EFFECTS OF HOT WATER–PREPARED *SIDA ACUTA* AQUEOUS LEAF EXTRACT SUPPLEMENTED WITH DIETS ON SERUM ENZYMES AND PROTEINS OF LAYING HENS

Ademola, S.G.[1], Arasi, K.K.[1]*, Adio, A.A.[1], Okimi R.A.[1], Odunsi, A.A.[1], Shittu, M.D.[2], Akinwumi, A.O.[1], Ige, D.O.[1], Akindele, A.M.[1] and Animashaun, A.A.[1]

[1]Department of Animal Nutrition and Biotechnology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State Nigeria

[2]Department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

*Corresponding author- kolapoarasi16@gmail.com 08033655995

ABSTRACT

One hundred and twelve (112) ISA Brown points of lay birds wererandomlydistributedinto7 treatment groups of 4 replicates per group with 4 birds in each replicate. Seven experimental diets were formulated for the study. A corn-soy bean meal- maize offal-based diet (basal diet) was the control. The basal diet was supplemented with 2ml and 4ml of hot water-prepared Sida acuta aqueous leaf extracts (100,125 and 150mg/L). Basal diet supplemented with 2ml and 4ml/kg hot water-prepared Sida acuta aqueous leaf extract (100g/L) respectively represented T1 and T2. Basal diet supplemented with 2ml and 4ml/kg hot aqueous extract (125g/L) respectively denoted T3 and T4. Basal diet supplemented with 2ml and 4ml/kg hot aqueous extract (150g/L) respectively tagged T5 and T6. The experimental birds were not vaccinated and medicated throughout the experimental period. However, the experimental hens had access to administration of multivitamin fortnightly of 5days weekly. Phytochemicals such asphytate, flavonoids, steroids and terpenoids were in higher concentrations in hot water-prepared Sida acuta aqueous leaf extracts than those of the leaf of S. acuta. There was significant interaction (P=0.044) effect of dosage and inclusion on serum ALP with the highest in T4. It was concluded that the supplementation of 4ml/kg of 125g/L of hot water-prepared Sida acuta aqueous leaf extract with the diet elevated serum ALP. This is suggestive of detrimental effect to the health of the hens when used for prolong period.

Keywords: *Sida acuta,* Layers, Medication, Phytochemical, Serum.

INTRODUCTION

Antibiotic use has increased as a result of the massive increase in poultry production to meet Nigeria's growing demand. This has raised concerns about the number of cases of antibiotic resistance in humans and animals that have been discovered through food consumption, environmental contamination, and direct contact. The result has been a high incidence of cancer and other illnesses. Over 100 different forms of cancer afflict people, according to the NCI (2012) (Diaz-Sanchez *et al*., 2015). This statistic led the European Union (2006) to suggest further options that fall under the category of natural growth promoters (NGPs). Nature has been a source of medical substances for thousands of years and an astounding number of modern medications have been obtained from natural sources. The usage of these substances in conventional medicine served as the basis for many of these isolations. In addition to performing numerous biological activities like antibacterial, antifungal, anti-inflammatory, anti-allergic, anti-helmintic, hepato-protective, analgelsic, neuroprotective, and immune-modulatory actions, bioactive chemicals, also known as phytochemicals, in plants have a variety of pharmacological effects on human and animal health (Shittu and Alagbe, 2020).

Currently, indigenous people employ *Sida acuta* Burm.f (Malvaceae) as one of those herbs to treat various health issues. This plant grows to a height of around 1.5 meters and is an erect, branching tiny perennial herb or shrub (Mohideen *et al*., 2002). According to Sreedevi *et al*. (2009), the plant has smooth, greenish bark, a thin, long, cylindrical, and extremely rough root, lanceolate, almost glabrous leaves, peduncles that are equal to the petioles, yellow flowers that can be solitary or in pairs, and smooth, black seeds. The plant is commonly known as “Udo”or “ire-agwo” in Igbo,“Osanpotu” in Yoruba, “Riegueyoto” in Bini and “Itseketu” in Ora (Akobundu andAgyakwa, 1998). The proximate composition of *S. acuta* (%) was reported by Raimi *et al*. (2014) as 9.03+0.06 moisture, 19.13+0.15 crude protein, 0.67+0.06 fat, 6.33+0.06 ash, 9.50+0.01 crude fibre and 55.30+0.10 carbohydrate. According to Nwankpa *et al*. (2015) *S. acuta* has a phytochemical composition (mg/100g) of 91.46±0.02 tannin, 1500.36±0.36 alkaloid, 530.27±0.03 saponin, 1163.86±0.1 flavonoid, 1454.50 ± 0.85 steroids, 115.29±0.05 terpenoids and 851.62±0.01 cardiac glycosides. Despite the usage of *S. acuta plants* in traditional medicine, its utilization in poultry is very limited. Hence, the current study explored the bioactive compounds in the plant as a substitute for drug in laying hens.

MATERIALSANDMETHODS

Experimental Site

The experiment was carried out at the Poultry Unit, Teaching and Research Farm, LAUTECH, Ogbomoso.

Collection of the Leaves and Processing of *Sida acuta* Extract

Fresh and matured leaves of *Sida acuta* were collected within the environment of Teaching and Research Farm. The leaves were cleaned under running water. The leaves were sundried for2hours daily for 5days. The leaves were then pulverized to coarse powder with an electric blender. The study adopted a modified method of (Shittu and Alagbe, 2020). Specifically, samples of *Sida acuta* powder (100, 125 and 150g) in conical flask (1000ml) were soaked in 1000ml distilled water, place in electric water bath at 600C for 60 minutes and allowed to cool. Thereafter, it was left for 18 hours, and it was agitated in an electric blender for 10minutes. After homogenization of the mixture of leaves with distilled water, the homogenized solution was sieved with muslin cloth. The collected hot aqueous leaf extracts of the *Sida acuta* were then kept in the refrigerator until it was required for supplementation to experimental diets.

Management of Experimental Birds

A total of 112 ISA Brown points of lay (POL) birds of 20 weeks of age were obtained from a reputable farm. The POL birds (at 20% daily egg production) were randomly distributed into7 treatment groups of 4 replicates per group. There were 4 birds in each replicate, making a total of 16 birds per treatment group. Feed and water were supplied ad libitum to the experimental birds. The experimental birds were not administered any medication and vaccination throughout the experimental period. However, the experimental hens had access to administration of Multivitamin fortnightly of 5days weekly.

Formulation of Experimental Diets

Seven experimental diets were formulated for the study. A corn soy bean meal maize offal-based diet (basal diet) was the control. The basal diet was supplemented with 2ml and 4ml of hot water-prepared *Sida acuta* aqueous leaf extracts (100,125and150mg/L). Basal diet supplemented with 2ml and 4ml/kg hot aqueous extract (100g/L) respectively represented T1 and T2, respectively. Basal diet supplemented with 2ml and 4ml/kg hot aqueous extract (125g/L) were respectively denoted as T3 and T4. Basal diet supplemented with 2ml and 4ml/kg hot aqueous extract (150g/L) respectively represented T5 and T6. The ingredient composition of experimental diet is shown in Table 1. The basal diet (%) contained 50 maize, 15 SBM, 17 maize offal, 5 wheat offal, 3 fishmeal (72%CP), 1.7 bone meal, 7.70 limestone, 0.1 methionine, 0.25 vitamin premix and 0.25 salt.

Experimental Design

A completely randomized design was adopted for the study. A 3x2 factorial arrangement was also used for the test ingredient. There were three (3) methods of preparation of hot aqueous extract of *Sida acuta* leaf namely, 100mg, 125mg and 150mg, each into 1000ml of distilled water. The inclusion levels of the hot aqueous extract were 2ml/kg and4ml/kg diet. The diet was characterized by 2726.3 kcal/kg ME and 16.89% CP.

Egg weight and Hen Day Production (HDP)

The weights of eggs laid were measured using a digital scale and recorded in grams (g). Hen day production was calculated using the formula below.

$$\text{Hen Day Production} = \frac{\text{Number of eggs laid X 100}}{\text{Number of hens X number of days}}$$

Serum Analysis

This was carried out on the70th day of the experiment. Two (2) laying hens from each replicate was used to determine the serum indices. Blood samples were collected using a 5.0ml needle and syringe through the brachial wing vein into bottle without anticoagulant. Serum total protein was determined using direct Biuret method as described by Lubran, (1978). The serum albumin was determined using Bromocresol green method (Doumas and Peters, 1997). Serum globulin was calculated as follows:

Globulin = total serum proteins – serum albumin (Coles, 1986).

The activities of alanineaminotransferase (ALT) and aspartateaminotransferase (AST) were determined using techniques described by Reitman and Frankel (1957). Alkaline phosphatase (ALP) enzyme action was measured in serum, as described by Kim and Wyckoff (1991).

Chemical Analysis

The photochemical constituent of leaf and hot water-prepared *Sida acuta* aqueous leaf extracts was determined using the standard procedures of AOAC, (2005).

Statistical Analysis

Data collected were analysed using One-Way Analysis of Variance of SAS (2004) software package. A 3x2 factorial ANOVA was used to estimate main and interaction effect of dosage of hot aqueous extract (100, 125 and 150g per litre of distilled water) and inclusion level (2 and 4 ml). Duncan's option of the same software was used to compare the means. A probability of 5 percent was considered significant.

RESULT AND DISCUSSION

The phytochemical constituents of leaf meal and hot aqueous extract of *Sida acuta* are presented in Table 2. The phytate content in hot aqueous leaf extracts was similar (166.32, 162.87and 155.73mg/100g) but higher than those of the leaf (120.92mg/100g). Oxalate was also similar in leaf and hot aqueous leaf extracts of *Sida acuta*. The highest tannins and flavonoids were noticed in 150g hot aqueous leaf extract of *Sida acuta*, while the lowest was revealed in *Sida acuta* leaf. The steroids of hot aqueous leaf extract (41.4,39.90,47.49mg/100g) were higher compared to the leaf (18.71mg). There were comparative terpenoids content in hot aqueous leaf extract of *Sida acuta* (47.31,62.42,60.02mg/100g) and the leaf (43.67mg/100g). Generally, phytochemicals such as phytate, flavonoids, steroids and terpenoids were in higher concentrations in hot *S. acuta* extract than those of the leaf of *S. acuta* .

Table2: Phytochemical constituents of leaf meal and hot aqueous extract *of Sida acuta*

Parameters	**Leaf**	**← Hot Aqueous Leaf Extracts of *S. acuta* →**		
		100g/L	**125g/L**	**150g/L**
Phytate(mg/100g)	120.92	155.73	162.87	166.32
Oxalate (mg/100g)	9.39	5.07	6.08	7.40
Tannins (mg/100g)	86.64	93.55	90.2	97.84
Flavonoids (mg/100g)	52.72	96.78	164.78	178.81
Alkaloids(mg/100g)	13.94	7.08	12.55	10.90
Steroids(mg/100g)	18.71	41.41	39.70	47.49
Terpenoids(mg/100g)	43.67	47.31	62.42	60.02

The concentration of various phytochemicals in the leaf of the present study was lower to the findings of Nwankpa et al. (2015) for *Sida acuta* leaf (mg/100g), 1500.36±0.36 alkaloid, 1163.86±0.1 flavonoid, 1454.50 ± 0.85 steroid,115.29±0.05 terpenoids and 851.62±0.01 cardiac glycosides except for 91.46±0.02 tannin. The composition of the leaf extract from the study of Shittu and Alagbe (2020) showed higher phytochemicals such as 4.25% (4250mg/100g) flavonoids, 0.23%(230mg/100g) phytate, 1.05% (1050mg/100g) steroids, 0.95% (950mg/100g) terpenoids than those of the present study. Earlier study by Ezeabara and Egenti (2018) reported 0.55mg/100gof alkaloids, 2.31mg/100g flavonoids and 1.85mg/100g steroids in methanol extracts of S.acuta. Shittu and Alagbe (2020) attributed the differences to age at harvest or the variety of the Sida acuta. These constituents in leaf and plants extracts vary from one plant to another. Other factors are geographical location, soil type, age of plants, method of harvesting and extraction (Alagbe et al., 2021). Phytochemicals have beneficial use in livestock production as antioxidants (specifically flavonoids, tannins) in animal feed, which will protect animals from oxidative damage caused by free radicals and are also regarded to have effect-enhancing and /or side effect neutralizing potential (Lillehoj and Lee, 2012). Flavonoids or bioflavonoids are potent antioxidant agents and protect the cells by scavenging and inhibiting the production and initiation of free radicals, superoxide anions and lipid peroxy radicals (Niu et al., 2017).

Table3: Laying performance, Serum proteins and enzymes of laying hens fed hot aqueous leaf extract of *Sida acuta*

Parameters	**Control**	**T1**	**T3**	**T4**	**T5**	**T6**	**P-value**	**SEM**	**Dosage Extract**	**Inclusion Level**	**Interaction**
		T2	**←125g/L→2ml**	**4ml**	**→150g/L←2ml**	**4ml**					

		→100g/L←										
		2ml	4ml									
HDP (%)	74.17	76.03	73.32	74.02	70.67	71.00	72.92	0.983	4.602	0.800	0.703	0.807
Egg weight (g)	55.63	57.60	57.40	55.84	55.43	57.40	59.03	0.121	0.946	0.051	0.684	0.549
AST (IU/L)	35.40[a]	20.13[c]	22.77[bc]	24.82[abc]	32.64[ab]	26.26[abc]	25.95[abc]	0.056	3.54	0.050	0.158	0.368
ALT(IU/L)	16.23	18.93	17.12	21.23	21.79	19.34	17.20	0.344	1.96	0.181	0.504	0.772
ALP(IU/L)	38.00	39.64	37.15	35.04	43.94	43.69	42.79	0.099	2.63	0.146	0.633	0.044
AST:ALT	2.18	1.06	1.33	1.17	1.50	1.36	1.51	-	-	-	-	-
TP(g/dl)	2.34	2.62	2.79	2.45	2.62	2.66	2.15	0.132	0.16	0.183	0.409	0.087
Albumin(g/dl)	1.81	1.62	1.84	1.60	1.71	1.56	1.38	0.131	0.12	0.079	0.596	0.212
Globulin(g/dl)	0.53	1.00	0.95	0.86	0.91	1.10	0.77	0.314	0.17	0.878	0.450	0.533

[abc]: Means along the same row with different superscripts are significantly different ($p<0.050$) AST =Aspartate aminotransferase; ALT=Alanineaminotransferase ALP=Alkalinephosphatase; TP = Total protein

Tannins contain antibacterial, anticarcinogenic, antiparasitic, and antioxidant properties. However, high amounts of tannins are well-known to be powerful toxins for the liver and kidneys, can lead to pathological changes in poultry tissues, including inflamed oesophageal mucosa, necrosis in the crop, gizzard, and duodenum, as well as bone abnormalities (Suleyman, 2017). Laying performance, serum proteins and enzymes of laying hens fed hot aqueous leaf extract of *Sida acuta* were presented in Table 3. Eggs from hens fed 2ml of 100g of hot aqueous extract of *Sida acuta* gave the heaviest egg weight, although dietary treatment did not impact significant (P=0.051) influence on egg weight. The phytochemicals in S. acuta extracts might have influenced egg weight as earlier observed by (Prasad and Ganguly, 2012) that phytochemicals present in Moringa leaf increased egg production and egg weight.

There was significant interaction (P=0.044) effect of dosage and inclusionon serum ALP with the highest in T4. The lowest serum albumin was noticed in hens fed 4ml/kg of 150g/L dosage of hot aqueous leaf extract of *S. acuta* (T6). Although significant reductions were observed in the serum AST of hens fed hot aqueous leaf extract of *S. acuta* supplemented diets. Much information is not available on the implication of low serum enzymes. However, it has been reported that serum aminotransferase (AST and ALT) are key enzymes in amino acid metabolism (Sepulveda, 2013). The serum total proteins were similar with control except T6. This indicated that serum Total protein of hens fed hot aqueous leaf extract of *S. acuta* did not interfere negatively on amino acid metabolism. However, the highest dosage of 150g/L of the hot aqueous leaf extract compromised the amino acid metabolism resulting in the lowest serum total protein. The ratio of AST/AST showed that the ratio was within the normal values (0.7 1.4) of healthy chickens (Sepulveda, 2013). Prolong or high doses of *S. acuta* may result in the development of neurological disorder when used for medicinal purposes (Eluwa et al., 2013).

CONCLUSION

The supplementation of 2ml/kgof100g/L of hot aqueous extract of *Sida acuta* with layers' diet increased egg weight while maintaining good HDP and serum total protein. However, feeding hot water-prepared aqueous leaf extract of *S. acuta* for 12 weeks period had detrimental effect on kidney function as there was elevated serum ALP.

REFERENCES

Akobundu, I .O., and Agyakwa, C. W.(1998).A Hand book of WestAfricanWeeds.2ndEdition,Intec printers, Ibadan. 348.

Alagbe, J. O., Shittu, M. D. and Eunice, A. O. (2021). Prospect of leaf extracts on the performance and blood profile of monogastric. *Biomedicine and Nursing.*7(3):14-20.

Association of Official Analytical Chemist AOAC. (2005). Official method of analysis. 19thEdition. Washington, D C., USA.

Coles, E. H. (1986). Veterinary Clinical Pathology. (EH Cole, editor), Fourth Edition. WB Saunders, NewYork.43–72.

Diaz-Sanchez, S., D'Souza, D., Biswas, D., and Hanning, I. (2015). Botanical alternatives to antibiotics for use in organic poultry production. *Poultry science*, *94*(6), 1419-1430.

Doumas, B. T. and Peters Jr, T. (1997). Serum and urine albumin: A progress report on their measurement and clinical significance. *Clinica Chimica Acta*,258(1):3–20.

Eluwa, M., Ofem, P., Asuquo, O., Akpantah, A. and Ekanem, T. B. (2013). Histological Study of the Effect of Ethanolic leaf extract of *Sida acuta* on the cerebral cortex of Adult Wistar Rats. *IOSR J. Dent. Med. Sci*, *8*, 60-63.

Ezeabara, C.A. and Egenti, M.O. (2018). Phytochemical and antimicrobial investigations on various parts of *Sida acuta* Burm. F. *Journal of Ayurvedic and Herbal Medicine,* 4(2):71-75.

Kim, E.E., and Wyckoff, H.W. (1991). Reaction mechanism of alkaline phosphatase based oncrystalstructures.Two-metalioncatalysis.*JournalofMolecularBiology*.218(2):449-64.

Lillehoj, H.S. and Lee, K.W. (2012). Immune modulation of innate immunity as alternatives-to-antibiotics strategies to mitigate the use of drugs in poultry production. *Journal of Poultry Science.*91(6):1286-1291.

Lubran,M.M.(1978).The measurement of total serum proteins by the Biuret method. *Annals of Clinical Laborat ory Science*.8(2):106//–110.

Mohideen, S., Sasikala, E. and Gopal, V. (2002). Pharmacognostic studies on *Sida acuta* Burm. f. *Ancient science of life*, *22*(1), 57-66.

NCI (National Cancer Institute). (2012).*Cancer Statistics*. Available online http://seer.cancer.gov/statfacts/html/urinb.html Date Accessed 14th December, 2024

Nwankpa, P., Chukwuemeka, O. G., Uloneme, G. C., Etteh, C. C., Ugwuezumba, P., and Nwosu, D. (2015). Phyto-nutrient composition and antioxidative potential of ethanolic leaf extract of *Sida acuta* in wistar albino rats. *African Journal of Biotechnology*, *14*(49), 3264-3269.

Niu, Y., Wan, X. L., Zhang, X. H., Zhao, L. G., He, J. T., Zhang, J. F. and Wang, T. (2017). Effect of supplemental fermented Ginkgo biloba leaves at different levels on growth performance, meat quality, and antioxidant status of breast and thigh muscles in broiler chickens. *Poultry Science*, *96*(4), 869-877.

Prasad, A. and Ganguly, S. (2012). Promising Medicinal Role of Promising Medicinal Role of *Moringa oleifera*: A Review. *Journal of Immunology and Immunopathology*, *14*(1).

Raimi, M. M., Oyekanmi, A. M., and Adegoke, B. M. (2014). Proximate, phytochemical and micronutrient composition of Sida acuta. *J. Appl. Chem*, *7*(2), 93-98.

Reitman, S. and Frankel, S. A. (1957). Colourimetric method for the determination of serumglutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of ClinicalPathology*,28:56–63.

SAS Institute Incorporated. (2004) SAS 9.1.3 Help and Documentation, Cary, NC.

Sepulveda, J. (2013). Challenges in routine clinical chemistry analysis: Proteins and Enzymes, In: *Accurate Results in Clinical Laboratory*.9.

Shittu, M.D. and Alagbe, J.O. (2020). Phyto-nutritional profiles of broom weed (*Sida acuta*) leaf extract. *Annals of Clinical Toxicoogy, 2020; 3 (2)*, *1032*.

Sreedevi, C. D., Latha, P. G., Ancy, P., Suja, S. R., Shyamal, S., Shine, V. J., ... and Rajasekharan, S. (2009). Hepatoprotective studies on *Sida acuta* Burm. f. *Journal of ethnopharmacology*, *124*(2), 171-175.

Suleyman, C. (2017). Tannins and their effects on poultry nutrition. In *International Conference Agriculture, forestry, food Science and Technology. Cappadocia, Turkey* (pp. 15-17).