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Study of the Bio-Tolerance of Aqueous extract of *Thonningia sanguinea* (Balanophoraceae) in Isa Brown Laying Hens

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Abstract

This experiment was conducted to evaluate the effect of aqueous extract of Thonningia sanguinea (THOS) on blood parameters and organs of Isa Brown laying hens. To do this, 220 laying hens of 16 weeks old (1113 ± 90 g lively mass) were randomly divided into two (02) groups of 110 hens each. The hens of group II (treated group) were treated with THOS for a week (07 days) at a rate of 10g/L while the hens of group I (control group) received only water without any additives. Blood samples were taken at day 0 (D0) (before treatment), D3 (third day of treatment) and D8, D14, D21 and D28 (after treatment) for determination of some biochemical and hematological parameters. Furthermore six hens in each group were slaughtered on day 8 (D8), D14, D21 and on D28. Vital organs (heart, liver, lungs, kidneys, spleen, duodenum, thymus and bone marrow) were then removed for histopathological analysis. THOS supplementation had no significant effect on hematological parameters as well as on the activity of serum enzymes such as ALT; AST; GGT and CPK but it significantly lowered the activity of ALP and LDH. Compared to the control group, THOS did not significantly affect electrolytes such as Na^+; Cl^-; Ca^{2+}; Mg^{2+} but significantly decreased potassium (K^+) at D21 and D28. Regarding metabolites (glucose, urea, creatinine, bilirubin, total protein and uric acid), THOS has not significantly altered carbohydrate; renal and protein metabolism but it has led to a significant decrease in triglycerides levels at D3 and D21. However, total cholesterol and total lipids levels have not undergone significant change. The analysis of histological sections of various organs showed nor tissue change, nor tissue damage. These results suggest that supplementation with THOS is well tolerated by laying hens organism and that its use would cause no major danger for the survival of laying hens.

Keywords: Laying hens, *Thonningia sanguinea*; THOS, hematological parameters, serum electrolytes, serum markers.

Introduction

Food security remains a cardinal challenge in sub-Saharan Africa, where nearly 201 million people, which represent one third of total population (23.7%), suffer from hunger and malnutrition[1]. The poultry sector is a major asset for food security. To do this, the managers in this sector are constantly looking for food additives in order to improve the quality of poultry feed and animal health. The use of additives allows not only control of pathogens such as salmonella but also strengthens the natural microflora[2]. Although antibiotics are playing this role, intensive useand sometimes misuse has led to appearance of more or less rapid multirésitantmicrobial strains[3]. Moreover, the emergence of new controversies about food safety and consumer safety as well as restrictions in the European Union for production without antimicrobial have led to a growing mistrust concerning proposed molecules for animal health[4].

The research was then sharpen on other prevention methods such as immunization and use of plant extracts, spices or essential oilsin poultry farming[5].

Several recent studies have shown beneficial effects of natural products for poultry health. These products may be food additives to improve animal health and productivity[6]. *Thonningiasanguinea* (balanophoraceae) a plant of Ivorian pharmacopoeia gave promoter results about poultry farming. In fact, the works of M'baïasbé *et al.*[7] and Ouattara *et al.*[8,9], revealed that the aqueous extract of *Thonningiasanguinea* (THOS) has a curative activity against avian salmonellosis and it improves production parameters such as egg production rate, egg weight and thickness of egg shell in laying hens.

This plant also has an outstanding antifungal activity and contains phenolic compounds in abundance[10]. According to N'Guessan *et al.,*these compounds with high antioxidant

properties help eliminate free radicals that accumulate in laying hens organs during their growth[11]. The brevifolin carboxylic acid and gallic acid were isolated from the aqueous extract of *Thonningia sanguinea*[11]. More recently, Kouakou *et al.*[12] and Konan *et al.*[13] revealed an anti coccidial effect *in vivo* and *in vitro* of THOS in laying hens.

Given these properties, this plant would be of great interest in improving productivity of poultry farms. But before any action being taken, a study of the biotolerance of THOS is required. The present study will evaluate the influence of THOS on the hens' body. To do this, in this study, various biochemical markers of different organs of chicken were evaluated and histopathological analyzes of these organs were also carried out to detect any tissue and cell damages.

Materials and Methods

Vegetal material: The inflorescences of *Thonningia sanguinea* (balanophoraceae) obtained from Yamoussoukro's region (central Côte d'Ivoire) were used. *Thonningia sanguinea* is an herb plant like, perennial and fleshy plant up to 20 centimeters high. It is also a nonspecific and exclusive parasite of various plants roots[14,15]. The thickened tubers and rhizomes are commonly sold in local markets for medicinal purposes. *Thonningia sanguinea* is present throughout the humid forest zone of Africa. In Côte d'Ivoire, it's found from the coast to Bamoro forest[16]. *Thonningia sanguinea* is found on plant markets of Abidjan under the name of "Blo-ablêlê" (bush pineapple) in local language "Agni"[17]. This plant is available from vendors of medicinal plants at all times but it's abundant in rainy season.

Preparation of THOS: All of the inflorescences were rid of any impurities by washing, cut into strips and dried in open air away from sunlight. These inflorescences were then pulverized in a blender (IKAMAG-RCT®, Labortechnick, USA). One hundred grams (100g) of powder were dissolved in 1 liter of deionized water and homogenized in a blender. The homogenate obtained was drained on a square of fabric and the filtrate was then filtered three times on absorbent cotton and once on wattman filter paper. This second filtrate thus obtained was put to dry in an incubator (45-50^{oC}) until complete evaporation of the solvent[18]. The resulting powder was put in an airtight jar and kept away from moisture.

Animals and experimental protocol: Two hundred and twenty (220) one day old Isa Brown hens (35 ± 3g lively weight) were obtained from Ivoire Poussins, a company of SIPRA group (Ivorian Society of Animal Production). The birds were acclimatized under standard conditions of poultry livestock in the laboratory of Veterinary Centre of Bingerville (LANADA) for sixteen (16) weeks. During the acclimatization period, birds were provided with standard food obtained from Ivograin Bingerville Company (ingredients shown on Table-1). After 16 weeks of acclimatization, hens (1113 ± 90g lively weight) were randomly allocated into two (02) groups of 110 birds each. The hens of group II (test or THOS supplemented group) were treated with THOS for a week (07 days) at a rate of 10g/L of water while the hens of group I (control group) received only water without any additives. Food and water were given *ad libitum* to hens. Blood samples were collected from the wing vein using a venoject or winged needle[19] at D0 (before treatment), D3 (third day of treatment) and D8, D14, D21 and D28 (8, 14, 21 and 28 days after treatment) for determination of biochemical and hematological parameters. Furthermore six hens in each group were slaughtered on day 8 (D8), D14, D21 and on D28. Vital organs (heart, liver, lungs, kidneys, spleen, duodenum, thymus and bone marrow) were then removed for histopathological analyses.

Hematological analyzes: About 3 milliliters (mL) of blood were collected in EDTA tubes at D0 (before treatment), D3 (third day of treatment) and D8, D14, D21 and D28 (8, 14, 21 and 28 days after treatment). These blood samples were then analyzed by a blood cell counter Swelab alpha (Boule Medical AB) which determines the number of white blood cells (WBC); red blood cells (RBC) and platelets (PLT). It also determines hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin content (MCH). Leukocyte formula (the different types of WBC) and eventual cellular abnormalities were determined by the method of thin smears stained with May Grunwald-Giemsa (MGG).

Biochemical analyzes: About 3 mL of blood was collected in a dry tube (additives free) at D0 (before treatment), D3 (third day of treatment) and D8, D14, D21 and D28 (8, 14, 21 and 28 days after treatment). After clotting, the samples were centrifuged immediately at 3500 revolution per minute (rpm) for 5 minutes using a centrifuge EAB 20 (Hettich centrifuges). Serum was then collected in sterile microtubes and stored at -20^{o}C until analyzed using a spectrophotometer (Mindray BA-88A: Mindray Medical International Limited) according to the protocol provided by the manufacturer of the different analysis kits.

Histopathological analyzes: During the experiment period, dead animals were autopsied to determine the cause of death. In addition, six hens in each group were slaughtered on day 8; D14, D21 and D28 after treatment. Organs (heart, liver, kidney, lungs, spleen, thymus, bone marrow duodenum) were removed to make cuts for histopathological analysis. The histopathological techniques are summarized in the various stages of classical histology consisted of preparing samples (organs removed and fixed in 10% formaldehyde) followed by standard colorations: HES and special: MGG, PAS, for observed by light microscopy. As for the bone marrow, it first undergoes decalcification in hydrochloric acid for 48 h before the fixation process in formaldehyde. MGG stainingand modified Wright staining were adopted for blood smear.

Statistical analyzes: Data Analyzes were carried out using Graph Pad Prism software version 5. The comparison of means two (2) by two (2) was made by Welch's t-test that analyses columns 2 by 2. While ANOVA (analysis of variance) one way followed by Dunnett's post-test was used to compare the different groups to the control group. The confidence interval is 95%, the average means were significantly different when $P < 0.05$.

Table-1
Composition of the basal diet used during the experiment

Ingredients	Stater diet	Grower diet	Laying diet
Metabolisable energy (kcal/kg)	3150	3127.87	2998.49
Crude protein (%)	20.5	18.02	17.01
Crude fat maters (%)	4.08	4.51	3.46
Crude ash (%)	6.14	6,72	13.2
Crude cellulose (%)	4.31	5.05	3.98
Calcium (g/kg)	9.44	10.26	34.91
Phosphorus (g/kg)	6.47	6.36	5.92
Sodium (%)	0.18	-	-
Vitamin A (UI/kg)	12500	0	0
Vitamin D3 (UI/kg)	2500	10000	7500
Vitamin E (UI/kg)	2500	10000	7500

Results and Discussion

Influence of dietary supplementation with THOS on hematological parameters: The influences of dietary supplementation with THOS on hematological parameters are summarized in Table-2.

Dietary supplementation with THOS did not significantly affect blood cells in both control and THOS supplemented groups throughout the experimental period. White blood cells (WBC) and red blood cells (RBC) counts as well as hematocrit levels didn't significantly varied were compared with their respective initial values at D0. This same observation was made at the mean corpuscular hemoglobin concentration (MCHC) and mean hemoglobin concentration (MHC). On the other hand, after three days of treatment, THOS supplementation resulted in a significant reduction in hemoglobin level (HGB) from the initial rate (D0). However no significant difference in hemoglobin values was finding in both control and THOS supplemented groups. There was also no significant change in the numbers of the leukocyte types (neutrophil, eosinophil, lymphocyte and monocyte) in control and THOS supplemented groups. In addition the analysis of thin smears revealed no morphological changes in the different blood cells.

Outcome of dietary supplementation with THOS on electrolytes: The results of dietary supplementation with THOS on electrolytes concentrations are summarized in Table-3.

During the experiment, serum sodium (Na^+) increased slightly in both control and THOS supplemented groups. But this increase is only significant at day 21 (D21) of the experiment ($P < 0.05$). There was also an increase in serum potassium (K^+) in control group from the initial rate at D0, but this increase was only significant at day 21. Serum potassium concentration did not increased significantly from D3 to D14 before normalized from D21. Furthermore, by comparing control group to THOS treated group, THOS supplementation caused a significant reduction in serum potassium from D21. In contrast to serum potassium and serum sodium, serum chloride (Cl^-) decreased significantly from D3 to D7 before normalized from the 14^{th} day of experimentation regardless of the treatment administered. As for the concentrations of calcium (Ca^{2+}) and magnesium (Mg^{2+}), they were not significantly affected during the experiment except a single significant increase in magnesium levels at D3 when hens are treated with THOS.

In sum, THOS supplementation had no significant impact on serum ion balance in hens.

Influence of dietary supplementation with THOS on serum enzymes activity: The outcomes of dietary supplementation with THOS on serum enzymes activities are summarized in Table-5.

The activities of serum enzymes AST (aspartate aminotransferase) and ALT (alanine aminotransferase) were significantly reduced compare to their initial levels (D0) regardless of the group considered. However, and during experiment, any significant different were observed on the activity of enzymes ALT and AST in both control and THOS treated groups. In opposite to AST and ALT, the activities of serum enzymes gamma-glutamyl transferase (GGT) and creatine phosphokinase (CPK) didn't vary significantly during experiment period.

Whereas aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase and creatine kinase activities remained relatively similar in both groups, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were significantly modified in the experimental groups compared to the controls ($P < 0.01$). In fact, ALP activity decreased gradually from its initial level (D0) with a significant decrease at D28. When compare to control group, ALP activity decreased significantly from D14 to the end of experiment (D28) in THOS

treated group. LDH activity also deceased from its initial value with a significant decrease from D7 to D14. THOS supplementation has significantly decrease LDH activity from day 21 (D21) when compare to control group.

In sum, the enzymatic activity of the different enzymes studied was relatively similar in both groups except LDH and ALP where THOS supplementation led to a significant decrease in enzyme activity.

Outcome of dietary supplementation with THOS on serum metabolite concentrations: The variation of various metabolites concentrations during the experiment is summarized in Table-4.

During the experimental period, blood glucose had significantly increased only at day 14 but it was quickly normalized since D21 in THOS treated group. Bilirubin (total and conjugated) concentrations were reduced significantly from D3 to the end of the experiment (D 28) in both control and THOS treated groups. However, there was no significant difference between control and THOS treated groups. This same effect was observed for concentrations of total protein; total cholesterol; triglycerides and total lipids. On the other hand, THOS supplementation did not significantly affect uric acid concentration. Furthermore the serum concentration of urea increased significantly on day 3 before normalized from D7 in both groups. As for creatinine concentration, it has also been significantly reduced at day 3 before normalized from D7. These effects were observed invariably in both control and TOHS supplemented groups regardless of the type of treatment.

Outcome of dietary supplementation with THOS on organ tissues: Analyzes of the histological sections revealed no minor or major tissue damage in the various organs studied (heart, liver, kidney, lung, spleen, thymus, bone marrow and duodenum) in both control and THOS supplemented groups over the experiment period. THOS supplementation did not modify tissue integrity of laying hens.

Table-2
Outcome of dietary supplementation with THOS on hematological parameter

Parameters	D0	D3		D7		D14		D21		D28	
		Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
WBC (10^9/L)	97.7^{a} ±2.92	102^{a}_{c} ±3.62	98.6^{a}_{c} ±2.96	98.0^{a}_{c} ±3.80	104.9^{a}_{c} ±4.03	101.3^{a}_{c} ±2.52	101.1^{a}_{c} ±3.51	97.09^{a}_{c} ±2.00	93.52^{a}_{c} ±3.04	98.7^{a}_{c} ±2.44	101.5^{a}_{c} ±2.71
RBC (10^{12}/L)	2.91^{a} ±0.05	2.74^{a}_{c} ±0.06	2.70^{a}_{c} ±0.05	2.74^{a}_{c} ±0.05	2.88^{a}_{c} ±0.05	2.74^{a}_{c} ±0.07	2.80^{a}_{c} ± 0.08	2.74^{a}_{c} ±0.04	2.74^{a}_{c} ±0.04	2.76^{a}_{c} ±0.04	2.80^{a}_{c} ±0.06
HGB (g/L)	16.9^{a} ±0.31	15.7^{a}_{c} ±0.37	15.3^{b}_{c} ±0.28	15.4^{b}_{c} ±0.23	16.6^{a}_{c} ±0.34	16.5^{a}_{c} ±0.27	16.3^{a}_{c} ±0.46	15.8^{a}_{c} ±0.29	15.8^{a}_{c} ±0.19	16.2^{a}_{c} ±0.21	16.5^{a}_{c} ±0.21
HCT (%)	39.0^{a} ±0.98	37.0^{a}_{c} ±0.70	36.9^{a}_{c} ±0.64	37.5^{a}_{c} ±0.59	38.46^{a}_{c} ±0.37	37.8^{a}_{c} ±0.90	38.5^{a}_{c} ±0.95	37.8^{a}_{c} ±0.75	38.1^{a}_{c} ±0.45	36.9^{a}_{c} ±0.52	37.8^{a}_{c} ±0.67
MGV (fl)	133.9^{a} ±2.51	135.3^{a}_{c} ±1.35	136.8^{a}_{c} ±0.79	135.1^{a}_{c} ±1.25	133.5^{a}_{c} ±1.69	138.9^{b}_{c} ±0.83	136.4^{a}_{c} ±0.92	137.3^{a}_{c} ±1.04	139.3^{b}_{c} ±0.83	133.6^{a}_{c} ±0.69	134.1^{a}_{c} ±1.19
MHC (pg)	58.4^{a} ±0.54	57.2^{a}_{c} ±0.76	56.6^{a}_{c} ±0.80	56.2^{a}_{c} ±0.50	57.4^{a}_{c} ±0.42	59.7^{a}_{c} ±0.81	58.74^{a}_{c} ±0.56	57.5^{a}_{c} ±0.41	57.8^{a}_{c} ±0.50	58.7^{a}_{c} ±0.45	58.4^{a}_{c} ±0.81
MCHC (g/dl)	43.8^{a} ±0.86	42.3^{a}_{c} ±0.65	41.5^{a}_{c} ±0.55	41.7^{a}_{c} ±0.43	43.1^{a}_{c} ±0.65	42.9^{a}_{c} ±0.57	43.1^{a}_{c} ±0.53	41.8^{a}_{c} ±0.39	41.5^{a}_{c} ±0.35	43.9^{a}_{c} ±0.40	43.7^{a}_{c} ±0.37
Neutrophil (%)	14^{a} ±1.9	16^{a}_{c} ±1.9	14^{a}_{c} ±2.9	15^{a}_{c} ±2.4	14^{a}_{c} ±2.3	14^{a}_{c} ±2.2	13^{a}_{c} ±1.9	13^{a}_{c} ±1.8	14^{a}_{c} ±2.0	14^{a}_{c} ±1.8	13^{a}_{c} ±2.1
Eosinophil (%)	3^{a} ±0.4	3^{a}_{c} ±0.3	4^{a}_{c} ±0.4	3^{a}_{c} ±0.5	5^{a}_{c} ±0.4	4^{a}_{c} ±0.5	3^{a}_{c} ±0.4	3^{a}_{c} ±0.3	4^{a}_{c} ±0.2	3^{a}_{c} ±0.3	3^{a}_{c} ±0.4
Lymphocyte (%)	81^{a} ±1.6	79^{a}_{c} ±2.9	81^{a}_{c} ±1.9	79^{a}_{c} ±2.2	81^{a}_{c} ±2.1	81^{a}_{c} ±1.6	80^{a}_{c} ±1.9	81^{a}_{c} ±1.6	80^{a}_{c} ±2.2	80^{a}_{c} ±1.5	81^{a}_{c} ±1.8
Monocyte (%)	3^{a} ±0.4	4^{a}_{c} ±0.4	3^{a}_{c} ±0.6	4^{a}_{c} ±0.5	3^{a}_{c} ±0.6	3^{a} ±0.6	4^{a}_{c} ±0.6	3^{a}_{c} ±0.4	4^{a}_{c} ±0.4	3^{a}_{c} ±0.6	4^{a}_{c} ±0.3

Means with different superscript letters (a / b) on the same line, are significantly different compared to means of D0 ($P < 0.05$). Means with different subscript letters (c / d) in the same cell, are significantly different compared to each other ($P < 0.05$). Results are expressed as mean ± standard error of the mean (SEM): (n = 110 in each group), WBC: white blood cells, RBC: red blood cells, HGB: Hemoglobin, HCT: Hematocrit, MGV: mean corpuscular volume, MHC: average hemoglobin concentration, MCHC: mean corpuscular hemoglobin concentration. Neutrophil, eosinophil, lymphocyte and monocyte are expressed in percentage of the number of white blood cells (%WBC).

Table-3
Outcome of dietary supplementation with THOS on electrolytes

Parameters	D0	D3		D7		D14		D21		D28	
		Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Na^+ (mEq/l)	149^{a} ±0.44	147^{b}_{c} ±1.02	148^{a}_{c} ±0.84	151^{a}_{c} ±0.71	150^{a}_{c} ±0.99	150^{a}_{c} ±0.75	150^{a}_{c} ±0.66	153^{b}_{c} ±0.22	153^{b}_{c} ±0.29	151^{a}_{c} ±0.51	151^{a}_{c} ±0.56
K^+ (mEq/l)	4.5^{a} ±0.04	5.0^{a}_{c} ±0.08	5.1^{a}_{c} ±0.10	4.9^{a}_{c} ±0.07	4.8^{a}_{c} ±0.10	5.1^{a}_{c} ±0.08	5.1^{a}_{c} ±0.09	5.4^{b}_{c} ±0.46	4.4^{a}_{d} ±0.04	5.0^{a}_{c} ±0.09	4.4^{a}_{d} ±0.08
Cl^- (mEq/l)	112^{a} ±0.30	106^{b}_{c} ±0.72	108^{b}_{d} ±0.60	110^{b}_{c} ±0.45	108^{b}_{c} ±0.55	111^{a}_{c} ±0.43	112^{a}_{c} ±0.43	112^{a}_{c} ±0.17	111^{a}_{c} ±0.18	112^{a}_{c} ±0.38	111^{a}_{c} ±0.32
Ca^{2+} (mg/L)	104^{a} ±0.94	107^{a}_{c} ±0.80	101^{a}_{d} ±2.21	103^{a}_{c} ±1.15	100^{a}_{c} ±1.18	97^{a}_{c} ±3.45	100^{a}_{c} ±2.39	109^{b}_{c} ±0.84	108^{a}_{c} ±1.03	101^{a}_{c} ±2.59	105^{a}_{c} ±2.16
Mg^{2+} (mg/L)	20^{a} ±0.15	20^{a}_{c} ±0.25	21^{b}_{d} ±0.29	20^{a}_{c} ±0.18	20^{a}_{c} ±0.18	20^{a}_{c} ±0.33	20^{a}_{c} ±0.29	21^{b}_{c} ±0.20	20^{a}_{d} ±0.19	20^{a}_{c} ±0.62	20^{a}_{c} ±0.51

Means with different superscript letters (a / b) on the same line, are significantly different compared to means of D0 (P <0.05). Means with different subscript letters (c / d) in the same cell, are significantly different compared to each other (P <0.05). Results are expressed as mean ± standard error of the mean (SEM): (n = 110 in each group)

Table-4
Influence of dietary supplementation with THOS on serum metabolites concentration

Parameters	D0	D3		D7		D14		D21		D28	
		Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Glycaemia (g/L)	1.89^{a} ±0.01	1.92^{a}_{c} ±0.02	1.93^{a}_{c} ±0.02	1.90^{a}_{c} ±0.01	1.91^{a}_{c} ±0.01	1.95^{a}_{c} ±0.02	2.04^{b}_{d} ±0.02	1.92^{a}_{c} ±0.01	1.91^{a}_{c} ±0.01	1.84^{a}_{c} ±0.03	1.87^{a}_{c} ±0.02
Urea (g/L)	0.07^{a} ±0.001	0.09^{b}_{c} ±0.005	0.09^{b}_{c} ±0.002	0.06^{a}_{c} ±0.001	0.07^{a}_{c} ±0.007	0.07^{a}_{c} ±0.002	0.07^{a}_{c} ±0.002	0.07^{a}_{c} ±0.001	0.07^{a}_{c} ±0.001	0.07^{a}_{c} ±0.001	0.07^{a}_{c} ±0.001
Creatinine (mg/L)	2.2^{a} ±0.05	2.0^{b}_{c} ±0.05	2.0^{b}_{c} ±0.06	2.1^{a}_{c} ±0.03	2.2^{a}_{c} ±0.04	2.2^{a}_{c} ±0.13	2.3^{a}_{c} ±0.11	2.0^{b}_{c} ±0.02	2.1^{a}_{c} ±0.03	2.3^{a}_{c} ±0.05	2.2^{a}_{c} ±0.05
Total Bilirubin (mg/L)	2.76^{a} ±0.10	2.96^{a}_{c} ±0.21	2.00^{b}_{d} ±0.14	2.20^{b}_{c} ±0.06	2.12^{b}_{c} ±0.09	1.78^{b}_{c} ±0.16	1.83^{b}_{c} ±0.19	1.87^{b}_{c} ± 0.06	1.75^{b}_{c} ±0.08	1.28^{b}_{c} ±0.07	1.53^{b}_{c} ±0.12
Conjugated Bilirubin (mg/L)	1.33^{a} ±0.05	1.13^{a}_{c} ±0.06	0.78^{b}_{d} ±0.04	1.13^{b}_{c} ±0.04	1.06^{b}_{c} ±0.04	0.94^{b}_{c} ±0.09	1.07^{b}_{c} ±0.09	0.83^{b}_{c} ±0.03	0.89^{b}_{c} ±0.04	0.71^{b}_{c} ±0.03	0.81^{b}_{c} ±0.07
Total Protein (g/L)	46.8^{a} ±0.49	39.7^{b}_{c} ±0.81	39.9^{b}_{c} ±0.89	37.0^{b}_{c} ±0.63	36.7^{b}_{c} ±0.56	42.6^{b}_{c} ± 0.49	41.4^{b}_{c} ±0.47	42.4^{b}_{c} ±0.30	41.9^{b}_{c} ±0.37	41.3^{b}_{c} ± 0.29	41.0^{b}_{c} ±0.26
Uric Acid (mg/L)	31.6^{a} ±0.71	36.1^{b}_{c} ±1.62	34.7^{a}_{c} ±1.30	31.3^{a}_{c} ±0.74	30.0^{a}_{c} ±0.78	29.4^{a}_{c} ±0.96	30.8^{a}_{c} ±1.10	30.0^{a}_{c} ±0.52	31.6^{a}_{c} ±0.64	27.2^{b}_{c} ±0.89	30.3^{a}_{d} ±0.96
Total Cholesterol (g/L)	1.10^{a} ±0.01	0.91^{b}_{c} ±0.03	0.99^{b}_{c} ±0.01	1.00^{b}_{c} ±0.02	1.02^{b}_{c} ±0.02	0.90^{b}_{c} ± 0.03	0.86^{b}_{c} ±0.03	1.04^{b}_{c} ± 0.01	1.01^{b}_{c} ±0.01	0.96^{b}_{c} ±0.04	1.03^{a}_{c} ±0.03
Triglycerides (g/L)	0.41^{a} ±0.01	0.55^{b}_{c} ±0.02	0.49^{b}_{d} ±0.02	0.28^{b}_{c} ±0.01	0.27^{b}_{c} ±0.01	0.26^{b}_{c} ±0.02	0.22^{b}_{c} ±0.02	0.58^{b}_{c} ±0.01	0.51^{b}_{d} ±0.02	0.33^{b}_{c} ±0.02	0.32^{b}_{c} ±0.02
Total Lipid (g/L)	3.09^{a} ±0.04	2.93^{a}_{c} ±0.06	2.95^{a}_{c} ±0.05	2.57^{b}_{c} ±0.03	2.61^{b}_{c} ±0.04	2.18^{b}_{c} ±0.10	2.16^{b}_{c} ±0.08	3.27^{b}_{c} ±0.03	3.14^{a}_{c} ±0.05	2.53^{b}_{c} ± 0.11	2.76^{b}_{c} ±0.09

Means with different superscript letters (a / b) on the same line, are significantly different compared to means of D0 (P <0.05). Means with different subscript letters (c / d) in the same cell, are significantly different compared to each other (P <0.05). Results are expressed as mean ± standard error of the mean (SEM): (n = 110 in each group)

Table-5
Outcome of dietary supplementation with THOS on serum enzymes activity

Parameters	D0	D3		D7		D14		D21		D28	
		Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
AST (UI/L)	199^{a} ±3.06	$181^{b}{}_{c}$ ±3.48	$171^{b}{}_{c}$ ±3.72	$192^{a}{}_{c}$ ±2.02	$187^{b}{}_{c}$ ±1.63	$162^{b}{}_{c}$ ±3.29	$167^{b}{}_{c}$ ±3.57	$152^{b}{}_{c}$ ±1.73	$156^{b}{}_{c}$ ±1.95	$155^{b}{}_{c}$ ±2.48	$159^{b}{}_{c}$ ±2.09
ALT (UI/l)	5^{a} ±0.18	$3^{b}{}_{c}$ ±0.20	$2^{b}{}_{c}$ ±0.52	$4^{a}{}_{c}$ ± 0.29	$4^{a}{}_{c}$ ±0.26	$3^{b}{}_{c}$ ±0.23	$3^{b}{}_{c}$ ±0.21	$4^{a}{}_{c}$ ±0.24	$3^{b}{}_{c}$ ±0.21	$3^{b}{}_{c}$ ± 0.25	$3^{b}{}_{c}$ ±0.18
GGT (UI/l)	30^{a} ±0.7	$29^{a}{}_{c}$ ±2	$30^{a}{}_{c}$ ±3	$30^{a}{}_{c}$ ±0.33	$31^{a}{}_{c}$ ±2	$32^{a}{}_{c}$ ±2	$30^{a}{}_{c}$ ±1	$31^{a}{}_{c}$ ±1	$29^{a}{}_{c}$ ±1	$30^{a}{}_{c}$ ±1	$31^{a}{}_{c}$ ±0.7
CPK (UI/l)	1507^{a} ±41	$1772^{a}{}_{c}$ ±64	$1796^{a}{}_{c}$ ±91	$1880^{a}{}_{c}$ ±12	$1907^{a}{}_{c}$ ±26	$1540^{a}{}_{c}$ ±9	$1560^{a}{}_{c}$ ±9	$1030^{a}{}_{c}$ ±17	$1000^{a}{}_{c}$ ±9	$1465^{a}{}_{c}$ ±85	$1522^{a}{}_{c}$ ±283
ALP (UI/l)	1160^{a} ±26	$1300^{a}{}_{c}$ ±43	$1261^{a}{}_{c}$ ±34	$1380^{a}{}_{c}$ ±109	$985^{a}{}_{d}$ ±2	$1731^{b}{}_{c}$ ±199	$1008^{a}{}_{d}$ ±88	$11132^{a}{}_{c}$ ±35	$913^{a}{}_{d}$ ±53	$997^{a}{}_{c}$ ±34	$814^{b}{}_{d}$ ±37
LDH (UI/l)	5557^{a} ±184	$6154^{a}{}_{c}$ ±90	$5320^{a}{}_{d}$ ±206	$3666^{b}{}_{c}$ ±622	$3643^{b}{}_{c}$ ±29	$4906^{a}{}_{c}$ ±549	$3946^{b}{}_{c}$ ±663	$5598^{a}{}_{c}$ ±141	$4834^{a}{}_{d}$ ±173	$6302^{a}{}_{c}$ ±17	$5499^{a}{}_{d}$ ±93

Means with different superscript letters (a / b) on the same line, are significantly different compared to means of D0 ($P < 0.05$). Means with different subscript letters (c / d) in the same cell, are significantly different compared to each other ($P < 0.05$). Results are expressed as mean ± standard error of the mean (SEM): (n = 110 in each group)

Discussion: Plasma electrolytes represent all the ions involved in control of the homeostasis and exchanges in the body. The most commonly evaluated in the body are calcium (Ca^{2+}) chloride (Cl^{-}), potassium (K^{+}), sodium (Na^{+}) and magnesium (Mg^{2+}). In this study, the mean values of these various constituents are all within normal ranges determined by Sturkie P.D.[20] and Mitruka B.M.[21]. Sodium is present in the extracellular compartment. It controls the interaction between this compartment and the intracellular compartment. It is also responsible for maintaining the osmotic pressure and controlling membrane permeability. During our experiment, the significant increase of sodium in both control et THOS treated groups (149 mEq/L to 153 mEq/L) is not outside the normal ranges: 144 mEq/L – 155 mEq/L; 139 – 155 mEq/L and 153 – 159 mEq/L respectively reported by Victoria *et al.*[22], Anonyme[23] and Aengwanich *et al.*[24]. This increase would likely be due to food intake. As a matter of fact, according to food intake causes a large fluctuation in serum sodium[25].

Potassium (K^{+}) as sodium controls the stability of water balance in the body. It is also involved in the transmission of nerve impulses. Its fluctuation therefore could be prejudicial to the hens' life. THOS supplementation has significantly normalized potassium levels of the laying hens. The aqueous extract of *T. sanguinea* therefore may act as a potassium overload eliminator. In fact, the cellular catabolism is known as a source of hyperkalemia, the aqueous extract of *T. sanguinea* because of its powerful antioxidant activity may regulate catabolism and correct hyperkalemia[26].

THOS supplementation did not significantly affect carbohydrates, lipid and protein metabolism. So were renal and biliary markers. The significant level decrease of lipids, bilirubin and protein observed when compared to the initial levels wouldn't be due to THOS supplementation since this phenomenon was observed in both THOS treated and untreated hens. In addition, no significant differences were revealed between THOS treated and untreated groups. Saponins present in THOS haven't had any effect on these different metabolisms although their hypoglycemic and low fat actions have been revealed in previous studies[27-32]. According to these authors, saponins reduce glucose's transport from the stomach to the small intestine's brush border[30]. As for the low-fat action, saponins from food intake would form complexes with sterols and bile acids thereby inhibiting the absorption of steroids by preventing them from crossing the intestinal wall[31,32].

In serum enzymology, the concentration level of enzymes used in the diagnosis of heart; liver and kidneys diseases (AST, ALT, GGT, CPK, LDH and PAL) is a reflection of the health status of these organs[33]. In this study, the significant decrease in ALT and AST activities would be due to normal physiological variation or food intake[25]. In fact, the significant decrease of these two enzymes was observed irrespective of the experimental group. In addition, there were no significant differences between the two experimental groups. The means values are consistent with those reported by Victoria A. Bowes[22] and Eren M.[34]. Concentrations of ALP and LDH were significantly reduced by THOS supplementation. This action was also observed by comparing both control and THOS treated groups.

This property of the aqueous extract of *T. sanguinea* could result from its hepatoprotective power. In fact, *T. sanguinea* aqueous extract has hepatoprotective action against aflatoxin B1 and an antioxidant power[26,35]. It would protect liver and muscles against poisonings xenobiotics and the harmful effects of certain drugs. This could also justify the fact that THOS supplementation has not caused significant changes on the cellular elements of blood revealed by blood count.

Conclusion

In sum, food supplementation with THOS (10 g/L) did not significantly alter the different metabolisms and the aptitudes of the hens' organs during the experiment period. It has conserved fluid balance so the osmotic pressure of fluid compartments of the hens' bodies. This supplementation is well tolerated by laying hens body and nor would cause pathologies or tissue and cell damages in hens.

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