

## Research Article



# Effects of Green Anise (*Pimpinella anisum*) Ration Supplementation on Haematological and Biochemical Parameters and Liver and Kidney Structure in Guinea Pigs

NKANA KONTCHIACHOU J. GWLADYS<sup>1</sup>, KOUAMO JUSTIN<sup>2</sup>, VEMO BERTIN NARCISSE<sup>3\*</sup>, MWEUGANG NGOUPO NATHALIE<sup>4</sup>, WANG-BAA TEMOA CHRISTOPHE<sup>1</sup>, AWANTU CHRISTIAN FUNWI<sup>1</sup>, SEMI YAM ALPHONSIUS<sup>1</sup>, KENNE NOUBISSIE CHRISTÈLE<sup>5</sup>, TENDONKENG FERNAND<sup>5</sup>

<sup>1</sup>Agronomic Research Institute for development (IRAD), Mankon, Bamenda, PO Box: 125, Bamenda, Cameroon; <sup>2</sup>University of Ngaoundéré, School of sciences and veterinary medicine, PO Box: 454, Ngaoundéré, Cameroon; <sup>3</sup>University of Buea, Faculty of Agriculture and Veterinary Medicine, Department of Animal Science, PO. Box: 63, Buea, Cameroon; <sup>4</sup>University of Ngaoundéré, Faculty of Sciences, Department of biological Sciences, PO Box: 454, Ngaoundéré, Cameroon; <sup>5</sup>University of Dschang, Faculty of Agronomy and Agricultural Sciences, Department of Animal Science, PO Box: 188, Dschang, Cameroon.

**Abstract** | This experiment was carried out to investigate the haematological and serum biochemical response of guinea pigs to green anise (*Pimpinella anisum*) powder ration supplementation. To achieve this aim, 60 adult female guinea pigs, weighing of  $450 \pm 50$  g were randomly distributed to three experimental groups (20 females per group). Experimental rations consisted of incorporating green anise powder in the basic ration (control) at 0.5% (GA0.5) and 0.75% (GA0.75) of feed. These female guinea pigs were fed with experimental rations, from mating to weaning at 3 weeks post-partum. Their weaned piglets continued to receive the respective experimental diets, until 8 weeks old. At this age, 30 young guinea pigs (15 males and 15 females) were randomly selected (5 males and 5 females per treatment) and slaughtered. Blood was collected in tubes containing anticoagulant (EDTA), for the evaluation of haematological parameters, and in dry tubes, for the dosage of serum biochemical parameters. The main results revealed that the number of total white blood cells, lymphocytes, monocytes and granulocytes increased significantly ( $p < 0.05$ ) in guinea pigs supplemented at 0.5% green anise, compared to the control. The haemoglobin concentration, packed cell volume, mean corpuscular volume of haemoglobin, the number of red blood cells and the concentration of placitinin were comparable ( $p > 0.05$ ) among rations. The number of platelets rose with an increasing level of green anise supplementation, but the significant ( $p < 0.05$ ) difference was observed only at 0.75%. The serum concentrations of total proteins, globulins and HDL increased at 0.50% green anise supplementation, referring to the control diet, nevertheless, the difference was significant ( $p < 0.05$ ) just for globulins concentration. Meanwhile, the concentrations of albumin, total cholesterol, triglycerides, glucose, creatinine, urea, ALAT and ASAT were comparable ( $p > 0.05$ ) among rations. The histological sections of the liver and kidney showed that their structures were not affected, following the green anise supplementation. Hence, the feed supplementation with green anise powder at 0.50% in guinea pigs boosted their immunity without side effects on the body functions.

**Keywords** | Green anise, Guinea pigs, Haemato-biochemical parameters, Kidney and liver histology, Supplementation

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**\*Correspondence** | Vemo Bertin Narcisse, University of Buea, Faculty of Agriculture and Veterinary Medicine, Department of Animal Science, PO. Box: 63, Buea, Cameroon; **Email:** vemobertin@yahoo.fr

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In Cameroon, caviaculture constitutes an important source of food for populations whose income does not allow easy access to meat (Kenfack et al., 2006). Currently this breeding is very promising, because it constitutes a secondary source of income. In addition, the prolificacy of the guinea pig, the simple management of its breeding and its economical feeding, are factors that make this animal an interesting subject for the fight against food insecurity (Niba et al., 2008), since it has a higher protein level than chicken, pork, rabbit, beef (Goering and Van Soest, 1970). However, in village breeding, it feeds on kitchen wastes and harvest residues, which often lack essential nutrients. This therefore results in low production and early mortality (Pamo et al., 2005). Improving the productivity of guinea pigs can be achieved, by improving their diet and, above all, by providing them a balanced diet (Pamo et al., 2005).

In order to improve their diet, many natural additives are used in animal feed as growth promoters, in order to circumvent the problems caused by antibiotics (Steiner, 2006). Their use is aimed at improving, directly or indirectly the efficiency of rations. These feed additives have played a major role in making intensive farming profitable and in providing consumers with access to healthy and nutritious poultry products (Chafai, 2006). Food additives are synthetic or natural substances which, when combined with food, perform various functions (colourings, preservatives, binders, emulsifiers, flavourings, etc.). These include natural plants, probiotics, prebiotics, symbiotics and organic acids (Doyle, 2003). Some plants have anti-microbial activities, thereby reducing harmful bacterial populations (Dorman and Deans, 2000), while others stimulate the immune system.

Green anise (*Pimpinella anisum* L.) is an annual aromatic herb, belonging to the *Apiaceae* family. It has been widely used to maintain or improve animal and human health (Al-Beitawi et al., 2009). It contains 2-6% of essential oils, phenolic acids, eugenol, estragole, and trans-anethole, which is a powerful phytoestrogen and the main active compound (Christaki et al., 2012). Green anise has been used over the years for its antioxidant (Gulcin et al., 2003), antimicrobial (Al-Kassie, 2008), antibacterial (Tabanca et al., 2003), antipyretic (Afifi et al., 1994) and antifungal (Soliman and Badea, 2002) properties. Anise has also been shown to have anti-cancer (Kadan et al., 2013), antihemolytic and anti-inflammatory (Iyer et al., 2013) properties. Studies have been conducted to evaluate the use of anise seed or oil in poultry nutrition, especially to replace antibiotics as growth promoters (Ciftci et al., 2005; Soltan et al., 2008; Al-Beitawi et al., 2009). Green anise seeds also improved blood profile in broiler chickens, as they stimulate the liver and kidneys to function more efficiently.

These benefits are due to the anethole present in anise, which has digestive stimulating effects and antimicrobial activity against pathogens that affect the gastrointestinal tract (Ciftci, 2005). However, there is hardly any work on the evaluation of the effects of this spice on the metabolic profile of animals. The objective of this study was to evaluate the effects of using green anise powder as feed supplement, on haematological and biochemical parameters, as well as its effects on the kidney and liver structure in guinea pigs.

## MATERIALS AND METHODS

### SITE OF STUDY

The study was conducted at the Teaching and Research Farm and the Laboratory of Animal Physiology and Health, of the Faculty of Agronomy and Agricultural Sciences of University of Dschang, Cameroon. The farm is located at an altitude of 1420 m above sea level, between Latitude 5°26'N and Longitude 10°26'E with an Equatoguinean climate.

### ANIMAL MATERIAL AND LODGING

For the present experiment, 75 adult pigs (15 males and 60 females) weighing  $450 \pm 50$  g were used. After reproduction of the previous adult animals, 30 young guinea pigs (15 males and 15 females) were used. Animals were lodged in pens made of plywood, measuring 1.25 x 0.60 x 0.30 m. Each cage was protected by a wire mesh cover preventing any threat of animals by predators. The animals were raised on the ground, covered with dry white wood chips about 5 cm thick, renewed every week. Each lodge was equipped with feeder and drinker.

### PLANT MATERIAL

The plant material was green anise (*Pimpinella anisum*) seeds. They were bought in a local market, sun dried, and ground into powder. The obtained powdered was stored in air-tired bags for further use in the experimental rations.

### EXPERIMENTAL RATIONS

The manufacture of the experimental rations took into consideration the nutritional needs of guinea pigs. *P. purpureum* was harvested around the farm in the campus, pre-sized, quantified and tightened to the animals of each batch. A basic ration was formulated (Table 1) and from that basic ration (control ration), two experimental rations were formulated by adding green anise (*Pimpinella anisum*) powder as follow:

Control ration: basic + *P. purpureum*;

GA 0.5% ration: basic diet + *P. purpureum* + 0.5% of green anise powder;

GA 0.75% ration: basic diet + *P. purpureum* + 0.75% of green anise powder.

**Table 1:** Percentage composition and analysed chemical composition of the basic ration.

Ingredients	% of inclusion
Maize	30
Re-molding	33
Fish meal	2
Soybean meal	2
Palm kernel cake	25
Cotton seed cake	5
Sea shell	2
*Premix 0.5	1
Total	100
<b>Analysed chemical composition of the basic ration</b>	
Dry matter (%)	94.92
OM (% DM)	85.99
Ash (% DM)	8.64
CP (% DM)	16.012
ME (Kcal/kg DM)	2427.78
CF (% DM)	13.14

\*Composition of the 0.5 premix: vit A: 3.000.000 IU, vit D: 50.0000 IU, vit E: 6.000 mg, vit K: 600 mg, vit B1: 600 mg, vit B2: 800 mg, vit B3: 1800 mg, vit B6: 400 mg, Vit12: 6 mg, folic acid: 250 mg, niacin: 600 mg, Cl: 86.500 mg, Fe: 12.000 mg, Cu: 1200 mg, manganese: 12.000 mg, Zn: 10.000 mg, I: 100 mg, Se: 40 mg, magnesium: 3397 mg, Na: 283 mg, CA: 215.166 mg, Methionine: 130.000 mg, lysine: 50.000 mg. 00 mg, Se: 40 mg, magnesium: 3397 mg, Na: 283 mg, CA: 215.166 mg, Methionine: 130.000 mg, lysine: 50.000 mg. RSM: rubber seed meal. DM= Dry Matter, OM= Organic Matter, CP,Crude Protein; CF, crude fiber.

## TRIAL CONDUCT OF TESTS

For this experiment, 60 females were identified using earring, and divided into 3 sets of 20 animals, each set having 5 subsets of 4 females. All the subsets were comparable in terms of live weight, and were randomly assigned to cages. Females of group one received the control (basic) ration, while those of the two other groups were fed with the basic ration supplemented respectively with 0.50% and 0.75% of green anise powder. For mating, 1 male was introduced into each cage. After 30 days of cohabitation, males were removed to allow females to complete the end of their gestation. After parturition, piglets were weaned at 3 weeks old. Piglets of each batch were then fed with the respective ration until 8 weeks old. At this last age, 30 young guinea pigs (15 males and 15 females), that is 10 per group (5 males and 5 females) were slaughtered and data were collected.

## DATA COLLECTION

### HAEMATOLOGICAL PARAMETERS

At 8 weeks old, ten (10) animals per batch were taken

randomly, fasted for 12 hours and slaughtered for collection of whole blood by section of the jugular vein. Blood samples collected in anticoagulant (EDTA) test tubes were used to determine the concentration of red blood cells, white blood cells, lymphocytes, monocytes, granulocytes, hemoglobin, platelets, the haematocrit, and placitonin, using a Genius electronic automatic blood reader (Yemdjie et al., 2017).

### BIOCHEMICAL PARAMETERS

Blood collected in tubes free from anticoagulant was allowed to rest for 24 hours, serum collected was stored at -20 °C for the analysis of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALAT), urea, creatinine, total cholesterol, HDL cholesterol, triglycerides, glucose, total protein, globulins and albumin, using the spectrophotometry method as prescribed by the Chronolab® (Barcelona, Spain) kits (Mahamat et al., 2020). Globulins concentrations were calculated as recommended by Abdel-Fattah et al. (2008).

### LIVER AND KIDNEY STRUCTURES

The kidneys and liver of each animal were fixed in Bouin's fluid, and then washed, dehydrated in alcohol bath of ascending grade, clarified in xylene immersion, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin. The tissue sections were observed under a light microscope at 400 x magnifications (Yemdjie et al., 2017).

### STATISTICAL ANALYSES

Data collected were subjected to two-ways analysis of variance (ANOVA) following the general linear model (GLM), using SPSS 20.0 software. Statistically significant differences observed among treatment were separated using the Duncan's test with 5% probability level (Mahamat et al., 2020).

## RESULTS AND DISCUSSION

### EFFECTS OF THE GREEN ANISE POWDER SUPPLEMENTATION ON HAEMATOLOGICAL PARAMETERS IN GUINEA PIG

#### WHITE BLOOD CELLS

The effects of feed supplementation of green anise powder on white blood cells in guinea pigs are presented in Table 2. The numbers of total white blood cells, lymphocytes, monocytes and granulocytes in males and females, as well as the overall mean increased significantly ( $p < 0.05$ ) in guinea pigs supplemented green anise seed powder at 0.5%, compared to the control. When sex is considered, the number of total white blood cells was significantly ( $p < 0.05$ ) higher in males than females, at 0.5% green anise. The same observation was done for granulocytes at 0.5% and 0.75% green anise. The contrary was noted for lymphocytes at 0.75%, and monocytes in control diet.

**Table 2:** Effects of feed supplementation with green anise powder on white blood cells in guinea pigs.

Parameters	Sex (n)	Control	Green anise		p
			0.50%	0.75%	
WBC (x10 <sup>3</sup> /ul)	♀ (5)	5.53±0.68 <sup>b</sup>	9.02±0.88 <sup>aB</sup>	7.10±1.69 <sup>ab</sup>	0.003
	♂ (5)	6.23±1.26 <sup>b</sup>	13.57±2.43 <sup>aA</sup>	5.85±0.72 <sup>b</sup>	0.000
	♀♂ (10)	5.88±1.01 <sup>b</sup>	11.04±2.89 <sup>a</sup>	6.27±1.14 <sup>b</sup>	0.000
p		0.365	0.006	0.243	
LYMPH (x10 <sup>3</sup> /ul)	♀ (5)	3.38±1.10	4.62±0.58	3.85±0.92 <sup>A</sup>	0.187
	♂ (5)	3.47±0.32 <sup>b</sup>	5.80±1.21 <sup>a</sup>	2.15±0.41 <sup>cB</sup>	0.000
	♀♂ (10)	3.42±0.75 <sup>b</sup>	5.14±1.05 <sup>a</sup>	2.71±1.02 <sup>b</sup>	0.000
p		0.878	0.096	0.028	
MO (x10 <sup>3</sup> /ul)	♀ (5)	1.20±0.29 <sup>bA</sup>	2.40±0.39 <sup>a</sup>	2.15±0.21 <sup>a</sup>	0.003
	♂ (5)	0.73±0.19 <sup>cB</sup>	2.65±0.59 <sup>a</sup>	1.67±0.42 <sup>b</sup>	0.000
	♀♂ (10)	0.97±0.33 <sup>c</sup>	2.51±0.48 <sup>a</sup>	1.83±0.42 <sup>b</sup>	0.000
p		0.037	0.473	0.227	
GRAN (x10 <sup>3</sup> /ul)	♀ (5)	0.97±0.18 <sup>b</sup>	1.67±0.34 <sup>aB</sup>	1.10±0.28 <sup>bB</sup>	0.000
	♂ (5)	1.23±0.38 <sup>b</sup>	5.12±1.39 <sup>aA</sup>	2.02±0.37 <sup>bA</sup>	0.000
	♀♂ (10)	1.10±0.31 <sup>b</sup>	3.20±2.02 <sup>a</sup>	1.71±0.57 <sup>b</sup>	0.001
p		0.276	0.001	0.040	

<sup>a, b, c</sup>: Means on the same line with the same superscripts do not differ significantly ( $p>0.05$ ). <sup>A, B</sup>: Means on the same column, for the same parameter with different capital letter differ significantly ( $p<0.05$ ). WBC: white blood cells; LYMPH: lymphocytes; MO: monocytes; GRAN: granulocytes. p: probability. (n): sample number.

**Table 3:** Effects of feed supplementation with green anise powder on red blood cells and haemoglobin concentration in guinea pigs.

Parameters	Sex (n)	Control	Green anise		p
			0.50%	0.75%	
RBC	♀ (5)	5.98±0.25	5.98±0.53	5.40±0.56	0.144
	♂ (5)	6.55±0.43	5.97±0.71	6.45±0.54	0.689
	♀♂ (10)	6.08±0.33	5.97±0.57	6.10±0.73	0.559
p		0.444	0.991	0.092	
HGB	♀ (5)	17.20±0.59 <sup>a</sup>	16.32±2.05 <sup>ab</sup>	14.15±0.91 <sup>b</sup>	0.041
	♂ (5)	16.97±1.20	16.50±1.55	17.00±1.33	0.930
	♀♂ (10)	17.08±0.88	16.40±1.74	16.05±1.84	0.551
p		0.748	0.889	0.057	
HCT	♀ (5)	40.90±3.11	42.70±3.37	38.98±1.90 <sup>B</sup>	0.360
	♂ (5)	43.87±2.76	43.47±5.13	44.35±1.96 <sup>A</sup>	0.863
	♀♂ (10)	42.38±3.15	43.04±3.96	42.55±3.28	0.923
p		0.203	0.792	0.033	
MCV	♀ (5)	67.87±2.71	71.30±2.94	72.00±4.10	0.352
	♂ (5)	70.50±1.32	72.42±4.69	68.30±3.45	0.139
	♀♂ (10)	69.18±3.31	71.88±3.59	69.53±3.76	0.145
p		0.132	0.672	0.304	

<sup>a, b</sup>: Means on the same line with the same superscripts do not differ significantly ( $p>0.05$ ). <sup>A, B</sup>: Means on the same column, for the same parameter with different capital letter differ significantly ( $p<0.05$ ). RBC: Red blood cells; HCT: Haematocrit; HGB: Haemoglobin; MCV: Means corpuscular volume of haemoglobin; p: probability. (n): sample number.

### RED BLOOD CELLS AND HAEMOGLOBIN

The effects of green anise powder supplementation on the number of red blood cells and haemoglobin are presented

in Table 3. Irrespective of the inclusion rate and sex, the supplementation of green anise powder in ration had no significant effect ( $p>0.05$ ) on the number of red blood



cells, haemoglobin concentration, mean corpuscular volume of haemoglobin and haematocrit, except of the haemoglobin concentration which decreased significantly ( $p<0.05$ ) at 0.75% green anise powder supplementation, referring to the control. Regarding the effects of the sex, only haematocrit revealed a significant ( $p<0.05$ ) increase in males at 0.75% green anise powder supplementation.

### THROMBOCYTES AND PLACITONIN

The effects of feed supplementation with green anise powder on thrombocytes number and placitonin in the guinea pigs are summarised in Table 4. Irrespective of the sex and in males, the platelets number rose with an increasing level of green anise powder supplementation. Nevertheless, the difference was significant ( $p<0.05$ ) only at 0.75%, when compared to the control ration. The sex had no significant ( $p>0.05$ ) effect on the thrombocytes number and placitonin, no matter the ration.

**Table 4:** Effects of feed supplementation with green anise powder on thrombocytes number and placitonin in guinea pigs.

Parameters	Sex (n)	Control	Green anise		p
			0.50%	0.75%	
PLT	♀ (5)	381.00±11.25	413.40±53.44	365.50±36.06	0.320
	♂ (5)	363.00±41.64 <sup>b</sup>	388.25±23.61 <sup>b</sup>	467.25±61.39 <sup>a</sup>	0.040
	♀♂ (10)	372.00±29.83 <sup>b</sup>	402.27±42.58 <sup>ab</sup>	433.33±72.68 <sup>a</sup>	0.047
	p	0.436	0.415	0.105	
PCT	♀ (5)	0.25±0.40	0.24±0.08	0.25±0.07	0.546
	♂ (5)	0.24±0.70	0.25±0.05	0.33±0.07	0.074
	♀♂ (10)	0.24±0.58	0.24±0.07	0.30±0.08	0.190
	p	0.871	0.853	0.244	

<sup>a, b</sup>: Means on the same line with the same superscripts do not differ significantly ( $p>0.05$ ). PLT: Platelets; PCT: Placitonin. p: probability. (n): sample number.

### EFFECTS OF GREEN ANISE POWDER SUPPLEMENTATION ON SERUM BIOCHEMICAL PARAMETERS IN GUINEA PIGS

#### PROTEINS PROFILE

Effects green anise powder supplementation on serum concentrations of total protein, albumin and globulins in guinea pigs are shown in Table 5. Irrespective of the sex and in males, the concentrations of total protein and globulins increased at 0.5% green anise powder supplementation, as compared to the control; but the difference was significant ( $p<0.05$ ) only for globulins. The concentration of albumin was comparable ( $p>0.05$ ) among rations, no matter the sex. Considering the sex, only albumin concentration showed a significant ( $p<0.05$ ) in females, at 0.5% supplementation.

**Table 5:** Effects of feed supplementation with green anise powder on protein profile in guinea pigs.

Parameters	Sex (n)	Control	Green anise		p
			0.50%	0.75%	
PT (g/dl)	♀ (5)	6.77±0.51	7.14±0.56	6.16±0.23	0.339
	♂ (5)	6.78±0.45 <sup>ab</sup>	7.24±0.20 <sup>a</sup>	6.67±0.28 <sup>b</sup>	0.042
	♀♂ (10)	6.78±0.29 <sup>ab</sup>	7.18±0.42 <sup>a</sup>	6.71±0.25 <sup>b</sup>	0.043
	p	0.988	0.749	0.629	
ALB (g/dl)	♀ (5)	2.98±0.20	2.90±0.09 <sup>A</sup>	2.81±0.22	0.642
	♂ (5)	2.82±0.30	2.59±0.12 <sup>B</sup>	2.73±0.12	0.437
	♀♂ (10)	2.90±0.25	2.76±0.19	2.75±0.14	0.579
	p	0.422	0.003	0.595	
GLOB (g/dl)	♀	3.79±0.21	4.28±0.59	3.98±0.20	0.296
	♂	3.95±0.30 <sup>b</sup>	4.65±0.27 <sup>a</sup>	3.93±0.37 <sup>b</sup>	0.020
	♀♂	3.87±0.25 <sup>b</sup>	4.42±0.50 <sup>a</sup>	3.95±0.28 <sup>b</sup>	0.045
	p	0.415	0.249	0.875	

<sup>a, b</sup>: Means on the same line with the same superscripts do not differ significantly ( $p>0.05$ ). <sup>A, B</sup>: Means on the same column, for the same parameter with different capital letter differ significantly ( $p<0.05$ ). PT: total protein, ALB: albumin, GLOB: globulins. p: probability. (n): sample number.

### LIPIDS PROFILE

The serum concentrations of HDL-cholesterol decreased significantly ( $p<0.05$ ) at 0.75% supplementation of green anise powder in the ration (Table 6) compared to the control, irrespective of the sex and in males. However, rations were comparable ( $p>0.05$ ) for the total cholesterol and triglycerides concentrations. The sex had no significant ( $p>0.05$ ) effect on lipids concentrations, no matter the ration.

**Table 6:** Effects of the supplementation with green anise powder on serum lipids concentrations in guinea pigs.

Parameters	Sex (n)	Control	Green anise		p
			0.50%	0.75%	
CHOLT (mg/dl)	♀ (5)	37.48±6.76	44.28±5.40	43.14±3.11	0.451
	♂ (5)	43.77±4.59	44.96±7.52	42.67±4.18	0.842
	♀♂ (10)	40.62±6.32	44.58±5.99	42.83±3.53	0.684
	p	0.174	0.879	0.897	
TGs (mg/dl)	♀ (5)	56.46±4.28	64.38±4.61	67.10±1.86	0.254
	♂ (5)	66.00±8.69	65.67±5.45	59.97±5.92	0.455
	♀♂ (10)	61.23±8.13	64.96±4.72	62.35±5.93	0.756
	p	0.096	0.711	0.189	
HDL (mg/dl)	♀ (5)	26.53±5.85	30.92±4.07	28.34±4.00	0.302
	♂ (5)	33.30±3.81 <sup>a</sup>	35.59±4.39 <sup>a</sup>	27.00±3.86 <sup>b</sup>	0.004
	♀♂ (10)	29.92±5.83 <sup>ab</sup>	33.04±4.64 <sup>a</sup>	27.45±3.56 <sup>b</sup>	0.041
	p	0.101	0.143	0.712	

<sup>a, b</sup>: Means on the same line with the same superscripts do not differ significantly ( $p>0.05$ ). CHOLT: total cholesterol, HDL: High density lipo-protein, TGs: triglycerides. p: probability. (n): sample number.

## CREATININE AND UREA

The effects of the supplementation with green anise powder on serum creatinine and urea concentrations are presented in Table 7. In females, the concentration of creatinine rose significantly ( $p < 0.05$ ) at 0.75% supplementation of green anise powder in the ration ( $p > 0.05$ ). Urea concentration was comparable ( $p > 0.05$ ) among diets, no matter the sex. The sex had no significant ( $p > 0.05$ ) influence on creatinine and urea concentrations, except at 0.5% green anise powder supplementation, where serum urea level increased significantly ( $p < 0.05$ ) in males.

**Table 7:** Effects of the supplementation with green anise powder on creatinine and urea concentrations in guinea pigs.

Parameters	Sex (n)	Control	Green anise		p
			0.50%	0.75%	
Creatinine (mg/dl)	♀ (5)	0.61±0.10 <sup>b</sup>	0.71±0.14 <sup>ab</sup>	0.84±0.14 <sup>a</sup>	0.009
	♂ (5)	0.65±0.13	0.78±0.19	0.61±0.09	0.399
	♀♂(10)	0.63±0.11	0.74±0.16	0.69±0.15	0.405
	p	0.673	0.513	0.068	
Urea (mg/dl)	♀ (5)	37.88±6.31	33.16±3.14 <sup>B</sup>	35.78±8.93	0.623
	♂ (5)	40.75±7.26	46.61±7.01 <sup>A</sup>	42.18±6.91	0.740
	♀♂(10)	39.31±6.48	39.14±8.58	40.05±7.45	0.863
	p	0.573	0.006	0.379	

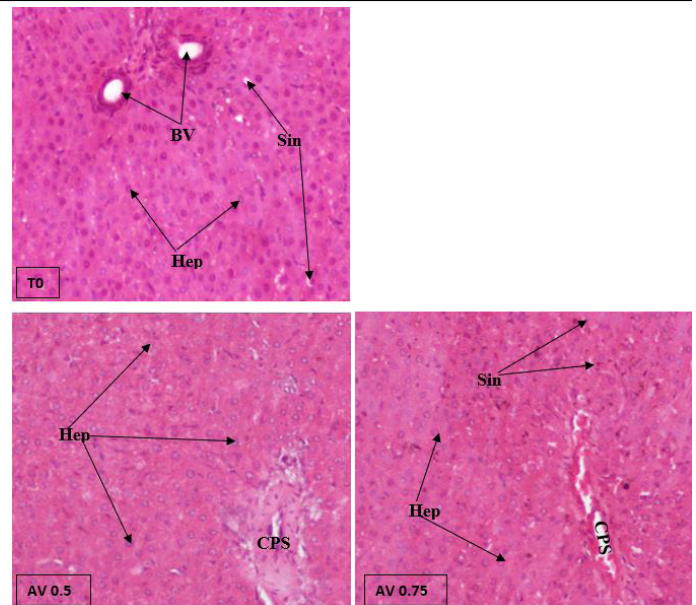
<sup>a, b</sup>: Means on the same line with the same superscripts do not differ significantly ( $p > 0.05$ ). <sup>A, B</sup>: Means on the same column, for the same parameter with different capital letter differ significantly ( $p < 0.05$ ). p: probability. (n): sample number.

## SERUM GLUCOSE AND TRANSAMINASES CONCENTRATIONS

The effects of supplementation with green anise powder on serum glucose and transaminases concentrations in guinea pigs are shown in Table 8. Irrespective of the sex, no significant ( $p > 0.05$ ) difference was registered among rations for the serum glucose and transaminases concentrations. In females, the glucose concentration decreased significantly ( $p < 0.05$ ) at 0.75% green anise powder supplementation, with reference to the control diet. The contrary was observed in males. At 0.75% green anise powder supplementation, males revealed a significant ( $p < 0.05$ ) increase in serum glucose concentration.

## EFFECTS OF GREEN ANISE POWDER SUPPLEMENTATION ON LIVER AND KIDNEY STRUCTURE IN GUINEA PIGS

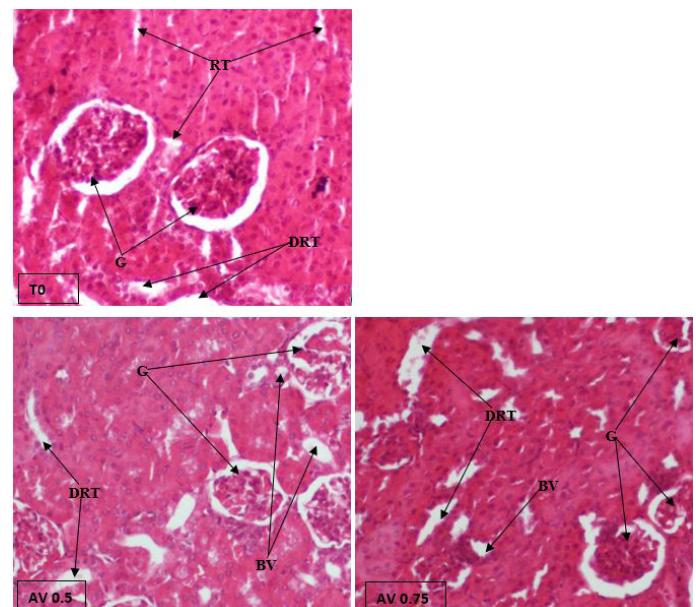
The Figure 1 illustrates the histological structure of the liver in guinea pig. The histological structure of the liver in control guinea pigs as well as in those fed with rations supplemented green anise was normal. It showed a hepatic parenchyma, constituted by many hepatic sinusoids, hepatocytes and blood vessels.



**Figure 1:** Histological sections of liver in guinea pig fed with rations supplemented green anise: HE x 400.

T0: basis ration (Control); AV 0.5 and AV 0.75: rations supplemented at 0.5 and 0.75% Green Anise. CPS: congested portal space; Hep: Hepatocytes; Sin: sinusoids; BV: blood vessels.

The Figure 2 presents the histological structure of kidney in guinea pig fed with rations supplemented green anise. The histological sections of the kidney in control guinea pigs and those receiving rations supplemented green anise revealed a classical structure et normal histological aspect. In fact, they presented a renal parenchyma containing many renal tubes, blood vessels and glomeruli.



**Figure 2:** Histological sections of kidney in guinea pig fed with rations supplemented green anise: HE x 400. T0: basis ration (Control); AV 0.5 and AV 0.75: rations supplemented at 0.5 and 0.75% Green Anise. G: glomerule; RT: renal tubes; DRT: dilated renal tubes; BV: blood vessels.

**Table 8:** Effects of green anise powder supplementation on glucose and transaminases concentrations in guinea pigs.

Parameters	Sex (n)	Control	Green anise		p
			0.50%	0.75%	
Glucose (mg/dl)	♀ (5)	74.33±14.22 <sup>a</sup>	76.21±9.77 <sup>a</sup>	62.75±2.81 <sup>bB</sup>	0.011
	♂ (5)	66.24±10.34 <sup>c</sup>	73.11±7.52 <sup>bc</sup>	82.17±3.41 <sup>aA</sup>	0.004
	♀♂(10)	70.28±12.29	74.83±8.46	75.70±10.44	0.212
p		0.393	0.619	0.002	
ALAT (IU)	♀ (5)	51.33±6.24	48.12±8.25	56.25±2.12	0.350
	♂ (5)	51.62±10.81	51.33±7.59	45.41±7.45	0.239
	♀♂(10)	51.47±8.18	49.55±7.65	49.02±8.09	0.416
p		0.964	0.568	0.128	
ASAT (IU)	♀ (5)	58.91±5.20	55.68±5.67	56.00±2.47	0.750
	♂ (5)	52.50±5.71	54.12±7.77	60.08±9.66	0.421
	♀♂(10)	55.70±6.11	55.88±6.23	58.72±7.85	0.683
p		0.148	0.925	0.607	

<sup>a, b</sup>: Means on the same line with the same superscripts do not differ significantly ( $p > 0.05$ ). <sup>A, B</sup>: Means on the same column, for the same parameter with different capital letter differ significantly ( $p < 0.05$ ). ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase. p: probability. (n): sample number.

Blood parameters are considered as the main pathological, nutritional and physiological indices for assessing the state of an organism (Isaac et al., 2013; Etim et al., 2014). Any change in the constituents of blood relative to the normal values is an important index for the interpretation of the physiological or metabolic state of the animal, especially the quality of feed (Babatunde et al., 1992). Haematological values are important indicators of health status of animals and indispensable tools in the diagnosis, treatment and prognosis of many diseases. From the results of this study, white blood cells (total white blood cells, lymphocytes, monocytes, granulocytes) increased at 0.5% supplementation of feed with green anise powder. Haemoglobin concentration decreased with supplemented rations, while the number of platelets increased with green anise supplementation. The red blood cells, the haematocrit, placitinin and mean corpuscular volume of haemoglobin were not affected by the supplementation. This indicates that supplementation of green anise powder did not have any negative consequences on the haematological parameters in guinea pigs. These results are similar to those recorded by Al-Shammari et al. (2017) who showed that the anise supplement significantly improved red blood cells, white blood cells, haemoglobin and haematocrit, after 28 days and 56 days of feeding.

Determining the level of biochemical parameters is found to be of great importance in assessing the toxic effects of a substance on the liver and kidneys (Kalender et al., 2005). Serum total protein and globulins concentrations increased at 0.50% supplementation with green anise. These results are in agreement with those of Tagwa (2010) and Mukhtar et al. (2013). It could be due to nutritional efficiency due to good assimilation of proteins. Al-Shammari et al. (2017) showed that the anise supplement significantly improve the total protein and globulins after 28 and 56 days of

feeding. In this study, serum albumin concentration was not affected. According to Eckersall (2008), low serum albumin might be due to renal dysfunction or hyper hydration.

The lipids concentrations were not affected by the supplementation of feed with green anise powder. This could be due to the fact that the functioning of the liver was not perturbed by the presence of green anise powder. These results correspond to those of Soltan et al. (2008), whose revealed that anise supplementation non-significantly decreased cholesterol concentration and of those of Badr (2019), who reported no significant different in blood plasma constituents of rabbits using anise leaves as supplement in feed. This could indicate that the supplementation with green anise powder did not have any effect on total cholesterol and triglycerides in guinea pigs.

The levels of creatinine and urea serve as indicators of the histological state and functioning of kidneys. In the present study, there were no significant differences between creatinine and urea values among rations. This might explain that the functioning of the kidneys of guinea pigs was not perturbed by the presence of green anise powder in the diets.

The concentrations of ALAT and ASAT constitute indicators of the state and functioning of hepatic cells (Ogunlade et al., 2012). The concentration of ALAT and ASAT were not affected by the supplementation with green anise powder. These results are in agreement with those of Al-Shammari et al. (2017), that showed the low activities of liver enzymes (ASAT and ALAT) of broilers supplemented with anise seeds. According to these authors, anise seed contains properties that may have stimulated



digestion of the broilers. Also, study carried out by Ciftci (2005), showed that green anise seeds improved the blood profile in broiler chickens, as they stimulate the liver and kidneys to function more efficiently. These benefits are due to the anethole, an active molecule present in anise, which has digestive stimulating effects and antimicrobial activity against pathogens that affect the gastrointestinal tract.

## CONCLUSIONS AND RECOMMENDATIONS

This study revealed that the administration of green anise seeds powder up to 0.5% in feed has no detrimental effects on haematological and biochemical parameters of guinea pigs, but stimulates the immune system in guinea pigs.

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## NOVELTY STATEMENT

Use of phytobiotics as feed additives in animal productions.

## AUTHORS' CONTRIBUTION

This work was carried out in collaboration between all authors. Authors NKJG, VBN, MNN, KJ and TF contributed substantially to the conception and the design of the study, data analysis and interpretation. Authors NKJG, VBN, KC, WTC, AC and SYA contributed in data acquisition. Authors NKJG and VBN contributed in drafting the article. All authors revised it critically for important intellectual content and approved the final manuscript.

## CONFLICT OF INTERESTS

The authors have declared no conflict of interest.

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