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Effect of Feeding Fortified Composite Cassava Stump Meal-based Diets on Organ Weights, Haematology and Serum Biochemical Indices in Broiler Chickens

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Authors' contributions

This work was carried out in collaboration among all authors. Authors VAA and MA suggested the study. Authors VAA and MA participated in the design and coordination. Authors VAA, MA, OGO and MAA carried out the field trial and laboratory studies. Authors VAA, MA, OGO and MAA carried out statistical data analysis and interpret the results. Authors VAA, MA, OGO and MAA search for literature and prepared the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study was designed to investigate the effects of using fortified composite cassava stump meal (FCCSM) as energy source in the diets of broiler chickens using organ, haematology and biochemical indices as response criteria. 5 diets were formulated in which FCCSM was added at 0, 5, 10, 15 and 20% inclusion levels to replace maize and designated diets I, II, III, IV and V, respectively. 150 chicks were assigned to 5 diets 3 replicates with 10 chicks per replicate in a Completely Randomized Design. At the end of the experiment, 2 birds per replicate were slaughtered and their blood collected for haematology and serum studies. Organ weights of the slaughtered birds were also measured. All data were subjected to analysis of variance. Results showed that among all the organ weights measured, only the liver, lung and proventriculus were

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significantly ($P < 0.05$) influenced by the dietary treatments. Among all the haematological parameters measured, the Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Haemoglobin (MCH), lymphocytes and basophils were significantly ($P<0.05$) influenced by the dietary treatments. For the serum biochemical indices measured, the albumin (ALB) and albumin/globulin (ALB/GLO) ratio were significantly ($P<0.05$) influenced by the dietary treatments. Generally, there was no deleterious effect of the dietary treatments on the organ weights, haematological and biochemical indices of the broiler chickens. Consequently, it was recommended that FCCSM could be included up to 20% in the diets for broiler-chickens without adverse effects.

Keywords: Cassava leaf and stump; fortified composite meal; haematology; organs; serum.

1. INTRODUCTION

In many developing countries, most of the cereal that is grown is for human consumption, and hopefully, surpluses are used for animal feeding. However, the reality is that whatever is available is competed for by humans and livestock for food and feed, respectively. This competition raises the cost of poultry production. A high cost of animal production, translates into a high cost of animal protein. Cost of energy sources in poultry feed has been reported to be about 70% of the total feed cost and about 40% of poultry production cost [1].

It is therefore imperative to find local agricultural residues and by-products that can be used as alternative feed ingredients in poultry production. These by-products should be cheap, readily available and not detrimental to the birds' health and productivity [2].

The exploitation of agro by-products and farm wastes such as cassava stump and leaf blend as alternative feed ingredient for chicken feeding trials will go a long way in reducing the cost of feeding chickens in developing countries where these by-products are largely available. The use of cassava and its by-products as an alternative feed ingredient of energy source goes a long way in reducing the feed cost in poultry production [3]. A well processed dried cassava meal stands readily as an alternative feedstuff [4]. Cassava root products are deficient in carotene and carotenoids, so supplementing diets containing cassava root with cassava leaf meal will improved this. Cassava leaves are highly nutritious, they have high protein levels ranging from 16.6% - 39.9% [5], and mineral levels, as well as being a valuable source of vitamin B1, B2, C and carotenes. and the ME ranges from approximately 1590 kcal/kg [6] to 1,800 kcal/kg [7].

Many studies investigating the effects of various feed on the haematology and serum biochemistry of livestock concluded that feed ingredients including alternative sources affect the physiology of animal. The blood contains several metabolites which provide useful information on nutritional status and clinical investigation on an individual. Thus, the use of blood parameters for medical and nutritional assessments has been recommended [8]. Based on these theoretical and practical backgrounds, this study is designed to evaluate the effects of feeding different inclusion levels of composite cassava stump meal fortified with cassava leaves as feed ingredient, on the relative organs weights, haematology and serum biochemistry of broiler chickens.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was carried out at the Poultry Unit of The Teaching and Research Farm (T&RF), The Federal University of Technology, Akure (Latitude $7^0$18''N and Longitude 5^0 10''E) Ondo State, Nigeria [9]. Akure falls within the rainforest zone of the humid tropics which is characterized by hot and humid climate. The mean annual rainfall is 1500mm and the rain period is bimodal with a short break in August. The altitude is about 350.52 m above sea level, the mean annual humidity is 75% and that of temperature is 27°C [10].

2.2 Collection of Test Ingredients

Cassava stumps (the ends trimmed off the cassava tubers as they are manually prepared for onward transmission into the rotary washer and peeler) were collected from garri industry at Igbatoro, Akure. This part contains a little content of cassava plus the peel and it was milled (without peeling), pressed so as to reduce the

moisture and it was sieved to remove excess fibre, and then it was subjected to heat treatment (frying) so as to reduce the cyanogenic glucosides level and to prevent spoilage. All these processes were carried out within 24 hours of harvest in order to prevent fermentation. Cassava leaves were sourced for in large quantity, air-dried and then milled to produce cassava leaf meal.

2.3 Fortified Composite Cassava Stump Meal Production

The composite cassava leaf and cassava stump meal were mixed together in ratio 1:9 (1 kg cassava leaf meal + 9 kg cassava stump meal) to produce the fortified composite cassava stump meal (the test ingredients in the study).

2.4 Experimental Diets

Five (5) broiler chicken experimental diets were formulated with the basal diet formulated for each phase (starter and finisher) to meet [11] minimum requirement of each of the phases. The five (5) broiler starter diets were formulated using fortified composite cassava stump meal (FCSM) as one of the feed ingredients at the inclusion levels of 0, 5, 10, 15 and 20 kg and designated diets I, II, III, IV and V, respectively and the diets were thoroughly mixed. The same procedure was followed for the finisher phase. The gross composition of the starter and finisher experimental diets are as presented on Tables 1and 2.

2.5 Experimental Layout and Feeding Trials

A total number of two hundred (200) day-old Arbor-acre breed of broiler chicks were purchased from Farm Support in Ibadan, Nigeria, out of which one hundred and fifty (150) were assigned to five (5) dietary treatments of three (3) replicates and ten (10) chicks per replicate in a Completely Randomized Design and the weight of each replicate was balanced (±1 g). The starter diets were fed *ad libitum* from 0 - 28days during which weekly feed consumption and weekly weight changes were measured and feed conversion ratio was calculated from these two parameters. Thereafter, the finisher diets were also fed to the started chicks *ad libitum* from 29 - 49days and the weekly feed consumption, weekly weight changes were recorded and feed conversion ratio was also calculated.

Table 1. Composition of broiler starter experimental diets (g/100 g)

Ingredients	Diet I	Diet II	Diet III	Diet IV	Diet V
Maize	54.00	49.00	44.00	39.00	34.00
Wheat offal	3.30	3.30	3.30	3.30	3.30
Soybean meal	21.00	21.00	21.00	21.00	21.00
Groundnut cake	12.00	12.00	12.00	12.00	12.00
Fish meal	5.00	5.00	5.00	5.00	5.00
Fortified composite cassava stump meal	0.00	5.00	10.00	15.00	20.00
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Di-calcium phosphate	1.00	1.00	1.00	1.00	1.00
Limestone	1.50	1.50	1.50	1.50	1.50
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Vegetable oil	1.50	1.50	1.50	1.50	1.50
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis:					
Crude protein (%)	22.70	22.68	22.63	22.58	22.53
Metabolizable energy (Kcal/kg)	3009.92	3006.31	3002.82	2999.27	2995.72
Calcium (%)	1.12	1.13	1.14	1.14	1.15
Available phosphorus (%)	0.55	0.56	0.57	0.58	0.59
Lysine (%)	1.26	1.26	1.26	1.26	1.27
Methionine (%)	0.48	0.48	0.47	0.47	0.46

**Contained vitamins A (8,500,000 IU); D3 (1,500,000 IU); E (10,000 mg); K3 (1,500 mg); B1 (1,600 mg); B2 (4,000 mg); B6 (1,500 mg); B12 (10 mg); Niacin (20,000 mg); Pantothenic acid (5,000 mg); Folic acid (500 mg); Biotin H2 (750 mg); Choline chloride (175,000 mg); Cobalt (200 mg); Copper (3,000 mg); Iodine (1,000 mg); Iron (20,000 mg); Manganese (40,000 mg); Selenium (200 mg); Zinc (30,000 mg); and Antioxidant (1,250 mg) per 2.5 kg*

Table 2. Composition of broiler finisher experimental diets (g/100 g)

Ingredients	Diet I	Diet II	Diet III	Diet IV	Diet V
Maize	54.00	49.00	44.00	39.00	34.00
Wheat offal	5.80	5.80	5.80	5.80	5.80
Soybean meal	21.00	21.00	21.00	21.00	21.00
Groundnut cake	12.00	12.00	12.00	12.00	12.00
Fish meal	0.10	0.10	0.10	0.10	0.10
Fortified composite cassava stump meal	0.00	5.00	10.00	15.00	20.00
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	1.50	1.50	1.50	1.50	1.50
Di-calcium phosphate	2.00	2.00	2.00	2.00	2.00
Limestone	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25
Salt	3.00	3.00	3.00	3.00	3.00
Vegetable oil					
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis:					
Crude protein (%)	19.70	19.65	19.60	19.55	19.50
Metabolizable energy (Kcal/kg)	3030.24	3026.69	3023.14	3019.59	3016.04
Calcium (%)	1.11	1.11	1.12	1.13	1.13
Available phosphorus (%)	0.50	0.51	0.52	0.53	0.60
Lysine (%)	1.10	1.10	1.10	1.10	1.10
Methionine (%)	0.40	0.40	0.40	0.40	0.40

**Contained vitamins A (8,500,000 IU); D3 (1,500,000 IU); E (10,000 mg); K3 (1,500 mg); B1 (1,600 mg); B2 (4,000 mg); B6 (1,500 mg); B12 (10 mg); Niacin (20,000 mg); Pantothenic acid (5,000 mg); Folic acid (500 mg); Biotin H2 (750 mg); Choline chloride (175,000 mg); Cobalt (200 mg); Copper (3,000 mg); Iodine (1,000 mg); Iron (20,000 mg); Manganese (40,000 mg); Selenium (200 mg); Zinc (30,000 mg); and Antioxidant (1,250 mg) per 2.5 kg*

2.6 Slaughtering and Blood Collection

At the end of the experimental period, the birds were starved overnight and two (2) birds were randomly selected per replicate. The birds were stunned, slaughtered by severing the jugular vein, for collection of blood used for the haematological and serum indices studies. For haematology, blood samples were collected into sterilized bottles containing Ethylene Diamine Tetra-acetic Acid (EDTA) and the following blood parameters were determined; erythrocyte sedimentation rates (ESR), packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC), absolute haemoglobin and differential white blood count. Blood samples for serum biochemical studies were collected into test tubes (without anticoagulant) and placed in test tube racks in the Microscopy Laboratory of Animal Production and Health Department, Federal University of Technology, Akure, Nigeria. After some hours, the serum was obtained by centrifugation and serum samples were stored in a deep freezer prior further study and the following parameters were also determined; aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, total protein, albumin, globulin, creatinine and cholesterol.

2.7 Organ Weight Measurement

After slaughtering, the birds were scalded at 60°C in a water bath for about 30 seconds before defeathering. Thereafter, the following visceral organs (liver, kidney, pancreas, gizzard, spleen, heart, and lungs) were harvested and weighed using a sensitive weighing balance and expressed in grams per kilogram body weight.

2.8 Data Analyses

All data collected on organs weights, haematology and serum biochemistry were subjected to Analysis of Variance (ANOVA) using SPSS version 17 package. Where significant difference existed, Duncan Multiple Range Test (DMRT) of the same package was used to separate the means.

3. RESULTS

3.1 Relative Organ Weights

The relative organ weights of broiler chickens fed fortified composite cassava stump meal-based diets are presented in Table 3. The relative

weights of liver, lung and proventriculus were significantly (P<0.05) influenced by the dietary treatments. The relative weight of the liver of the birds fed control diet (17.80±1.62 g/kg body weight) was similar (P>0.05) to those fed all the test diets (i.e. diets II - V). Also, the relative weight of the lung of the birds fed diet III (4.3±0.19 g/kg body weight) was higher than those fed diets I, II, and IV, but similar (P>0.05) to those fed all the test diet V. For the proventriculus, the relative weight recorded for birds fed diet I, III, IV and V were similar and they were higher than those fed diet II (3.6±0.32 g/kg body weight). The gizzard weights varied: (15.00±0.99 – 18.30±2.00 g/kg body weight) and belly fat varied: (10.74±1.01 – 15.51±2.25 g/kg body weight).

3.2 Haematological Studies

Table 4 shows the haematology of broiler chickens fed fortified composite cassava stump meal-based diets. Among all the parameters measured, only the Mean cell haemglobin concentration (MCHC), Mean cell haemglobin (MCH), lymphocytes and basophils were significantly (P<0.05) influenced by the dietary treatments. The MCHC of birds fed diet IV (33.41±0.05 mm/hr) was significantly (P<0.05) higher than those fed diet III (33.21±0.01 mm/hr) but similar to those fed diets I, II and V. The MCH on the other hand for birds fed diet III (30.11±0.01 pg of Hb) was higher than those fed diet IV (30.03±0.04 pg of Hb) but statistically similar to those fed diets I, II and V. Highest Packed cell volume (PCV) (30.00±0.55%), highest Red blood cell, RBC ($2.6\pm0.09x10^6mm^{-3}$) and highest Haemoglobin concentration (Hbc) (9.98±0.18g/100ml), were recorded in birds fed diet V (20% FCCSM) while lowest PCV (23.50±3.92%), lowest Rbc ($1.91\pm0.48x10^6mm^{-3}$) and lowest Hbc (7.83±1.32g/100ml) were recorded in birds fed diet I (0%FCCSM). Highest Mean cell volume (MCV) ($168.12\pm40.28\mu^3$) was observed in birds fed diet I and lowest MCV ($114.162\pm2.12\mu^3$) was observed in birds fed diet V. Lymphocyte value varies from 59.60±0.51 – 62.00±0.37%, heterophils; 19.67±1.36 – 25.80±1.56%, monocytes; 11.20±1.02 - 13.67±0.96%, basophils; 2.40±0.25-3.33±0.21% and eosinophils;1.00±0.00-1.33±0.21%.

3.3 Biochemical Indices

Table 5 shows the serum metabolites of broiler chickens fed fortified composite cassava stump meal. Among all the parameters measured only the albumin and albumin/globulin ratio were significantly (P<0.05) influenced by the dietary treatments. The highest albumin (2.19±0.18 g/dl%) and highest value (1.21±0.32%) for albumin/globulin ratio were observed in birds fed diet II, while the lowest albumin (1.62±0.09/dl%) and lowest value (0.51%) for albumin/globulin ratio were observed in birds fed diets IV and V respectively. Highest total protein (5.53±0.79g/dl) was recorded in birds fed diet V, while lowest total protein (4.25±0.22g/dl) was recorded in birds fed diet I. Highest cholesterol (71.15±14.89mg/dl) was recorded in birds fed diet V, while the lowest cholesterol (47.75±11.97mg/dl)was observed in birds fed diets I. Alkaline phosphatise varies; 15.17±3.20–19.90±2.69IU/l, Aspartate aminotransferase; 59.17±7.77 – 120.82±28.28IU/l and total bilirubin; 15.17±3.2 – 19.90±2.69IU/l.

4. DISCUSSIONS

Organs are body parts, composed of several types of tissues, capable of carrying out specialized function [12]. The relative organs measured were comparable in all the dietary treatments except liver, lungs and proventriculus that were significantly (P<0.05) influenced by the dietary treatments. The values recorded for all

Table 3. Relative organs weights (g/kg body weight) of broiler chickens fed fortified composite cassava stump meal-based diets

Parameters	Diet I	Diet II	Diet III	Diet IV	Diet V
Liver	17.80 ± 1.62^{ab}	14.36 ± 1.05^{b}	16.47 ± 1.37^{ab}	18.69 ± 1.01^{a}	15.83 ± 0.38^{ab}
Heart	3.71±0.19	3.87±0.24	4.26±0.31	3.73±0.12	3.55±0.21
Lung	4.15 ± 0.21^{b}	4.00 ± 0.20^{b}	4.93 ± 0.19^{a}	4.09 ± 0.30^{b}	4.42 ± 0.20^{ab}
Pancreas	1.83±0.09	1.87±0.09	1.73±0.16	1.98±0.12	2.05±0.17
Spleen	1.05±0.09	1.03±0.14	1.00±0.11	0.96±0.07	1.12±0.15
Gizzard	15.00±0.99	15.39±1.18	16.76±1.66	16.56±1.62	18.30±2.00
Belly fat	13.35±1.00	10.74±1.01	15.51±2.25	14.00±1.62	13.38±1.37
Proventriculus	5.22 ± 0.41^{a}	3.69 ± 0.32^{b}	4.30 ± 0.27^{ab}	4.45 ± 0.22^{ab}	5.18 ± 0.35^{a}

a'b: Mean within rows having different superscripts are significantly different (P<0.05)

Table 4. Effects of varying levels of fortified composite cassava stump meal on haemotological indices of broiler chickens

Parameters	Diet I	Diet II	Diet III	Diet IV	Diet V
Erythrocyte sedimentation rates m/hr)	5.67±1.73	4.17±1.58	3.25±0.63	4.67±0.80	3.00±0.01
Pack cell volume (%)	23.50±3.92	26.33±3.44	28.00±1.23	28.50±1.88	30.00±0.55
Red blood cell ($\times 10^6 mm^{-3}$)	1.91±0.48	2.26±0.44	2.36±0.18	2.48±0.23	2.62±0.09
Haemoglobin concentration (g/100ml)	7.83±1.32	8.77±1.15	9.30±0.41	9.52±0.62	9.98±0.18
Mean cell haemoglobin concentration (%)	33.31±0.08^{ab}	33.30±0.06^{ab}	33.21±0.01^{b}	33.41±0.05^{a}	33.27±0.03^{ab}
Mean cell haemoglobin (pg of Hb)	30.05±0.07^{ab}	30.06±0.06^{ab}	30.11±0.01^{a}	30.03±0.04^{b}	30.06±0.03^{ab}
Mean cell volume (μ^3)	168.12±40.28	148.75±35.05	119.78±3.96	135.54±8.23	114.62±2.12
Lymphocytes (%)	61.00±0.63^{ab}	60.67±0.88^{ab}	60.00±0.82^{ab}	62.00±0.37^{a}	59.60±0.51^{b}
Heterophils (%)	20.83±1.97	22.17±2.26	23.25±2.29	19.67±1.36	25.80±1.56
Monocytes (%)	13.67±0.96	13±1.24	13±1.29	13.67±0.67	11.2±1.02
Basophils (%)	3.17±0.31^{ab}	3.00±0.26^{ab}	2.75±0.25^{ab}	3.33±0.21^{a}	2.4±0.25^{b}
Eosinophils (%)	1.33±0.21	1.17±0.17	1.00±0.00	1.33±0.21	1.00±0.00

a-b: Mean within rows having different superscripts are significantly different (P<0.05)

Table 5. Effects of varying levels of Fortified Composite Cassava Stump Meal on serum metabolites of broiler chickens

Parameters	Diet I	Diet II	Diet III	Diet IV	Diet V
Total Protein (g/dl)	4.25±0.22	4.4±0.31	4.56±0.66	4.38±0.51	5.53±0.79
Albumin (g/dl)	1.97±0.12^{ab}	2.19±0.18^{a}	1.62±0.17^{b}	1.62±0.09^{b}	1.78±0.16^{ab}
Globulin (g/dl)	2.28±0.19	2.21±0.33	2.93±0.68	2.75±0.44	3.75±0.64
Albumin/Globulin Ratio	0.89±0.09^{ab}	1.21±0.32^{a}	0.70±0.13^{b}	0.64±0.07^{b}	0.51±0.05^{b}
Cholesterol (mg/dl)	47.75±11.97	67.02±19.13	69.32±10.65	52.42±12.61	71.15±14.89
Creatinine (mg/dl)	2.64±0.6	2.88±0.72	4.19±1.15	3.49±0.78	4.53±1.06
Alkaline Phosphatise (IU/l)	18.93±3.21	15.17±3.2	15.57±4.86	17.00±1.9	19.9±2.69
Aspartate Aminotransferase (IU/l)	59.17±7.77	76.75±19.21	111.75±30.59	93.17±20.81	120.83±28.28
Total Bilirubin (IU/l)	18.93±3.21	15.17±3.2	15.57±4.86	17.00±1.9	19.90±2.69

a-b: Mean within rows having different superscripts are significantly different (P<0.05)

the dietary treatments falls within the normal range, suggesting that the diets were not detrimental to the birds. The presences of anti-nutritional factors have been associated with abnormal enlargement of organs such as liver and pancreas due to detoxifying activities [13]. All the values recorded for the organs did not follow a particular trend in relationship with the dietary treatments. The liver, lungs and proventriculus, although sharing significant variation among the treatment mean values, did not have a particular trend.

Blood plays an important role in the body of the animals. It defends the body against diseases, maintain water balance in the body, transport urea and prevents excess loss of blood by clotting over an injury and these various blood functions are made possible by its constituents [14]. Blood also represents a means of assessing clinical and nutritional health status of animals in feeding trials [15]. In this study, the values for MCHC, MCH, Lymphocytes and basophils were significantly affected by the dietary treatments. The MCHC values varied; 33.21 – 33.41% and MCH values varied;30.03 – 30.11% and falls within the range; 26 - 35% and 33 - 47% respectively [16,17,18] indicating that the fortified composite cassava stump meal could have contributed positively to the utilization of the dietary protein for health being. [19] reported that when the haematological values fall within the

normal range reported for the animal, it is an indication that the diets did not show any adverse effect on haematological parameters during the experimental period but when the values fall below the normal range, it is an indication of anaemia. The PCV from this study varied; 23.50 – 30.00%, which falls within the normal range of 25 - 45% for chickens as reported by [17] and [18]. PCV value below normal range is an indication of anaemia [20] and poor quality of protein of the diets [16]. Thus, with the values obtained in this study (23.50 – 30.00%), it could be suggested that the nutritional quality of the test diets compared favourably with the control. The RBC count of all the birds ranged; 1.91 – 2.62 x 10^6 mm^{-3} and falls within the normal range of 2.0 – 4.0x$10^6$$mm^{-3}$ reported for broiler chickens [21], [17], [18]. Albumin, globulin and creatinine are measures of adequacy of protein quality, quantity and degree of protein utilization [22].The higher the albumin value, the higher the clotting ability of the blood and hence the prevention of haemorrhage (Robert *et al.,* 2003). Elevated globulin levels are mostly due to production of antibodies to fight infections [23]. This study reveals that total protein, globulin, cholesterol, creatinine, alkaline phosphatase, aspartate aminotransferase and total bilirubin were not significantly affected by the inclusion of FCCSM and this therefore suggests that the test ingredients might not pose any deleterious health challenges to the chickens. The value for total protein varied in all the treatments ranging from 4.25–5.53 g/dl. The significant variation was observed for globulin but with no particular trend. For the total protein and globulin, the values obtained in birds fed diets I – V was lower than the normal ranges; 5.00 – 7.00 g/dl and 3.00 – 3.5g/dl respectively. But the values recorded for birds fed diet V (5.53 g/dl and 3.75 g/dl) falls within the normal range for total protein and globulin.

5. CONCLUSION

From this study, using the fortified composite cassava stump meal as feed ingredient in broiler chicken diets compared favourably with the control diet which indicates that the FCCSM do not pose any deleterious effects on the health status of the birds.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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