International journal of Advanced Biological and Biomedical Research
Available online at http:www.ijabbr.com
Volume 7, Issue 3 (2019) pp. 237-248

Original Article

Effects of Dried *Centella Asiatica* Leaf Meal as a Herbal Feed Additive on the Growth Performance, Heamatology and Serum Biochemistry of Broiler Chicken

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Received: 3 February 2019, Revised: 20 March 2019, Accepted: 24 March 2019

ABSTRACT

A 42 days experiment was carried out to evaluate the effects of dried *Centella asiatica* leaf meal (CSP) as an herbal feed additive on the growth performance, haematology and serum biochemistry of broiler chicken. A total of 200 Ross 308 day old broiler chicks of mixed sex was divided into four (4) treatment groups of fifty (50) birds, each group was further divided into five replicates each containing ten (10) birds. Group A was fed basal diet + 0% CPS (control), group B,C and D were fed basal diet + 2%, 4% and 6% CPS, respectively.The basal diet was formulated to meet the nutritional requirements of birds according to NRC (1994). Feed and water were provided unrestricted and a completely randomized design and birds were vaccinated according to the prevailing disease in the environment. The obtained results showed that there were significant ($p<0.05$) differences among all treatments in the values of final live weight, body weight gain and feed conversion ratio. Diet containing 6% CPS had the highest weight gain of 2072.9grams, while broilers fed 0% CPS had the lowest weight gain of 1534.7 grams. Supplementation of CPS did not affect ($p>0.05$) the daily feed intake and mortality rate. All the hematological (PCV, Hb, RBC, MCV, MCH, MCHC, WBC and its differentials) and serum biochemical parameters (Albumin, globulin, total protein, SGPT and SGOT) were not significantly ($p>0.05$) different among the treatments.
It can be concluded that the inclusion of CPS at 6% enhanced the overall performance of the birds without causing any deleterious effect on the health of the animals.

Key words: *Cantella asiatica* leaf meal, Performance, Hematology, Serum biochemistry, Broilers.

Introduction

When animals with good genetic potential are kept in a conducive environment and fed a balanced ration, they attain weight early. Some of the cardinals in livestock management are health, housing and feeding. In Nigeria, the use of antibiotics in feed has become a recent trend in treating various bacterial infection and as growth promoters. Unfortunately, most of these drugs (antibiotics) are very expensive and they increase the total cost of production, cause disease resistant and their residues are found in the final carcass of animals after slaughter especially when it is administered in high dose thereby causing health problems to humans. Therefore, with the increasing awareness on food safety by the government and other non-governmental organizations, farmers are now educated on the use

of medicinal or herbal plants which are more suitable for animal and human health, with high safety margin and low cost.

Herbal (medicinal) plants are widespread throughout the world and have been reported to contain phytochemicals which produce definite physiological action in animals, strengthen the body and help to fight against diseases (Edeoga *et al*., 2005 and Mirazaei-Aghsaghali., 2012). Phytochemicals are chemicals that contain bioactive constituents such as tannins, steroids, terpenoids, alkaloids, flavonoids, phenols and glycosides which perform several functions such as antibacterial (Mangalanayaki and Sivaneshwari, 2018; Suyogra *et al*., 2017; Sumaiya *et al*., 2015 and Umer *et al*., 2015), antifungal (Gong *et al*., 2012; Vijay *et al*., 2013), antimicrobial (Khan *et al*., 2012 and Negamani *et al*., 2014), antioxidant (Sharififar *et al*., 2009), antihelmintic (Mohankumar *et al*., 2015; Deore *et al*., 2009), anti-inflammatory (Goyal *et al*., 2013; Jaya *et al*., 2012). According to Langhout (2000) a medicinal plant must be capable of being used for therapeutic purposes or possess chemicals for the synthesis of drugs. There are more than 20,000 species of herbal plants found worldwide, few species have been extensively used traditionally for the treatment of various ailments in many countries, the practice had still continued because of its immense contribution in maintaining the health of human and animals. One of the commonly found herbal plant in gwagwalada is *Centella asiatica* leaf.

Centella asiatica is native to India and it belongs to the family Apiaceae. The leaves are green small, and fan shaped. According to Singh (2012), the leaves, stems, and roots of the plants are highly medicinal. *C. asiatica* contains different biologically active chemicals likeasiaticosides, medasiatic acid, madecassoside, saponins, centelloside, pectin, tannins, phytosterols, mucilages, resins, vallerine, flavonoids and many other derivatives (Ablise *et al*., 2004; Ahmed and Bassuony, 2009; Al-Bayati, 2008; Lateef, 2013). The plant has been traditionally used to treat hypertension, skin infection, leprosy and fever (Gupta and Sharma, 2007). *C. asiatica* exhibits important pharmacological activities such as antibacterial, anti-ulcer, anti-inflammatory, antifungal and antioxidant (Mariappan Senthilkumar, 2018; Liu *et al*., 2008).

Several researches have been carried out on the use of different medicinal plants in poultry, for instance garlic, turmeric and thyme powder which have been shown to possess excellent antimicrobial properties making them effective against bacteria like Escherichia coli, Bacillus careus, Staphylococcus spp, Salmonella enterritidis in birds (Levic *et al*., 2011; Rota, 2004; Sakovic *et al*., 2010; Al-bayati, 2008). Supplementation of Rosemary leaves at 0.5% in the diet of Ross 308 broilers improved the final live weight and immune response of the birds Ghazalah and Ali (2008). According to (Alagbe, J.O, 2017). *Polyalthia longifolia* leaf meal at 2% caused a significant ($p<0.05$) increase in the final live weight of birds. But there is little information on supplementation of *Centella asiatica* leaf meal in poultry diet, an evaluation of this test material in broiler feed will give a clue on the safe inclusion level, its effect on growth and blood parameters.

Methodology

Experimental Site

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Farm, Gujarat, (Western India).

Collection and processing of *centella asiatica* leaves

Fresh healthy leaves of *Centella asiatica* were collected from the farm premises and it was authenticated by Dr. Kumar. M. at the University of Gurat. The collected matured leaves were washed in running tap water and then rinsed in distilled water. It was later air dried for 14 days and then hammer milled to produce *Centella asiatica* powder (CSP).

Pre-experimental operations

A deep litter poultry house was used for the experiment, the pen was swept, cleaned and well disinfected, feed and water troughs were also washed. The electrical fittings (bulb) 200 watts were properly fixed and a vaccination programme was designed before the commencement of the study.

Animal management

One day old 200 (Ross 308) broilers of mixed sex were obtained from a commercial hatchery in India. The chicks were weighed and assigned into four dietary treatment group, anti-stress (Strexia) was added in the drinking water of the birds. Each group was further divided into five replicates each of ten (10) birds. Vaccines were administered according to the prevailing vaccination schedule in the environment (Lasota vaccine on 3rd and 11th day, Gumboro vaccine on 7th& 18th day for 1st and 2nd dose respectively). Vitamins (Miavit) was added in water a day before and after each vaccination. The light (electric bulb) was continuous, clean feed and water was provided unrestricted throughout the experimental period which lasted for 42 days.

Experimental diets and design

Four diets were formulated to meet the nutritional requirements of birds according to NRC (1994). Control diet (T1) did not contain CSP, while diets T2, T3 and T4 contained CSP at 2.0%, 4.0% and 6.0% respectively. The used experimental design was a completely randomized design. Daily feed intake (g) was calculated by difference between the offered feed and the left over, feed conversion ratio was determined as feed intake divided by body weight gain, water consumption and mortality were recorded daily throughout the experimental period. Mortality was recorded daily and all management practices were strictly observed throughout the experimental period.

Other Parameters measured

Blood analysis

At the 6th week of the experiment, blood samples were collected from three randomly selected birds per replicate, the samples were collected via the branchial vein to aspire 5mls of blood from each bird into bottles containing Ethylene Diamine Tetra Acetate (EDTA) for haematological analysis. Some of the measured haematological parameters are Pack cell volume (PCV), Red blood cell (RBC), White blood cell (WBC), Haemoglobin concentration (Hb) and absolute counts of neutrophils, lymphocytes, monocytes and eosinophils which were all computed according to standard techniques as reported by Jain (1986). Mean corpuscular volume (MCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration were computed according to Ritchie (1994).
Blood samples that were meant for serum biochemistry were collected into other bottles free from any EDTA. The serum total protein, Albumin and Globulin were computed according to (Doumas and Briggs, 1972), Glutamic oxaloacetate transaminase (SGOT), Glutamic phosphatase transaminase (SGPT) was determined according to Reitman and Frankel (1957).

Laboratory Analysis

Proximate analysis of diets and CSP were determined according to AOAC (2000). The phytochemical screening was determined according to procedures outlined by Harbone (1973) and Odebiyi and Safowora (1978). The mineral analysis were carried out using Atomic Absorption Spectrophotometer (AAS).

Statistical Analysis

All the generated data were subjected to a one way analysis of variance (ANOVA) and treatment means were compared using GLM procedures of SAS (1997). Differences among treatment means were separated by Duncan's multiple range test (Duncan, 1955).

Table 1. Percentage Composition of Broiler Starter Diet

Ingredients	Diets			
	1	2	3	4
Maize	63.00	62.00	61.00	60.00
Soya meal	25.00	25.00	25.00	25.00
Groundnut cake	6.60	6.60	6.60	6.60
Bone meal	3.00	3.00	3.00	3.00
Limestone	1.50	1.50	1.50	1.50
Lysine	0.15	0.15	0.15	0.15
Methionine	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30
CSP	-	2.00	4.00	6.00
Total	100.0	100.0	100.0	100.0
Determined analysis				
ME (Kcal/kg)	3101.5	3102.3	3102.2	3102.1
Crude protein (%)	23.68	23.63	23.62	23.60
Ether extract (%)	5.48	5.51	5.57	5.59
Crude fibre (%)	3.14	5.22	5.28	5.41

* Premix supplied per kg diet :- Vit A, 10,000 I.U; Vit E, 5 mg; Vit D3, 3000I.U, Vit K, 3 mg; Vit B2, 5.5 mg; Niacin, 25 mg; Vit B12, 16 mg; Choline chloride, 120 mg; Mn, 5.2 mg; Zn, 25 mg; Cu, 2.6 g; Folic acid, 2 mg; Fe, 5 g; Pantothenic acid, 10 mg; Biotin, 30.5 g; Antioxidant, 56 mg

Table 2. Percentage Composition of Broiler Finisher Diet

Ingredients	Diets			
	1	2	3	4
Maize	63.00	62.00	61.00	60.00
Soya meal	25.00	25.00	25.00	25.00
Groundnut cake	6.60	6.60	6.60	6.60
Bone meal	3.00	3.00	3.00	3.00
Limestone	1.50	1.50	1.50	1.50
Lysine	0.15	0.15	0.15	0.15
Methionine	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30
CSP	-	2.00	4.00	6.00
Total	100.0	100.0	100.0	100.0
Determined analysis				
ME (Kcal/kg)	3004.9	3006.5	3006.4	3006.1
Crude protein (%)	20.68	20.64	20.61	20.63
Ether extract (%)	6.41	6.57	6.52	6.60
Crude fibre (%)	3.44	3.22	3.28	3.41

* Premix supplied per kg diet :- Vit A, 8,000 I.U; Vit E, 3 mg; Vit D3, 6000I.U, Vit K, 5 mg; Vit B2, 5.5 mg; Niacin, 25 mg; Vit B12, 16mg; Choline chloride, 120 mg; Mn, 5.2 mg; Zn, 25 mg; Cu, 2.6 g; Folic acid, 2 mg; Fe, 5 g; Pantothenic acid, 10 mg; Biotin, 30.5 g; Antioxidant, 56 mg

Table 3. Proximate composition of *Centella asiatica* leaf meal (CSP)

Components	% Composition
Dry matter	90.44
Crude protein	13.06
Crude ash	4.07
Ether extract	2.01
Crude fibre	16.14

Table 4. Phytochemical composition of *Centella asiatica* leaf meal (CSP)

Constituents	(%) Composition
Flavonoids	6.99
Phenols	12.21
Alkaloids	2.03
Tannins	3.03
Saponins	2.07
Oxalate	0.08
Phytate	0.77

Table 5. Mineral composition of *Centella asiatica* leaf meal (CSP)

Minerals	% (mg/100 g)
Copper	1.78
Iron	0.02
Zinc	13.02
Calcium	10.02
Magnesium	9.06
Potassium	5.12
Sodium	8.04
Phosphorus	0.31
Manganese	0.18

Table 6. The effect of supplementing *Centella asiatica* leaf meal (CSP) on the performance of broiler chickens

Parameters	Treatments				SEM
	1	2	3	4	
			Live body weight (g)		
Initial weight	40.10	40.01	40.03	40.08	0.41
7th day	110.9	110.2	110.0	110.4	0.35
28th day	891.3	845.1	850.6	856.8	10.18
49th day	1574.8^{c}	2001.7^{b}	2088.2^{ab}	2113.0 ^{ab}	29.71
			Body weight gain (g)		
0-7days	70.80	70.19	69.97	70.32	2.07
7-28 days	780.4	734.9	740.6	746.4	11.10
0-49days	1534.7^{c}	1961.7^{a}	2048.2^{ab}	2072.9^{a}	61.44
			Feed intake (g/bird)		
0-7days	1402.44	1489.88	1478.90	1473.88	33.07
7-28days	2145.56	2103.85	2105.13	2108.13	90.97
0-49days	3548.00	3593.73	3584.03	3582.01	0.07
			Feed conversion ratio (feed/gain)		
7-28days	2.41^{a}	2.49^{b}	2.47^{a}	2.46^{b}	0.13
0-49days	2.25^{a}	1.78^{ab}	1.72^{ab}	1.70^{b}	-
			Mortality		
	0/50	0/50	0/50	0/50	

[abc] means different superscript along rows differs significantly at p<0.05

Table 7. The effect of supplementing different levels *Centella asiatica* leaf meal (CSP) on the haematology of broiler chickens

Parameters	Diets				
	1	2	3	4	SEM
PCV (%)	33.88	36.79	37.56	37.33	10.88
Hb (g/dl)	10.31	10.21	10.24	10.22	0.24
RBC $\times 10^6 (mm^{-1})$	3.03	3.09	3.11	3.31	0.01
MCV (fl)	89.61	90.07	92.33	94.45	7.03
MCH (pg)	33.01	33.27	33.67	33.88	5.01
MCHC (%)	34.10	34.25	34.34	34.31	3.12
WBC $\times 10^6 (mm^{-1})$	31.06	33.61	33.81	39.11	0.04
Lymphocytes (%)	40.11	41.22	44.46	44.38	4.34
Monocytes (%)	4.01	4.21	4.07	4.03	0.09
Heterophil (%)	43.44	44.01	45.71	45.07	9.31
Basophils (%)	1.30	1.38	1.25	1.40	0.06
Eosinophils (%)	4.07	4.11	4.21	4.32	0.54

[abc] means different superscript along rows differs significantly at p<0.05

Table 8. The effect of supplementing different levels of *Centella asiatica* leaf meal (CSP) on the serum biochemistry of broiler chickens

Parameters	Diets				
	1	2	3	4	SEM
Albumin (g/dL)	1.81	1.83	1.82	1.80	0.35
Globulin (g/dL)	1.98	1.99	1.97	1.96	0.22
Total protein (g/dL)	3.79	3.82	3.79	3.76	0.04
Albumin/globulin ratio	0.91	0.92	0.92	0.92	0.05
SGOT (U/L)	100.8	100.4	100.2	100.3	43.01
SGPT (U/L)	18.55	18.33	18.21	18.18	3.11

[abc] means different superscript along rows differs significantly at P<0.05
SGPT: Serum glutamic phosphatase transaminase
SGOT: Serum glutamic oxaloacetate transaminase

Results and Discussion

The results on the proximate composition of *Centella asiatica* leaf meal (CSP) are presented in Table 3. Dry matter content of CSP was 90.44% while those of crude protein, crude fibre, ether extract and total ash are 13.06%, 16.14%, 2.01% and 4.07%, respectively. Similar observations were made by (Paramageetham *et al.*, 2004) and (Chew Shio Heong, 2011). The authors reported that CSP has a higher nutritive value and it's free from toxic elements, thus making its inclusion in poultry feed safe and increasing the palatability.

Phytochemical analysis of *Centella asiatica* leaf meal (CSP) revealed the presence of tannins, flavonoids, saponin, alkaloids, phytate and phenolic compounds. The phytochemical constituents isolated from CSP have been reported to have hypotensive, anti-inflammatory, antioxidant, antifungal, antimicrobial and antibacterial activity (Mariappan Senthilkumar, 2018; Loganathan *et al.*, 2017; Abhishek and Saini, 2013; Chew *et al.*, 2011; Adegoke *et al.*,

2010 and Hassan *et al*., 2004). (Bačkor *et al*., 2009; Mustafa *et al*., 2010), found that phenol is a key antioxidant in plants, flavonoids also play a part in bacteria inhibition (Shahid-Ud-Duaula, 2009; Subhashini *et al*., 2010; Tasneef *et al*., 2013 and Edeoga *et al*., 2005). The result showed that CSP contained a higher level of phenol (12.21%) followed by flavonoids, tannins, saponin, alkaloids, phytate and oxalate with 6.99%, 3.03%, 2.07%, 2.03%, 0.77% and 0.08% respectively, this report is also in agreement with the findings of (Hussin *et al*., 2009) who reported that CSP is abundant in phenol as expressed in Table 4.

The mineral composition of CSP shows that it is one of the most promising medicinal plants because of its antioxidant ability and presence of macro and micro elements which are needed for animal survival and productivity (Hargano *et al*., 1999). Results on mineral constituents showed that copper, iron, zinc, calcium, magnesium, potassium, sodium, phosphorus and manganese were 1.78, 0.02, 13.02, 10.02, 9.06, 5.12, 8.04, 0.31 and 0.81 (mg/100g). The results obtained on the mineral composition of CSP are not in concurrence with Moyo *et al*. (2011) who reported thatdried Moringaoleifera leaves contained calcium (3.65%), phosphorus (0.30%), magnesium(0.50%), potassium (1.50%), sodium (0.164%), sulphur (0.63%), zinc (31.03 mg/kg), boron (49.93 mg/kg), selenium (363.0 mg/kg), Iron (490 mg/kg) and copper (8.25mg/kg).CSP contains significant amount of calcium, zinc, potassium, sodium and phosphorus.

The effect of supplementing CSP at different levels on feed intake, body weight change and feed conversion ratio of broilers is presented in Table 6.Average initial body weight was similar across treatments, feed intake was higher for birds in T2 (3593 g/bird) and T3 (3584.03 g/bird) as compared to the others, T1 had the lowest feed intake of 3548.0g/bird. The highest feed intake recorded in this experiment was greater than that reported by (Sarica *et al*., 2005) but contrary to the reports of Safa M.A. (Eltazi *et al*., 2014), this could be due to differences in the breed of birds, environmental condition as well as palatability of the diets. The final live weight of the birds were within 1574.8g – 2113.0 g for broilers on 6% and 0% CSP. Supplementation of CSP at 6% showed a significant ($p<0.05$) effect on their final live weight, body weight change and feed conversion ratio (FCR) in the current study. This was in agreement with the findings of (Hanan E. Al-Mashhadani, 2015) who reported that addition of 0.6% turmeric in broilers diet increases these parameters. Similarly observation was made by (Tarek M. Shafey, 2013) who reported higher body weight in broilers fed 1.80g Olive leaves extract (OLE) but contrary to the findings of (Agbabiaka *et al*., 2012).

Table 7 revealed the haematological traits of broilers fed different levels of CSP. The PCV, Hb, RBC, WBC, MCV, MCH and MCHC variables were not ($p>0.05$) significantly different among the treatments. According to (Quintavalla *et al*., 2001) haematology are vital tools for disease and nutrition verification. The values of PCV, Hb, RBC, WBC, MCV, MCH and MCHC were ranged as 33.58-37.56 (%), 10.21-10.31 (g/dl), 3.03-3.3110^6 (mm^{-1}), 89.61-94.45 (fl), 33.01-33.88 (pg), 34.10-34.34 (%) and 31.06-39.11 ($10^6(mm^{-1})$ respectively while the percentage of lymphocytes, monocytes, heterophil, basophil and eosinophil were ranged as 40.11-44.46 (%), 4.01-4.21 (%), 43.44-45.71 (%), 1.25-1.40 (%) and 4.07-4.32 (%) respectively. All the haematological values obtained were within the reference range for birds recorded by (Abdi-Hachescoo *et al*., 2011; H.A.A. Elagib and A.D.A. Ahmed (2011); Talebi *et al* (2005) and 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).

The serum biochemical parameters of broilers fed different levels of diet supplemented with CSP are shown on Table 8. The albumin, globulin, SGOT and SGPT were ranged as 1.80-1.83 (g/dl), 1.96-1.99 (g/dl), 100.2-100.8 (U/L) and 18.80-18.55 (U/L) respectively. All the evaluated serum parameters were not significantly ($p>0.05$) influenced with the inclusion of CPS in the diet. The total protein range was 3.76-3.82 (g/dl), this shows that the protein in the diet is enough to support the growth of the animal without any negative effect on their health. However, all the serum parameters values were in accordance to those previously reported by (Kecci and Col, 2011 and Ladokun *et al*., 2008). The SGOT and SGPT values reduced as the inclusion of CPS increased, this means that the integrity of the bird's liver is preserved, because anti-nutrients in CPS did not affect the animals making the test material safer to be added in broilers ration.

Conclusion

This experiment has clearly shown that when CPS is supplemented up to 7.0%, the growth performance is enhanced and the integrity of the blood profile (hematology and serum) is maintained.

Recommendation

- Similar experiment needs to be carried out to evaluate the effect of CPS on carcass and immune response.
- CPS can be processed and packed in 100g sachets like water soluble antibiotics and sold to livestock farmers.

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How to cite this article: J.O. Alagbe*, Effects of Dried Centella Asiatica Leaf Meal as a Herbal Feed Additive on the Growth Performance, Heamatology and Serum Biochemistry of Broiler Chicken. *International Journal of Advanced Biological and Biomedical Research*, 2019, 7(3), 237-248. Link: http://www.ijabbr.com/article_34768.html