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Liver Nutrient Concentrations of *Clarias gariepinus* (Teugels) Adults Fed Graded Levels of Dietary Unprocessed Soyabean Meal

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ABSTRACT: The liver nutrient concentrations of *Clarias gariepinus* (Teugels) adults (350.28 ± 0.16g) fed graded levels of dietary unprocessed soyabean meal (USBM) were studied for 56 days. Five replacement percentage levels of the processed soyabean meal (SBM) by the USBM were adopted to provide five test diets: D_1, D_2, D_3, D_4 and D_5 at 10.00, 20.00, 30.00, 40.00 and 50.00% USBM respectively. A control diet (D_0) without USBM but with the processed SBM was also used. All the diets were formulated isonitrogenous at 38% crude protein. Significant decreases (P < 0 05) in the values of crude protein (CP), either extract (EE), ash (AS) and nitrogen-free-extract (NFE) in the liver as the dietary USBM level increased imply that the provision of these nutrients in the liver was probably moderated by the quantity of USBM in the diets. The insignificant (P > 0.05) decreases in the values of the macro-elements in the liver i.e. sodium (Na), potassium (K), magnesium (Mg), calcium (Ca) and phosphorus (P) still imply that the rate of absorption of these minerals from the diet was probably dependent on the quantity of USBM in the diets. The increasing levels of dietary USBM (10.00-50.00%) might have imposed negatively on the storage of these minerals in fish livers. The storage of the trace elements (Zn, Fe, Se, Cu and Mn) in the liver followed the pattern shown by the macro-elements except that they decreased significantly (P < 0.05) as the dietary USBM increased.

Keywords: *Clarias gariepinus*, Unprocessed soyabean meal, Liver nutrients, Macro-elements.

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Introduction

The liver plays a significant role in the metabolism of nutrients in animals (including fish). It is the metabolic centre for detoxification of chemicals. Stone *et al.* (2002) enumerated some of the liver's many and varied functions to include: carbohydrate, lipid and protein metabolisms; the storage of minerals and vitamins such as iron, copper, potassium, vitamins A, D and B_{12} and the detoxification of compounds such as alcohol and drugs. Other functions include: the conversion of the heamoglobin of dead red blood corpuscles into green and yellow pigment which give the bile its yellowish-green colour; and the retention of the iron content of haemoglobin for its reuse in the body. The formation of bile and the provision of considerable amount of heat through the chemical activities of the liver enable homiothermic animals maintain a high body temperature (Stone *et al*., 2002).

Pollutants and/or inhibitory compounds to normal physiological functions in fish might cause some distortions in the activities of the key organs in the body of the fish. Liver damage for example was confirmed by changes in the activities of certain key enzymes in the fish such as Glutamate-Pyruvate Transaminase (GPT) and Glutamate-Oxaloacetate Transaminase (GOT) (Asztalos and Nemesok, 1985). Increase in blood glucose levels is a general response of fish to acute pollutant effects including organophosphates and pyrethroids (Luskova *et al*., 2002). Oluah and Njoku (2001) reported that there was a significant high positive correlations (b = 0.21; r = 0.9958) between liver glucose and paraquat concentrations. The authors posited that the rate of glucose metabolization in both the liver, blood and body tissues of *Clarias gariepinus* (Teugels) due to paraquat concentrations was dose-dependent.

Plant protein supplements in the fish diets are generally cheaper sources of protein per unit of nutrient than animal protein sources. Of all sources of plant proteins, soyabean meal has been extensively evaluated and found palatable to fish. Nonetheless, unprocessed soyabean meal contains anti-nutritional factors which hinder growth in fish and must be heat-treated to ameliorate this problem. The dietary protein content on body composition of various sizes of catfish has been evaluated in different production systems. Faturoti *et al*. (1986) reported a range of 37-40% dietary protein as optimum for the growth of advanced fry of *Clarias (lazera) gariepinus*. James (2000) also reported that the body fat component of catfish decreases as the dietary protein increases.

Traditionally, animal protein is regarded as the foundation of any aquaculture feed formulation programme. Nevertheless, the utility of plant proteins and most especially soyabean meal has been adequately recognized because of its high digestibility and ample indispensable amino acids (Edwin and Tom, 1995). The processed soyabean meal (SBM) has a high metabolizable energy value of 96% and its oil content is relatively stable due to the high level of naturally occurring enzymes in it (Holmes, 1999). Rackis and Liener (1998) reported that the unprocessed soyabean meal is highly palatable to catfish and meets a majority of its amino acids requirements. The unprocessed soyabean meal (USBM) on the other hand has anti-nutritional factors that affect catfish growth and performance (Rackis and Liener, 1998). Smith (1999) therefore stated that for maximum metabolizable energy in fish, the unprocessed SBM should be processed at a maximum temperature of 175ºC. Against this background, this study investigated the effects of feeding graded levels of dietary unprocessed soyabean meal to *Clarias gariepinus* on the concentration of nutrients in the liver tissues of the fish. The essence was to investigate the extent of metabolic distortions of such liver nutrients as carbohydrates, proteins, fats and minerals owning to the anti-nutritional factors in unprocessed soyabean meal.

Materials And Methods

Experimental Procedure

Three hundred and sixty (360) adults of *Clarias gariepinus* (Teugels) (mean weight, 350.28 ± 0.16g) were purchased from Phinomar Fish Hatchery in Enugu State, Nigeria and transported to the Fisheries Laboratory of Ebonyi University, Abakaliki, Nigeria. The fish specimens were acclimatized for 14 days on chick starter diet, fed at 3% body weight per day (bw.d-1). Records of fish weights were taken with the aid of a top-loading electronic Mettler balance (Model P T 600). The fish were randomly stocked in 18 aerator-fitted glass aquaria (55 x 30 x30 cm^3) at 20 fish per aquarium and supplied with 25cm^3 dechlorinated tap water. The fish were stocked in six sets of aquaria replicated thrice (6 x 3) to provide 18 experimental treatments. Five (5) experimental diets (D_1, D_2, D_3, D_4 and D_5) were formulated at 38% crude protein (Table 1) to include 5 levels: 10.00, 20.00, 30.00, 40.00 and 50.00% of unprocessed soyabean meal (USBM) respectively. These five levels of the unprocessed soyabean meal in the diets were percent replacements of the processed soyabean meal (SBM) in the control diet (D_0).

Dietary ingredients (Table 1) were thoroughly mixed, precooked and pelletized with a locally fabricated pelletizer and oven-dried. The formulated diets were packed in small cellophane bags, labelled according to treatment, and stored in pest-free cupboard within the laboratory. Proximate analyses of the test diets (D_1-D_5) and control (D_0) were carried out. Feeding was carried out thrice daily at four hourly intervals (8am, 12noon and 6pm) at 5% bw.d^{-1} for 56 days (8 weeks). The filtration systems of the aquaria helped in the elimination of feaces and other residues. Water temperature readings (27 ± 0.08ºC) and the pH values (6.60 ± 0.16) were recorded with a maximum thermometer and a pH meter (Model Ph-J-201L) respectively. Control weighing was carried out every 14days and feed administered the subsequent 14days adjusted in accordance with the body weight of fish.

Method of Analysis

The five test diets (D_1-D_5) and the control (D_0) were analyzed in the laboratory for their proximate compositions according to the method described by Windham (1996) (Table 2). The percent nitrogen content was determined by the micro-kjeldahl method and converted to total protein equivalent by multiplying by 6.25 (Windham, 1996). The crude fat was measured in a soxhlet apparatus of lipid by protein ether (b. pt. 40-60ºC) extraction. The moisture content was determined by drying 2.00g triplicate samples at 105ºC to constant weight and calculating the percentage moisture by using the formula:

$$\frac{x - y}{x} \times \frac{100,}{1}$$

where: x = weight of wet sample, y = dry weight of sample. Ash was determined by combusting 2.00g triplicate samples for 12 hour at 550ºC. The percentage nitrogen-free-extract (NFE) was determined by difference.

Two fish specimens per treatment were dissected for the excision of their liver lobes on days 14, 28, 42 and 56 of the experimental period. The liver lobes were thoroughly washed with diluted water and kept in 250cm³ glass bottles containing 0.09% physiological saline solution. The proximate and mineral compositions of the fish livers were carried out in accordance with the methods described by Windham (1996). The flame photometric method was used to determine the values of sodium (Na) and potassium (K); while ethyleneamine tetraacetic acid (EDTA) titrations were used for those of calcium (Ca) and magnesium Mg). Complexometric titration method was used for zinc (Zn). For other all other minerals tested, the spectrophotometric method of assessment was used (Windham, 1996) and these were compared with calibrated series. The proximate composition of the fish livers was determined as already described. All the data obtained were analyzed using descriptive statistics and analysis of variance (ANOVA) to indicate statistical significance ($P < 0.05$) (Steel and Torrie, 1990). The Duncan’s (1955) Multiple Range Test method was employed to partition the difference.

Results

The gross compositions of the unprocessed soyabean meal incorporated diets (D_1-D_5) and the control (D_0) are shown in the Table 1. The proximate compositions of the six diets (D_0-D_5) are also shown in Table 2. Table 3 shows the proximate compositions of the *Clarias gariepinus* adults fed graded levels of dietary unprocessed soyabean meal (USBM) for 56 days; while Tables 4 and 5 show the macro and trace element components of the mineral composition of the liver of the fish within the experimental period (56 days).

The control fish exhibited significantly ($P < 0.05$) higher values of the crude protein (CP), ether extract (EE), ash (AS), dry matter content (DM) and nitrogen-free-extract (NFE) in their livers than those fed with USBM diets within the study period (days 14-56) (Table 1). Additionally, the vales of CP, EE, AS, DM and NFE in the liver of fish fed the USBM diets decreased significantly ($P < 0.05$) with the increasing concentrations of USBM (10.00-50.00%). This situation was evident when the fish livers were analyzed for the above-mentioned nutrients on the days 14, 28, 42 and 56 (Table 3).

Table 1. Gross Compositions of Unprocessed Soyabean Meal Incorporated Diets (38% Crude Protein) Fed to *Clarias gariepinus* Adults for 56 Days

Ingredients (%)	Control D_0 (0% USBM[1])	Unprocessed Soyabean Meal Incorporated Diets				
		D_1 (10% USBM)	D_2 (20% USBM)	D_3 (30% USBM)	D_4 (40% USBM)	D_5 (50% USBM)
Yellow maize	11.61	11.61	11.61	11.61	11.61	11.61
Unprocessed soyabean meal	54.76**	5.48	10.95	16.43	21.87	27.30
Groundnut cake	-	49.28	43.81	38.33	32.89	27.46
Fishmeal	16.43	16.43	16.43	16.43	16.43	16.43
Blood meal	10.95	10.95	10.95	10.95	10.95	10.95
Palm oil	5.00	5.00	5.00	5.00	5.00	5.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin mix[2]	0.60	0.60	0.60	0.60	0.60	0.60
Mineral mix[3]	0.40	0.40	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1]*Unprocessed soyabean meal,* [2]*Vitamin mix provided the following diluted in cellulose (mg/kg in diet); Thamine, 10; Riboflavin, 20; Pyridoxine, 10; Folacin, 5; Pantothenic acid, 40; Choline Chloride, 3000; Naicin, 150; Vitamin* B_{12}*, 0.06; Retinyl acetate (500,000 IU/g), 12; Choline copherol, 50; Cholecalciferol (1,000,000 IU/g), 6; Menadione-Na-bisulphate, 80; Inositol, 400; Biotin, 2; Vitamin C, 200; and Ethoxyquine, 200.*

[2]*Contained as g/kg of premix:* $FeSO_4.7H_20$*, 5;* $MgSO_4.7H_2O$*, 132;* K_2SO_4*, 329.90, KI, 0.15; NaCl, 45;* $NaSO_4$*, 44.88;* $AlCl_3$*, 0.15;* $CoCl_2.6H_2O$*, 5;* $CuSO_4.5H_2O$*, 5;* $NaSeO_3$*, 0.11;* $MnSO_4.H_2O$*, 0.7; and cellulose, 380.97.*

*** Value of processed soyabean meal in the control diet.*

Table 2. Proximate Compositions of Unprocessed Soyabean Meal Incorporated Diets (38% Crude Protein) Fed to *Clarias gariepinus* Adults for 56 Days

Ingredients (%)	Control D_0 (0% USBM[1])	Unprocessed Soyabean Meal Incorporated Diets D_1 (10% USBM)	D_2 (20% USBM)	D_3 (30% USBM)	D_4 (40% USBM)	D_5 (50% USBM)
Crude protein (%)	37.66	36.83	36.84	37.52	37.60	37.64
Ether extract (%)	5.44	4.60	5.03	5.63	4.58	4.56
Ash (%)	11.64	11.52	11.60	11.80	10.58	10.60
Moisture content (%)	10.58	10.51	10.62	10.55	11.46	11.46
NFE[2] (%)	34.68	36.54	35.91	34.50	35.78	35.74
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1]*Unprocessed soyabean meal,* [2]*Nitrogen-free-extract*

Table 3. Proximate Compositions of *Clarias gariepinus* Adults Fed Graded Levels of Dietary Unprocessed Soyabean Meal for 56 Days

Duration (Days)	Nutrient (%)	Control D_0 (0% USBM[1])	Unprocessed Soyabean Meal Incorporated Diets					Overall Mean $\bar{x}$
			D_1 (10% USBM)	D_2 (20% USBM)	D_3 (30% USBM)	D_4 (40% USBM)	D_5 (50% USBM)	
14	CP[2]	16.62 ± 0.07^{a}	15.79 ± 0.06^{b}	15.01 ± 0.07^{c}	14.26 ± 0.06^{d}	13.55 ± 0.06^{e}	12.87 ± 0.07^{f}	14.68 ± 0.06
	EE[3]	5.61 ± 0.02^{a}	5.33 ± 0.04^{b}	5.06 ± 0.03^{c}	4.81 ± 0.02^{d}	4.57 ± 0.02^{e}	4.34 ± 0.03^{f}	4.95 ± 0.03
	AS[4]	1.03 ± 0.04^{a}	0.98 ± 0.03^{a}	0.93 ± 0.03^{a}	0.88 ± 0.03^{a}	0.84 ± 0.03^{ab}	0.80 ± 0.02^{b}	0.91 ± 0.03
	DM[5]	23.08 ± 0.70^{a}	26.93 ± 0.06^{b}	30.58 ± 0.70^{c}	34.05 ± 0.08^{d}	37.35 ± 1.01^{e}	40.48 ± 1.21^{f}	32.08 ± 1.06
	NFE[6]	53.66 ± 1.21^{a}	50.97 ± 1.24^{b}	48.37 ± 1.11^{c}	46.00 ± 1.10^{d}	43.69 ± 1.12^{e}	41.51 ± 1.13^{f}	47.36 ± 1.14
28	CP	17.45 ± 0.06^{a}	15.33 ± 0.06^{b}	14.44 ± 0.05^{c}	13.68 ± 0.04^{d}	12.96 ± 0.03^{e}	12.23 ± 0.04^{f}	14.35 ± 0.04
	EE	5.89 ± 0.02^{a}	5.18 ± 0.02^{b}	4.92 ± 0.03^{c}	4.65 ± 0.03^{d}	4.43 ± 0.02^{e}	4.12 ± 0.02^{f}	4.86 ± 0.02
	AS	1.08 ± 0.05^{a}	0.95 ± 0.03^{b}	0.84 ± 0.03^{c}	0.81 ± 0.04^{c}	0.75 ± 0.03^{d}	0.71 ± 0.03^{d}	0.86 ± 0.03
	DM	24.68 ± 0.60^{a}	30.47 ± 1.03^{b}	34.67 ± 1.11^{c}	39.48 ± 1.13^{d}	36.44 ± 1.10^{e}	43.46 ± 1.14^{f}	34.87 ± 1.02
	NFE	50.90 ± 1.22^{a}	48.07 ± 1.21^{b}	45.13 ± 1.13^{c}	41.38 ± 1.14^{d}	45.42 ± 1.13^{e}	39.48 ± 1.14^{f}	51.33 ± 1.21
42	CP	17.52 ± 0.07^{a}	13.03 ± 0.05^{b}	12.27 ± 0.05^{c}	11.63 ± 0.04^{d}	11.02 ± 0.04^{e}	10.40 ± 0.04^{f}	12.65 ± 0.05
	EE	5.91 ± 0.03^{a}	4.40 ± 0.04^{b}	4.18 ± 0.03^{c}	3.95 ± 0.03^{d}	3.76 ± 0.02^{e}	3.50 ± 0.03^{f}	4.28 ± 0.03
	AS	1.32 ± 0.05^{a}	0.81 ± 0.04^{b}	0.71 ± 0.03^{c}	0.69 ± 0.03^{c}	0.64 ± 0.03^{cd}	0.61 ± 0.03^{d}	0.80 ± 0.03
	DM	23.39 ± 0.06^{a}	40.90 ± 1.14^{b}	44.47 ± 1.15^{c}	45.97 ± 1.16^{d}	48.56 ± 1.16^{e}	51.94 ± 1.15^{f}	42.51 ± 1.06
	NFE	51.86 ± 1.21^{a}	40.86 ± 1.13^{b}	38.37 ± 1.12^{c}	37.76 ± 1.14^{d}	36.02 ± 1.06^{e}	33.55 ± 1.04^{f}	39.74 ± 1.03
56	CP	17.63 ± 0.06^{a}	12.38 ± 0.04^{b}	11.66 ± 0.04^{c}	11.05 ± 0.03^{d}	10.48 ± 0.02^{e}	9.88 ± 0.03^{f}	12.18 ± 0.04
	EE	5.94 ± 0.02^{a}	4.18 ± 0.02^{b}	3.97 ± 0.02^{c}	3.75 ± 0.03^{d}	3.57 ± 0.03^{e}	3.33 ± 0.03^{f}	4.12 ± 0.02
	AS	1.42 ± 0.06^{a}	0.77 ± 0.03^{b}	0.67 ± 0.03^{c}	0.65 ± 0.02^{c}	0.61 ± 0.02^{cd}	0.58 ± 0.03^{e}	0.78 ± 0.03
	DM	23.03 ± 1.03^{a}	43.85 ± 1.14^{b}	47.25 ± 1.14^{c}	48.67 ± 1.15^{d}	51.13 ± 1.16^{e}	54.34 ± 1.16^{f}	44.71 ± 1.13
	NFE	51.98 ± 1.21^{a}	38.82 ± 1.12^{b}	36.45 ± 1.04^{c}	35.88 ± 1.05^{d}	34.21 ± 1.13^{e}	31.87 ± 1.14^{f}	38.20 ± 1.13

[1]Unprocessed soyabean meal, [2]Crude protein, [3]Ether extract, [4]Ash, [5]Drymatter content, [6]Nitrogen free-extract, values in the same row followed by the same superscripts are not significantly different ($P > 0.05$); values in the same row followed by different superscripts differ significantly ($P < 0.05$).

Table 4. Macro-elements of the Mineral Compositions of the Liver of *Clarias gariepinus* Adults Fed Graded Levels Dietary Unprocessed Soyabean Meal for 56 Days

Duration (Days)	Nutrient (%)	Control D_0 (0% USBM[1])	Unprocessed Soyabean Meal Incorporated Diets D_1 (10% USBM)	D_2 (20% USBM)	D_3 (30% USBM)	D_4 (40% USBM)	D_5 (50% USBM)	Overall Mean $\bar{x}$
	Na[2]	0.08 ± 0.03^{a}	0.07 ± 0.03^{a}	0.06 ± 0.02^{a}	0.05 ± 0.02^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.06 ± 0.02
	K[3]	0.12 ± 0.04^{a}	0.10 ± 0.04^{a}	0.09 ± 0.03^{a}	0.08 ± 0.03^{a}	0.07 ± 0.02^{a}	0.06 ± 0.02^{ab}	0.09 ± 0.03
14	Mg[4]	0.08 ± 0.03^{a}	0.08 ± 0.03^{a}	0.07 ± 0.02^{a}	0.06 ± 0.02^{a}	0.06 ± 0.02^{a}	0.05 ± 0.02^{a}	0.07 ± 0.02
	Ca[5]	0.19 ± 0.02^{a}	0.16 ± 0.02^{a}	0.14 ± 0.04^{a}	0.12 ± 0.03^{a}	0.10 ± 0.03^{ab}	0.09 ± 0.03^{a}	0.13 ± 0.03
	P[6]	0.25 ± 0.04^{a}	0.21 ± 0.04^{a}	0.18 ± 0.03^{ab}	0.15 ± 0.04^{b}	0.13 ± 0.03^{b}	0.11 ± 0.03^{b}	0.17 ± 0.03
	Na	0.08 ± 0.03^{a}	0.07 ± 0.03^{a}	0.05 ± 0.02^{a}	0.04 ± 0.02^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{ab}	0.05 ± 0.02
	K	0.11 ± 0.02^{a}	0.09 ± 0.03^{a}	0.08 ± 0.03^{a}	0.07 ± 0.03^{a}	0.06 ± 0.02^{a}	0.05 ± 0.02^{ab}	0.08 ± 0.02
28	Mg	0.08 ± 0.02^{a}	0.07 ± 0.02^{a}	0.06 ± 0.02^{a}	0.05 ± 0.02^{a}	0.05 ± 0.02^{a}	0.04± 0.01^{a}	0.06 ± 0.02
	Ca	0.18 ± 0.04^{a}	0.15 ± 0.03^{a}	0.13 ± 0.04^{a}	0.11 ± 0.03^{ab}	0.09 ± 0.02^{ab}	0.08 ± 0.03^{b}	0.12 ± 0.03
	P	0.24 ± 0.04^{a}	0.20 ± 0.04^{a}	0.17 ± 0.04^{ab}	0.14 ± 0.03^{b}	0.12 ± 0.03^{b}	0.10 ± 0.03^{bc}	0.16 ± 0.03
	Na	0.07 ± 0.02^{a}	0.06 ± 0.02^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 001^{a}	0.01 ± 0.00^{ab}	0.04 ± 0.01
	K	0.09 ± 0.03^{a}	0.08 ± 0.03^{a}	0.07 ± 0.02^{a}	0.06 ± 0.02^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{ab}	0.06 ± 0.02
42	Mg	0.07 ± 0.03^{a}	0.06 ± 0.02^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.05 ± 0.02
	Ca	0.15 ± 0.03^{a}	0.13 ± 0.03^{a}	0.11 ± 0.03^{a}	0.09 ± 0.03^{a}	0.08 ± 0.02^{ab}	0.07 ± 0.02^{b}	0.11 ± 0.03
	P	0.20 ± 0.04^{a}	0.17 ± 0.04^{a}	0.14 ± 0.04^{a}	0.12 ± 0.03^{b}	0.10 ± 0.03^{b}	0.09 ± 0.03^{b}	0.14 ± 0.03
	Na	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.03 ± 0.01
	K	0.08 ± 0.03^{a}	0.06 ± 0.02^{a}	0.05 ± 0.01^{a}	0.05 ± 0.02^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.05 ± 0.02
56	Mg	0.05 ± 0.02^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}	0.04 ± 0.01
	Ca	0.11 ± 0.03^{a}	0.10 ± 0.03^{a}	0.08 ± 0.03^{a}	0.07 ± 0.03^{a}	0.06 ± 0.02^{a}	0.06 ± 0.02^{a}	0.08 ± 0.03
	P	0.15 ± 0.04^{a}	0.13 ± 0.03^{a}	0.11 ± 0.03^{a}	0.09 ± 0.03^{a}	0.08 ± 0.03^{a}	0.08 ± 0.03^{a}	0.11 ± 0.03

[1]Unprocessed soyabean meal, [2]Sodium, [3]Potassium, [4]Magnesium, [5]Calcium, [6]Phosphorus, values in the same row followed by the same superscripts are not significantly different (P > 0.05); values in the same row followed by different superscripts differ significantly (P < 0.05).

Table 5. Trace-elements of the Mineral Compositions of the Liver of *Clarias gariepinus* Adults Fed Graded Levels Dietary Unprocessed Soyabean Meal for 56 Days

Duration (Days)	Nutrient (%)	Control D_0 (0% USBM[1])	Unprocessed Soyabean Meal Incorporated Diets D_1 (10% USBM)	D_2 (20% USBM)	D_3 (30% USBM)	D_4 (40% USBM)	D_5 (50% USBM)	Overall Mean $\bar{x}$
	Zn[2]	6.51 ± 0.05^{a}	6.18 ± 0.05^{b}	5.87 ± 0.04^{c}	5.58 ± 0.04^{d}	5.30 ± 0.03^{e}	5.03 ± 0.03^{f}	5.75 ± 0.04
	Fe[3]	5.32 ± 0.05^{a}	5.05 ± 0.03^{b}	4.80 ± 0.04^{c}	4.56 ± 0.03^{d}	4.33 ± 0.03^{e}	4.12 ± 0.03^{f}	4.70 ± 0.04
14	Se[4]	0.11 ± 0.01^{a}	0.10 ± 0.01^{a}	0.09 ± 0.01^{a}	0.08 ± 0.01^{a}	0.07 ± 0.01^{a}	0.06 ± 0.01^{a}	0.09 ± 0.01
	Cu[5]	0.34 ± 0.01^{a}	0.29 ± 0.01^{a}	0.28 ± 0.02^{ab}	0.26 ± 0.02^{b}	0.25 ± 0.02^{b}	0.24 ± 0.03^{b}	0.28 ± 0.02
	Mn[6]	0.96 ± 0.04^{a}	0.91 ± 0.04^{a}	0.87 ± 0.03^{ab}	0.82 ± 0.03^{b}	0.78 ± 0.03^{bc}	0.74 ± 0.04^{c}	0.85 ± 0.04
	Zn	6.68 ± 0.06^{a}	5.87 ± 0.05^{b}	5.58 ± 0.05^{c}	5.30 ± 0.04^{d}	5.04 ± 0.04^{e}	4.79 ± 0.03^{f}	5.54 ± 0.04
	Fe	5.45 ± 0.05^{a}	4.80 ± 0.03^{b}	4.56 ± 0.03^{c}	4.33 ± 0.03^{d}	4.11 ± 0.03^{e}	3.91 ± 0.02^{f}	4.53 ± 0.03
28	Se	0.12 ± 0.01^{a}	0.09 ± 0.01^{a}	0.08 ± 0.01^{a}	0.07 ± 0.01^{a}	0.06 ± 0.01^{ab}	0.05 ± 0.01^{b}	0.08 ± 0.01
	Cu	0.38 ± 0.02^{a}	0.28 ± 0.02^{b}	0.27 ± 0.02^{b}	0.25 ± 0.02^{b}	0.24 ± 0.02^{b}	0.23 ± 0.02^{b}	0.28 ± 0.02
	Mn	0.98 ± 0.04^{a}	0.86 ± 0.04^{b}	0.83 ± 0.03^{b}	0.78 ± 0.03^{c}	0.74 ± 0.03^{c}	0.70 ± 0.03^{cd}	0.82 ± 0.03
	Zn	6.72 ± 0.07^{a}	4.99 ± 0.05^{b}	4.74 ± 0.05^{c}	4.51 ± 0.04^{d}	4.28 ± 0.03^{e}	4.07 ± 0.03^{f}	4.89 ± 0.04
	Fe	5.56 ± 0.06^{a}	4.08 ± 0.04^{b}	3.88 ± 0.03^{c}	3.68 ± 0.02^{d}	3.49 ± 0.02^{e}	3.32 ± 0.03^{f}	4.00 ± 0.03
42	Se	0.14 ± 0.01^{a}	0.08 ± 0.02^{b}	0.07 ± 0.01^{b}	0.06 ± 0.01^{b}	0.05 ± 0.01^{b}	0.04 ± 0.01^{b}	0.07 ± 0.01
	Cu	0.42 ± 0.02^{a}	0.24 ± 0.02^{b}	0.23 ± 0.02^{b}	0.21 ± 0.02^{b}	0.20 ± 0.01^{b}	0.20 ± 0.01^{b}	0.25 ± 0.02
	Mn	0.99 ± 0.06^{a}	0.73 ± 0.05^{b}	0.71 ± 0.04^{b}	0.66 ± 0.04^{c}	0.63 ± 0.03^{c}	0.60 ± 0.03^{cd}	0.72 ± 0.04
	Zn	6.76 ± 0.06^{a}	3.74 ± 0.03^{b}	3.56 ± 0.03^{c}	3.38 ± 0.03^{d}	3.21 ± 0.03^{e}	3.05 ± 0.03^{f}	3.95 ± 0.04
	Fe	5.60 ± 0.04^{a}	3.06 ± 0.04^{b}	2.91 ± 0.02^{c}	2.76 ± 0.02^{d}	2.62 ± 0.02^{e}	2.49 ± 0.02^{f}	3.24 ± 0.03
56	Se	0.18 ± 0.01^{a}	0.06 ± 0.01^{b}	0.05 ± 0.01^{b}	0.05 ± 0.01^{b}	0.04 ± 0.01^{b}	0.03 ± 0.01^{b}	0.07 ± 0.01
	Cu	0.46 ± 0.02^{a}	0.18 ± 0.01^{b}	0.17 ± 0.02^{b}	0.16 ± 0.02^{b}	0.15 ± 0.01^{b}	0.15 ± 0.01^{b}	0.21 ± 0.02
	Mn	1.10 ± 0.03^{a}	0.54 ± 0.02^{b}	0.53 ± 0.03^{b}	0.50 ± 0.02^{b}	0.47 ± 0.02^{bc}	0.45 ± 0.02^{c}	0.60 ± 0.02

[1]Unprocessed soyabean meal, [2]Zinc, [3]Iron, [4]Selenium, [5]Copper, [6]Manganese, values in the same row followed by the same superscripts are not significantly different ($P > 0.05$); values in the same row followed by different superscripts differ significantly ($P < 0.05$).

The study revealed that the unprocessed soyabean meal (USBM) in the fish diets had some deleterious effects on the values of CP, EE, AS, DM and NFE as the duration of the study extended from day 14 to day 56. It was recorded that the values of these nutrients in the fish livers depleted to the magnitudes of 5% between day 14 and day 28; 15% on day 42 and 5% on day 56 (Table 3). This trend was reflected in the mean values of these nutrients, where for example, CP values decreased from 14.68 ± 0.06% (day 14) to 14.35 ± 0.04% (day 28), 12.65 ± 0.05% and 12.18 ± 0.04% (day 56) (Table 3).

The mineral compositions of the livers of fish fed the diets and the control (Tables 4 and 5) also indicated that the values of the macro-element components in the control fish namely: sodium (Na), potassium (K), magnesium (Mg), calcium(Ca) and phosphorus (P) were higher than those in fishes fed the USBM diets (Table 4). However, differences in the nutrient values between the control fish livers and the test fish livers were insignificant ($P > 0.05$). Although the values of Na, K, Mg, Ca and P decreased with the increasing concentrations of USBM in the diets, such decreases were statistically insignificant ($P > 0.05$). However, there were significant ($P < 0.05$) decreases in the values of the trace-elements of the livers i.e. zinc (Zn), iron (Fe), selenium (Se), copper (Cu) and manganese (Mn) as the dietary USBM increased from 10.00% to 50%; and as the study period lasted between days 14 and 56 (Table 5). It was also observed that these trace-elements increased in the magnitudes of 5% on day 28, 15% on day 42 and 25% on day 56 (Table 5).

Discussion

The presence of a high of naturally occurring enzymes in the processed soyabean meal (SBM) contributes immensely to the stability of its oil content (Holmes, 1999). Other positive attributes of the SBM as a useful plant protein source in fish feeding include: its high metabolizable energy value (96%), its high palatability to catfish and its ability to meet majority of the amino acid requirements of the fish. When soyabean meal is fed raw and unprocessed to catfish the negative effects of this action on fish performance has been attributed to the presence of anti-nutritional factors inimical to fish growth (Rackis and Liener, 1998). Therefore, fish nutritionists are constrained to use the method provided by Smith (1999) which ensures that soyabean meal is processed at a minimum temperature of 175ºC before being incorporated in the fish diets.

The results of this study indicated the consequences of the unconventional application of unprocessed soyabean meal (USBM) at different levels of inclusion in catfish diets: especially as it affected the proximate and mineral compositions of the fish liver. The liver is the seat of nutrients metabolism in fish as well as the centre for the detoxification of chemicals (Stone *et al*., 2002). Hence, it is imperative that studies on the nutrients status of the fish liver be carried out consequent upon the provision of feed ingredients with a propensity to inhibit growth. Therefore, the significant decreases ($P > 0.05$) in the values of CP, EE, AS, DM and NFE in the fish livers (Table 3) as the dietary USBM levels increased that the provision of these nutrients in the liver was probably moderated by the quantity of USBM in the diets. The result of this study is consistent with the report of Steffens (1989) which stated that the unprocessed soyabean meal could hinder the provision of amino acids necessary for growth and production of enzymes. Since the values of CP decreased as the level of dietary USBM increased from 10.00% (D_1) to 50.00% (D_5), it could be that the hindrance caused by the USBM to provide the necessary amino acids for the synthesis of applied (as replacement of the processed SBM un the diets) increased.

The negative effect of anti-trypsin factors in USBM (Rackis and Liener, 1998) in this study might have prevented the provision of the necessary amino acids from the digestion of the ingested protein in the fish diet (Table 1), and which ultimately might have prevented the metabolism of proteins in the livers. Similar deductions could be made of the factors responsible for the metabolism of crude fat (EE) and digestible carbohydrate (NFE) in the fish. From these results, it could be stated that anti-lipase and anti-amylase factors were at play due to the presence of USBM in the fish diets. This implies that apart from its anti-trypsin properties, dietary USBM also has both anti-lipase and anti-amylase factors that hinder the metabolism of fats and carbohydrates in the fish.

Decreases in macro-element compositions of the fish livers although insignificant ($P > 0.05$) imply that the rate of absorption of Na, K, Mg, Ca and P from the ingested USBM diets decreased as the levels of dietary USBM increased from 10.00%to 50.00% since fishes are noted to absorb minerals from food and ambient water environment (Lagler *et al.*, 1977) it was apparent that the ability to execute this function was dependent on the concentrations of USBM in the diets. Increasing levels of dietary USBM (10.00-50.00%) might have impacted negatively on the storage of these minerals (macro-elements) in the fish livers (Table 4). Welcome (1979) reported that nitrate ions, as well as calcium, magnesium, phosphorus and chloride ions/elements could enhance the growth, survival and reproduction of fish in its water medium. Hence, the utility of dietary and aqueous minerals by fishes in this study for growth and survival was better enhanced at low inclusion levels of dietary USBM (10.00%) than at higher inclusion levels (50.00% USBM). The same explanation provided for the absorption and storage of macro-elements in the fish livers might also be applicable to the trace -elements (Zn, Fe, Se, Cu and Mn). The only difference was the significant decreases ($P < 0.05$) in the values of these nutrients as the dietary USBM increased (Table 5).

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