Nig. J. Anim. Prod. 2022, 49(2):46-55.https://doi.org/10.51791/njap.v49i2.3461 *Nigerian Journal of Animal Production*

Response of broilers chickens to commercial feed supplemented with cocoa powder as antioxidant under tropical environment

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Abstract

Cocoa is an excellent source of most essential minerals and vitamins, polyphenol compound, flavanols and procyanidins that exert it as a strong antioxidant. This study was conducted to determine the performance characteristics, haematological indices and internal organs of broilers fed commercial feed supplemented with commercially prepared cocoa seed powder (CSP) as an antioxidant. Cobb 500 broiler chickens (n=192), at two-week old were randomly allotted into four dietary treatments which include concentrate diet only (control group), concentrate +5g/kg of CSP, concentrate +10g/kg of CSP, concentrate +15g/kg of CSP, constituting treatments 1 – 4. Each treatment was replicated three times with eight birds per replicates. At eighth week of age, two birds were selected from each replicate and their blood samples were collected into EDTA bottles for haematological analysis. Also, two birds per replicate were selected and slaughtered and their internal organs carefully removed and weighed. Parameters were collected for performance, haematological indices and internal organs. Results revealed that the final weight, weight gain, feed intake and feed conversion ratio were significantly increased with increase in CSP ($p<0.05$). Birds fed diets containing15gramme supplementation with cocoa powder had the highest significant values ($p<0.05$)for the average final weight and weight gain (3283.00 and 2893.17g/bird, respectively). Feed intake and feed conversion ratio were also significant ($p<0.05$), broilers fed 10g supplementation had the highest feed intake (6120.83g/bird while treatment 4 had the best value of feed conversion ratio (2.08). The packed cell volume (PCV), haemoglobin, white blood cell (WBC), lymphocytes and MCH were significantly affected by supplementation of cocoa powder. The PCV and haemaglobin decreased with increasing levels of cocoa powder in the diets, while white blood cell increased with increasing levels of cocoa powder in the diets. Broilers fed control diets had the highest PCV, haemoglobin and lowest for WBC (38.00%, 12.73 x10^6µland 9.40x10^6µl, respectively), The values recorded for internal organs varied among the treatments. The ranges for liver, kidney, lung, spleen and GIT length were 1.21 – 1.41g, 0.36 – 0.95g, 0.04 – 0.05g and 183 – 215.00g, respectively. It was concluded that dietary supplementation of cocoa powder up to 15g/kg of commercial feed, improved performance characteristics and was not detrimental to haematological indices and weights of broiler chicken internal organs.

Keywords: Broiler, cocoa powder, haematological parameters, growth and organs weights

Réponse des poulets à griller à l'alimentation commerciale complétée par de la poudre de cacao comme antioxydant sous environnement tropical

Résumé

Le cacao est une excellente source de minéraux et de vitamines les plus essentiels, composé

de polyphénol, de flavanols et de procyanidines qui l'exercent comme un antioxydant fort. Cette étude a été menée pour déterminer les caractéristiques de performance, les indices hématologiques et les organes internes des poulets de poulets nourris à la production commerciale complétés par une poudre de graines de cacao (PGC) préparée dans le commerce comme antioxydant. Cobb 500 poulets à griller (n = 192), à deux semaines, a été alloué au hasard dans quatre traitements diététiques comprenant un régime de concentration uniquement (groupe de contrôle), concentré + 5g / kg de csp, concentré + 10g / kg de csp, concentré + 15g / kg de PGC, constitue des traitements 1 à 4. Chaque traitement a été reproduit trois fois avec huit oiseaux par réplicats. À la huitième semaine d'âge, deux oiseaux ont été choisis parmi chaque réplique et leurs échantillons de sang ont été collectés dans des bouteilles EDTA pour l'analyse hématologique. En outre, deux oiseaux par réplication ont été sélectionnés et abattus et leurs organes internes soigneusement éliminés et pesés. Les paramètres ont été collectés pour la performance, les indices hétérologiques et les organes internes. Les résultats ont révélé que le taux de poids final, du gain de poids, de la prise d'alimentation et de la conversion d'alimentation a été considérablement augmenté avec l'augmentation du PGC (P <0,05). Les régimes alimentaires de la Fed des oiseaux contenant15gramme une supplémentation avec de la poudre de cacao présentaient les valeurs significatives les plus importantes (p <0,05) pour le poids final et le gain de poids final moyen (3283,00 et 2893,17g / oiseau, respectivement). Le rapport de conversion d'alimentation et de conversion d'aliments a également été significatif (p <0,05), la supplémentation de poulets de 10g nourris à la production de poulets de poulets (6120,83 g / oiseau tandis que pendant le traitement 4 avait la meilleure valeur du rapport de conversion d'alimentation (2.08). Le volume de la cellule emballé (VCE), hésizoglobine, globule blanche (HGB), lymphocytes et la MCH ont été significativement affectés par la supplémentation de poudre de cacao. Le PCV et l'hémaglobine ont diminué avec des niveaux croissants de poudre de cacao dans les régimes, tandis que les globules blancs ont augmenté avec des niveaux croissants de poudre de cacao dans Les régimes alimentaires. Les régimes de contrôle de la Fed des poulets de chair ont eu le VCE le plus élevé, l'hémoglobine et le plus bas pour HGB (38,00%, 12,73 x 106 µl et 9.40x106µL, respectivement), les valeurs enregistrées pour les organes internes variaient entre les traitements. Les gammes pour le foie, les reins, les poumons, la rate et la longueur du git étaient de 1,21 à 1,41 g, 0,36 à 0,95 g, de 0,04 à 0,05 g et 183 - 215.00g, respectivement. Il a été conclu que la complémentation alimentaire de poudre de cacao jusqu'à 15 g / kg d'alimentation commerciale et une amélioration de la performance caractéristiques et n'étaient pas préjudiciables aux indices hématologiques et aux poids des organes internes du poulet à gril.

Mots-clés: griller, poudre de cacao, paramètres hématologiques, poids de croissance et d'organes

Introduction

Heat stress is one of the major problems facing poultry production in the tropical and subtropical regions, it inflicts heavy economic losses on poultry production as a result of stunted growth (Sahin *et al.,* 2001), decrease in hen-day production (Eid *et al.*, 2008) increased cost of production, high rate of mortality due to depressed immunity, and reproductive failure (Obidi *et al*., 2008). The strain generated by heat stress may also trigger changes in intestinal development by reducing the small intestine weight, the number of villi, and the proliferation rate of enterocytes (Uni *et al.*, 2001). It directly affects the bird capacity to digest and absorb the nutrients

required for maintenance and production (Quintero-Filho *et al.*, 2010). Donald (1998) stated that a greater number of physiological activities undergo specific changes in birds exposed to a hot environment. These facts were further supported by the report of Becker *et al.* (2003) who elaborated on the negative impacts of heat stress on the neuroendocrine, cardiovascular, respiratory and behavioral responses adopted by animals to adjust to altered environmental influences. Stressors activate the Sympathetic Adreno Medullary (SAM) and Hypothalamic-Pituitary-Adrenal (HPA) axes, resulting in the release of catecholamines and glucocorticoids, respectively (Belda *et al*., 2015). Post ***et al.*** **(2003)** reported that excessive environmental temperature leads to excretion of some minerals like calcium, iron, zinc and results in decreased bone strength. For optimal feed utilization and weight gain of broiler chickens, environmental temperature should be about 23˚C. However, the ambient temperature in the tropics ranges from 24˚C to 34˚C (NRC, 1995).Study by *Al-Aqil et al.(2009) established that broiler raised within the comfort zone present optimal feed intake and weight gain.* Ben *et al*. (1976) reported that heat stress found to decrease lymphocyte numbers and increase plasma corticosterone levels in cockerels. Broilers exposed to heat stress generated during road transportation significantly increased the basophil counts (Mitchell *et al*., 1992). The exposure of chickens to high temperature causes a decrease in blood hematocrit values (Maxwell *et al*., 1992) Studies have shown that heat stress induces reactive oxygen species (ROS) in various cells types (Katschinski *et al*., 2000; Davidson and Schiestl, 2001). It has also been suggested that over production of ROS is caused by the reduction of mitochondrial respiratory chain activity due to heat stress (Yang *et al*., 2010). Horv´ath and Babinszky (2018) stated that heat stress can lead to harmful impacts on birds including increase in the production of ROS, the formation of malondialdehyde (MDA) as an indicator for lipid peroxidation, and decreased vitamin concentrations. Thus, optimal broiler production in the hot season therefore requires an adequate and appropriate management system that can reduce the effects of heat stress to the minimum (Abioja *et al., 2011).Donkoh (1989) stated that antioxidants alleviate heat stress in poultry and* Sahin *et al*. (2001) also stated that body weight in heat-stressed broilers was significantly lower than in birds administered with antioxidant vitamins A and E. Vitamin E, a fat soluble vitamin is a biological chain-breaking antioxidant that protects cells and tissues from lipoperoxidative damage induced by free radicals (Mcdowell, 1989).

Cocoa is an excellent source of most essential minerals and vitamins, the minerals include calcium, copper, iron, manganese, magnesium, phosphorus, potassium, zinc. It also contains vitaminsA, E, B1, B6, riboflavin, niacin and essential amino acid. It is very rich in polyphenol compound (DRI, 2003).Cacao is known to contain very high levels of flavan-3-ols (flavanols) that occur both as monomers of epicatechin and catechin and as polymerized flavanols, or procyanidins (Payne *et al*., 2010; Hooper *et al*., 2012). Flavanols from cocoa and procyanidins exert strong antioxidant, it reduced the production of ROS by activated leukocytes (Sanbongi *et al*., 1997), to protect against erythrocyte haemolysis (Zhu *et al*., 2002). Rats treated with an oral dose of cocoa powder, showed an increased total plasma antioxidant capacity (Wan *et al*., 2001). Olayemi *et al*.(2020) reported an increased in weight gain when broilers were fed diet containing biologically upgraded cocoa

pod husk. Therefore, this study was carried out to evaluate the effect of feeding cocoa powder on growth, internal organ and hematological indices of broiler under tropical environment.

Materials and methods

The experiment was carried out at the Poultry Unit, Teaching and Research Farm, Oyo State College of Agriculture and Technology, Igbo-ora. Igboora is situated at 80 kilometres (50 miles) north of Lagos with Latitude 70 26' 1.79" N and Longitude 30 17' 16.37" E. The town's average annual rainfall is 139.39mm, humidity 76% and temperature of 22–290C (www.worldweatheronline.com). The experiment lasted for eight weeks between October 4 and November 29, 2020. Two hundred broilers (Cobb 500) were purchased from a reputable hatchery in Ibadan. After two weeks of brooding and acclimatization,192 birds were randomly allotted into four (4) treatments of varying levels of cocoa powder with three (3) replicates of 16 birds each in a completely randomized design. All management practices, necessary vaccination and medication were observed. The birds were fed with commercially prepared feed (broiler starter and finisher feed) throughout the period of the experiment. Cocoa powder used for the experiment was purchased from a reliable store in Ibadan and mixed with the commercial feed as shown below.

The experimental diets

Treatment 1 -Zero (0) cocoa powder (control)

Treatment 2-5g of cocoa powder/kg of feed

Treatment 3-10g of cocoa powder/kg of feed

Treatment4-15g of cocoa powder/kg of feed

Data collection

Data were collected on feed intake, body weight gain, feed conversion ratio and mortality. Feed intake was obtained by subtracting the weight of the left-over from the weight of the feed offered the previous day. Body weight gain was obtained by subtracting initial weight from the final weight while, feed conversion ratio was obtained by dividing feed intake by body weight gain. Mortality was measured in percentage and calculated as number of birds that died per replicate divided by the number housed multiply by 100. At week 8 of the experiment,3ml of blood were collected from two birds per replicate into sterile ethylene diamine tetra acetic acid bottle to assay haematological parameters that included packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), lymphocyte, heterophil and haemoglobin as described by Schalm, *et al.(*1975).Mean cell volume (MCV), mean corpuscular haemoglobin (MCH) ,mean corpuscular haemoglobin concentration (MCHC) were computed as follows. Mean cell volume (MCV) = Heamatocrit% × 10/RBC; Mean corpuscular haemoglobin (MCH) = haemoglobin × 10/RBC and Mean corpuscular haemoglobin concentration (MCHC) = haemoglobin × 10/Heamatocrit. Also at week 8, two birds per replicate were selected, slaughtered, scalded and internal organs removed. The following internal organs were carefully weighed or measured; liver, heart, gizzard with content, kidney, lung, empty gizzard, proventriculus, spleen, bile, GIT weight and GIT length.

Data were subjected to analysis of variance using general linear model according to the procedure of Statistical Analysis System(SAS, 2008). Differences among the means were subjected to Duncan Multiple Range Test of the statistical package at 5% level of significant.

Results

Table 1 shows the results of the effect of supplementing cocoa powder in the diets

on the growth performance of broiler chickens. The initial weight was not significantly affected ($p>0.05$), values recorded for treatments 1 – 4 were 406.07, 394.77, 412.25 and 389.83g/bird, respectively. The average final weight, weight gain feed intake and feed conversion ratio were significantly affected by the dietary treatments. Birds fed diets containing15g supplementation with cocoa powder had the higher significant ($p<0.05$) final weight and total weight gain (3283.00 and 2893.17g/bird, respectively). These values were however similar ($p>0.05$)to values for those fed 10g cocoa powder (3200.00 and 2787.73g/bird, respectively), but significantly higher ($p<0.05$) than those on treatment 2 and the control diet. Feed intake of broilers fed 10g supplementation was the highest (6120.83g/bird), however similar($p>0.05$)- to treatment 4 (6008.33g/bird), but significantly higher ($p<0.05$) than those fed 5gramme cocoa powder (5735.95g/bird) and the control diet (5826.88g/bird). Result of feed conversion ratio (FCR) was also significantly different ($p<0.05$). All the birds fed diets supplemented with cocoa powder were similar FCR ($p>0.05$). Control diet had the highest value (2.32) that was significantly higher ($p<0.05$) than 2.08 recorded for treatment 4 (15g cocoa powder per kg feed). The result of mortality was similar ($p<0.05$) for all the treatments.

Table 1: Growth performance of broiler chicken fed supplemented diet containing cocoa powder

Parameters	T1 (0g cocoa powder)	T2 (5g cocoa powder)	T3 (10g cocoa powder)	T4 (15g cocoa powder)	SEM
Average initial weight(g/bird)	406.07	394.77	412.25	389.83	4.44
Average final weight (g/bird)	2917.00^{c}	3100.00^{bc}	3200.00^{ab}	3283.00^{a}	52.23
Average total weight gain (g/bird)	2510.93^{c}	2705.25^{bc}	$2787.75a^{ab}$	2893.17^{a}	55.00
Average total feed intake (g/bird)	5826.88^{b}	5735.95^{b}	6120.83^{a}	6008.33^{a}	40.23
Feed conversion ratio (g)	2.32^{a}	2.12^{ab}	2.20^{ab}	2.08^{b}	0.05
Mortality	0.00	0.00	0.00	0.00	0.00

^{abc} *means in the same row with different superscripts differed significantly ($p<0.05$)*

Haematological indices of the experimental broilers chicken are presented in Table 2. The results revealed that PCV, haemoglobin, WBC, lymphocytes and MCH were significantly affected by the supplementation of the commercial feed with cocoa powder. The PCV and haemoglobin decreased with increasing levels of cocoa powder in the diets, while white blood cell increased with increasing levels of cocoa powder in the diets. Broilers fed the control diet had the highest PCV, haemoglobin and lowest for WBC (38.00%, 12.73 $x10^{6}$µland 9.40$x10^{6}$µl, respectively), while the reverse were for those fed 15g test ingredient (32.67%,10.37 $x10^{6}$µland 10.90$x10^{6}$µl, respectively). The corresponding values were significantly different ($p<0.05$). lymphocyte and mean corpuscular hemoglobin values were also significantly different ($p<0.05$).Treatment 3 had the highest values (69.33% and 44.63%, respectively) that were significantly higher ($p<0.05$) than obtained for treatment 4 (60.00% and38.47%, respectively), however, similar ($p>0.05$) to those of treatments 1, and 2. However, RBC, heterophil, MCV and MCHC were not significantly affected by dietary supplementation of cocoa powder in the experimental diets.

Table 2: Haematological indices of broilers fed supplemented diet containing cocoa powder

Parameters	T1(0g cocoa powder)	T2 (5 g cocoa powder)	T3 (10 g cocoa powder)	T4 (15 g cocoa powder)	SEM
Park cell volume (%)	38.00[a]	37.00[b]	34.67[ab]	32.67[b]	1.04
Heamoglobin (g/dl)	12.73[ab]	12.30[ab]	11.70[b]	10.37[b]	0.44
Red blood cell ($_X10^6$/µl)	3.03	3.10	2.63	2.70	0.11
White blood cells ($_X10^6$/µl)	9.40[b]	10.85[a]	10.83[a]	10.90[a]	0.32
Heterophil (%)	32.33	35.00	29.33	38.67	1.72
Lymphocytes (%)	65.67[a]	65.00[ab]	69.33[a]	60.00[b]	1.66
Mean cell volume(%)	125.87	119.40	132.50	121.00	2.55
Mean corpuscular hemoglobin (%)	42.10[ab]	39.70[ab]	44.63[a]	38.47[b]	1.18
Mean corpuscular hemoglobin concentration (%)	33.53	33.20	33.70	31.77	0.38

[abc] Means in the same row with different superscript differed significantly (P<0.05

Characteristics of the internal organ weights of broilers fed the experimental diets are presented in Table 3.All measured indices were not significantly affected (p>0.05) by the dietary treatments. All the parameters varied among the treatments except the gastro-intestinal track length that tends to slightly increase numerically (p>0.05) with increasing inclusion of cocoa powder in the diets. The ranges for liver, kidney, lung, spleen and GIT length were 1.21 – 1.41g, 0.36 – 0.95g, 0.04 – 0.05g and 183 – 215.00cm, respectively.

Table 3: Internal organ weights of broilers fed diets supplemented with cocoa powder

Parameters	T1(0g of cocoa powder)	T2 (5 g of cocoa powder)	T3 (10 g of cocoa powder)	T4 (15 g of cocoa powder)	SEM
Liver (g)	1.34	1.35	1.41	1.27	0.02
Heart (g)	0.32	0.43	0.51	0.33	0.04
Gizzard with content (g)	2.33	2.31	2.29	2.19	0.03
Kidney (g)	0.27	0.31	0.31	0.28	0.00
Lung (g)	0.95	0.40	0.54	0.36	0.12
Empty gizzard (g)	1.84	1.78	1.75	1.66	0.03
Proventriculus (g)	0.33	0.17	0.31	0.30	0.04
Spleen (g)	0.04	0.05	0.04	0.04	0.00
Bile (g)	0.08	0.12	0.15	0.10	0.00
GIT weight (g)	75.30	81.60	110.00	79.00	6.88
GIT Length (cm)	183.00	214.60	214.00	215.00	42.79

Discussion

The weight gain of broiler was higher with increase dietary inclusions of cocoa powder. This indicated that cocoa powder had a growth promoting properties (DRI, 2003). This agreed with findings of Olayemi *et al*. (2020) of increased weight gain of broiler chickens when fed diets containing **biologically upgraded cocoa pod husk at 10% inclusion level.** The increased weight could be attributed to contents of vitamins A, E, B1, B6, riboflavin, niacin and essential amino acid and polyphenol compound in cocoa powder (DRI, 2003). Flavanols from cocoa and procyanidins exert strong antioxidant, which reduced the production of reactive oxygen species by activated leukocytes (Sanbongi *et al*., 1997), which could lead to increase in metabolic activities. In addition, the presence of minerals and vitamins in cocoa powder may have improved the

resistance of birds to oxidative stress thus enhancing maximum utilization of nutrient. The increase in feed intake of birds fed cocoa powder may be due to suppressing effect of cocoa powder on heat stress. Heat stress is implicated in direct effects on the bird capacity to digest and absorb the nutrients required for maintenance and production (Quintero-Filho *et al.*, 2010). Donald (1998) stated that a greater number of physiological activities undergo specific changes in birds exposed to a hot environment. The better FCR recorded for T4 might be due to the presence of minerals in cocoa powder which could lead to increase metabolic activities. However, this disagreed with finding of Olayemi *et al* (2020) who recorded poor FCR at higher inclusion level of **biologically upgraded cocoa pod husk**feed.

Haematological parameters are important indices of physiological and pathological status for both animals and humans (Adeneye *et al.,* 2006). Blood plays a vital role in the examination of physiological, nutritional and pathological status of the animal because it serves as the medium of transportation of substances in the body (Doyle, 2006). Supplementation of cocoa powder to the diets reduced the PCV, haemoglobin, WBC and lymphocytes. However, the values were within normal documented range (you need reference here, please). The decreased PCV and haemoglobin with increased supplemental levels of cocoa powder could be indications of anti-nutrients in cocoa powder (Aremu and Abara, 1992) Also, the increased WBC with increased dietary cocoa powder indicated reaction of immune system to injection of anti-nutritional factors in cocoa powder. However, PCV values obtained were within the normal range (24.90 – 45.00%) for healthy birds as reported by Wikivet (2008). For haemoglobin, the value obtained were within the range (7–18g/dl) for healthy birds as reported by Muturaka *et al.*(1997). The red blood cell values obtained were within the normal range (1.80-3.81) as reported by Nworgu *et al* (2003). Excessive high WBC beyond normal has been reported in cases of infection, inflammatory conditions allergy and stress (Soetan *et al*., 2013).The MCV, MCH, and MCHC and heterophils were not influenced by dietary treatments and the values were within the normal range for healthy birds (….refs).

It is a common practice in feeding studies to use the weight of some internal organs like kidney and liver as indicator of toxicity. The spleen and liver weights are indices of changes in immune system caused by heat stress (Moein *et al.,* 2012). The weight of organs (kidney, liver, gizzard with content and empty, heart, spleen) were not influenced by the treatments. This established the safety of the treatment and that the experimental birds were able to effectively combat the anti-nutrients in cocoa powder. The result agreed with Imaseun and Ijeh (2018) who reported non-significant differences in measured weights of internal organs when diets of broiler chickens were supplemented with two sources of antioxidants as feed additives. The results of GIT weight at 10g supplementation could be due to strain generated by heat stress which may have triggered changes in intestinal development by reducing the small intestine weight, the number of villi and the proliferation rate of enterocytes (Uni *et al.*, 2001).

Conclusion

It was concluded from this experiment that dietary supplement of cocoa seed powder in the diets of broiler chickens at 15g/kg of feed improved performance characteristics and was not detrimental to haematological indices and internal organ weights of broiler chicken. Therefore, it is recommended that cocoa seed powder can

be included in the commercial feed up to 15g/Kg feed to improve the performance of broiler chicken.

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Received: 19th November, 2021
Accepted: 26th February, 2022