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Effect of *Centella asiatica* as feed additive on blood profile, egg quality and gut microbial contents of ISA brown laying birds

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ABSTRACT

The use of medicinal plants is gaining popularity all over the world; hence there is a need to exploit various plants that could be of economic importance to animal. This study was conducted to investigate the utilization of *Centella asiatica* leaf meal (CALM) as feed additive in layers production. The study was carried out in two phases, the grower phase and layer phase. A total number of 150 birds were used at 10 birds per replicate of 3 replicates per treatments of a total of 5 treatments. The diets contained CALM at five levels of inclusion 0.0 % (control), 0.2%, 0.4%, 0.6% and 0.8% at grower and layer phases. The experiment was arranged in a completely randomized design layout. Hence, CALM showed potentials of being natural growth promoting additive. CALM used as additive at 0.2-0.8% inclusion level significantly increased ($p<0.05$) final live weight, weight gain and improved feed conversion ratio at grower and layer phases. 0.6% inclusion of CALM increased packed cell volume (PCV) and haemoglobin (Hb) and 0.8% inclusion of CALM increased red blood cell (RBC) values at grower phase. At layer phase, 0.6% inclusion of CALM significantly increased ($p<0.05$) PCV and RBC while 0.8% CALM significantly increased ($p<0.05$) haemoglobin (Hb) value but there was significant reduction ($p<0.05$) in total bacteria count (TBC) at the caecum and ileum of birds at grower and layer phases respectively. 0.8% CALM inclusion resulted in significantly highest ($p<0.05$) weight of eggs, hen day egg production at 4th, 8th and 12th week in lay. 0.6% inclusion of CALM significantly improved ($p<0.05$) egg weight and yolk weight score at 12th weeks in lay. It can be concluded that the inclusion of CALM in the diets of Isa Brown birds, resulted in better blood profile, haematological and serum biochemical parameters, gut microbial population, egg production and quality of laying hens

Introduction

Food is one of the basic physiological needs of plants and animals. The provision of good, wholesome and adequate nutrient through food enhances the growth, development, reproduction and productivity of living organisms (FAO, 2016).

Nutritionists are becoming increasingly interested in agro-industrial by-products as poultry feed ingredients. Modern intensive poultry has made incredible progress in terms of producing high-quality, safe chicken meat, eggs, and poultry by-products in an efficient and cost- effective manner. The industry had to optimize the health and well-being of the birds while minimizing the industry's environmental impact (Alloui, 2013).

Products from poultry are rich sources of high-quality protein, minerals and vitamins which are readily available and inexpensive source of animal protein for human consumption (FAO, 2016). For poultry, *Centella asiatica* leaf meal has certain functional properties that can serve as growth promoters, anti- microbial and antiparasitic factors, immunomodulatory and anti-inflammatory agents. Taking these facts into account, *Centella asiatica* leaf meal may be used as a feed ingredient to minimize feed costs as well as a practical feed to improve poultry health and well- being (Taha, 2019; Abo Ghanima *et al.*, 2020).

Ajayi et al. (2020) also concluded that *C. asiatica* leaf meal could serve as a good source of vitamins

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and mineral supplements to human and animal especially monogastric animals. The leaves also contain phytochemical components such as flavonoids, alkaloids which are of good health benefit to both human and animal when consume for therapeutic purpose and could be of economic importance to the pharmaceutical industry (Ajayi *et al.,* 2020).

Centella asiatica is native to India and belongs to the family Apiaceae. The leaves are green small, and fan shaped, the leaves, stems, and roots of the plants are highly medicinal. *C. asiatica* contains different biologically active chemicals, saponins, centelloside, pectin, tannins, phytosterols, mucilages, resins, vallerine, flavonoids and many other derivatives. The plant has been traditionally used to treat hypertension, skin infection, leprosy and fever. *C. asiatica* exhibits important pharmacological activities such as antibacterial, anti-ulcer, anti-inflammatory, antifungal and antioxidant (Alagbe, 2017).

Despite the huge potentials of *Centella asiatica* especially with the phytochemicals, minerals and vitamins contents the plant is underutilized in poultry production hence the need to investigate the effect of *C. asiatica* as additive in chicken layers production. Therefore, the objective of the present study was to investigate the utilization of *Centella asiatica* leaf meal (CALM) as feed additive in layers production

Materials and Methods

Experimental design

In a completely randomized design, a total number of 150 Isa Brown growing pullets (12 weeks) were randomly allocated to five dietary treatments in a colony cage system and fed experimental diets from 12 weeks to 28 weeks. Ten birds were grown in each replicate in a battery cage system. The birds were fed and given water *ad libitum* according to standard management methods. The experiment lasted for 16 weeks. The dietary treatments were T1: basal diet without additive, Treatment 2: basal diet with 0.2% additive, Treatment 3: basal diet with 0.4% additive, Treatment 4: basal diet with 0.6% additive and Treatment 5: basal diet with 0.8% additive.

Source and processing of test ingredient

Fresh leaves of *Centella asiatica* were harvested within Babcock University campus, Ilishan Remo, Ogun State, South-Western, Nigeria. The leaves were carefully air-dried for four days at room temperature (25°C) to obtain a constant weight and were ground individually in kitchen blender to fine particle sizes and stored in air-tight containers until incorporated into formulated diet. The grinded leaves were incorporated into basal diets of the birds at each phase of the experiment (grower and layer phases). Birds on control had basal diet only. A total number of 150 grower pullets (12 weeks) were purchased from Farm Support in Oyo State, Nigeria. The experiment was carried out in a private farm at Ibadan in Badeku village, Oyo State, Nigeria under the supervision of the Department of Agriculture and Industrial Technology, Babcock University, Ilisan-Remo, Ogun State, Nigeria. Badeku is located in the South- Western rainforest belt of Nigeria with annual rainfall of 1500 mm and mean daily temperature 23°C. It is located at longitude 4° 4'E of the Greenwich meridian and latitude 7° 21'N of the equator. The study lasted 16 weeks. *Centella asiatica* leaf meal (CALM) was subjected to a proximate analysis (AOAC, 1990) and the result is presented in Table 1.

Management of experimental birds

The diet was formulated to suit the nutritional needs of the birds as determined by the National Research Council (NRC) (as stated in Tables 2 and Table 3). Before the birds arrived, the pen houses were properly cleaned. Body weight of the birds was measured, assigned to different treatments, and afterwards on a weekly basis for 16 weeks. The amount of feed consumed was monitored on a daily basis. The feed conversion ratio (FCR) was calculated by dividing the total amount of feed consumed by the amount of weight gained (Yi *et al.*, 2018). The treatment group's average values for these parameters were calculated and reported.

Haematology and serum biochemistry indices

At the end of 16 weeks, two birds per replicate totaling 6 birds per treatment were selected for blood sampling**.** Blood sample (2mls/bird) was collected via the jugular vein using sterile needles and syringes into EDTA bottles for haematological parameters while another 2mls of blood was collected into heparinized bottles to investigate the serum biochemistry and lipid profile using the standard procedures described by Fafiolu *et al.* (2014). Haematological indices such as Red Blood Cell (RBC), White Blood Cell (WBC) and its differentials, Packed Cell Volume (PCV), and Hemoglobin Concentration (HB) were investigated. Serum biochemical indices determined include: Total Protein, Serum Glucose, Serum Cholesterol, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). The birds were fasted prior to blood collection for two hours but water was made available. Serum triglycerides, Serum enzymes: Aspartate amino transferase (AST), Alanine amino transferase (ALT) and alkaline phosphatase (ALP) were determined using spectrophotometric methods.

Cholesterol fractions were categorized as low density lipoproteins (LDL), very low-density lipoproteins (VLDL) and high density lipoproteins (HDL).

Table 1. Proximate composition (g/100g DM) of *Centella asiatica* leaf (CALM).

Parameters	Composition (%)
Dry matter	90.80
Crude protein content	17.80
Ether extract content	2.43
Ash content	9.48
Crude Fibre content	11.50
Metabolizable energy (J).	9.94

Egg production parameters

Age at first lay: The number of days from point of lay to the day the first egg is laid

Body weight of hen at first lay: These was measure as weight of each live pullet average over the number of pullets' weight per group at first lay.

Weight of first egg: weight of each first egg average over the weight of first eggs laid per group using a balance sensitivity of 0.01g

Hen day production: Number of eggs collected divided by number of birds alive as at that day multiplied by 100%

Egg quality evaluation

Two eggs per replicate were sampled for egg quality evaluation at first lay, week 4, 8 and 12 of the laying periods.

Internal egg qualities

The following measurements were determined:

i. Albumen height: The eggs were gently broken and the maximum albumen height was measured with tripod spherometer.
ii. % Albumen weight: This was calculated as the percentage of the albumen weight to the egg weight.
iii. Yolk weight: This was measured using Mettler top-loading weighing balance.
iv. Yolk colour: Egg yolk color was measured with the 15 bands of the "Roche Yolk Color Fan.
v. Haugh Unit (HU): This was calculated using the values obtained for the egg weight and albumen height using the formula:

$$HU = 100\log (H+7.5-1.7W^{0.37})$$

Where, H= Albumen height in mm, W= Egg weight in gram

vi. Yolk Cholesterol profile: For yolk cholesterol content, 3 eggs per treatment were collected at12th week of lay. The yolk was carefully separated from the albumen using a separating pan. The samples were stored at - 20°C until analysis. One gram of yolk was placed into a centrifuge tube. Twenty milliliters of chloroform: methanol was added in a ratio of 2:1. The mixture was blended on a vortex. A 10litre volume of clear extract was used for total cholesterol assay. Cholesterol Assay Kit by Dialab (D00119, Dialab GmbH, Austria) was used for the cholesterol estimation

External egg qualities

i. Egg weight: This was measured using Mettler® top-loading sensitive scale.
ii. Egg length and width: The length and width of each egg were measured using vernier calipers. The width was measured as the distance between two ends of the egg at the widest cross-sectional region using vernier calipers. The length was measured as the distance between the broad and narrow ends of the eggs.
iii. Egg shape Index (ESI): This was calculated as the percentage of the egg breadth (width) to the egg length. This formula is as follows:

$$\text{Egg Shape Index} = \frac{\text{Width of egg (mm)} \times 100}{\text{Length of egg (mm)}}$$

iv. Shell thickness: The thickness of individual air-dry shells was measured to the nearest 0.01mm using micrometer screw gauge.
v. Shell weight: Egg shells were air-dried in the crates. The relative shell weight was calculated by relating the shell weight to the weight of the egg.

Intestinal microbiology

Samples of the contents from the ileum and cecum were collected from slaughtered birds (three birds per treatment) at the end of the experiment into glass containers, sealed, and were put on ice in the laboratory for enumeration of microbial populations. One gram of mixed contents was blended into 9 mL of reduced sterile dilution blank solution (RSDBS). Further serial dilutions were made in RSDBS for aerobic bacterial enumeration. The initial dilution in RSDBS was also used as a source for serial dilutions in PBS for enumeration of aerobic bacterial populations. The samples from the ileum and caeca were diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, $10^{-5}$and 10^{-5}From each dilution, 0.1 mL was inoculated on agar plates for aerobics. Bacteria was enumerated on Nutrient Agar (NA), Eosin Methylene Blue agar (EMB) for

coliform and PDA (Potato Dextrose Agar) for fungal growth.

For bacterial growth, all the plates were incubated at 37°C. MRS agar plates was incubated anaerobically for 48 h (gas-pack container, AnaeroPackTM, Tokyo, Japan), and other plates were incubated aerobically for 24 h. Total numbers of bacterial colonies were counted at each incubation period and expressed as log10 cfu g^{-1} digesta. The spread plate method for plate count determination was performed in accordance with the procedure recommended by the APHA.

For fungal growth, PDA (Potato Dextrose Agar) was allowed to cool to about 45°C after which 2.5g/1 of streptomycin antibiotics was added into it in order to inhibit bacterial growth on the medium. This medium was poured into sterile petri dishes for fungal isolation. 1ml of microbial suspension from 10^{-2} dilution tube was added. The petri dishes were swirled and their content was allowed to solidify. Plates were incubated upside down at room temperature for 72hrs. Total number of colonies was counted at the end of incubation period. The spread plate method for plate count determination was performed in accordance with the procedure recommended by the APHA 1993. Macroscopic features like colony colour, texture and margins were examined for species differentiation.

Statistical analysis

Analysis of Variance (ANOVA) was done using SAS (2010) package to analyse data on growth performance (feed intake, changes in body weight, feed conversion ratio) and haematological indicators. New Duncan's Multiple Range Test (DNMRT) of the same package was used to separate different means.

Results

The effect of *Centella asiatica* leaf meal as additive on serum biochemical parameters of the experimental birds at growing phase is presented in Table 4. The initial haematological parameters showed no significant difference ($p>0.05$) on packed cell volume, haemoglobin, platelet, white blood cells count, lymphocytes, eosinophils, basophils, monocytes and heterophils.

The packed cell volume (PCV) ranged from 30.50% in T3 to 33.00% in T4, haemoglobin concentration (Hb) ranged from 9.77g/dl in T3 to 10.47g/dl in T4. Red Blood Cell (RBC) ranged from 2.92x1012 /mm3 in T3 to 3.11.15x1012 /mm3 in T4 and White Blood Cell (WBC values) ranged between 15.12 -15.56 x109 /mm3 among the experimental birds, WBC values among treatments were not statistically different ($p>0.05$), though they increased numerically as levels of *Centella asiatica* leaf meal increased. Monocytes count ranged between 3.17% - 3.17%, lymphocytes count ranged between 62.17% to 64.17% while heterophils count ranges from 31.33% to 30.17% respectively. Monocytes, lymphocyte and heterophils were significantly different ($P<0.05$).

Table 4 showed the effect of the inclusion of *Centella asiatica* as feed additive on blood profile of laying birds. The inclusion of *Centella asiatica* significantly affected ($p<0.05$) the red blood cells count, packed cell volume and haemoglobin concentration. There was no significant difference ($p>0.05$) for platelets, MCHC, MCV, MCH, white blood cells count, heterophils, lymphocytes, monocytes, eosinophils and basophils. The control (T1) had significantly higher ($p<0.05$) values for RBC, PCV and haemoglobin concentrations.

There was a slight contrast with the findings of this result as RBC counts; PCV and haemoglobin were significantly influenced ($P<0.05$) by the inclusion of *Centella asiatica* leaf meal. The values of PCV, Hb, RBC, WBC, MCV, MCH and MCHC ranged as follows: 47.00-41.67%, 15.63-13.87 g/dl, 5.53-4.87mm, 115666.67- 8133.33, 8.49-8.56%,2.83-2.85% and 33.26-33.2810^{6}mm-1 respectively while the percentage of lymphocytes, monocytes, heterophil, basophil and eosinophils ranged as 64.33-44.46 %, 8.00-10.67, 26.33-29.33, 0.67-1.40 and 0.67-0.33 respectively. Table 5 showed the results of the serum biochemical parameters of the birds fed *Centella asiatica* as additive at the grower stage. Significant difference ($p<0.05$) were observed only in glucose. The values of glucose decreased significantly ($p<0.05$) as the levels of *Centella asiatica* was increasing. However, the values of glucose ranged from 171.62 – 199.64 mg/dL.

Table 6 showed the serum biochemical parameters of layer birds fed graded levels of *Centella asiatica* as feed additives. The inclusion of the additive only significantly influenced ($p<0.05$) glucose and aspartate transaminase (AST). Birds that received higher levels of *Centella asiatica* (T4 and T5) had significantly higher ($p<0.05$) values of glucose and AST. The additive did not affect ($p>0.05$) the levels of cholesterol, total protein, low density lipoprotein, high density lipoprotein, globulin, ALT, very low-density lipoprotein and albumin. Biochemical constituents in serum are closely associated with health status of the birds. In this study, no differences were detected among the treatment groups ($P>0.05$), except for glucose and AST.

The mean external and internal egg quality parameters of the experimental birds fed *Centella asiatica* leaf meal as additive at fourth week of lay are presented in Table 7. The parameters considered were; egg weight, egg length, egg width, egg shape index, shell thickness, shell weight, albumen weight, albumen height, percentage albumen weight, yolk weight, yolk height, and Haugh Unit. The inclusion of *Centella asiatica* as feed additives had significant effects ($p<0.05$) on albumen height, yolk weight, yolk height, Haugh unit, egg weight, egg width and egg shape index but no significance differences ($P>0.05$) were observed for egg length, shell thickness, shell weight, albumen weight and percentage albumen weight. Birds that received *Centella asiatica* leaf meal had significantly higher ($p<0.05$) egg weight and yolk weight.

Albumen height, yolk weight, yolk height, haugh unit, egg weight, and egg shape index ranged between 1.25g-1.32g, 14.33g-16.17, 1.77-1.95mm, 9.81g-3.26g, 4.75-0.08mm, 0.75-0.82mm respectively. Albumen height, yolk weight, yolk height, Haugh unit, egg weight, egg width and egg shape index of the laying hens fed the experimental diets were significantly ($P<0.05$) influenced by the different experimental diets. The laying hens fed *Centella asiatica* leaf meal diets (T3- T5) showed the highest egg weight and egg length compared to control (T1). Generally, the egg weights and egg lengths from the laying hens fed *Centella asiatica* leaf meal as additive are higher than those fed T1 (0% CALM) .

Table 8 showed the intestinal microbial count of Isa Brown hens fed diets containing different levels of *Centella asiatica* leaf meal as additive at laying phase. The results showed that the inclusion of the test ingredients significantly influenced ($p<0.05$) the microbial populations at the caecum and ileum of the chickens. At the caecum, birds that received higher levels of *Centella asiatica* (T4 and T5) had significantly higher population of bacteria, coliform and fungi. At the ileum, birds that received *Centella asiatica* leaf meal had significantly higher ($p<0.05$) population of bacteria, coliform and fungi.

Table 2. Gross composition of grower diet containing varying levels of *Centella asiatica*

Ingredients (Kg)	Control T1	0.2% (CA) T2	0.4% (CA) T3	0.6%(CA) T4	0.8% (CA) T5
Maize	50.00	50.00	50.00	50.00	50.00
Wheat offal	28.80	28.80	28.80	28.80	28.80
Soya meal	16.00	16.00	16.00	16.00	16.00
Oyster shell	2.00	2.00	2.00	2.00	2.00
Bone meal	1.50	1.50	1.50	1.50	1.50
Fish meal	1.00	1.00	1.00	1.00	1.00
Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00
Crude Protein %	19.90	19.94	19.97	20.01	20.04
M.E (kca/kg)	2600.04	2612.13	2621.63	2635.89	2654.89
Ca (ppm)	10061.00	10061.00	10061.01	10061.03	10061.05
P(ppm)	5534.00	5534.00	5534.00	5534.01	5534.00

Table 3. Gross Composition of Layers Diet Containing Varying Levels of *Centella asiatica*

Ingredient (kg)	Control T1	0.2% (CA) T2	0.4% (CA) T3	0.6%(CA) T4	0.8%(CA) T5
Maize	55.00	55.00	55.00	55.00	55.00
Soya meal	20.00	20.00	20.00	20.00	20.00
Wheat offal	15.00	15.00	15.00	15.00	15.00
Oyster shell	4.00	4.00	4.00	4.00	4.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00	1.00
Layers/v/m Vit/mineral	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Crude protein (%)	17.30	17.34	17.37	17.41	17.44
M.E (kca/kg)	2600.04	2600.08	2600.11	2600.15	2600.18
Ca (ppm)	10061.00	10061.00	10061.01	10061.03	10061.05
P (ppm)	5534.00	5534.00	5534.00	5534.01	5534.01

Table 4. Haematological parameters of hens fed *Centella asiatica* leaf meal as additive at growing phase (day 141-224)

Parameters	T1	T2	T3	T4	T5
PCV (%)	31.17±0.1	29.50±1.0	30.50±0.3	33.00±1.1	30.50±0.7
Hb (g/dl)	9.80±0.4	9.55±0.4	9.77± 0.2	10.47±0.3	9.87±0.3
RBC (106ul)	3.03±0.1^{b}	2.89±0.1^{a}	2.92 0.1^{a}	3.11±0.1^{ab}	2.91±0.1^{ab}
WBC (103ul)	14516.6±342.94	15125.0±626.07	15008.3±244.75	15558.3±404.02	15008.3±286.48
PLATELET	118333.3±3470.5	126833.3±14772.5	136333.3±13288.3	138333.3±13052.9	132166.±8400.1
LYM (%)	62.50±1.8	60.00±2.3	62.17±0.5	64.17±1.0	62.17±1.3
HET (%)	30.17±1.7	32.33±2.3	31.33±1.0	28.00±0.9	30.17±1.7
MON (%)	4.17±0.31	4.00±0.52	3.17±0.31	3.50±0.22	3.17±0.40
EOS (%)	2.67±0.49	3.50±0.72	3.33±0.71	4.00±0.45	4.33±0.49
BAS (%)	0.50±0.22	0.17±0.17	0.00±0.00	0.33±0.21	0.17±0.17

Note: PCV- Packed Cell Volume, Hb- Haemoglobin, RBC- Red Blood Cell, WBC- White Blood Cell, Lymph – Lymphocyte, HET- Heterocytes, MON- Monocytes,EOS- Eosinophils, BAS-Basophils.

Table 5. Serum biochemical parameters of pullets fed *Centella asiatica* leaf meal as additive at growing phase (day 84- 224)

Parameters	T1	T2	T3	T4	T5
Glucose (mg/dL)	199.64± 4.38^{ab}	195.87± 7.56^{a}	190.70± 5.06^{bc}	171.62± .55^{c}	184.48±6.08^{c}
Cholesterol (mg/dL)	98.42±5.55	113.7± 7.8	118.76± 4.32	98.08± .24	109.4± 3.19
AST (LU/L)	94.06±2.24	91.10±7.89	97.81±2.06	93.12±6.27	94.79± 5.69
ALT (LU/L)	4.32±0.63	4.45±0.77	4.72±0.82	4.55±0.58	5.31±0.37

Note: AST- Aspartate Aminotransaminase, ALT–Alanine Transaminase

Table 6. Serum biochemical parameters of pullets fed *Centella asiatica* leaf meal as additive at laying phase (day 141- 224)

Parameters	T1	T2	T3	T4	T5
Glucose (mg/dl)	120.33 ± 0.88^{ab}	117.67± 1.45^{a}	122.33± 0.88^{bc}	124.67± 0.88^{c}	125.67 ± 0.88^{c}
Total protein (mg/dl)	8.47± 0.15	8.57± 0.34	8.77± 0.34	8.03± 0.15	8.23± 0.09
Albumin (mg/dl)	5.07± 0.08	5.15± 0.21	5.27± 0.19	4.83± 0.09	4.95± 0.04
Globulin (mg/dl)	3.39± 0.06	3.42± 0.13	3.50± 0.15	3.21± 0.05	3.28± 0.05
Cholesterol (mg/dl)	376.00± 7.02	374.67± 13.87	386.33 ± 6.84	388.33 ± 8.82	390.67 ± 8.17
VLDL (mg/dl)	49.67± 4.37	50.47± 2.36	47.33± 2.67	49.33± 0.33	47.33± 1.45
LDL (mg/dl)	134.00± 22.03	133.20± 23.21	157.00 ± 16.17	147.20 ± 15.34	155.33 ± 10.93
HDL (mg/dl)	190.33± 12.72	191.00± 8.02	182.00 ± 6.81	192.00 ± 6.56	188.00 ± 4.36
ALT(U/l)	19.00± 5.13	23.67± 4.81	29.67± 4.67	32.33± 1.67	31.67± 1.33
AST(U/l)	22.67± 6.89^{a}	24.67± 8.99^{ab}	49.00± 16.52^{ab}	57.67± 5.81^{b}	44.33± 6.69^{ab}

Note: LDL-low-density lipoprotein, HDL-high density lipoprotein, ALT- alanine aminotransferase, AST-aspartate aminotransferase, VLDL-very low density lipoprotein

Table 7. Effect of *Centella asiatica* leaf meal inclusion on egg quality (4th weeks in lay)

Parameters	T1	T2	T3	T4	T5
Albumen Height (cm)	1.25 ± 0.10^{ab}	1.35 ± 0.06^{b}	1.27 ± 0.02^{ab}	1.13 ± 0.03^{a}	1.32 ± 0.09^{ab}
Yolk Weightt (g)	14.33 ± 0.92^{a}	16.00 ± 0.86^{ab}	16.50 ± 0.50^{ab}	16.83 ± 0.75^{b}	16.17 ± 0.60^{ab}
Egg Weight (g)	56.33 ± 2.08^{a}	62.17 ± 0.95^{b}	65.17 ± 1.62^{b}	64.00 ± 1.77^{b}	64.17 ± 1.89^{b}
Egg Length (cm)	6.40 ± 0.24	6.48 ± 0.17	6.30 ± 0.09	6.25 ± 0.11	6.15 ± 0.10
Egg Width (cm)	4.75 ± 0.08^{a}	4.92 ± 0.05^{ab}	4.93 ± 0.10^{ab}	5.10 ± 0.07^{b}	5.05 ± 0.16^{ab}
Shell Thickness (mm)	0.17 ± 0.01	0.22 ± 0.05	0.22 ± 0.05	0.26 ± 0.05	0.25 ± 0.07
Shell Weight (g)	7.33 ± 0.56	6.67 ± 0.21	6.50 ± 0.50	7.67 ± 0.56	7.33 ± 0.67
Yolk Height (cm)	1.77 ± 0.10^{a}	2.05 ± 0.04^{b}	1.88 ± 0.04^{ab}	1.90 ± 0.06^{ab}	1.95 ± 0.03^{ab}
Albumen Weight (g)	37.50 ± 3.34	37.17 ± 1.56	41.50 ± 1.88	36.17 ± 1.17	39.17 ± 1.66
Haugh Unit	9.81 ± 3.27^{c}	3.37 ± 1.85^{bc}	6.64 ± 2.61^{ab}	12.23 ± 5.53^{a}	-3.26 ± 5.42^{ab}
%Albumen Weight (g)	66.16 ± 4.59	59.87 ± 2.65	63.60 ± 1.90	56.74 ± 2.42	61.21 ± 2.74
Egg Shape Index	0.75 ± 0.03^{a}	0.76 ± 0.02^{ab}	0.78 ± 0.01^{ab}	0.82 ± 0.01^{b}	0.82 ± 0.03^{b}

Table 8. Intestinal microbial population of Isa Brown hens fed *Centella asiatica* leaf meal as additive at laying phase (day 140-224)

ILEUM	T1	T2	T3	T4	T5
TBC (cfu/g) x 10-5	2.95± 0.15^{a}	6.20± 0.30^{bc}	6.25±0.35^{bc}	5.40±0.20^{b}	7.10±0.20^{c}
Isolates	*MV, SA, BM, SL*	*BR, SAU, SR, EA*	*BR, PV, KA, EA, KA*	*BR, MV, MV, CL, EA*	*SF, BR, CV, KA*
TCC (cfu/g) x 10-5	1.75±0.15^{a}	2.20±0.30^{ab}	2.20±0.20^{ab}	2.00±0.30^{ab}	2.90±0.20^{b}
Isolates	*EC*	*EC*	*EC*	*EC*	*EC*
TFC (cfu/g) x 10-5	0.00±0.0^{a}	0.05± 0.1^{a}	0.40±0.0^{b}	0.40±0.1^{b}	0.30 ± 0.1^{b}
Isolates	-	*FO*	*PO, AN*	*FO, SC*	*FO, AN*

Note: TBC = Total bacteria count, TCC: total coliform count, TFC: total fungi count. MV= *Micrococcus varian, SA= Succinimonas amylotica,* BM= *Bacteria macerans, SL= Streptococcus lactis,* BR= *Bacteria ruminicola, Staphyloccocus aureus, SR= Selenomonas ruminatum, EA= Enterobacter aerogens, PV= Proteus vugaricus, KA= Klebsiella aerogens, MV= Micrococcus varian, CL= Clostridium lochheadii, SF= Streptococcus faecium, CV= Clostridium welchii, EC= Escherichia coli, FO= Fusarium oxysporum, PO= Penicillium oxysporium, AN= Aspergillus niger, SC= Saccharomyces cerevisa*

Discussion

Results for haematology of ISA brown laying birds on varying levels of *Centella asiatica* leaf meal showed that Hb concentration of experimental birds were within the normal range of 9.77 to 10.47g/dl (Mitruka and Rawnsley, 1997). The lowest Hb concentration was recorded in T2 (9.55g/dl), while birds in T4 had the highest value (10.47 g/dl). In this study, there was no case of abnormal rise in the values of WBC. Results revealed that birds in T4 had the highest WBC count (15.56 × 109/mm^{3}), with lowest value of (15.01 × 109/mm^{3}) in T3. Lymphocytes are important in forming barriers against local disease conditions and may be involved in antibody formation.

According to Quintavalla *et al.* (2001), haematology is vital tools for disease and nutrition verification. The results obtained in this study were in agreement with the findings of Alagbe (2019) who studied the effects of dried *Centella asiatica* leaf meal as herbal feed additive on the growth performance, heamatology and serum biochemistry of broiler chickens. Alagbe (2019) concluded that dried *Centella asiatica*leaf meal inclusion as feed additive had no effect on the haematology of chickens. In contrast, Ghasemi *et al.* (2010) found the addition of 0.2% medicinal herbs spices (garlic and thyme) in a ration for laying hens increased lymphocytes, whereas other haematological traits remained unchanged while Rahman *et al.* (2021) observed no significant difference in the haematology of laying birds fed with phytogenic feed additives.

All the haematological values obtained were within the reference range for birds recorded in the previous reports (Abdi-Hachescoo *et al.*, 2011; Elagib and Ahmed, 2011; Talebi *et al.*, 2005; Simaraks *et al.*, 2004). According to Soetan *et al.* (2013), hemoglobin aids in oxygen transportation to animal tissues. WBC and its differentials assist to fight infections and produce antibodies to protect the body (Adeyinka and Bello, 2013). Togun *et al.* (2007) reported that when hematological parameters fall within the range for an animal, it is an indication that the diet (test material) has no adverse effect on the health of the animals during the experimental period, but when they fall below normal range, it is a sign of anemia or harmful effects of dietary contents. A PCV less than 35% is a sign of anaemia and a PCV greater than 55% is suggestive of dehydration or polycythemia (Etim *et al.*, 2014). Hematological studies are used to investigate the number and morphology of the cellular elements of the blood. They are also clear indicators to disease prognosis and feed stress monitoring (Akintunde *et al.*, 2019; Soetan *et al.*, 2013; Simaracks *et al.*, 2004; Togun and Oseni, 2005). They are medium for measurements of potential biomarkers, because its collection is relatively non-invasive and it encompasses an enormous range of physiological process in the body at any given time (Togun and Oseni, 2005).

The results of this study agree with the reports of Saki *et al.* (2014) who reported that dietary inclusion of phytogenic feed additives did not change the serum cholesterol levels in laying hens. Mansoub (2011) found that supplementation with thyme powder in layer diets did not influence the immunity in laying hens and produced no change in serum HDL and LDL. The findings from this study was also in agreement with the reports of Alagbe *et al.* (2018) that phytogenic feed additives did not influence serum total protein, albumin, globulin and uric acid. These results simply show that the protein in the diets is enough to support the growth and development across the treatments.

It should be noted that the liver is the center of several digestive, metabolic and productive activities, and as such, is susceptible to a varying degree of chemical and biological damages. Such damages are made obvious by the serum levels of specific enzymes originating from the liver. These enzymes, depending on their levels may cause some disruptions to bodily functions, thereby resulting in poor health and production performance. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the blood are bioindicators of liver function and damage (Yildirim *et al.*, 2011). Increased levels of these enzymes are associated with liver or muscle damage, resulting from the body's response to stress (Lumeij, 2008). The values of these enzymes in the present study showed significant differences (P<0.05) among treatment groups. Fernandez *et al.* (1994) observed

that induced liver damage using aflatoxin in broilers and layers led to an increase in serum ALT. Therefore, the reduction in AST in T2, T3 and T5 can be deduced as an indication of better liver function.

The egg weights obtained at 4th week in lay in this study were within the standard weight (56g) reported by (Akintunde and Toye, 2022).

Phytobiotics have shown positive effects by increasing the beneficial bacteria and displacing the pathogenic bacteria. The colonization of the gastrointestinal tract (GIT) by the beneficial bacteria (*Bacillus, Escherichia coli and Pseudomonas*) and fungi *(Penicillium, Aspergillus, Fusarium)* suppress the activity and habitation of potentially pathogenic species (Rinttila and Apajalahti, 2013). The result suggests that *Centella asiatica* helps to colonize the GIT with beneficial bacteria and fungi and reduces the virulence of pathogenic bacteria and fungi. This work is in agreement with the report of Murugesan *et al.* (2015) who reported that phytogenic feed additive significantly reduced the cecal population of coliforms and fortified the gut microbiota with beneficial bacteria, such bacillus spp. Once the Bacillus spp. are established, they might selectively exclude the pathogens from adhering due to their fast colonization, proliferation, and acidification properties in the GIT (McReynolds *et al.*, 2009).

Conclusion

This study has revealed that the inclusion of *Centella asiatica* leaf meal in layers diet has no detrimental effect on performance profile. However, the inclusion of *Centella asiatica* in layers diet at the rate of 0.8% improved the overall health of the birds. It also improved the activities of beneficial bacteria in the gastro intestinal tract of layer birds thereby making more nutrients available from the feed consumed. The inclusion of CALM also increased egg weight, yolk weight and improved albumen quality expressed in Haugh units.

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