

GROWTH PERFORMANCE, HAEMATOLOGICAL, SERUM INDICES AND HISTOPATHOLOGY OF FINISHER BROILER CHICKENS OFFERED NUTRITIONAL SUPPLEMENT OF COMFREY (*Symphytum officinale* L) LEAVES EXTRACT.

Abstract

Present study was carried out to investigate the effect of nutritional supplement of Comfrey (*Symphytum officinale* L) leaves extract on the performance, haematological, serum indices and histopathology of finisher broilers. 200g of Comfrey symphytum leaves were squeezed in 1 liter of H₂O with a juicer to produce comfrey symphytum leaves extract. The leaves extract were further mixed (diluted) in clean drinking water at the rate of 0ml/liter of H₂O, 50ml/liter of H₂O, 100ml/liter of H₂O and 150ml/liter of H₂O designated as T₁, T₃, T₄ and T₅. T₂ was given conventional supplement (Divertamin). Five groups of 30 broiler chicks (28 days old) of Agritech breed were randomly assigned to one of the supplementary Comfrey symphytum leaves extract and the conventional supplement for 28 days in a completely randomized design. Each group was subdivided into three replicates of 10 birds each. The birds were fed normal broiler finisher diet for all the groups. At the end of the 28 days feeding trial, performance indices result showed that T₅ (150ml extract/liter of H₂O) gave best value ($P < 0.05$) for feed conversion ratio, average weight changes and average daily weight gain but statistically similar ($P > 0.05$) to the control. The indices for economics of production showed that T₅ had high earnings for revenue and gross margin with reduced cost/kg weight gain. The haematological and serum indices were not affected significantly ($P > 0.05$). Histopathological examination revealed that comfrey leaves extract caused effaced tissue stromal elements, congestion and marked cellularity of the liver. It was concluded that Comfrey symphytum leaves extract could be used as a nutritional supplement to improve performance of broiler finishers at 150ml of extract/liter of H₂O due to its cost effectiveness and increased revenue and profit margin.

Key words: Comfrey leaves, Extract, **Broiler**, Performance, Haematology, Serum, Histopathology, **Nutritional supplement**

INTRODUCTION

The need to produce high quality poultry meat in Nigeria to feed the ever growing population is hampered due to high cost of feed, high cost of veterinary services for the health care of the animals and other necessary nutritional supplements to enhance heavier body weight gain and efficiency of feed utilization. This has led to low or reduced consumption of animal protein. 53g

of protein per caput per day was recommended by FAO (2010) out of which 35g is to be consumed from animal source. Contrary to the recommendation of FAO, Nigeria consumes only 10 to 15g per caput per day from animal source. Low dietary intake of poultry meat stems from the prohibitive price of poultry products resulting from high cost of production. It is a common practice that however, rich a feed may be farmers still purchase synthetic vitamins and minerals fortified with some essential amino acids to enhance the nutritional status of the animals which consequently increase the cost of production. "The reason is to stimulate faster growth rate, heavier body weight gain and better efficiency of feed utilization. These synthetic nutrients are usually very costly. It is important to note that Leaf vegetables supply minerals, proteins and vitamins which could complement the inadequacies of most feed stuffs" (Hon and Basir, 1980). It is also worthy of note that most of these nutritional plants and vegetables are available, free and cheap growing in the homes as flowers or in the nearby forests; it can also be cultivated at your backyard or farm for use and very rich in nutrient. Fasuyi and Nonyerem, (2007) reported that "Protein from plant leaves source is perhaps the most naturally abundant and the cheapest potential source of protein. Therefore, it is necessary to take advantage of the benefits of these vegetables as a nutritional supplement in the poultry industry to reduce cost of production and gain heavier body weight at a cheap cost. This will help to reduce the cost of using conventional supplements". Leaves have high fibre content despite its rich nutritional content and hence a burden to the digestive system when consumed as leaf meal; it is better to explore the consumption of the nutrients in leaf meal in the form of juice extract in other to share the rich nutritional benefit of it. Gala *et al.* (2003) reported that "a healthy body with sound digestive system has the capacity to disintegrate about 35% of the ingredients from vegetables and assimilate them where as the body is able to absorb 95% of substances contained in juice. The use of juice extract from plant leaves therefore is the most effective and efficient way of deriving the nutrients and enzymes in leaves of plants required in the body for proper metabolism and utilization in the tissues for growth".

"Comfrey plant leaves appear to have the potential to supply needed proteins, vitamins and minerals and could serve as a nutritional supplement to enhance growth. Comfrey (*Symphytum officinale* L) is native to Europe, Central Asia and prevalent in the South Eastern part of Nigeria, where it is used in bone healing, treating of anemia and as a blood booster. Comfrey leaves is used in making salad as a rich source of protein and vitamin B₁₂. It is very high in crude protein (between 21 to 31% crude protein) and is a forage of high digestibility (between 37 to 77%)" (Robert, 1983). "The fresh leaves of comfrey are eaten by sheep, pigs and poultry but are often not palatable for cattle and rabbits" (Robert, 1983). "Comfrey leaf contains 18 amino acids, rich in minerals and particularly high in K and is higher than many other forage crops in Ca, P, Fe and Cu" (Robert, 1983). Similarly, it was reported that comfrey leaf meal contains 18.37% crude protein, 11.87% nitrogen free extract, 5.25% ether extract, 47.12% fiber and 6.90% ash. It also contain minerals such as Cu (47.85mg/kg), Zn (374.2mg/kg), Mn (41.85mg/kg), Fe (349.65mg/kg), Ca (3242.2mg/kg), Mg (262.8mg/kg), Na (2433.4mg/kg) and K (2568.5mg/kg). Anti-nutritional factors contained in comfrey leaf include alkaloids (5.40%), flavonoid (18.36%),

saponin (14.95%) and tannin (0.543%) (Esiegwu and Obi, 2021). “Despite the nutritional benefits of comfrey, it contains anti-nutritional substances such as pyrrolizidine alkaloids (which is carcinogenic). Cases of hepatic veno-occlusive disease that were produced by using pyrrolizidine alkaloid capsules in USA has been reported” (Huxtable *et al.*, 1986). “Comfrey has been reported to cause liver damage and cancerous tumours in rats” (Teynor *et al.*, 1992). “Alkaloids extracted from Quaker comfrey leaves and injected in rats at dosage of 9-71mg of alkaloid/kg body weight three times per week for several weeks caused liver damage or death” (Culvenor *et al.* 1980). In Nigeria, many families and herbal doctors are still harnessing the benefits of comfrey leaves for treatment of various ailments, and as a rich source of vegetable and blood tonic. There is dearth of information on the nutritional and health benefit of comfrey leaves by researchers and its potential toxic risks in Nigeria.

Therefore, the aim of this research was to investigate the effect of comfrey symphytum leaves extract on the growth performance, haematological and serum biochemical indices and Histopathology of finisher broiler chickens.

MATERIALS AND METHOD

Experimental site

“This experiment was carried out at the poultry unit of teaching and research farm, Imo State University, Owerri, which is located within the Southern-Eastern agro ecological zone of Nigeria. Owerri lies between latitude 5⁰29’North and longitude 7⁰20’East. It is about 91m above sea level with annual rainfall, temperature and humidity ranging from 1,500mm to 2,200mm, 20.0 - 27.5⁰c and 75 - 90% respectively” (Accuweather, 2015).

Source and processing of comfrey symphytum leaves extract

The comfrey plant was planted in Imo State University teaching and research farm, Owerri. 200g of the fresh leaves were squeezed in 1 liter of water with a juicer to produce comfrey symphytum leaves extract. The comfrey leaves extract were mixed in drinking water at the rate of 0ml/liter of H₂O, 50ml of extract/liter of H₂O, 100ml of extract/liter of H₂O and 150ml of extract/liter of H₂O. Initially, the comfrey leaves meal were dried and sent to the laboratory for proximate, phytochemical and mineral analysis according to AOAC (2010).

Experimental diets

The birds were fed normal broiler finisher diet containing 19% crude protein and 2900kcal/kg of energy as shown in Tables 1. T₁ was the control and contained no comfrey leaves extract. T₂ contained conventional supplement (Divertamin) and used at the factory recommended level (4g to 4litres of H₂O). T₃, T₄ and T₅ contained 50ml comfrey leaves extract/liter of H₂O, 100ml of comfrey leaves extract/liter of H₂O and 150ml of comfrey leaves extract/liter of water, respectively. The Divertamin contained multivitamine, electrolyte and amino acids and was used

at the factory recommended level. “The Divertamin used contained Vitamin A, 375000 IU; Vitamin D₃, 110000 IU; Vitamin E, 1000 IU; Vitamin K₃, 4350 mg; Vitamin B₂, 4350 mg; Vitamin B₆, 2350 mg; Vitamin B₁₂, 11350 mg; Vitamin C, 10000 mg; Nicotinic acid, 16700 mg; DL-Methionine, 10000 mg; L-lysine, 15000 mg; Potassium chloride, 87000 mg; Manganese sulphate, 12000 mg; Sodium sulphate, 212000 mg; Sodium Chloride, 50000 mg; Copper sulphate, 12000 mg; Zinc sulphate, 12000 mg. It is used in poultry to achieve optimum growth and production in the management of stress related to high temperature etc. It is indicated in the treatment of vitamin and mineral deficiency and in the management of stressful condition” Esiegwu et.al (2021).

Table 1: Ingredient and calculated nutrient composition of the experimental diet

Ingredient	Quantity
Maize	60.00
Soya bean	20.00
Fish meal	2.00
Blood meal	1.00
Palm kernel cake	4.25
Palm oil	1.0
Wheat offal	8.00
Bone meal	3.00
Salt	0.25
Vitamin premix	0.25
Lysine	0.15
Methionine	0.10
Calculated nutrient composition	
Crude protein (%)	19.00
ME kcal/g (%)	2926.33
Ether extract (%)	13.95
Ash (%)	3.08
Ca (%)	1.28
P (%)	0.97
Lysine (%)	1.08
Methionine (%)	0.70

Experimental design

“One hundred and fifty (150) day old Agritech broiler chicks were purchased from a certified poultry vendor in Owerri. The chicks were brooded for four weeks. Thereafter, the birds were divided into five groups of 30 birds each in a completely randomized design. Each group was further divided into three replicates of 10 birds each. Each of the groups was assigned to one of

the comfrey leaves extract. All the treatment groups were fed same normal broiler finisher diet. The initial live weights of the birds were taken and weekly thereafter. The birds were fed *ad libitum*. The birds in each treatment were served six (6) litres of the comfrey leaves extract mixture per day for the comfrey groups, six (6) litres of the Divertamin mixture per day for the Divertamin group and normal drinking water for the control. Clean fresh drinking water was supplied to the birds after the consumption of the comfrey leaves extract and the Divertamin water. The trial lasted for four weeks” Esiegwu et.al (2021).

Data collection

Feed intake was recorded daily and the birds were weighed weekly after the initial body weights were taken. Feed intake was determined by weighing the feed offered and the left over the next morning. The difference between the two values was the feed consumed. Feed conversion ratio was determined by dividing average daily feed intake by average daily body weight gain.

Economics of production

Economic parameters determined were; Average weight changes; Average daily weight gain, Average daily feed intake, cost/kg weight gain, cost of total feed consumed

Cost of production (₦) = cost/kg weight gain multiplied by average weight changes.

Price/kg meat (₦) = price of selling one kg of meat

Revenue (₦) = price/kg meat multiplied by average weight change

Gross margin (gain) = Revenue minus cost of production

Haematological and serum indices studies

“At the last day of the feeding trial, three birds per treatment were randomly selected to determine their haematological and serum indices. 5ml blood samples were collected from the wing vein of the birds using syring and needle and placed in the specimen bottles with EDTA (Ethylene Diamine Tetra Acetate) for haematological studies. Blood was analysed within three hours of collection for haemoglobin (HB), packed cell volume (PCV), red blood cell (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and white blood cell (WBC) as outlined” by Ochie and Kolhatkar (2000). “Another 5ml of blood samples were collected and placed in the specimen bottles without EDTA for serum analysis. Blood samples placed in the specimen bottles without EDTA were used to analyse the serum biochemical parameters such as urea, total protein, creatinine, cholesterol, serum electrolytes and liver enzymes as outlined” by Ochie and Kolhatkar (2000).

Histopathological examinations

Histopathological examinations were carried out according to Baker *et al.* (1989). **Collected specimen** were fixed in 10% **neutral buffered formalin** for 24 hours and dehydrated in ascending

grades of alcohol (70, 80 and 90% absolute) and de-alcoholized in xylene for 30 minutes. The tissues were impregnated in molten paraffin wax and subsequently embedded using disposable plastic embedding moulds. The embedded tissues were sectioned with haematoxylin and eosin (H/E) staining procedure. The sections were examined under microscope (DM 500 leica binocular microscope) and observations noted. The examined slides were photo-graphed with leica DM 500 binocular microscope with photomicrographic accessories.

Statistical analysis

“Data collected were subjected to analysis of variance using the SPSS software (2012). Where analysis of variance indicated significant treatment effects, means were compared using Duncan’s New Multiple Range Test (DNMRT)” (SPSS, 2012)

Results

Performance characteristics of finisher broilers offered comfrey leaves extract are presented in Tables (2). There were no significant treatment effect ($P>0.05$) on the average initial weight, average final weight and average daily feed intake. Average weight changes, average daily weight gain and feed conversion ratio were significantly ($P<0.05$) influenced by dietary treatments. The data on economics of production are shown in Tables (3). Cost of feed (₦220.11) was increased at T₂ (Divertamin group) compared to other treatments. The results of the haematological and serum biochemical indices of broiler finishers offered comfrey leaves extract are presented in Tables 4 and 5. The haematological indices analysed did not show any treatment effect ($P>0.05$) except the eosinophil and mean cell haemoglobin. The biochemical parameters showed that only the alkaline phosphate (ALK) and chloride were significantly ($P<0.05$) affected while all other parameters were not influenced by dietary treatments ($P>0.05$). Alk showed a significant difference. T₂ (Divertamine group) was significantly increased compared to T₄ (100ml extract/liter of H₂O) but statistically similar to the control. T₁ (control), T₃ (50ml extract/liter of H₂O) and T₅ (150ml extract/liter of H₂O) were similar ($P>0.05$) statistically. The histopathological examination of some organs are shown in plates 1 to 5 of each organ.

Discussion

The average daily feed intake which were not influenced by dietary treatment ($P>0.05$) implied that the anti nutritional factors (ANF) in comfrey leaves extract did not depress the appetite of the birds. It could suggest that the ANF present in the juice extract were within tolerable level. The performance of the broilers improved as the inclusion level of the comfrey leaves extract increased. The comfrey leaves extract influenced the average weight gain of the birds in T₅ (150ml extract/liter of H₂O) resulting in better feed conversion ratio although, statistically similar to the control (T₁) and the Divertamin group (T₂). This is attributed to the rich nutrient contained in comfrey leaves especially proteins and its high amino acid profile and minerals which is necessary for growth and replenishment of lost tissues. Moreso, the nutrient in comfrey leaves has high digestibility (between 37 to 77%) (Robert, 1983). The feed conversion ratio was lower than the value 3.33 to 3.70 for finisher broilers offered Moringa oleifera leaf water (Esiegwu, 2019) but within the range 2.07 to

2.56 for broiler chickens fed varying levels of *Seasum indicum* leaves (Adedeji, 2019) and higher than the reference value 1.7 to 2 for broilers (Ghosh, 2015). The performance of T₅ (150ml comfrey extract/liter of H₂O) for average weight changes (1876.67g), average daily weight gain (65.719) and feed conversion ratio (2.08) was best across treatments indicating effective metabolism and utilization of amino acids in the comfrey leaves extract. The heavier body weight gain of broilers offered comfrey leaves extract could be attributed to a likely higher intake of amino acids (Tijani *et al.*, 2016). This shows that the inclusion or use of comfrey leaves extract improves performance considerably. Comfrey leaves extract enhanced performance better than conventional supplement which could be due to improved digestibility and nutrient utilization.

The high cost of feed (₦220.11) in T₂ (Divertamin group) was due to the high cost of conventional supplements and this is what we want to curtail and this is the reason for this research. Cost per kg weight gain was also highest at T₂ (₦495.25) compared to other treatments as a result of high cost of feed and poor feed conversion ratio, comparatively. Cost of production rose very high above others at T₂ due to high cost per kg weight gain and relatively low average weight changes. T₅ (150ml extract/liter of H₂O) revealed a higher revenue earnings (₦2439.67) and better gross margin (profit) (₦1782.84) as a result of the better performance of the comfrey leaves extract in producing heavier average weight changes and better feed conversion ratio (low value for feed conversion ratio). In financial terms, T₅ revealed a lower cost per kg weight gain, higher revenue (₦2439.67) and higher gross margin (₦1782.84) comparatively. In the market every money counts. The factor responsible for the financial merit of T₅ (150ml extract/liter of H₂O) were better value of feed conversion ratio due to the efficacy of the non conventional supplement (comfrey leaves extract) to produce heavier body weight change and also the reduced cost of comfrey leaves extract. It could be concluded from economics of production that the use of comfrey leaves extract as a nutritional supplement resulted in heavier body weight gain, and better feed conversion ratio leading to lower cost per kg weight gain, higher revenue and gross margin (profit) earning. Therefore, it is more economical to use some natural sources of nutrient supplement such as comfrey leaves extract to improve performance of broilers and have higher returns on investment due to cost effectiveness.

Haemoglobin (Hb) did not show any significant treatment effect ($P > 0.05$), however the values were within normal reference range, 7-13g/100ml (Banergee, 1998 and Jain, 1989) and 6.5-9g/100ml (Swenson, 1977). The packed cell volume (PCV) were not significantly different ($P > 0.05$) across treatments but fall within the normal reference value 25- 45% (Banergee 1998); 22-35% (Jain 1989) and 30-33% or 35-40% for male chickens (Swenson, 1977). The red blood cells (RBC) were also within normal reference value $7-1.2 \times 10^{12}/l$ (Jain, 1989).

Table 2: Performance characteristics of broiler finisher chickens Offered comfrey leaves extract.

PARAMETER	T ₁	T ₂	T ₃	T ₄	T ₅	SEM
Average Initial weight (g)	479.33	470.00	478.00	479.00	476.67	17.45
Average final weight (g)	2286.67	2126.67	2038.67	2299.33	2353.33	94.62
Average weight changes (g)	1807.34 ^{ab}	1656.67 ^{ab}	1560.67 ^b	1820.00 ^{ab}	1876.67 ^a	86.79
Average daily weight gain (g)	64.55 ^{ab}	59.17 ^{ab}	55.74 ^b	65.01 ^{ab}	67.02 ^a	3.02
Average daily feed intake (g)	135.23	133.19	139.07	133.13	134.22	3.35
Feed conversion ratio	2.09 ^{ab}	2.25 ^{ab}	2.49 ^a	2.05 ^b	2.00 ^b	0.14

Ab means within the same row with different superscripts are significantly different (P<0.05).

T₁ = Control (Normal water, 0ml/liter of H₂O); T₂ = conventional supplement (Divertamin)

T₃=50ml extract/liter of H₂O; T₄=100ml extract/liter of H₂O; T₅=150ml extract/liter of H₂O

Table 3: Economics of production of broiler finishers offered comfrey leaves extract.

Parameters	T ₁	T ₂	T ₃	T ₄	T ₅
Cost/kg of feed (₦)	170.20	220.11	173.01	174.03	175.00
Cost of feed consumed (₦)	642.91	820.47	672.32	649.30	656.83
Cost/kg weight gain (₦)	355.72	495.25	430.79	356.76	350.00
Cost of production (₦)	642.91	820.47	672.32	649.30	656.83
Revenue (₦)	2349.54	2153.67	2028.87	2366.00	2439.67
Gross margin (₦)	1706.63	1333.20	1356.55	1716.70	1782.84

Note: Selling Price per kg meat is ₦1300

Awodi *et al.* (2005) and Chineke *et al.* (2006) reported that packed cell volume, haemoglobin and mean cell haemoglobin are major indices for evaluating circulating avian erythrocytes and are very useful in the diagnosis of anaemia and also serve as a useful indices of the bone marrow capacity to produce red blood cells as in mammals. The values of the PCV, Hb, and RBC showed that the birds were not anaemic and the bone marrow was intact. The non significant treatment effect was a pointer to the fact that comfrey leaves extract supplied the birds with additional Fe and proteins for the haemoglobin to circulate adequate erythrocytes in the blood thereby keeping the birds immune system intact. The comfrey leaves extract did not indicate any deleterious effect on the haematological indices of the birds as the values did not fall below the normal reference range. Kepeme *et al.* (2011) reported that when the PCV values are below the normal range, the chickens were anaemic. The mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were not significantly different (P>0.05). The MCHC fall within normal range (Jain, 1989). This non significant treatment (P>0.05) effect on these indices implies that the production of red blood cells by the bone marrow was adequate across treatment. White blood cell fall within normal range $9-31 \times 10^3/\text{mm}^3$ (Banergee, 1998). This suggests that there was no toxin or infection arising from the ingestion of the comfrey leaves extract. It has been

reported that leukocyte counts as well as heterophil and lymphocytes ratio were used as indicators of stress responses and sensitive bio markers that are crucial to immune functions (Shaniko, 2003). The white blood cells and the differentials are good indicators of infection or presence of toxin and rises in the presence of infection.

Table 4: Haematological indices of broiler finishers offered comfrey leaves extract

PARAMETER	T ₁	T ₂	T ₃	T ₄	T ₅	SEM
Haemoglobin(g/dl)	12.77	13.00	12.90	12.63	12.60	0.24
Packed cell volume (%)	39.67	41.33	40.00	38.00	37.67	1.98
White blood cell(t)	11.60	11.93	11.33	11.60	11.40	0.21
Neutrophil%	54.67	56.33	53.33	55.00	53.33	1.83
Eosinophil%	1.33 ^{ab}	1.00 ^b	1.33 ^{ab}	1.00 ^b	2.00 ^a	0.21
Basophil%	0.00	0.00	0.00	0.00	0.00	0.00
Lymphocytes%	42.67	41.33	43.33	42.00	43.00	1.71
Monocytes%	1.33	1.33	2.00	2.00	1.67	0.26
Red blood cell (x10 ^{12/L})	12.90	13.13	13.13	12.63	12.77	0.24
ESR(MM ³ /1 st hour)	26.67	23.33	26.67	33.33	36.67	6.15
MCV(PI)	30.73	31.47	30.43	30.03	29.43	0.99
MCH(Pg)	9.87 ^b	9.90 ^{ab}	9.83 ^b	10.00 ^a	9.83 ^b	0.04
MCH(g/l)	32.27	31.57	32.27	33.33	33.67	1.07

Ab means within the same row with different superscripts are significantly different (P<0.05).

The number of the WBC increases in acute infection or toxicity. Eosinophil increases in allergy and in parasitic infection (Banergee, 1998). The white blood cells showed that the comfrey leaves extract had no deleterious effect on the blood of the birds. The biochemical indices, urea, creatinine, cholesterol, total proteins and the liver enzymes (alkaline phosphate (ALK), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT)), showed no significant difference (P>0.05) across treatments. Serum urea is a strong indices used in monitoring protein quality and the extent of amino acid balance. It has been reported that high level of serum urea was an indicator of low protein quality as a result of imbalance of amino acids (Nworgu, *et al.*, 2007). The non-significant difference (P>0.05) of the serum urea was a pointer to normal serum protein quality which means that the comfrey leaves extract did not import any contamination or exact any negative effect to protein-amino acid metabolism. Total protein, albumin and globulin were not affected by treatment significantly (P>0.05). This is a pointer to the balance of nutrients, consumption and metabolism of adequate protein and amino acids by the birds as seen in the performance indices. The protein needs of the birds across treatments were met. The non-significant difference of the globulin and albumin across treatments was a pointer to intact immune system and healthy birds. Sanchez Monge *et al.* (2004) reported that increased globulins are seen in chronic infections, liver damage and kidney dysfunction. Serum albumin is a strong predictor of health; a low albumin concentration is a sign of poor health (Kastow, 2009). Aletor and Egberongbe (1992) reported that the lower the value

of creatinine the higher the protein quality. Ross *et al.* (1978) also reported that abnormal high blood creatinine would indicate muscle wastage and imply that the animal was surviving at the expense of body reserves which also results in weight loss. The non-significant value ($P>0.05$) of the creatinine was a sign of nutritional adequacy of protein in both quality and quantity. The cholesterol values were not significantly different ($P>0.05$). This was an indication of proper fat metabolism and utilization. Fat was adequately mobilized to the tissues and utilized for all the treatments. In order words, there was no interference on fat metabolism by any anti-nutritional factor. Ukpabi *et al.* (2015) reported that serum enzyme activities are used for checking toxicity as well as monitoring protein quality and damages done to the liver. The enzymes (ALK, SGPT, SGOT), were statistically similar to the control. It was a sign that there was no pronounced toxic effect on the blood and liver of the birds. That means, the comfrey leaves extract did not impact any negative or deleterious effect on the blood of the birds.

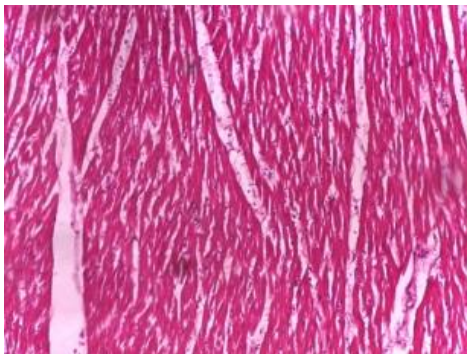
Table 5: Biochemical Indices of broiler finishers offered comfrey leaves extract

PARAMETERS	T ₁	T ₂	T ₃	T ₄	T ₅	SEM
Urea (mg/100ml)	6.83	7.20	7.10	6.67	6.77	0.34
Creatinine (mg/100ml)	62.67	65.67	63.67	59.33	60.67	3.34
Cholesterol (mmol/L)	8.27	8.63	8.47	7.93	8.13	0.37
Total protein (mg/100ml)	60.33	63.00	61.33	58.33	59.33	2.86
Albumin	23.00	23.67	22.33	21.33	22.00	1.53
Globulin	37.33	39.33	39.00	37.00	37.33	1.95
Sodium Na ⁺ (mmol/L)	41.33	42.00	42.33	40.33	41.33	1.08
Potassium K ⁺ (mmol/L)	1.27	1.27	1.23	1.23	1.23	0.04
HCO ₃ ⁻ (mmol/L)	11.27	11.33	11.10	11.17	11.13	0.14
Chloride Cl ⁻	55.67 ^a	22.67 ^a	23.00 ^a	22.00 ^a	22.33 ^a	26.31
ALK (iu/L)	1.23 ^{ab}	1.37 ^a	1.23 ^{ab}	1.20 ^b	1.30 ^{ab}	0.04
SGOT (iu/L)	11.23	11.40	11.30	11.20	11.23	0.14
SGPT (iu/L)	6.47	6.67	6.73	6.73	6.60	0.16

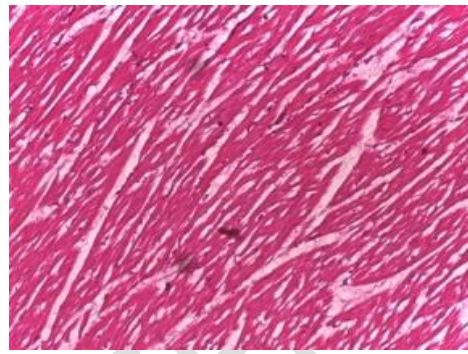
Ab means within the same row with different superscripts are significantly different ($P<0.05$).

The histology section of the heart from Plate (P) 1:T₁ to Plate (P) 5: showed cardiac muscle with unremarkable myocyte nuclei, muscle fibre and myocardium. There was no observable histologic changes in the heart across treatments. The heart was normal indicating that the antinutritional factors in comfrey leaves extract were not deleterious to the heart. The kidney tissue did not show any histologic change or alteration in the architecture. Histology section showed kidney tissue with unremarkable glomeuli, bowmans capsule, tubules tufts and stroma. This still was an indication that the comfrey leaves extract had no negative effect on the kidney cells within the dosage level used. Histology section of the liver tissue for T₁ and T₂ showed hepatic tissue with unremarkable central vein, sinuroids and lamina of hepatocytes. The hepatocytes and other stroma elements appear normal. T₁ and T₂ representing the control and the divertamin group, respectively had no issue with the liver. T₃ and T₄ (Plate 3 and 4) revealed

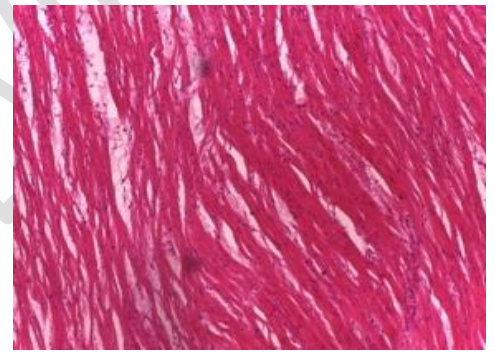
hepatic tissue with unremarkable central vein and effaced tissue stromal elements. T₅ (plate 5) showed effaced tissue stromal elements with congestion and marked cellularity. The effaced tissue stromal elements, congestion and marked cellularity observed in T₅ were earlier signs of an onset of toxicity or abnormality and could be attributed to the anti nutrients contained in the comfrey leaves extract especially alkaloids. The findings support the report of Culvenor *et al.* (1980) that alkaloids extracted from Quaker comfrey leaves and injected in rats at dosage of 9-71mg of alkaloid/kg body weight three times per week for several weeks caused liver damage or death and Teynor *et al.* (1992) reported that comfrey caused liver damage and cancerous tumours in rats.



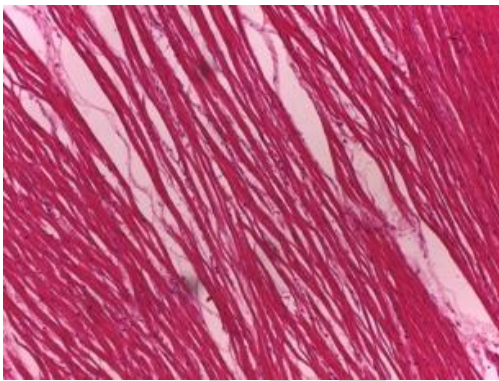
P1: Heart T₁



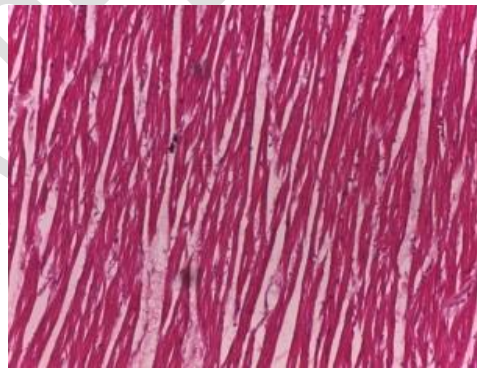
P2: Heart T₂



P3: Heart T₃

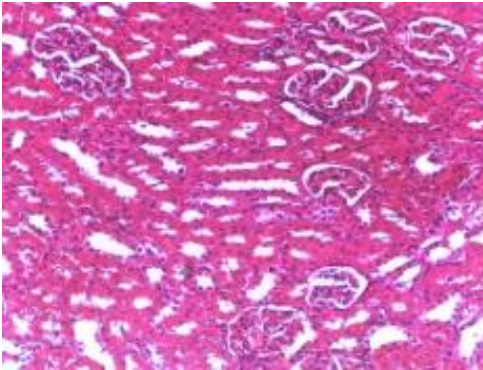


P4: Heart T₄

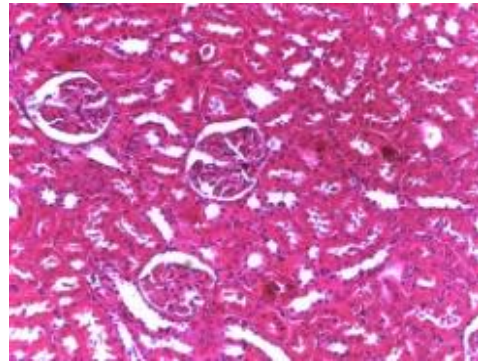


P5: Heart T₅

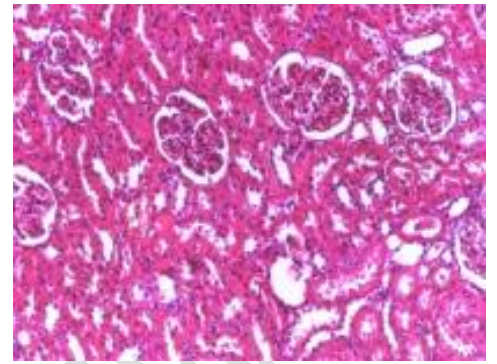
The histology section of the heart



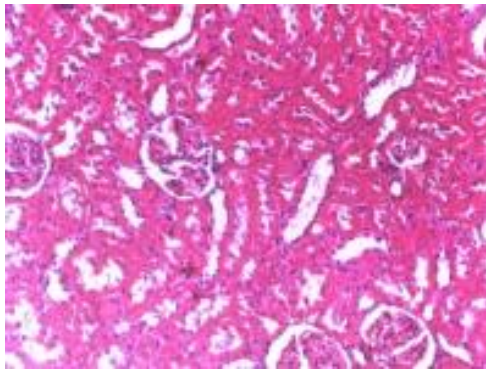
P1: kidney T₁



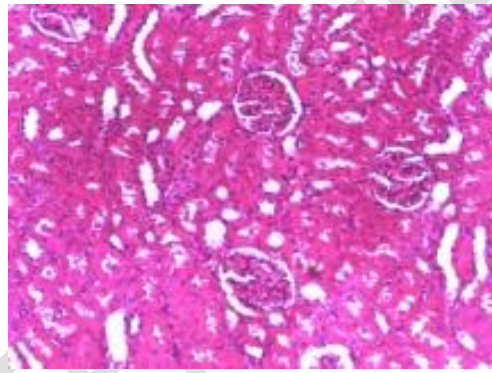
P2: kidney T₂



P3: kidney T₃

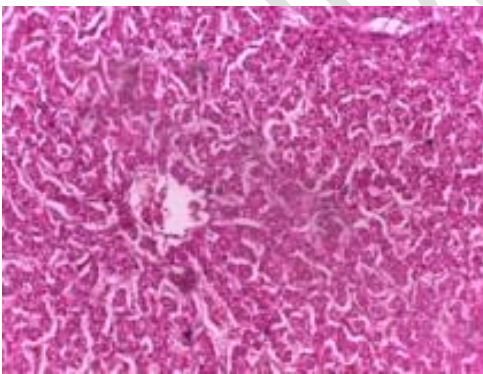


P4: kidney T₄

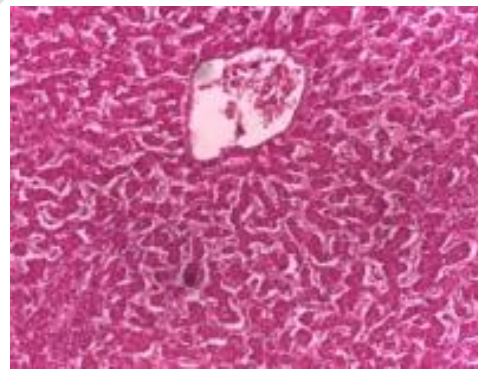


P5: kidney T₅

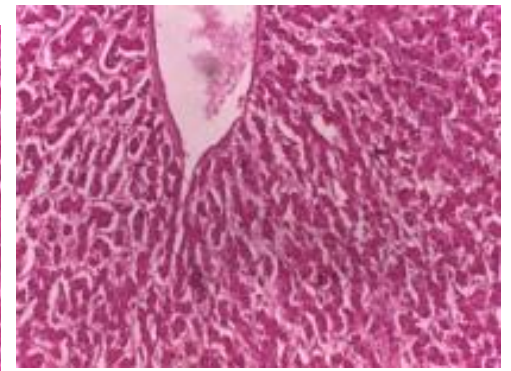
Histology section of the kidney



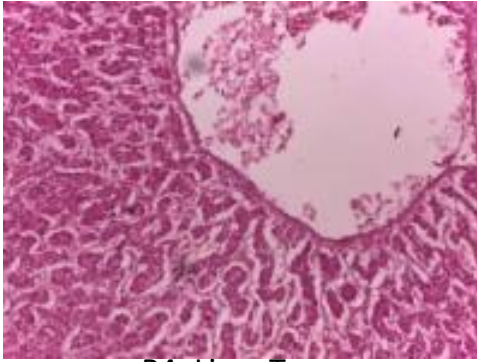
P1: Liver T₁



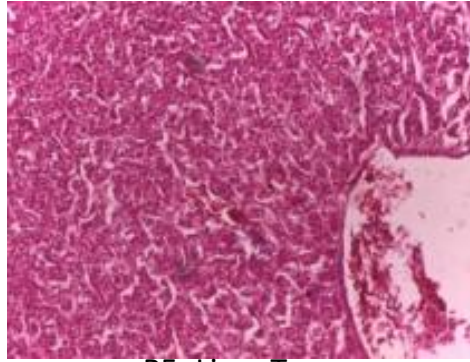
P2: Liver T₂



P3: Liver T₃



P4: Liver T₄



P5: Liver T₅

Histology section of the liver tissue

Note: P means Plate

Conclusion

This study showed that the use of comfrey leaves extract as a nutritional supplement in broiler finisher chicken was beneficial in improving average weight gain and feed conversion ratio at 100ml to 150ml of comfrey leaves extract/litre of H₂O.

The study also revealed that the use of cheap available natural sources of supplements such as comfrey leaves extract reduced cost/kg weight gain, increased the revenue earnings and gross margin (profit).

The study also showed that comfrey leaves extract had no visible deleterious effect on the haematological and serum indices of broiler finisher chickens within the dosage level used.

The study revealed that comfrey leaves extract had no damaging effect on the heart and the kidney.

Histopathological examinations of the organs revealed that comfrey leaves extract initiated gradual histological alteration of the liver cells in the form of effaced tissue stromal elements, congestion and marked cellularity of the liver.

It was concluded that comfrey leaves extract was not only cost effective but improved performance of broiler finishers at 100ml to 150ml of extract/litre of water

Recommendations

It was therefore recommended that 100ml to 150ml of comfrey leaves extract per liter of H₂O should be used for oral supplementation on broiler finisher chickens. Its daily use should not exceed four weeks to avoid liver degeneration.

In the future, it may be necessary to develop a packaging method to store comfrey leaves extract for use in the broiler industry to reduce cost and improve productivity. There is need also to try this dosage level on other poultry and livestock. There is need to find a way of reducing the anti-nutritional factors in the extract to avoid any deleterious effect on any animal.

Studies should be carried out on other leaf vegetables as a replacement for conventional supplements.

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Conflict of interest

I declare that there is no conflict of interest in this paper. The work is original document of the authors.

Authors' contribution

Esiegwu, AC and Obih, TKO., jointly designed this work. Data collection was done by the two authors. Obih, TKO carried out the statistical analysis while Esiegwu, AC did the write up.

REFERENCES

- Accuweather (2015). Weather for Owerri, Nigeria. Available at <http://www.accuweather.com/en/ng/owerri/253317/weather-forecast/253317>. Retrieved on November 15, 2015.
- Adedeji, O.S., Amao, S.R. and Olugbemiga, J.S. (2019). Effect of *Sesamum indicum* leaves on growth performance and carcass characteristics of broiler chickens. *Nigerian J. Anim. Sci.* 21 (1): 222-232.
- Aletor, V.A. and Egberongbe.O. (1992). Feeding differently processed soybean. Part 2: An assessment of haematological responses in the chicken diet. *Nahrung*, 36(4): 364 - 369.
- AOAC (2010). Official methods of analysis.19th edition. *Association of official Analytical chemists*. Washington D. C. USA.

- Akubue, P., Kar, A., Nnacheta, F. (1980). Toxicity of extracts of roots and leaves of *Telfairia occidentalis*. *Planta Medica*, 38(4): 339 – 343.
- Awodi, S., Ayo, J.O., Atodo, A.D. and Dezende, T. (2005). Some haematological parameters and the erythrocyte osmotic fragility in the laughing dove (*Streptopella senegalensis*) and the village weaver bird (*Ploceus cuculoptus*). In: Dairo, F.A., Fajemilehin, S.O.K. and Onibi, G.E. (Eds), Proceedings of 10th annual conference of Animal Science Association of Nigeria, University of Ado Ekiti, Ekiti State, Nigeria, 384-387.
- Baker, F. J., R. E. Silvertown and C. J. Palister (1989). *Introduction to medical laboratory technology*, Bounty Press Ltd. Pp:148
- Banerjee, G.C. (1998). *A textbook of animal husbandry*. 8th edition, Oxford and IBH pub ltd. Pp.118 - 143.
- Chineke, C.A., Ologun, A.G., and Ikeobi, C.O.N (2006). Haematological parameters in rabbit breeds and crosses in humid tropics. *Pakistan Journal of Biological Science*. 9 (11): 2102-2016.
- Culvenor, C.C.J., Clark, M., Edgar, J.A., Frahn, J.L., Jago, M.V., Peterson, J.E. and Smith, L.W. (1980). Structure and toxicity of the alkaloids of Russian comfrey (*Symphytum x uplandilum* Nyman), a medicinal herb and item of human diet. *Experientia*, 36: 377-379.
- Esiegwu, A.C. (2019). The supplementary effect of moringa oleifera leaf water on performance and blood indices of finisher broilers. *Direct Research Journal of Veterinary Medicine and Animal Science*, Vol. 4 pp. 27-32.
- Esiegwu, A. C. and Obih, T. K. O. (2021). Growth performance, haematological and serum biochemical indices of broiler starter chickens offered dietary supplement of comfrey leaves extract. *Journal of Agriculture and Food Sciences*. Volume 19 Number1, pp 48 – 62.
- F.A.O. (2010). The state of food insecurity in the world. Annual report. P62.
- Fasuyi, A.O. and Nonyerem, A.D (2007). Biochemical, nutritional and haematological implications of *Telfaina occidentalis* leaf meal as protein supplement in broiler starter diet. *African Journal of biotechnology*, Vol. 6 (8), pp. 1055-1063.
- Gala, D.R., Gala, D. and Gala, S. (2003). Juice-Diet for perfect health. Navneet publication (India) limited., Dantali, Gujarat.
- Ghosh, N. (2015). *Poultry Science and Practice. A Textbook*. CBS Publishers and Distributors pvt Ltd.

- Hills, L.D. (1976). *Comfrey Fodder, food and remedy*. Universe books, Ny. Pp 253.
- Huxtable, R.J., Luthy, J. and Zweifel, U. (1986). Toxicity of comfrey-pepsin preparations. *New Eng. Journal of Med.* 315(17):1095.
- Ifon, E.T. and Basir, O. (1980). The nutritive value of some Nigerian leafy vegetables, particularly vitamin and mineral contents". *Food chemistry*, Vol 4, pp. 263-267.
- Jain, N.C. (1989). *Veterinary Haematology*, 4th edition, Lea and Febiger, Philadelphia, USA.
- Kastow (2009). Globulin, Total proteins available at <http://www.drkastow.com/htm/protein.albumin.globulin.htm>.
- Kayode, A.A.A., Kayode, O.T., Odetola, A. A. (2010). *Telfairia occidentalis* ameliorates oxidative brain damage in malnourished rats. *International Journal of Biological Chemistry*, 4: 10 – 18.
- Kepeme, E.V., Elaluu, V., Koofreh, M.E and Udensis, O. (2011). Phytochemistry and haematology potential of ethanol seed, leaf and pulp extracts of carioca papaya (inn). *Pakistan Journal of Biological Sciences*, 14: 408-411.
- Nwanna, E. Oboh, G. (2007). Antioxidant and hepatoprotective properties of polyphenol extracts from *Telfairia occidentalis* (Fluted pumpkin) leaves on acetaminophen induced liver damage. *Pakistan Journal of Biological Sciences*, 10(16): 2682 – 2687.
- Nworgu, F.C., Ogunberro, S.A, and Solesi S.S. (2007). Performance and some blood chemistry indices of broiler chickens served fluted pumpkin (*Telfaina occidentalis*) leaf extract supplement. *American Eurasian Journal of Agric and Environ* 2(1): 90 - 98.
- Ochie, J. and Kolhatkar, A. (2000). *Medical laboratory science. Theory and practice*. Tata McGraw-Hill company limited New Delhi.
- Robert, G.R. (1983). *Comfrey-A controversial crop*. Minnesota Report, Item No. AD-MR-2210. Agricultural Experiment Station University of Minnesota MR-191.
- Ross, J.G., Halliday, W.G. and Jones, R.M. (1978). Haematological and blood chemistry comparison values for clinical pathology. *Poultry veterinary record*, 102: 29 - 39.
- Sanchez, M., Rilopez-torrejou G., Paschal C.Y., Vaulag Martin-Estaben, M.J. and Sakedoi, G. (2004). *Clinical and experimental allergy*. 34(11): 1747-1753.
- Shaniko, S. (2003): Physiological responses of laying hens to the alternative housing system. *International Journal of Poultry Science* 2: 357 - 360.

SPSS (2012). *Statistical package for social sciences*, version 21. USA IBM Corporation.

Swenson, M.J. (1977). *Dukes physiology of domestic animals*. 9th edition CBS publishers ed distributors, PVT Ltd, India, pp. 14-35.

Teynor, T.M., Putnam, D.H., Doll, D.J., Kelling, K.A., Oelka, E.A., Undersander, D.J. and Oplinger, E.S. (1992). *Comfrey: alternative field crops manual*. <https://hort.purdue.edu>.

Tijani, L. A., Akanji, A.M., Agbalaya, K. and Onigemo, M.O. (2016). Haematological and serum biochemical profiles of broiler chickens fed diets containing moringa leaf meals. *Journal of Tropical Agriculture, Food, Environment and Extension*. Volume 14 number 3, 7-11

Ukpabi, U. H., Mbachu, C. I., and Nwazue, B. (2015). Effect of inclusion of different levels of raw Adenantherapavoninaseed meal (RAPSM) on haematology and blood biochemistry of finisher broiler. *Nigerian Journal of Animal Science*, 17 (1), 28-36.

Esiogwu, A. C., & Obih, T. K. O. (2021). Growth performance, haematological and serum biochemical indices of broiler starter chickens offered dietary supplement of comfrey leaves extract. *Journal of Agriculture and Food Sciences*, 19(1), 48-62.