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Histology, serum biochemistry and haematological profiles of *Clarias gariepinus* fed diets containing *Luffa cylindrica* seedmeal

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Abstract. *A 56-day feeding trial was conducted to evaluate the effect of processing time, inclusion level and/or their interactions on serum biochemistry, haematology and histology of the liver and kidney of Clarias gariepinus fed diets containing Luffa cylindrica seedmeal. The five formulated diets were designated as control (CTR) and tested, replaced at 15 and 30% by 5- and 10-min toasted Luffa cylindrica seedmeal (D515T, D530T, D1015T and D1030T). The experimental design followed a 2x2 factorial experiment in a completely randomised design; the processing time of 5- and 10-min toasting and inclusion level of 15 and 30% serves as factors. Triplicate groups of each treatment were made. Blood sampling, harvesting of organs, serum biochemistry, histology and haematological studies followed standard procedures. The results of the study showed that the effect of processing time, inclusion level and their interactions had significant impact ($p<0.05$) on Clarias gariepinus. A decrease in RBC, PCV and Hb was observed when compared with control for inclusion level, processing time and/or their interaction. Total protein, albumin and globulin of the blood of fish fed diet CTR was not significantly different ($p>0.05$) from that of the blood of the fish fed D1015T. Mild to moderate vacuolation of the hepatocytes were recorded among the livers of fish fed control and test dietary treatments except those fed D1030T that recorded very severe vacuolation of the hepatocytes. The kidney, being the excretory organ, was also affected. However, normal cell architecture was recorded in fish fed CTR, D530T and D1030T.*

Keywords: *Clarias gariepinus*, *Luffa cylindrica*, liver, kidney, histology, haematology

Introduction

Aquaculture is rapidly growing not only in sub-Saharan Africa but also in Asia and Latin America (FAO, 2009). Tacon et al. (2006) reported that aquaculture sector alone uses larger percentage of the fishmeal produced in the world. This is unsustainable because harvest from fishery fluctuates year in year out (Huntington, 2004).

The fish feed production industries that largely depend on the use of fishmeal find a great challenge its use as a cost-effective source of animal protein in their feed (Huntington et al., 2004). Fagbenro et al. (2003) reported that seed meal from leguminous plants could serve as a good replacer of ever expensive and scarce fish meal. Soybean meal have been largely reported to have the capacity to replace most of the fishmeal components in fish feed without leaving a detrimental effect on the animal (Lovell, 1988; Storebakken et al., 2000) due to its high nutrient profile (Lim and Akiyama, 1992; El-Sayed, 1999). However, human beings and other livestock industries utilize soybean meal as their protein source making its use not being sustainable for sustainable aquaculture. Thus the need to find good alternative to soybean meal used in fish feed industries especially at the village level by searching for cheaper and readily available plant proteins with little or no cost and comparable nutrient profiles to replace soybean meal in fish feed (Yue and Zhou, 2008). Such use of alternative plant protein sources must be eco-friendly, nutritionally balanced and economically sustainable.

Luffa cylindrica is said to have nutrient composition that is comparable to legumes like soybean meal, groundnut cake that are commonly used in aqua feed as plant protein sources (Oshodi et al., 1993; Sanchez-Vioque et al., 1998). Oyetayo and Ojo (2012) reported that *L. cylindrica* belongs to the family cucurbitacea and its seed has a high quantity of amino acid that is essential to be supplied to the body system. It, however, contains some phytochemicals such as saponin and alkaloids, etc. It is commonly found in Nigeria. To the best of our knowledge, there has been little information on the use of *Luffa cylindrica* seedmeal as protein source dietary ingredient for fish (Jimoh et al., 2012). Jimoh et al. (2013) constituted a first report in journal on the use

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of *L. cylindrica* in fish feed; 15% level of inclusion of cooked *L. cylidrica* seedmeal in *Clarias gariepinus* diet had a comparable growth and nutrient utilization results with that of control.

Processing time and inclusion level are two major factors that could affect nutrient utilization by fish (Adeparusi and Jimoh, 2002). Excessive heating time may denature protein in feed ingredient while too low heating time might not be effective to remove anti-metabolite in feed ingredients. Effective thermal treatment must be able to degrade anti-metabolites in plant feed ingredients without destroying its amino acid profile. Thermal treatments have been reported to inactivate some anti-metabolites in plant feed ingredients (Fagbenro et al., 2010; Jimoh et al., 2011). Francis et al. (2001) postulated that inclusion of plant feed ingredients containing some form of anti-nutrients or the other could be tolerated by fish up to certain inclusion level beyond which the physiological mechanism of the fish may malfunction. Hence, it could be said that thermal treatment and/or inclusion level jointly determine the success of using plant feed ingredients in fish feed and feeding. Till date paucity of information exists on the use of processing time and/or inclusion level of *L. cylidrica* seedmeal in *Clarias gariepinus* diets, especially the effect it has on haematological, histological alterations and biochemical parameters which are good pointers to health status of not only farmed fish but also farmed animals and even humans (Bahmani et al., 2001; Ferreira et al., 2007) because they supply a dependable information on metabolic disroders and deficiency. It is against this background that an attempt was made in this study to examine the effect of processing time, inclusion level and their interaction on the histology, haematology and serum biochemistry of *Clarias gariepinus* fed diets containing dry heat-treated *Luffa cylindrica* meal.

Material and methods

Seed collection and processing

Luffa cylindrica seeds, 1.5 kg were obtained from Institute of Agricultural Research and Training Moor plantation Ibadan, Nigeria. The seeds were divided into two parts. The first part was toasted in an oven (Emel Electric Oven-20L) at 150ºC for 5 min and the other part at the same temperature for 10 min. The two processed samples were allowed to cool and were ground to a powdery form. Representative parts of each of these samples and other practical feedstuffs were analyzed for their proximate composition (AOAC, 1990).

The results of the proximate composition depicted in Table 1 were used to formulate five diets; a control diet which consists of soybean meal replacing 50% fishmeal and four test diets which consist of 5- and 10-minute toasted *Luffa cylindrica* seedmeal replacing soybean meal at 15 and 30% level (Table 2). The five formulated diets were designated as CTR, D515T, D530T, D1015T and D1030T. All the feed ingredients in each dietary treatment were blended and sieved to a powdery form. They were thoroughly mixed with hot water to facilitate cohesion among the dietary ingredients. The mixed dietary ingredients were pelleted into 2mm pellets and oven dried at 55ºC for 24h. They were thereafter kept frozen in a refrigerator at -20ºC.

Table 1. Proximate composition (g/100g Dry Matter) of some feed ingredients

Ingredients (g/100g DM)	SBM	5-min TLCM	10-min TLCM	Maize
Moisture	9.23	8.00	9.44	10.19
Crude protein	38.04	32.00	30.50	9.87
Crude lipid	18.13	26.50	28.00	4.28
Crude fibre	2.46	2.38	2.47	3.67
Ash	6.13	3.62	3.94	2.73
NFE	26.01	27.53	25.65	69.26
Total	100.00	100.00	100.00	100.00

*SBM: Soybean Meal; TLCM- Toasted *Luffa cylindrica* Meal.

Experimental design, system and feeding trial

The experimental design followed a two-factor factorial experiment in a completely randomized design wherein the first factor; Inclusion level of 15 and 30% of *Luffa cylindrica* and the second factor; processing time of 5 and 10 min toasting were adopted. The experimental system consists of a set of rectangular plastic tanks each with a capacity of 70 L of water. Triplicate groups of each treatment were made consisting of 15 fish per tank. *Clarias gariepinus* juveniles were gotten from the Department of Fisheries Technology, Federal College of Animal Health and Production Technology, Ibadan Oyo state, Nigeria. Acclimation of the fish for two weeks was done while being fed on commercial diet. All the fish were starved for 24h prior to the commencement of the feeding trial to create appetite and prepare the fish for good acceptance of the experimental diet. The feeding trial was conducted in the wet laboratory of Federal College of Animal Health and Production Technology, Ibadan. All fish were group fed twice daily at a fixed feeding rate of 5% of their body weight per day. Feeding to apparent satiation was generally done in the morning at 09:00 am and 05:00 pm in the evening for 56 days.

Blood sampling

At the end of the experiment, 3 ml blood per replicate was obtained by caudal vein puncture using a 25G needle and 1 ml disposable syringe. 1 ml blood sample was collected in ethyline diamine tetraacetic acid (EDTA) treated bottle for haematological examination while the 2 ml blood sample was collected in untreated sampling bottle. Blood samples untreated bottles were centrifuged at 8.000 rpm for 10 min after allowing it 30 min to clot and the serum was collected following the procedure of Tomlinson et al. (2013) for onward serum biochemistry determination.

Haematological test

Packed cell volume (PCV) was determined by the microhaematocrit method, while haemoglobin concentration (Hb) was estimated by the cyanmethaemoglobin method (Schalm et al., 1975; Coles, 1986). Red blood cell (RBC) count and total white blood cell (WBC) counts were analysed by the use of haemocytometer. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Schalm et al., 1975; Coles, 1986). Serum cholesterol was measured by colourimetric method (Allain et al., 1974).

Table 2. Gross and nutrient composition (g/100g Dry Matter) of experimental diets containing differently timed dry heat treated *Luffa cylindrica* seedmeal fed to *Clarias gariepinus*

Gross composition (g/100g DM)	Diet treatment				
	CTR	D515T	D530T	D1015T	D1030T
Fishmeal	27.78	27.78	27.78	27.78	27.78
Soybean	50.00	42.50	35.00	42.50	35.00
5-min toasted LCM	-	8.91	17.81	-	-
10-min toasted LCM	-	-	0	9.34	18.69
Cornmeal	10.00	10.00	10.00	10.00	10.00
Fish premix	2.50	2.50	2.50	2.50	2.50
Fish oil	5.00	5.00	5.00	5.00	5.00
Starch	4.72	3.31	1.91	2.88	1.03
Proximate composition (%)					
Moisture	7.91	7.97	8.04	8.09	8.27
Crude protein	40.19	40.22	40.25	40.22	40.25
Crude lipid	19.69	19.59	19.48	19.80	19.91
Crude fibre	2.13	2.68	3.23	2.79	3.46
Ash	6.57	6.90	7.24	7.06	7.55
NFE	23.51	22.64	21.76	22.04	20.56
Amino acid composition (%)					
Arginine	3.16	3.83	4.50	3.87	4.59
Histidine	1.00	1.13	1.26	1.14	1.28
Isoleucine	1.95	2.13	2.32	2.15	2.35
Leucine	3.21	3.48	3.74	3.50	3.79
Lysine	2.95	3.24	3.52	3.26	3.57
Methionine	0.90	1.05	1.20	1.06	1.22
M+C	1.46	1.69	1.92	1.70	1.95
Phenylalanine	1.88	2.12	2.36	2.14	2.40
P+T	3.26	3.56	3.87	3.59	3.92
Threonine	1.88	1.97	2.06	1.98	2.08
Tryptophan	0.50	0.55	0.60	0.55	0.61
Valine	2.12	2.35	2.59	2.37	2.63
Fatty acid composition (%)					
LOA (18:2n-6)	5.63	4.85	4.07	4.85	4.07
LNA (18:3n-3)	0.80	0.69	0.58	0.69	0.58
ARA (20:4n-6)	0.09	0.09	0.09	0.09	0.09
EPA (20:5n-3)	0.54	0.54	0.54	0.54	0.54
DHA (22:6n-3)	1.06	1.06	1.06	1.06	1.06
Total n-3	2.41	2.30	2.19	2.30	2.19
Total n-6	5.72	4.94	4.16	4.94	4.16
n3:n6	0.42	0.46	0.53	0.46	0.53
Total phospholipid, %	3.08	2.93	2.78	2.93	2.78

Specification: each kg contains: Vitamin A - 4.000.000IU; Vitamin B - 800.000IU; Vitamin E - 40.000IU, Vitamin K_3 - 1.600mg; Vitamin B_1 - 4.000mg; Vitamin B_2 - 3.000mg; Vitamin B_6 - 3.800mg, Vitamin B_{12} - 3 mcg; Nicotinic Acid - 18000mg; Pantothenic Acid - 8.000mg; Folic Acid - 800mg; Biotin - 100 mcg; Choline Chloride - 120.000mg; Iron - 8,000mg; Copper - 800mg; Manganese - 6.000mg; Zinc - 8.000mg; Iodine - 400mg; Selenium - 40mcg; Vit. C - Coated 60.000mg, Inositol - 10.000mg; Cobalt - 150m, Lysine - 10.000mg; Methionine - 10.000 mg, Antioxidant - 25.000mg manufactured by Bi-mix Brrand, Corporate head office/factory: 1, Odo-Olowu Street, Ijesatedo, Lagos, Nigeria.
LOA - Linoleic Acid; LNA - Linolenic Acid; ARA - Arachidonic Acid; EPA - Eicosapentanoic Acid; DHA - Docosahexanoic Acid
Proximate, fatty acid and amino acid profiles of the experimental diets were calculated using a program (excel) developed by NACA (2008).

Biochemical tests

The serum total protein was analyzed by the use of a commercial kit (Randox Laboratories Ltd, U.K) following Biuret method (Reinhold, 1953; Doumas et al., 1971; Doumas and Peters, 1997) while bromocresol green method was used to obtain albumin value (Doumas et al., 1971). Serum globulin value was obtained by the difference between the serum total protein and serum albumin (Colville, 2002). The method of Toro and Ackermann (1975) was used to determine blood glucose.

Histological examination of the test organs

The liver and kidney of three fish (n=3) from each replicate tank were removed and fixed in phosphate buffered 10% formalin after they had been previously anaethesised with 100 ml/L clove oil. Dehydration of the tissue was done in graded levels of alcohol. The organs were then cleared in xylene and embedded in paraffin wax following the method of (Huges and Perry, 1976). Sectioning of the tissues (5-7 µm) and eventual staining with Harris haematoxyllin-eosin (H&E) stain were done according to the methods of Bancroft and Cook (1994). The stained slides were observed under a light microscope at varying magnification (x40) and photographed with a minimum of 7 photomicrographs taken per replicate slide.

Data analysis

Data were subjected to two-way analysis of variance (ANOVA) using SPSS 16.0 version for the source and level effect. The interactive effect was subjected to one-way ANOVA. Duncan multiple range test was used to compare differences among individual treatment means where the ANOVA reveals significant difference ($p<0.05$).

Results and discussion

Effect of processing time, inclusion level and their interactions on the haematological profiles of Clarias gariepinus

Table 3 shows the effect of processing time, inclusion level and their interactions on the haematological profiles of *Clarias gariepinus* fed diets containing dry heat-treated *Luffa cylindrica* seedmeal. Processing time had significant impact ($p<0.05$) on the haematology of *C. gariepinus* exposed to different dietary treatments. In the haematology parameters examined, the blood of fish fed diet CTR had the highest values of Hb, PCV and RBC which was significantly different ($p<0.05$) from the values recorded for the blood of fish exposed to other dietary treatments. The blood of fish fed diets containing 15-min toasted *L. cylindrica* seedmeal had the lowest values of Hb, PCV and RBC. A contrast was the trends of results recorded for WBC, MCV and MCH, the highest values were found in the blood of fish fed diets containing 15-min toasted *L. cylindrica* seedmeal while the lowest value of these parameters were found in the blood of fish fed diet CTR.

Table 3. Effect of processing time, inclusion level and their interactions on the haematological profiles of *Clarias gariepinus* fed diets containing dry heat treated *Luffa cylindrica* seedmeal

Parameter	Hb	PCV	RBC	WBC	MCV	MCH	MCHC
Sources (PT)							
CTR	9.95^{a}	24.33^{a}	3.12^{a}	14.57^{c}	77.94^{b}	31.89^{b}	40.91^{a}
5-min PT	9.28^{c}	21.41^{c}	2.42^{c}	17.10^{a}	88.87^{a}	38.35^{a}	46.02^{b}
10-min PT	9.56^{b}	23.59^{b}	2.64^{b}	15.38^{b}	89.50^{a}	36.21^{a}	40.57^{c}
Level (IL)							
0%	9.95^{a}	24.33^{a}	3.12^{a}	14.57^{c}	77.94^{c}	31.89^{b}	40.91^{a}
15%	9.82^{b}	23.50^{b}	2.75^{b}	15.54^{b}	84.85^{b}	35.71^{a}	41.79^{a}
30%	9.02^{c}	21.50^{c}	2.38^{c}	16.94^{a}	92.88^{a}	40.31^{a}	44.75^{b}
PT*IL							
CTR	9.95^{a}	24.33^{a}	3.12^{a}	14.57^{c}	77.94^{c}	31.89^{b}	40.91^{a}
D515T	9.81^{b}	23.20^{c}	2.73^{b}	16.42^{b}	85.04^{b}	35.94^{a}	42.28^{a}
D530T	8.76^{d}	19.62^{d}	2.25^{d}	17.78^{a}	92.71^{a}	39.20^{a}	44.67^{b}
D1015T	9.83^{b}	23.80^{b}	2.77^{b}	14.65^{c}	85.95^{b}	35.50^{a}	41.30^{a}
D1030T	9.28^{c}	23.38^{bc}	2.51^{c}	16.11^{b}	93.04^{a}	36.96^{a}	39.72^{c}
SEM	0.12	0.45	0.08	0.32	1.60	0.21	0.25

Hb - Haemoglobin content (gm/100ml); PCV - Packed Cell Volume (%); WBC - White Blood Cell Count ($10^{9}mm^{3}$); RBC - Red Blood Cell Count ($10^{6}mm^{3}$); MCHC - Mean Corpuscular Haemoglobin Concentration (%); MCV - Mean Corpuscular Volume (u^{3}); MCH - Mean Corpuscular Haemoglobin (pg); PT*IL - Interaction effect of processing time (PT) and Inclusion Level (IL); Means with different superscripts in the same column are significantly different ($p<0.05$) from each other.

Inclusion level also had significant impact ($p<0.05$) on the haematology of *Clarias gariepinus* fed diets containing dry heat-treated *Luffa cylindrica* seedmeal. The same trends of results as observed for effect of processing time on the Hb, PCV and RBC values were also observed for effect of inclusion level. However, the blood of fish fed 30% inclusion level had the highest values of WBC, MCV and MCH. The MCH of the blood of fish fed diets containing 30% inclusion of toasted *L. cylindrica* seedmeal was not significantly different ($p>0.05$) from the MCH of the blood of fish fed diets containing 30% inclusion level of toasted *L. cylindrica* seedmeal. The trends in Hb, RBC and PCV showed superior

performance of 10-min processing time and 15% inclusion level but not comparable to control giving higher values of these parameters. The interaction of processing time and inclusion level impacted significantly ($p<0.05$) on the haematology of *Clarias gariepinus* fed diets containing dry heat treated *Luffa cylindrica* seedmeal. The Hb, PCV and RBC values of the blood of fish fed diet CTR were the highest, which is significantly different from the Hb, PCV and RBC of the blood of those of other dietary treatments. WBC, MCV and MCH values of the blood of fish fed D530T were the highest while the lowest values were found in fish fed diets CTR. There was no significant difference ($p>0.05$) in the MCH of the blood of fish fed all the dietary treatment except control. A contrasting trend was noted of RBC and WBC. The interactive effect of processing time and inclusion level revealed that irrespective of processing time, inclusion level yielded a blood profile that has level to level comparison, hence the non-significant difference recorded in the blood profile of fish fed D515T and D1015T. A divergent trend of RBC and WBC recorded in this study in which as RBC increases, WBC decreases, was also established by Zhou et al. (2009). Francesco et al. (2012) and Fazio et al. (2013) reported inverse relationship between RBC and WBC. Satheeshkumar et al. (2011) explained this that high RBC amount reduces the need for large quantity of WBC.

The results of this study indicated that processing time, inclusion level and/or their interactions had significant impact on the blood profiles, metabolic and excretory organs of *Clarias gariepinus*. A decrease in RBC, PCV and Hb was observed when compared with control for inclusion level, processing time and/or their interaction. For processing time effect, fish exposed to 10 min processing time had significantly higher RBC, PCV and Hb. Although the values of haematological parameters obtained in this study were within the reference intervals reported in Adeyemo et al. (2014) for *Clarias gariepinus*, the effectiveness of higher processing time indicated that some of the anti-metabolites in the feed ingredients were destroyed.

Effect of processing time, inclusion level and their interactions on the serum biochemistry of Clarias gariepinus

Table 4 shows the effect of processing time, inclusion level and their interactions on the serum biochemistry of *Clarias gariepinus* fed diets containing dry heat-treated *Luffa cylindrica* seedmeal. Processing time had effect which is significant ($p<0.05$) on the serum biochemistry. Fish fed diet CTR had the highest values in all the parameters of serum biochemistry measured which is not significantly different ($p>0.05$) from the values recorded for blood of fish fed diets containing 10-min toasted *Luffa cylindrica* seedmeal. The blood of fish fed diets containing 5-min toasted *Luffa cylindrica* seedmeal had the lowest values of the parameters of serum biochemistry measured except blood glucose.

Table 4. Effect of processing time, inclusion level and their interactions on the serum biochemistry of *Clarias gariepinus* fed diets containing dry heat treated *Luffa cylindrica* seedmeal

	TP	Alb.	Glob.	Pl. Chol.,	Bld. Gluc.
Sources (PT)					
CTR	3.73^{a}	1.58^{a}	2.15^{a}	142.79^{a}	115.30^{c}
5-min PT	3.29^{b}	1.21^{b}	2.08^{a}	119.3^{b}	131.69^{a}
10-min PT	3.66^{a}	1.46^{a}	2.20^{a}	135.3^{a}	122.30^{b}
Level (IL)					
0%	3.73^{a}	1.58^{a}	2.15^{a}	142.79^{a}	115.30^{c}
15%	3.62^{a}	1.43^{a}	2.19^{a}	137.30^{a}	122.30^{b}
30%	4.02^{b}	1.24^{b}	2.09^{a}	117.30^{b}	131.69^{a}
PT*IL					
CTR	3.73^{a}	1.58^{a}	2.15^{a}	142.79^{a}	115.30^{c}
D515T	3.53^{b}	1.24^{b}	2.30^{a}	127.30^{b}	126.97^{b}
D530T	3.04^{c}	1.18^{b}	1.86^{b}	111.34^{c}	136.29^{a}
D1015T	3.70^{a}	1.63^{a}	2.08^{ab}	147.37^{a}	117.61^{c}
D1030T	3.62^{ab}	1.30^{b}	2.33^{a}	123.17^{b}	127.00^{b}
SEM	0.07	0.05	0.06	3.68	2.12

TP- Total protein (g/dL); Alb- Albumin (g/dL); Glob– Globulin (g/dL); Pl. Chol.- Plasma cholesterol (mg/dL); Bld Gluc.- Blood glucose (g/dL); PT*IL- Interaction effect of processing time (PT) and Inclusion Level (IL); Means with different superscripts in the same column are significantly different ($p<0.05$) from each other.

Inclusion level also had significant effect ($p<0.05$) on the serum biochemistry of *Clarias gariepinus* fed diets containing dry heat-treated *Luffa cylindrica* seedmeal. The highest values of serum biochemistry parameters were recorded in the blood of fish fed 0% inclusion level of *Luffa cylindrica* seedmeal which is not significantly different ($p>0.05$) from the values recorded for the blood of fish fed diets containing 15% inclusion level of *Luffa cylindrica* seedmeal. Fish fed diets containing 30% inclusion level had the lowest values of serum biochemistry parameters except total protein and blood glucose. The values reported for parameters of serum biochemistry agrees with the normal range for *Clarias gariepinus* reported in the work of Okorie-kanu and Unakalamba (2015). Age difference could be attributed to the variations that were recorded in our haematological results with that of Okorie-kanu and Unakalamba (2015). Another pointer could be the environment in which fish is sampled. Ellsaesser and Clem (1987) reported that variation is always exhibited in the serum parameters of laboratory acclimatized fish when compared to that of production pond or those in the wild. Stress could also be a contributory factor to the variations recorded between the laboratory cultured fish and wild fish (Myburgh et al., 2008).

The interaction of processing time and inclusion level effected a significant ($p<0.05$) impact on the serum biochemistry of fish fed dietary treatments containing toasted *Luffa cylindrica* seedmeal. Fish fed CTR has the highest values of total protein and the lowest values of blood glucose, while the rest parameters values were in the ranges for the other tested diets. However, total protein, albumin and globulin of the blood of fish fed diet CTR was not significantly different ($p>0.05$) from that of the blood of fish fed D1015T. Total cholesterol of the blood of fish fed D530T was the lowest and its blood glucose was the highest. The serum biochemistry parameters of the blood of fish fed D515T and D1030T were not statistically different ($p>0.05$).

The serum biochemistry values in this study are within the normal range. Higher than normal serum total proteins are indices of liver damage (Coz-Rakovac et al., 2005) decimating its deamination capacity by reducing its aminotransferase ability. Barcellos et al. (2003) reported that higher than normal serum protein could mean that some protein has been mobilized as substrate for hepatic glyconeogenesis. The values of total proteins in this study fall within the normal range of 20-40 g l^{-1} reported by Svobodova and Vykusova (1991). Excess blood glucose than normal was also noted among the fish exposed to test dietary treatments when compared to that of control. This was also found by Kikuchi et al. (1994), Kikuchi (1999) and Kumar et al. (2010). Aggregate plant ingredients in the test dietary treatments were more than control which consequently increases the carbohydrate contents of the diets explaining why serum glucose was more in the test dietary treatment group than the control group. Artacho et al. (2007) established that serum glucose is a metabolite of carbohydrate metabolism. Though, Yang and Chen (2003) reported that excessive than normal serum glucose could be related to renal and/or liver damage. Hypoxic condition could increase plasma glucose (Svoboda et al., 2001; Lermen et al., 2004). The level of serum glucose is considered to be a specific indicator of sympathetic activation during stress condition. The serum cholesterol reduces among test dietary treatment groups when compared with control. The processing time of 10 min and inclusion level of 30% and the interactive effect of 5 min and 30% inclusion gave the lowest values of blood cholesterol. Reduction in blood cholesterol was also found by Jimoh et al. (2015a) that it was due to it being used in the manufacture of cortisol arising from stress created by consumption of diets containing higher plant ingredients. De Schrijver (1990) reported that plant ingredients possess hypercholesteremic effect. Kaushik et al. (1995) and Yamamoto et al. (2007) also found serum cholesterol of fish being reduced by plant feed ingredients in their diets.

Effect of the dietary treatments on liver histology of Clarias gariepinus

Photomicrographs of the liver of *Clarias gariepinus* exposed to different dietary treatments containing differently processed *Luffa cylindrica* seedmeal included at different levels are presented in Figure 1. There was moderate diffuse vacuolation of hepatocytes in the liver of fish fed diet CTR, though many normal hepatocytes without vacuolations were also present; Fish exposed to diet D515T had a mild diffuse vacuolation of hepatocytes. A moderate diffuse vacuolation of hepatocytes,with many normal hepatocytes without vacuolation, was observed in the liver of fish fed diet D530T. Fish fed diet D1015T had mild periportal cellular infiltration in its liver; while a moderate to severe diffuse vacuolar degeneration of hepatocytes was found in fish exposed to diet D1030T.

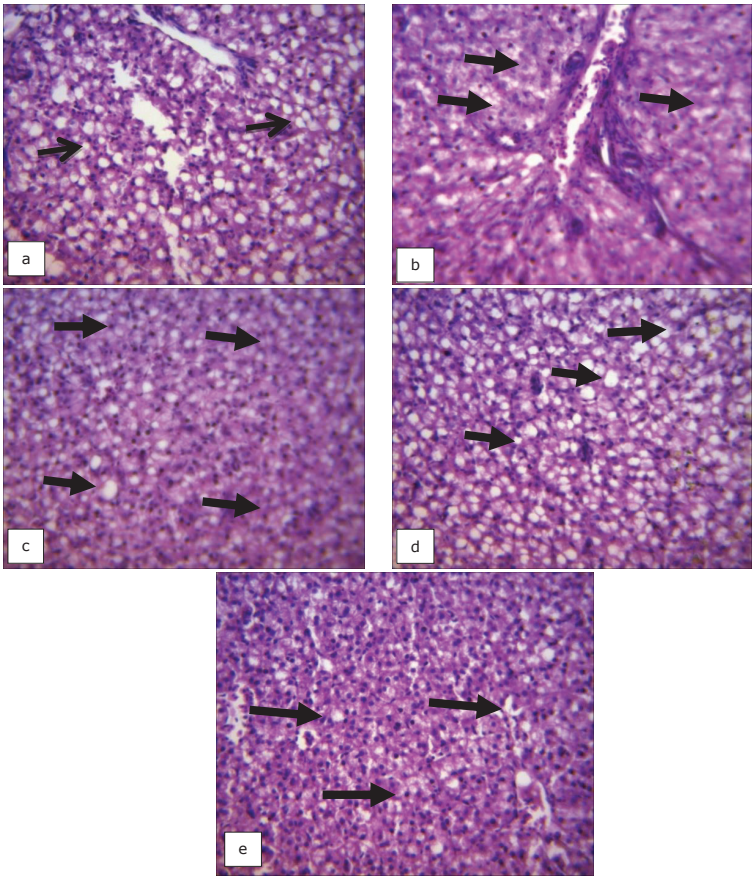

Figure 1. Photomicrographs (H&E Stained) of the Liver of *Clarias gariepinus* fed; a) diet CTR- there is a moderate diffuse vacuolation of hepatocytes, many normal hepatocytes without vacuolations are also present; b) diet D515T- there is a mild diffuse vacuolation of hepatocytes; c) diet D530T- there is a moderate diffuse vacuolation of hepatocytes (arrows), many normal hepatocytes without vacuolation are also present, there is mild periportal cellular infiltration; d) diet D1015T- there is a moderate to severe diffuse vacuolar degeneration of hepatocytes (arrows); e) diet D1030T- there is also a moderate diffuse vacuolation of hepatocytes X400.

Effect of the dietary treatments on kidney histology of Clarias gariepinus

Figure 2 shows the effect of feeding diets containing differently processed *Luffa cylindrica* meal at different inclusion levels on the histology of the kidney of *Clarias gariepinus*. Fish fed D515T had areas of glomerular degeneration (black arrows), and mild hemorrhage in the renal parenchyma (white arrow); there was massive haemorrhage of the entire parenchyma (arrows) in the kidney of fish fed diet D1015T. No visible lesions were seen in the micrographs of fish fed CTR, D530T and D1030T.

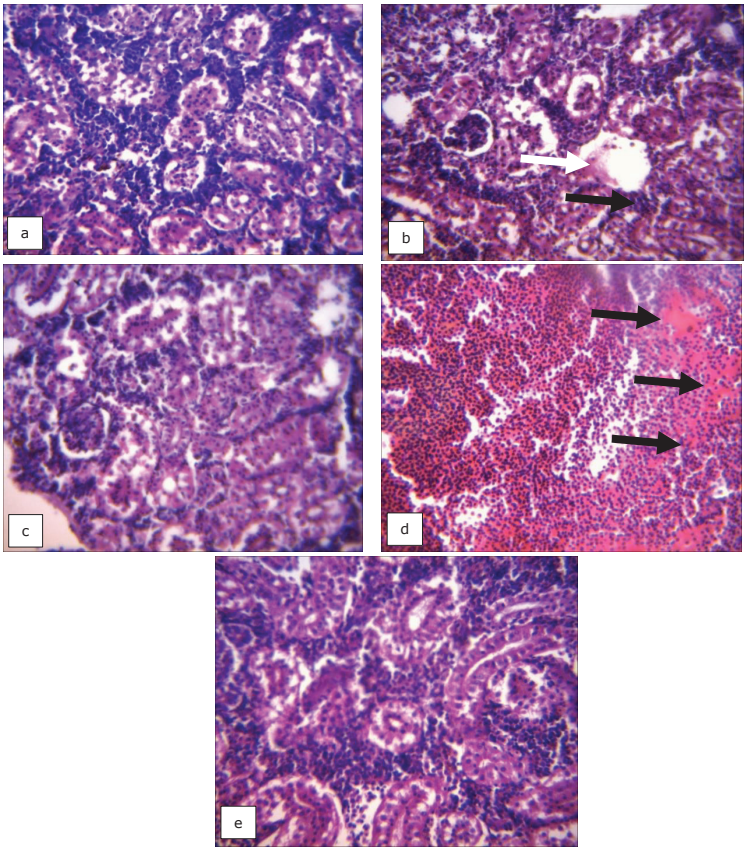

Figure 2. Photomicrographs (H&E Stained) of the kidney of *Clarias gariepinus* fed; a) diet CTR - no visible lesions seen; b) diet D515T - there are areas of glomerular degeneration (black arrows), and mild hemorrhage in the renal parenchyma (white arrow); c) diet D530T - no visible lesions seen; d) diet D1015T - there is a massive haemorrhage of the entire parenchyma (arrows);e) diet D1030T - no visible lesions seenx400.

Mild to moderate vacuolation of the hepatocytes recorded among the livers of fish fed control and test dietary treatments except those fed D1030T that recorded very severe vacuolation of the hepatocytes was due to the fact that liver is the centre of metabolism; regulating the various nutritional substances consumed by the fish. Cabot (2000) and Nero et al. (2006) reported that liver is predisposed to damage by different xenobiotic substances in food substances consumed by fish. Moderate to severe vacuolation observed in this study were in consonance with the reports of Pereira et al. (2002); Hansen et al. (2006); Martins et al. (2007); Mérida et al. (2010) and Valente et al. (2011) that recorded the presence of numerous vacuolation in the liver. Jimoh et al. (2015b) recorded marked vacuolation of hepatocytes of the liver of the fish exposed to different dietary treatments containing soybean meal that is replaced by watermelon seedmeal. Livers of the fish exposed to different dietary treatments under this study could be said to belong to intermediate condition based on semi-quantitative analysis of histological alteration in the liver developed by Martínez-Llorens et al. (2012). The same authors categorized the extent of liver damage into three; for the livers, healthy livers with normally shaped and sized hepatocytes with uncongested vessels and normal sinusoid organization referred to as grade 1 liver. Grade 2 liver is the intermediate condition characterized with displaced nuclei and/or lipofuscin-like material. Grade 3 is referred to as degraded liver with necrosis, pyknotic nuclei, hyaline cytoplasm and/or large sinusoid spaces. The kidneys, being the excretory organ, were also affected. However, normal cell architecture was recorded in fish fed CTR, D530T and D1030T.

Conclusion

From the foregoing, it could be concluded that processing time (5 and 10 min), inclusion level of toasted *Luffa cylindrica* seedmeal (15 and 30%) in diets and their interactions had significant impact ($p<0.05$) on the hematology and serum biochemistry of *Clarias gariepinus*. A decrease in RBC, PCV and Hb was observed when compared with control for inclusion level, processing time and/or their interaction. Total protein,

albumin and globulin of the blood of fish fed diet CTR was not significantly different (p>0.05) from that of the blood of fish fed D1015T. Mild to moderate vacuolation of the hepatocytes were recorded among the livers of fish fed control and test dietary treatments except those fed D1030T that recorded very severe vacuolation of the hepatocytes. The kidney, being the excretory organ, was also affected. However, normal cell architecture was recorded in fish fed CTR, D530T and D1030T.

References

Adeparusi EO and Jimoh WA, 2002. Digestibility coefficients of raw and processed lima bean diet for Nile tilapia, *Oreochromis niloticus*. Journal of Applied Aquaculture, 12, 89-98. doi:https://doi.org/10.1300/J028v12n03_09

Adeyemo BT, Obande RA and Solomon SG, 2014. Haematological reference ranges of cultured Clarias gariepinus in the Lower Benue River Basin, Nigeria. Comparative Clinical Pathology, 23, 361-366. doi:https://doi.org/10.1007/s00580-012-1624-1

Allain CC, Poon LS, Chan CS, Richmond W and Fu PC, 2000. Enzymatic determination of total serum cholesterol. Clinical Chemistry, 20, 470-5.

Artacho P, Soto-Gamboa M, Verdugo C and Nespolo RF, 2007. Blood biochemistry reveals malnutrition in black-necked swans (*Cygnus melanocoryphus*) living in a conservation priority area. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 146, 283-290.

Bahmani M, Kazemi R and Donskaya P, 2001. A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). Fish Physiology and Biochemistry, 24, 135-140. doi:https://doi.org/10.1023/A:1011911019155

Bancroft JD and Cook HC, 1994. Manual of histological techniques and their diagnostic application. Churchill Livingstone.

Barcellos G, Kreutz LC, Rodrigues LB, Fioreze I, Quevedo RM, Cericato L, Conrad J, Soso AB, Lacerda LDA and Terra S, 2003. Haematological and biochemical characteristics of male jundiá (*Rhamdia quelen* Quoy & Gaimard *Pimelodidae*): changes after acute stress. Aquaculture Research, 34, 1465-1469. https://doi.org/10.1111/j.1365-2109.2003.00972.x

Cabot S, 2000. The liver and detoxification. https://www.google.com/rch?q=Cabot+S%2C+2000.+The+liver+and+detoxification&oq

Coles E, 1986. Veterinary clinical pathology. 4th Edition, WB Saunders company Philadelphia, London, Toronto, Mexico, Riodejenario, Sydney, Tokyo & Hong Kong, pp. 136-170.

Colville J, 2002. Blood chemistry. Laboratory Procedures for Veterinary Technicians. 4th Edition, St. Louis, Mosby, USA, pp. 75-103.

Coz-Rakovac R, Strunjak-Perovic I, Hacmanjek M, Lipej Z and Sostaric B, 2005. Blood chemistry and histological properties of wild and cultured sea bass (*Dicentrarchus labrax*) in the North Adriatic Sea. Veterinary research communications, 29, 677-687. doi:https://doi.org/10.1007/s11259-005-3684-z

De Schrijver R, 1990. Cholesterol metabolism in mature and immature rats fed animal and plant protein. The Journal of nutrition, 120, 1624-1632. doi:https://doi.org/10.1093/jn/120.12.1624

Doumas BT and Peters JrT, 1997. Serum and urine albumin: a progress report on their measurement and clinical significance. Clinica Chimica Acta, 258, 3-20. doi:https://doi.org/10.1016/S0009-8981(96)06446-7

Doumas BT, Watson WA and Biggs HG, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chimica Acta, 31, 87-96. doi:https://doi.org/10.1016/0009-8981(71)90365-2

El-Sayed A-FM, 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis spp*. Aquaculture 179, 149-168. doi:https://doi.org/10.1016/S0044-8486(99)00159-3

Ellsaesser CF and Clem L, 1987. Blood serum chemistry measurements of normal and acutely stressed channel catfish. Comparative biochemistry and physiology. A Comparative physiology, 88, 589-594. doi:https://doi.org/10.1016/0300-9629(87)90087-9

Fagbenro O, Akande T and Fapounda O, 2003. Use of roselle (*Hibiscus Sabdariffa*) seed meal as a soybean meal replacer in practical diets for fingerlings of African Catfish (*Clarias gariepinus*). In: Proceeding of the Third international conference on African fish and Fisheries, Cotonou, Benin, pp. 10-14.

Fagbenro OA, Adeparusi EO and Jimoh WA, 2010. Effect of processing on the nutrient, mineral and antinutrients composition of sunflower (*Helianthus annus*) seed meal. International Journal of Chemistry, 20, 165-170.

FAO, 2009. Fish as feed input for aquaculture: practices, sustainability and implications. FAO Fisheries and Aquaculture Technical Paper.

Fazio F, Marafioti S, Arfuso F, Piccione G and Faggio C, 2013. Comparative study of the biochemical and haematological parameters of four wild Tyrrhenian fish species. Veterinární medicína, 58, 576-581. doi:https://doi.org/10.17221/7139-VETMED

Ferreira J, Hawkins A and Bricker S, 2007. Management of productivity, environmental effects and profitability of shellfish aquaculture-the Farm Aquaculture Resource Management (FARM) model. Aquaculture, 264, 160-174. doi:https://doi.org/10.1016/j.aquaculture.2006.12.017

Francesco F, Satheeshkumar P, Senthil Kumar D, Caterina F and Giuseppe P, 2012. Comparative study of hematological and blood chemistry of Indian and Italian Grey Mullet (*Mugil cephalus* Linneaus 1758). HOAJ Biology, 1-5. doi:https://doi.org/10.7243/2050-0874-1-5

Francis G, Makkar HP and Becker K, 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199, 197-227. doi:https://doi.org/10.1016/S0044-8486(01)00526-9

Hansen AC, Rosenlund G, Karlsen Ø, Olsvik PA and Hemre

GI, 2006. The inclusion of plant protein in cod diets, its effects on macronutrient digestibility, gut and liver histology and heat shock protein transcription. Aquaculture Research, 37, 773-784. doi:https://doi.org/10.1111/j.1365-2109.2006.01490.x

Huges G and Perry S, 1976. Morphometric study of trout gills: a light-microscopic method suitable for the evaluation of pollutant action. Journal of Experimental Biology, 64, 447-460.

Huntington T, 2004. Feeding the fish: sustainable fish feed and Scottish aquaculture. Report to the Joint Marine Programme (Scottish Wildlife Trust and WWF Scotland) and RSPB Scotland, pp. 26-28.

Huntington T, Frid C, Banks R, Scott C and Paramor O, 2004. Assessment of the sustainability of industrial fisheries producing fish meal and fish oil. Report to The Royal Society for the Protection of Birds, Poseidon Aquatic Resource Management Ltd and The University of Newcastle-upon-Tyne, UK.

Jimoh WA, Fagbenro OA and O AE, 2011. Effect of processing on the nutrient, mineral and antinutrients composition of sesame (*Sesamum indicum*) seed meals. Electronic Journal of Environmental Agricultural and Food Chemistry, 10, 1858-1864.

Jimoh W, Aderolu A, Ayeloja A and Shodamola M, 2012. Haematological response of *Clarias gariepinus* (Burchell 1822) fed diets containing *Luffah cylindrical* seed meal. In: Proceeding of the 27th Annual Conference of Fisheries Society of Nigeria (FISON), Banquet Hall, Government House, Yenagoa, Bayelsa State, pp. 392-396.

Jimoh W, Aderolu Z, Ayeloja A and Shodamola M, 2013. Use of *Luffah cylindrica* seedmeal as a soybean meal replacer in diet for fingerlings of African catfish, *Clarias gariepinus* (Burchell 1822). International Journal of Applied Agricultural and Apicultural Research, 9, 98-105.

Jimoh WA, Ayeloja AA, Ajasin FO, Okemakin FY, Abdusalami SA and Adekunle OF, 2015a. Some haematological and biochemical profile of blood of Nile tilapia *(Oreochromis niloticus*) fed on diets containing watermelon (*Citrullus lanatus*) seedmeal. Bayero Journal of Pure and Applied Sciences, 8, 109-114. doi:https://doi.org/10.4314/bajopas.v8i1.19

Jimoh WA, Shittu MO, Ayeloja AA, Okemakin FY, Abdulsalami SA, Adekunle OF and Banjoko OJ, 2015b. Histological changes in the liver of Nile tilapia (*Oreochromis niloticus*) fed diets containing watermelon (*Citrullus lanatus*) at varying replacement levels. Journal of Science and Technology, 6, 85-92.

Kaushik S, Cravedi J, Lalles J, Sumpter J, Fauconneau B and Laroche M, 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 133, 257-274. doi:https://doi.org/10.1016/0044-8486(94)00403-B

Kikuchi K, 1999. Partial replacement of fish meal with corn gluten meal in diets for Japanese flounder *Paralichthys olivaceus*. Journal of the World Aquaculture Society, 30, 357-363.doi:https://doi.org/10.1111/j.1749-7345.1999.tb00686.x

Kikuchi K, Furuta T and Honda H, 1994. Utilization of soybean meal as a protein source in the diet of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquaculture Science, 42, 601-604.

Kumar V, Makkar HP, Amselgruber W and Becker K, 2010. Physiological, haematological and histopathological responses in common carp (*Cyprinus carpio* L.) fingerlings fed with differently detoxified *Jatropha curcas* kernel meal. Food and Chemical Toxicology, 48, 2063-2072. doi:https://doi.org/10.1016/j.fct.2010.05.007

Lermen CL, Lappe R, Crestani M, Vieira VP, Gioda CR, Schetinger MRC, Baldisserotto B, Moraes G and Morsch VM, 2004. Effect of different temperature regimes on metabolic and blood parameters of silver catfish *Rhamdia quelen*. Aquaculture, 239, 497-507. doi:https://doi.org/10.1016/j.aquaculture.2004.06.021

Lim C and Akiyama D, 1992. Full-fat soybean meal utilization by fish. Asian Fisheries Science, 5, 181-197.

Lovell RT, 1988. Use of soybean products in diets for aquaculture species. Journal of Aquatic Production, 7, 27-52.

Martínez-Llorens S, Baeza-Ariño R, Nogales-Mérida S, Jover-Cerdá M and Tomás-Vidal A, 2012. Carob seed germ meal as a partial substitute in gilthead sea bream (*Sparus aurata*) diets: amino acid retention, digestibility, gut and liver histology. Aquaculture, 338, 124-133. doi:https://doi.org/10.1016/j.aquaculture.2012.01.029

Martins DA, Valente LM and Lall SP, 2007. Effects of dietary lipid level on growth and lipid utilization by juvenile Atlantic halibut (*Hippoglossus hippoglossus*, L.). Aquaculture, 263, 150-158. doi:https://doi.org/10.1016/j.aquaculture.2006.10.017

Mérida SN, Tomás-Vidal A, Martínez-Llorens S and Cerdá MJ, 2010. Sunflower meal as a partial substitute in juvenile sharpsnout sea bream (*Diplodus puntazzo*) diets: amino acid retention, gut and liver histology. Aquaculture, 298, 275-281. doi:https://doi.org/10.1016/j.aquaculture.2009.10.025

Myburgh JG, Botha CJ, Booyse DG and Reyers F, 2008. Provisional clinical chemistry parameters in the African Sharptooth catfish (*Clarias gariepinus*). Journal of the South African Veterinary Association, 79, 156-160. doi:https://doi.org/10.4102/jsava.v79i4.265

Nero V, Farwell A, Lister A, Van Der Kraak G, Lee L, Van Meer T, MacKinnon M and Dixon D, 2006. Gill and liver histopathological changes in yellow perch (*Perca flavescens*) and goldfish (*Carassius auratus*) exposed to oil sands process-affected water. Ecotoxicology and environmental safety, 63, 365-377. doi:https://doi.org/10.1016/j.ecoenv.2005.04.014

Okorie-kanu CO and Unakalamba NJ, 2015. Normal haematological and blood biochemistry values of cultured Clarias gariepinus in Southeast, Nigeria. Comparative Clinical Pathology, 24, 1445-1450. doi:10.1007/s00580-015-2093-0

Oshodi AA, Olaofe O and Hall GM, 1993. Amino acid, fatty acid, mineral composition of pigeon pea (*Cajnus cajan*). International Journal of Food Nutrition, I43, 178-191. doi:https://doi.org/10.3109/09637489309027541

Oyetayo FL and Ojo BA, 2012. Food value and phytochemical

composition of *Luffa cylindrica* seed flour. American Journal of Biochemistry, 2, 98-103. doi:https://doi.org/10.5923/j.ajb.20120206.02

Pereira O, Rosa E, Pires M and Fontaınhas-Fernandes A, 2002. Brassica by-products in diets of rainbow trout (*Oncorhynchus mykiss*) and their effects on performance, body composition, thyroid status and liver histology. Animal Feed Science and Technology, 101, 171-182. doi:https://doi.org/10.1016/S0377-8401(02)00223-7

Reinhold JG, 1953. Glucose. Standard methods of clinical chemistry, 1, 65-70.

Sanchez-Vioque R, Clement A, Vioque J, Bautista J and Millan F, 1998. Polar lipids of defatted chickpea (*Cicer arietinum* L) flour and protein isolates. Food Chemistry, 63, 357-855. doi:https://doi.org/10.1016/S0308-8146(98)00015-6

Satheeshkumar P, Ananthan G, Senthilkumar D, Khan AB and Jeevanantha K, 2011. Comparative investigation on haematological and biochemical studies on wild marine teleost fishes from Vellar estuary, southeast coast of India. Comparative Clinical Pathology, 21, 275-281. doi:https://doi.org/10.1007/s00580-010-1091-5

Schalm O, Jain N and Carrol E, 1975. Veterinary Hematology. Lea and Febiger, Philadelphia, 87, USA.

Storebakken T, Refstie S and Ruyter B, 2000. Soy products as fat and protein sources in fish feeds for intensive aquaculture. In: Soy in Animal Nutrition (ed. J.K. Drackley). Federation of Animal Science Societies, Champaign, pp. 127-170.

Svoboda M, Kouřil J, Hamáčková J, Kalab P, Savina L, Svobodova Z and Vykusova B, 2001. Biochemical profile of blood plasma of tench (*Tinca tinca* L.) during pre-and postspawning period. Acta Veterinaria Brno, 70, 259-268. doi:https://doi.org/10.2754/avb200170030259

Svobodova Z and Vykusova B, 1991. Diagnostics, prevention and therapy of fish diseases and intoxications. Manual for International Training Course on Fresh-Water Fish Diseases and Intoxications (in Czech). Research Institute of Fish Culture and Hydrobiology, Vodnany.

Tacon AGJ, Hasan MR and Subasinghe RP, 2006. Use of fishery resources as feed inputs for aquaculture development: trends and policy implications. FAO Fisheries Circular No. 1018, Rome, Italy.

Tomlinson L, Boone LI, Ramaiah L, Penraat KA, von Beust BR, Ameri M, Poitout-Belissent FM, Weingand K, Workman HC and Aulbach AD, 2013. Best practices for veterinary toxicologic clinical pathology, with emphasis on the pharmaceutical and biotechnology industries. Veterinary Clinical pathology, 42, 252-269. doi:https://doi.org/10.1111/vcp.12059

Toro G and Ackermann PG, 1975. Practical clinical chemistry. Little Brown & Company.

Valente L, Linares F, Villanueva J, Silva J, Espe M, Escórcio C, Pires M, Saavedra M, Borges P and Medale F, 2011. Dietary protein source or energy levels have no major impact on growth performance, nutrient utilisation or flesh fatty acids composition of market-sized Senegalese sole. Aquaculture, 318, 128-137. doi:https://doi.org/10.1016/j.aquaculture.2011.05.026

Yamamoto T, Suzuki N, Furuita H, Sugita T, Tanaka N and Goto T, 2007. Supplemental effect of bile salts to soybean meal-based diet on growth and feed utilization of rainbow trout *Oncorhynchus mykiss*. Fisheries Science, 73, 123-131. doi:https://doi.org/10.1111/j.1444-2906.2007.01310.x

Yang J-L and Chen H-C, 2003. Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology. Chemosphere, 53, 877-882. doi:https://doi.org/10.1016/S0045-6535(03)00657-X

Yue Y-R and Zhou Q-C, 2008. Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indexes for juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture, 284, 185-189.

Zhou X, Li M, Abbas K and Wang W, 2009. Comparison of haematology and serum biochemistry of cultured and wild Dojo loach *Misgurnus anguillicaudatus*. Fish Physiology and Biochemistry, 35, 435-441. doi:https://doi.org/10.1007/s10695-008-9268-4