg^{-1} CPH, respectively, in the diet.

Experimental design, birds and management

Two hundred and fifty-two 21-day old COBB 500 broiler chicks of similar weights were used in a completely randomised design experiment with seven treatments (diets) and three replicates (a total of 21 experimental units). There were 12 chicks (six males and six females) per replicate. The seven diets, formulated to meet the minimum protein requirements for standard broiler finishers (NRC, 1994) were control diet with no

CPH (0 CPH), three diets containing graded levels of *P. ostreatus*-fermented CPH (PF-CPH) at 100-g, 200-g and 300-g kg^{-1} (designated as 100PF-CPH, 200PF-CPH and 300PF-CPH, respectively), and three diets containing graded levels of enzyme-treated CPH (E-CPH) at 100-g, 200-g and 300-g kg^{-1} (designated as 100E-CPH, 200E-CPH and 300E-CPH, respectively) (Table 1). Thus, the PF-CPH or E-CPH in the diet replaced proportions of maize, wheat bran and soyabean in the diet.

Replicate groups of birds were placed in separate pens (each measuring 24 m × 8 m × 3 m) in a concrete-floored deep litter house (using wood shavings). The upper half of the building was of wire mesh on a lower half (0.7 m high) of cement blocks to ensure adequate ventilation. Water and feed were supplied *ad libitum* over the experimental period of 35 days, with a continuous light regime provided during dark hours. Management practices such as routine vaccination, drug administration, and maintenance of cleanliness in and out of the poultry house were observed as recommended for intensively housed poultry.

Assessment of blood parameters

At the end of the experiment, two birds (one male and one female) were selected at random from each replicate group and their live weights measured after starving them (of feed but not water) overnight to empty their crops. The birds were slaughtered and bled. Approximately 5 ml of blood per bird was collected into each of two clean labelled vacutainers. One vacutainer contained ethylene diamine tetraacetic acid (EDTA) anticoagulant while the other was without EDTA. The blood samples in the EDTA–containing vacutainers were then processed for determination of haematological parameters like haemoglobin (Hb) concentration and packed cell volume (PCV), while blood in the vacutainers without EDTA was processed for serum.

Haematological parameters (Hb concentration and PCV) were determined using microhaematocrit and colorimetry-cyanomethhaemoglobin methods, respectively (Cheesbrough, 2000). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) UV enzymatic method using a diagnostic kit (Linear Chemicals S.L., Barcelona, Spain). Serum total protein and albumin were determined according to the Biuret method and BCG-method, respectively, using analytical kits (Human Gesellschaft fur Biochemica und Diagnostica mbH, D-65205 Wiesbaden Germany). Uric acid and total cholesterol were determined by the enzymatic endpoint colorimetric method using diagnostic reagent kits (Linear Chemicals S.L., Barcelona, Spain). Globulin level in blood was calculated as the difference between total protein and albumin.

Statistical analysis of data

All data collected were subjected to analysis of variance using GENSTAT Release 9 (2007) computer software. Initial weight of broilers was used as a covariate in the analysis of variance for final weight of broilers. Significant ($P < 0.05$) treatment means were separated using LSD.

Results and discussion

Table 1 shows the composition of ingredi-

Table 1

Composition of Experimental Broiler Finisher Diets

Ingredient (g kg^{-1})	*Treatments*						
	Control diet	P. ostreatus *fermented CPH based diets*			*Enzyme supplemented CPH based diets*		
	0 CPH	*100PF-CPH*	*200PF-CPH*	*300PF-CPH*	*100E-CPH*	*200E-CPH*	*300E-CPH*
PF-CPH	-	100.0	200.0	300.0	-	-	-
E-CPH	-	-	-	-	100.0	200.0	300.0
Yellow maize	600.0	540.0	480.0	420.0	540.0	480.0	420.0
Wheat bran	137.4	95.5`	53.5	11.6	88.2	38.9	0.0
Fish meal	127.6	139.5	151.5	163.4	146.8	166.1	175.0
Soya bean meal	100.0	90.0	80.0	70.0	90.0	80.0	70.0
Oyster shell	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Dicalcium phosphate	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vita-min premix 1	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mycofix 2	0.5	0.5	0.5	0.5	0.5	0.5	0.5
TOTAL	1000	1000	1000	1000	1000	1000	1000
Calculated nutrient content (g kg^{-1})							
Crude protein	198.5	202.6	206.8	210.9	202.7	207.0	206.3
Lysine	11.53	11.80	12.08	12.35	12.17	12.81	12.99
Methionine	4.50	4.61	4.72	4.82	4.71	4.91	4.93
Calcium	15.93	17.51	19.10	20.68	17.35	18.77	19.82
Available phosphorus	5.09	5.84	6.59	7.34	5.81	6.55	7.05
M.E.3(MJ kg^{-1})	11.7	11.3	10.9	10.7	11.1	11.0	10.8
Analysed nutrient components (g kg^{-1} DM)							
Organic matter	918	907	900	900	891	900	901
Crude protein	199	218	211	208	221	220	215
Ether extract	21	18	19	22	18	19	23
Crude fibre	28	50	71	86	47	66	77
Nitrogen-free extract	545	501	481	461	485	478	460
Calcium	30.22	27.40	34.11	34.04	49.20	31.58	22.00
Phosphorus	19.08	19.65	18.59	18.50	20.33	23.87	16.45

Notes

1. Vitamin premix (Vital Broiler & Chick Premix-G; 2g/kg) contains: vitamin A (11,000 I.U.); vitamin E (5 mg); Folic acid (0.36 mg); Calcium panthothenate (0.4 mg); vitamin B12 (12 mcg); vitamin B6 (1.6 mg); vitamin D (2,000 I.U.); vitamin K (1.2 mg); Niacin (1.6 mg); vitamin B2 (2.4 mg); vitamin B1 (0.6 mg); Biotin (0.08 mg); Choline chloride (24 mg); Manganese (50 mg); Zinc (40 mg); Copper (2.8 mg); Iodine (32 mg); Selenium (0.16 mg); cobalt (0.4 mg); Ethoxyquin and BHT. 2. Mycofix® (BIOMIN GmbH, Herzogenburg, Austria) is a commercial mould fixing agent which is added to feeds at a rate of 0.5 g per kg of feed. It binds mycotoxins in vivo preventing them from causing harm to the animals. 3. Metabolizable energy

TABLE 2

Haematological-Serum Indices of Broilers fed Finisher Diets Containing Graded Levels of PF-CPH or E-CPH

Parameter	*Dietary treatments*							*SED5*
	C 0 CPH	*100 PF-CPH*	*200 PF-CPH*	*300 PF-CPH*	*100 E-CPH*	*200 E-CPH*	*300 E-CPH*	
Hb1 (g dl^{-1})	12.13a	12.18a	12.30a	11.92a	11.85a	11.75a	10.83b	0.365
PCV2 (%)	37.61a	37.77a	38.13a	36.94a	36.73a	36.43a	33.58b	1.132
Cholesterol (nmol l^{-1})	3.05a	2.90ab	2.88ab	2.45bc	2.30c	2.40bc	2.85ab	0.243
Uric Acid (µmol l^{-1})	122.8c	126.2bc	137.2bc	192.0a	142.5b	96.5d	81.3d	11.38
Total Protein (g l^{-1})	44.33	46.67	43.83	41.33	42.50	44.33	42.00	2.323
Albumin (g l^{-1})	15.83	16.00	14.83	14.67	15.33	14.17	13.17	0.976
Globulin (g l^{-1})	28.50	30.67	29.00	26.67	27.17	30.17	28.83	1.881
AST3 (U l^{-1})	540.0a	470.2bc	433.8cd	409.2de	490.8b	437.7cd	389.0de	19.58
ALT4 (U l^{-1})	5.50	6.17	5.50	5.33	6.00	5.83	6.33	0.086

Notes
a-e – Means in same row with at least one letter superscript in common are not significantly different ($P > 0.05$).
1Hb = Haemoglobin concentration; 2PCV = Packed Cell Volume; 3AST = aspartate aminotransferase; 4ALT = alanine aminotransferase; 5SED = Standard error of difference between two means.

ents in experimental diets and their analysed proximate components. The crude protein level in diets ranged from 199.0 to 221.1 g kg^{-1} while metabolisable energy levels were between 10.7 and 11.7 MJ kg^{-1}. Means for blood parameters from birds in the various treatments are shown in Table 2.

In general, blood examinations are performed for several reasons: 1) as a screening procedure to assess general health 2) to diagnose clinico-pathological conditions, and 3) to detect nutritional deficiencies (Simaraks, Chinvasri & Aengwanich, 2004). Several reports have shown significant effects of feed on the haematology and serum biochemistry of livestock, and these have concluded that feed ingredients including unconventional sources do affect animal physiology (Moharrery, 2006; Jumoke *et al*., 2006; Anitha *et al*., 2007). The Hb, PCV, CH, uric acid and AST activity values changed with CPH dietary level ($P < 0.05$) (Table 2).

For the Hb and PCV values observed among the chickens, treatment 300E-CPH recorded the least values of 10.83 g dl^{-1} and 33.58 per cent, respectively, while treatment 200PF-CPH gave the highest values of 12.30 g dl^{-1} and 38.13 per cent, respectively. Except for treatment 300E-CPH, the Hb and PCV values of birds on test diets varied marginally ($P > 0.05$) from that of the control diet (Table 2). PCV, also known as the hematocrit or erythrocyte volume fraction (EVF), is the volume percentage of red cells in the blood.

The primary function of red blood cells is to transport haemoglobin, which in turn carries oxygen from the lungs to the tissues

(Waugh & Grant, 2001). Measures of Hb, PCV and red blood cell count (RBC) give an indication of the oxygen carrying capacity of the blood and are useful in diagnosing anaemia and polycythemia in broiler chickens (Goodwin, Davis & Brown, 1992; Aduloju, 2000). However, the Hb and PCV values observed for all treatment groups in the study fell within the normal ranges for healthy broiler chicken (9-13 g dl^{-1} for Hb and 30 – 40% for PCV), as earlier reported (Anon, 1980; MVM, 1986; Igene, Imoeti & Aletor, 2001). This suggests that the test diets provided adequate nourishment (including dietary adequacy of mineral, vitamins and amino acids) to the birds. Also the observed trends for the results generally suggest that dietary incorporation of CPH at >30 per cent may be detrimental to the blood quality, and general health of broiler chickens in the finisher phase. Reduction in the Hb usually translates into a fall in the RBC and PCV (very low readings for RBC), Hb and hematocrit can indicate anaemia (Moss, 1999). Important for the production of haemoglobin molecules and subsequently red blood cells are nutrients including proteins, iron and folic acid, which are rich in *P. ostreatus* (Çağlarırmak, 2007), and, thus, *P. ostreatus* fermentation had enriched CPH with these nutrients. This possibly accounted for the PT-CPH treatments recording relatively higher Hb and PCV levels compared to that of the E-CPH treatments.

Serum uric acid contents of the birds were within the range of 80 – 195 μmol l^{-1}, with birds on the 100PF-CPH and 200PF-CPH dietary treatments showing comparable levels ($P > 0.05$) to that observed for the control treatment. Dietary concentration and pre-treatment of CPH influenced the level of uric acid, which increased with increase in PF-CPH, but decreased with increase in E-CPH in the diet (Table 2). In birds, uric acid is a major product of nitrogen catabolism and its blood concentration may be influenced by age and diet. Although the presence of uric acid in the serum of animals is an important antioxidant for the neutralisation of over 50 per cent of free radicals in the blood stream, elevated level of uric acid (hyperuricaemia) is the primary risk factor for gout (Glantzounis *et al*., 2005).

Phytochemical screening of the cocoa by-products, such as the hulls and CPH has revealed the presence of flavonoids, tannins, among other phenolics (Arlorio *et al.*, 2005; Alemawor *et al*., 2009). Apart from the several biological effects (including anti-inflammatory) they exhibit, flavonoids are a group of polyphenolic compounds that have been reported to reduce or inhibit the activity of xanthine oxidase, the enzyme that catalyzes the final step in uric acid synthesis (Costantino *et al.*, 1992; Pauf & Hille, 2009). This probably accounted for the declining trend observed for the serum uric acid levels in the broiler chickens as dietary level of E-CPH is increased (Table 2).

However, the observed increase in serum uric acid levels in birds on higher PF-CPH dietary treatment may be partly due to the lower polyphenolic flavonoid content of the PF-CPH material compared to the unfermented CPH, thus, having a lower influence on uric acid synthesis. *P. ostreatus* solid-state fermentation has been previously reported to significantly ($P < 0.05$) decrease the total phenolics of CPH by over 85 per cent (Alemawor *et al*., 2009). The observed increase also could be partly due to the metabolism of purine substances in the dried

residual mycelia of *P. ostreatus*, a component of the PF-CPH ingredient. Purine compounds found in *P. ostreatus* species are precursors for uric acid formation (Kaneko *et al.*, 2008).

Although some of the treatments recorded serum uric acid values higher than that for the control treatment (Table 2), these observed values can be regarded as normal as they fall within the reference range of 1.9 – 12.5 mg dl^{-1} (113.05 – 743.75 μmol l^{-1}) for mature birds (Clinical Diagnostic Division, 1990). Data by Al-Ankari (2006) also indicated that serum uric acid in broiler chickens range from 3.3 to 10.12 mg dl^{-1}. Thus, the results suggest that both types of treated CPH up to the 30 per cent inclusion rate can be safely fed to broiler chickens without negatively affecting the birds' serum uric acid concentration.

Serum levels of AST and ALT belonging to the group of enzymes called transaminases are good indicators of the health status of organs, particularly the liver. The values of these enzymes are normally low in blood. However, necrotic activity in the liver causes a release of abnormally high quantities of the enzymes into the blood where they can be measured. The control diet was significantly ($P < 0.05$) different from 100PF-CPH, 200PF-CPH, 100E-CPH and 200E-CPH diet which were also significantly different ($P < 0.05$) from the 300PF-CPH and 300E-CPH diets for AST (Table 2). Level of AST in the blood decreased with increasing level of PF-CPH or E-CPH (Table 2). The observed declining trend for serum AST concentration with increasing level of dietary CPH ($P < 0.05$) indicates that PT-CPH or E-CPH demonstrated profound antioxidant and hepatoprotective activities in the birds, and these activities could probably be due to some inherent phytochemicals or components of the treated CPH. The serum levels of ALT and AST suggest that dietary incorporation of PF-CPH or E-CPH up to 300 g kg^{-1} finisher diet had no deleterious effect on the liver function of the broiler chickens.

All treatments involving dietary supplementation of PF-CPH or E-CPH recorded serum cholesterol concentrations lower than that for the control treatment (Table 2). However, the PF-CPH treatment showed a dose-dependent decreasing trend whilst the *vice-versa* was observed for the E-CPH treatment. Increasing PF-CPH level increased the proportion of dietary fibre (Table 1), and this possibly contributed to the subsequent decreasing trend assumed by the cholesterol concentration for the PF-CPH treatment category. CPH and the residual *P. ostreatus* mycelia (components of the PF-CPH ingredient) have been found to contain non-starch polysaccharides (NSPs) (Serra & Aragay, 1998; Manzi & Pizzoferrato, 2000; Vriesmann *et al*., 2011, 2012). These NSPs, which are primary components of dietary fibre, can lower serum level of cholesterol due to their binding effect on bile acids (Marlett *et al*., 1994; Lia *et al*., 1995; Delaney *et al*., 2003; Owen, Amakiri & Ngodigha, 2009),

Soluble fibre or NSPs (such as beta-glucans, pectin and gums) can bind to bile acids or salts preventing their reabsorption but increasing their exclusion or excretion. This reduces the amount of bile salts in the liver and causes the liver to use cholesterol to make new bile salts (beta-glucans' binding of bile acids activates cholesterol 7-hydroxylase and up-regulates LDL-receptor, thus, increasing the transport of LDL-cholesterol into hepatocytes and the conversion

of cholesterol into bile acids), reducing the amount of cholesterol in the blood (Nilsson *et al*., 2007; Bryant, 2008). This observation agrees with previous finding that an increase in dietary NSP in the intestinal content reduced cholesterol absorption and plasma cholesterol concentration in broiler chickens (Smits *et al*., 1997). Fibre may also exhibit their hypocholesterolemic effect by altering the serum concentration of hormones or short-chain fatty acids that affect lipid or cholesterol metabolism (Shinnick, Mathews & Ink, 1991).

The increasing trend for the cholesterol concentration with increase in the dietary E-CPH may be due to inherent CPH phytochemical components influencing the regulation of fatty acid metabolism (such as control of activity of acetyl CoA carboxylase, an enzyme which mediates in the rate-limiting step of carboxylation of acetyl CoA to malonyl CoA in fatty acid synthesis) or cholesterol biosynthesis. Increase in total serum cholesterol may either be desirable or undesirable depending on the type of cholesterol, HDL (regarded as 'good' cholesterol) or VLDL (a.k.a. 'bad' cholesterol), being affected. Aside total serum cholesterol determination, proportions of the different cholesterol types in the blood of the birds were, however, not determined in the study. Future work may have to consider monitoring which cholesterol type contributed to the most change in total cholesterol as influenced by the E-CPH or PT-CPH dietary treatment. In any case, cholesterol values obtained for birds in the study did not exceed the upper limit of 3.63 nmol l^{-1} quoted as safe for broilers by Meluzzi *et al*. (1992). Generally, low serum total cholesterol correlates with production of lean meat (Anitha, Moorthy & Viswanathan, 2007), and the low values obtained in the study are encouraging as people begin to be more conscious of cholesterol levels in the kind of meat they eat.

The results of the study indicated that the inclusion of up to 300 g kg^{-1} PF-CPH or E-CPH in a broiler finisher diet had no negative effect on levels of the blood and biochemical indices measured. El-Deek & Al-Harthi (2004) and Attia *et al*. (2001) have also reported that multi-enzyme addition to broiler diet had no adverse effects on blood serum constituents. Notwithstanding, the favorable blood values obtained for the broiler chickens fed finisher diets containing either E-CPH or PF-CPH at $\leq$ 30 per cent, in an earlier study (Alemawor *et al*., 2010), demonstrated best results for growth and feed conversion efficiency at 20 per cent PF-CPH and 10 per cent E-CPH dietary incorporation rates.

Conclusion

No adverse effects of feeding the two kinds of pre-treated CPH up to 300 g kg^{-1} in finisher diets were observed in the blood values and health of broiler chickens. Other blood parameters can be analysed in future studies to complement the values. Since there is, currently, no literature information on the levels of the measured indices considered unsafe, specifically for broiler chickens fed CPH-based rations, results from the study may serve as a primary reference for any future studies on dietary effect of CPH on blood indices of broiler chickens.

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