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Serum metabolites and weights of internal organs of broilers fed on varying levels of *Vachellia angustissima* leaf meal

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**Serum metabolites and weights of internal organs of broilers fed on varying levels of
Vachellia angustissima leaf meal**

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Abstract

The objective of the study was to determine the relationship between inclusion levels of *Vachellia angustissima* leaf meal against nutritionally-related blood metabolites, activity of liver enzymes and scaled internal organs in broilers. One hundred and twenty Ross 308 broiler chicks with initial bodyweight of 0.90 ± 0.043 kg were randomly allotted to six diets containing different inclusion levels namely 0, 30, 60, 90, 120 and 150 g/kg DM of *V. angustissima* leaf meal. Each inclusion level of *V. angustissima* leaf meal was replicated four times. Five birds were randomly assigned to each replicate. There was a linear decrease in cholesterol ($P < 0.05$) with increasing levels of *V. angustissima* leaf meal. There was a linear increase in scaled gizzard weight (SGW) ($P < 0.05$), scaled heart weight (SHW) ($P < 0.001$) and intestines weight ($P < 0.001$). Scaled spleen weight (SSW) ($P < 0.01$) had a positive quadratic relationship with levels of *V. angustissima* leaf meal. There was a positive quadratic response in alkaline phosphatases activity with increasing levels of *V. angustissima* leaf meal ($P < 0.001$). The concentration of cholesterol and liver enzymes demonstrate the potential of leaf meals to be incorporated in poultry diets. The optimum inclusion level of *V. angustissima* was attained at 60 g/kg for ALP, 90 g/kg on for SHW and SHW.

Keywords: Proanthocyanidins, Gizzard, Liver, Alkaline phosphatase, Cholesterol

Introduction

Changes in climate and increasing human population, which lead to intense competition with animals for food, have resulted in high prices of conventional protein sources for chickens (Melesse *et al.*, 2013; Mpofu *et al.*, 2016). This has prompted the utilisation of non-conventional protein sources as feed for chickens. In tropical regions, changes in climate is expected to increase the availability of tree and shrubs compared to grasses (Hlatini *et al.*, 2018). There is a wide range of leguminous trees, such as *Vachellia* species that have the

potential to be used as feedstuffs for chickens. *Vachellia angustissima* is among *Vachellia* species that are abundant and have potential to be used as feed resources for broilers. *Vachellia angustissima* is rich in protein (229 g/kg DM) and moderate levels of minerals (Rubanza *et al.*, 2007). Utilisation of *V. angustissima* is likely to be economical particularly to emerging and smallholder poultry farmers.

Besides feed intake and growth performance (Gudiso *et al.*, 2018), which have a direct bearing on the digestive physiology, it is crucial to determine the relationship between inclusion levels of *V. angustissima* against nutritionally-related blood metabolites, enzymes activity and weights of internal organs. Blood metabolites, liver enzymes activity and weights of internal organs play a vital role in assessing physiological, pathological and nutritional status of animals (Nworgu *et al.*, 2007; Iheukwumere *et al.*, 2007; Hlatini and Chimonyo, 2016). *Vachellia angustissima* contains polyphenols such as proanthocyanidins that may have a propensity to increase the production of liver enzymes and the size of internal organs (Khanyile *et al.*, 2016). Proanthocyanidins found in *V. angustissima* consist largely of 5-deoxyflavan 3-ols and proanthocyanidin dimers and hexamers (Smith *et al.*, 2012). It also distorts the concentrations of some nutritionally-related metabolites (Masoudi *et al.*, 2011; Makanjuola *et al.*, 2014). Weights of internal organs are used to determine the abnormalities associated with anti-nutritional factors (Ahamefule *et al.*, 2006; Nkukwana *et al.*, 2014). Thus, the investigation of *V. angustissima* in relation to nutritionally-related metabolites, enzyme activity and internal organ weights is crucial. The objective of the study was, therefore, to determine the response of internal organs, liver enzymes and blood metabolites to increasing levels of *V. angustissima* leaf meal. It was hypothesised that increasing inclusion levels of *V. angustissima* leaf meal caused a linear increase in scaled internal organ weights, liver enzymes and nutritionally-related metabolites.

Materials and methods

Study site

The experiment was done at Ukulinga Research Farm, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The farm is located at 30° 24'S, 29° 24'E and 700 to 775m above the sea level. The area receives the mean rainfall of 735 mm yearly, of which high percentages occurs between October and April months. The mean average daily temperature is about 17.2 °C.

Harvesting and preparation of leaf meal

The leaves were harvested from North – East of Harare city, in Domboshawa (17° 35'S, 31°14'E) region, Zimbabwe. Fresh tree branches were cut from the growing points at the stage of maturity. They were harvested by carefully beating branches with a stick, before removing twigs. The leaves were air-dried for five days in a shade to prevent nutrient deterioration. During drying, the leaves were often turned to prevent moulding. After drying, dried leaves were ground using a hammer mill to pass through a 2 mm sieve to remove foreign particles, bagged and placed in a well-ventilated room. The total tannins for *V. angustissima* leaf meal was 24.4 g/kg DM.

Bird management, design and diet formulation

Care and use of birds were done following the certificate of authorisation to experiment on living animals from the University of KwaZulu-Natal Animal Ethics Committee (Reference Number: AREC/ 093/015PD). A total of 120 day-old Ross 308 broiler chicks, purchased from National Chicks, were reared in the brooder house and offered commercial starter feed for 21 days. On day 22 (finishing phase), birds were weighed and allocated to floor pens

containing floor shavings to the depth of 10 cm in an environmentally controlled house. They were offered experimental diets containing *V. angustissima*. The finishing phase started from day 22 and ended on day 35. Birds with an initial average body weight of 0.90 ± 0.043 kg were used. The tube feeders and nipple drinkers were used to provide feed and water, respectively. Temperature in the house was maintained by the exchange of air provided by the tunnel ventilation system. The 12-hour dark and 12-hour light photoperiod was applied. There was no vaccination during the experimental period.

A completely randomized design with six diets containing different levels of *V. angustissima* was used. Each inclusion level of *V. angustissima* leaf meal was replicated four times. Five birds were randomly assigned to each replicate. The feed was formulated to meet the nutrient requirements for broilers, according to the Ross 308 Broiler Nutrition Specifications (2014). *Vachellia angustissima* leaf meal were incorporated in a broiler diets at 0, 30, 60, 90, 120 and 150 g/kg DM inclusion level. The experimental diets were iso-nitrogenous and iso-energetic. The average daily feed intake of broilers fed on 0, 30, 60, 90, 120 and 150 g/kg DM of *V. angustissima* was 0.11, 0.11, 0.09, 0.08, 0.08, 0.07 kg DM/ d, respectively (Gudiso *et al.*, 2018). The average daily gain was 0.06, 0.06, 0.03, 0.03, 0.02 and 0.02 kg BW/d, respectively.

Chemical analyses

The dry matter, ash, crude protein and ether extract were determined using AOAC (1990) Official Methods 945.15, 942.05, 979.09 and 920.39, respectively. Nitrogen content was determined using the Dumas Combustion method in a Leco Truspec Nitrogen Analyser, St Joseph MI, USA. Crude protein was then calculated as N x 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) levels were analysed using ANKOM Fibre Analyser

(Ankom Macedon, NY, USA), according to Van Soest (1973) and Van Soest et al. (1991). Samples for mineral analyses were ashed at 450°C overnight (Abdou et al., 2011). The water holding capacity (WHC) was determined according to the method described by Robertson et al. (1980). The swelling capacity was determined according to the method described by Canibe and Bach-Knudsen (2002). The bulk density was determined according to the method described by Kyriazakis and Emmans (1995). Total tannin concentrations were estimated calorimetrically using the butanol-HCL method (Reed *et al.*, 1982). The ingredient composition of the diets with different levels of leaf meal is given in table 1. The chemical analysis of the *V. angustissima* leaves and diets is illustrated in Table 2.

Blood collection and analyses

About 5 ml blood was collected from the neck of each bird into non-coagulated vacutainer tubes containing sodium heparin. Collected blood samples were allowed to coagulate at room temperature (25°C) and centrifuged for 10 minutes at 1000 x g within two hours of collection. Serum was extracted and transferred into polypropylene tubes and kept at – 20°C for preservation until analysis. The serum was analysed for total protein (TP) using method by Doumas and Biggs (1972). Iron (Fe) was assayed using spectrophotometry as explained by Tietz (1976). Phosphorus and alkaline phosphatase (ALP) were analysed according to the colometric method (Tietz et al., 1993). The enzymatic method was used for the determination of both cholesterol (Allain et al. 1974) and uric acid (Tietz 1995). The ultraviolet method was followed for the determination of aspartate aminotransferase (AST) (Bergmeyer et al., 1986) and alanine aminotransferase (ALT) (Horder et al., 1991).

Slaughtering procedure and measurements

After overnight fasting, the average slaughter weight of birds from diets containing 0, 30, 60, 90, 120 and 150 g/kg DM of *V. angustissima* was 1.85, 1.74, 1.24, 1.24, 1.23 and 1.07 kg, respectively. Birds were humanely treated according to the routine abattoir protocols which include ante-mortem inspection. In each replicate, three birds were slaughtered. Birds were electrically stunned before bleeding, scalding and plucking. They were then eviscerated and separated into liver, gizzard, heart, spleen, proventriculus and intestines. They were then weighed using a digital scale. Then, the weights of the organs were divided by the slaughter body weight to determine scaled liver weight (SLW), gizzard weight (SGW), heart weight (SHW), spleen weight (SSW), proventriculus weight (SPW) and intestines weight (SIW). The internal organs were scaled to account for differences in body weights.

Statistical analyses

Polynomial regression (PROC REG) procedure of (SAS 2010) was used to determine the relationships between *V. angustissima* levels and the internal organs, nutritionally-related blood metabolites and liver enzymes. The model: $Y = B_0 + B_1A + B_1A^2 + E$

Where: Y = the response variables (Internal organs, Liver enzymes and Blood metabolites)

$B_0 + B_1 + B_1^2$ = regression component

A = *Vachellia angustissima* inclusion levels

E = Error

The piecewise regression (broken-stick) analysis using PROC NLIN procedure was used to determine the threshold value at which the inclusion level for *V. angustissima* leaf meal caused ALP, gizzard weight and heart weight to be constant or decrease.

The model: $Y_i = y_0 + y_1 + y_2 (I_{xc}) (x_i - x_c) + \varepsilon_i$,

Using parameters (y_0 , y_1 , y_2) and the x_c , the two segmented simple regression functions

where; $Y_j = y_0 + y_1 (x_i)$, for $x_i \leq x_c$; and $Y_k = y_0 + (y_1 + y_2) x_i$, for $x_i \geq x_c$

Where; Y_i is the response variable when *V. angustissima* leaf meal inclusion level is constraining performance;

Y_j is the response variable before *V. angustissima* leaf meal inclusion level constrains performance;

Y_k is the response variable when *V. angustissima* leaf meal inclusion level exceeds the optimum inclusion level; $Y_o = y_o - y_2 x_c$; when $x_i = 0$;

y_o is the intercept or minimum Y_i when $x_c < 0$;

y_1 is the rate of change of Y_i when $x_i < x_c$;

y_2 is the rate of increase in Y_i when $x_i > x_c$;

x_i is the inclusion level of *V. angustissima*;

x_c is the optimum level of inclusion beyond which ALP is constrained by increase in *V. angustissima* leaf meal; and

I_{xc} is a dummy variable with value 0 when $x_i < x_c$ and 1 when $x_i \geq x_c$.

All measurements were considered significant at $P < 0.05$.

Results

Table 3 illustrate the relationship between levels of *Vachellia angustissima* (*V. angustissima*) leaf meal against liver enzymes and blood metabolites. There was a positive quadratic relationship between increasing levels of *V. angustissima* leaf meal diet and ALP concentration ($P < 0.001$; 53.59 ± 14.63). The optimum inclusion level for ALP activity was attained at 60 g/kg of leaf meal. Incremental levels of *V. angustissima* leaf meal had no significant relationship with ALT and AST concentration ($P > 0.05$). A linear decrease was observed between increasing levels of the *V. angustissima* leaf meal and cholesterol concentration ($P < 0.05$; -7.13 ± 8.81). As the level of *V. angustissima* leaf meal increases the cholesterol concentration decreased. There was no significant relationship between the

albumin, iron, phosphorus, total proteins and uric acid with increasing levels for the leaf meal ($P > 0.05$).

Table 4 shows the relationship between increasing levels of *V. angustissima* leaf meal and scaled weights of the liver (SLW), heart (SHW), spleen (SSW), gizzard (SGW), proventriculus (SPW) and intestines (SIW). There was a positive quadratic relationship between levels of *V. angustissima* leaf meal and SPW ($P < 0.01$; $8.26 \times 10^{-5} \pm 0.27 \times 10^{-4}$). A linear increase in SHW ($P < 0.01$; $1.26 \times 10^{-3} \pm 0.25 \times 10^{-3}$) of broilers when fed inclusion levels of *V. angustissima* leaf meal was observed. A positive linear response was observed in SGW ($P < 0.05$; $3.38 \times 10^{-2} \pm 0.15 \times 10^{-2}$) and SIW ($P < 0.001$; $1.46 \times 10^{-3} \pm 0.11 \times 10^{-2}$). As inclusion level of *V. angustissima* leaf meal increases SGW and SIW increased. The level of *V. angustissima* leaf meal in a broiler diet had no significant effect on both SLW and SSW ($P > 0.05$). The optimum inclusion level of *V. angustissima* for scaled weights of the heart and gizzard was 90 g/kg DM, respectively.

Discussion

Determining relationship between inclusion levels of *V. angustissima* leaves against nutrition-related blood biochemistry and internal organs is crucial to evaluate impact of tanniferous feedstuff on wellness and performance of broilers. Khan and Zafar (2005) reported that blood profiles are important indices of the physiological state including diet of animals. Ahamefule *et al.* (2006) reported that liver enzymes activities are sensitive to toxic substances in the feed. The ALP, ALT and AST activities are collectively used to assess the homeostatic response of the liver to metabolites that pass through the portal blood and as indicators of liver damage (Esonu *et al.*, 2007; Masoudi *et al.*, 2011). The observed quadratic increase in concentration of ALP with increasing levels of *V. angustissima* leaf meal can be

attributed to the obstruction of the bile flow due to proanthocyanidins. Increased ALP suggests that some abnormality was induced in the liver. The optimal response from ALP response suggested that broilers could tolerate up to 60 g/kg DM of the leaf meal beyond liver function gets compromised. The response of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of broilers fed on *V. angustissima* leaf meal-based diet indicate acceptable physiological status and diet. Elevation of serum AST and ALT can occur as a result of exposure to toxins leading to toxemia, inflammation and alteration in permeability of hepatocellular membrane (Khanyile *et al.*, 2016). The significant reduction in cholesterol concentration with increasing levels of *V. angustissima* leaf meal might indicate presence of hypocholesterolemic properties. This response could also be associated with decrease in lipid mobilization. Similarly, Donkoh *et al.* (1999) observed reduction in broilers cholesterol with increasing levels of *Cnidioscolus aconitifolius* leaf meal in diets. In contrast, serum cholesterol levels were not affected by increasing levels of the leaf meal (Esonu *et al.*, 2002; Iheukwumere *et al.*, 2007). The response in total protein albumin, iron, phosphorus and uric acid of broilers offered varying levels of *V. angustissima* leaf meal indicates they were not distorted from their normal concentrations.

The total protein concentration from the serum is the indirect way for showing adequacy of dietary proteins to meet animal requirements (Hlatini and Chimonyo, 2016). The quality of the protein in a diet constitutes of the addition of the protein quality from individual constituents (Ahamefule *et al.*, 2006). The study agrees with Agbede and Aletor *et al.* (2003) who observed that increasing levels of Glyricidia leaf meal had no effect on total serum protein. In contrast, Iheukwumere *et al.* (2007) and Melesse *et al.* (2013) observed an increase in total serum protein with increasing levels of leaf meals.

It is a common practice to use weights of the internal organs as indicators of the effects of anti-nutritional factors to animals (Ahamefule *et al.*, 2006). The response of gizzard (SGW), proventriculus (SPW) and intestines (SIW) to increasing levels of *V. angustissima* leaf meal can be attributed to fibre compaction including bulky effect together with mechanism for adaptation (Mpofu *et al.*, 2016). The birds with larger and stronger internal organs like gizzard can become better to utilise fibrous diets. A linear increase in scaled heart weight (SHW) with inclusion levels of *V. angustissima* leaf meal can be explained by an attempt to supply oxygen and an increase in blood circulation. Agbede and Aletor (2003) and Nkukwana *et al.* (2014) also reported an increase in heart weight of chickens when fed increasing levels of *Glyricidia sepium* and *Moringa Oleifera* leaf meal, respectively. The enlarged heart could also be linked to high fat deposition around the pericardium of the heart. This can be related to high fat concentration from the leaf meal or addition of fat to compensate for energy deficits encountered with increasing levels of the leaf meals when formulating the diets. The observed increase in SHW in broilers when fed *A. angustissima* agrees with Ayssiwede *et al.* (2011) who reported an increase internal organs of chickens fed *Leucaena leucocephala* leaf meal.

Conclusions

Vachellia angustissima leaf meal can be added up to 90 g/kg in the broiler diet without constraining gizzard and heart weights. The significant reduction observed in cholesterol and ALP is associated with the inclusion of *V. angustissima* leaf meal. The reduction in serum cholesterol concentration of the broilers fed *V. angustissima* leaf meal is an indication that leaf meals can decrease cholesterol in the meat. The positive response of ALP activity indicates that *V. angustissima* is free of toxic substances when fed to broilers. Intestinal organs increased linearly with levels of *V. angustissima* leaf meal.

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Table 1. Feed ingredients (g/kg DM) for the diets with increasing inclusion levels for *Vachellia angustissima* leaf meal

Feed ingredient	<i>V. angustissima</i> inclusion levels (g/kg DM)					
	0	30	60	90	120	150
Yellow maize	64.38	64.2	59.07	62.2	56.85	54.15
Soya oilcake ^(44- 47%)	30.50	28.00	27.17	22.10	23.90	23.90
Sunflower oil	1.50	1.80	1.60	1.90	1.90	3.00
Limestone	0.91	0.50	0.50	0.20	0.20	0.20
Monocalcium phosphate	1.52	1.15	0.01	0.10	0.10	0.10
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin & mineral premix	0.25	0.25	0.25	0.25	0.25	0.25
L-lysine HCL	0.32	0.50	3.00	2.30	2.70	2.70
DL- methionine	0.22	0.20	2.00	1.55	1.70	0.30

1 **Table 2.** Chemical composition for the diets (as fed) with increasing inclusion levels of *Vachellia angustissima* leaf meal

Components	<i>V. angustissima</i>	<i>V. angustissima</i> inclusion levels (g/kg DM)					
		0	30	60	90	120	150
Dry matter	979.2	872.85	874.50	873.22	877.86	880.72	875.10
Crude protein	249.0	193.43	204.51	208.11	186.48	204.76	231.1
Crude fat	27.8	24.80	26.89	21.63	44.59	48.85	42.80
Ash	40.1	48.41	48.58	30.75	31.39	35.44	31.40
<i>Physical properties</i>							
NDF	287.0	58.86	55.24	53.73	54.45	52.35	61.35
ADF	153.8	13.93	14.49	14.77	16.26	15.66	18.81
ADL	40.38	12.48	13.45	13.77	14.64	14.55	18.29
Bulk density (g/ml)	0.98	1.55	1.58	1.51	1.51	1.51	1.44
WHC (g/g)	0.24	0.16	0.17	0.18	0.18	0.17	0.17
SWC (ml/g)	9.5	4.40	4.51	4.51	4.5	4.6	5.05
Total tannins	23.6	-	-	-	-	-	-

2 NDF= neutral detergent fibre; ADF= acid detergent fibre; ADL= acid detergent lignin; WHC = water holding capacity; SWC = swelling water
 3 capacity.
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8 **Table 3.** Liver enzymes and blood metabolites from *Vachellia angustissima* increasing inclusion levels

Variable	<i>V. angustissima</i> inclusion levels (g/kg DM)						SEM	Significance levels		
	0	30	60	90	120	150		Linear	Quadratic	
ALP (U/L)	837.9	948.6	679.8	707.4	753.1	1322.1	88.09	NS	***	13
ALT (U/L)	-0.30	-0.5	2.79	2.00	-1.70	0.60	1.10	NS	NS	14
AST (U/L)	225.8	236.4	252.1	217.3	252.6	269.7	20.23	NS	NS	15
Albumin (g/dl)	2.14	2.18	2.29	2.33	2.11	2.16	0.07	NS	NS	16
Cholesterol (mg/dl)	210.20	206.8	194.9	189.1	184.5	186.2	55.00	*	NS	17
Iron (ug/dl)	248.0	260.3	275.0	243.3	267.6	260.3	12.28	NS	NS	18
Calcium (mg/dl)	7.43	7.23	10.64	38.27	9.05	8.30	7.44	NS	NS	19
Phosphorus (mg/dl)	8.13	9.25	8.57	8.43	8.51	9.06	0.34	NS	NS	20
Total Protein (g/dl)	3.94	4.00	4.12	4.39	3.75	4.27	0.17	NS	NS	21
Uric acid (mg/dl)	15.61	4.06	4.10	5.08	3.39	4.30	4.90	NS	NS	22
										23
										24
										25
										26
										27

MSE=

28 mean standard error; Level of significance.*** P < 0.001; ** P < 0.01; *P < 0.05; NS, not significant P > 0.05; ALP = alkaline phosphatase;
29 ALT = alanine aminotransferase; AST = aspartate aminotransferase 1Normal ranges. ; cholesterol (mg/dL), 18 to 79; BUN = blood urea nitrogen
30 (mg/dL), 6 to 30; TP = total protein (g/dL), 6.0 to 8.0; ALB = albumin (g/dL), 1.8 to 3.3; GLOB = globulin (g/dL), 3.9 to 6; Ca = calcium
31 (mg/dL), 6.5 to 11.4; P = phosphorus (mg/dL), 3.6 to 9.2; ALT = alanine aminotransferase (units/L), 9 to 43; ALP = alkaline phosphatase
32 (units/L), 92 to 294.

33 **Table 4. Effects of inclusion levels of *V. angustissima* leaf meal on the scaled weights of internal organs in broilers in a finisher phase**

Item (g/kg BW)	<i>V. angustissima</i> inclusion levels (g/kg DM)						SEM	Significance levels ³⁴ ³⁵		³⁶
	0	30	60	90	120	150		Linear	Quadratic	
SLW	2.1x10 ⁻²	2.1x10 ⁻²	2.2x10 ⁻²	2.2 x10 ⁻²	2.1 x10 ⁻²	2.1 x10 ⁻²	6.72x10 ⁻⁴	NS	NS	³⁷
SGW	3x10 ⁻²	3 x10 ⁻²	3.5 x10 ⁻²	3.4 x10 ⁻²	3.3 x10 ⁻²	3.3 x10 ⁻²	1.27x10 ⁻³	*	NS	³⁸
SHW	4 x10 ⁻³	5 x10 ⁻³	6 x10 ⁻³	5 x10 ⁻³	5 x10 ⁻³	5 x10 ⁻³	2.16x10 ⁻⁴	***	NS	³⁹
SSW	1 x10 ⁻³	0 x10 ⁻³	1 x10 ⁻³	1 x10 ⁻³	1 x10 ⁻³	1 x10 ⁻³	1.75x10 ⁻⁴	NS	NS	⁴⁰
SPW	5 x10 ⁻³	5 x10 ⁻³	6 x10 ⁻³	5 x10 ⁻³	5 x10 ⁻³	5 x10 ⁻³	1.67x10 ⁻⁴	NS	**	⁴¹
SIW	2.3 x10 ⁻²	2.3 x10 ⁻²	2.6 x10 ⁻²	2.7 x10 ⁻²	2.6 x10 ⁻²	2.8 x10 ⁻²	9.39x10 ⁻⁴	***	NS	⁴² ⁴³

44 MSE= mean standard error; Level of significance. *** P < 0.001; ** P < 0.01; *P < 0.05; NS, not significant P > 0.05; SLW = scaled liver
 45 weight; SGW = scaled gizzard weight; SHW = scaled heart weight; SSW = scaled spleen weight; SPW = scaled proventriculus weight; SIW =
 46 scaled intestines weight, BW = body weight.