DIETARY REPLACEMENT OF SOYBEAN MEAL BY TOASTED SUNFLOWER SEEDMEAL IN THE DIET OF *Clarias gariepinus*: EFFECT ON GROWTH, BODY COMPOSITION, DIGESTIBILITY, HAEMATOLOGY AND HISTOPATHOLOGY OF THE LIVER.

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ABSTRACT

Dietary replacement of soybean meal by toasted sunflower meal in *Clarias gariepinus* diet was investigated in a 56-day feeding trial using growth, body composition, digestibility, haematology and liver histology as indices of assessment. Sunflower seed meal was toasted at 204^0C for 5, 10 and 15 minutes. Control diet consist of soybean meal which was replaced by differently toasted sunflower seed meal at a rate 15, 30 and 45% to produce nine 40% crude protein, 18kj/kg test diets. Triplicate groups of fish in 70-litre capacity aerated rectangular plastic tanks were assigned to each dietary treatment at a stocking rate of 15 fingerlings (4.97±0.42g average weight) per tank in a completely randomized design. Fish were fed to satiation. Data generated from the experiment were subjected to one-way analysis of variance. The results of the experiment revealed that up to 30% replacement level of the differently toasted sunflower had a statistically similar results with soybean-based control diets using growth, body composition, digestibility and haematology as indices. Liver histology however show that massive fatty infiltration recorded in the liver of fish fed TSF145 and TSF 345 and diffuse vacuolar degeneration of hepatocytes and kupffer cell hyperplasia observed in the liver of fish fed TSF245 and TSF330.

Keywords: histology, toasted, triplicate groups, seed meal.

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تاثير الاستبدال الجزئي لبذور عباد الشمس بديلا عن كسبة فول الصويا في عليقة سمكة *Clarias gariepinus*
على النمو ومكونات الجسم والهضم وبعض معايير الدم ونسيج الكبد

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المستخلص

نفذت تجربة استبدال جزئي لبذور عباد الشمس بديلا عن كسبة فول الصويا في عليقة سمكة *Clarias gariepinus* لمدة 56 يوما لدراسة النمو ومكونات الجسم والهظم وبعض معايير الدم ونسيج الكبد.عرضت بذور عباد الشمس الى درجات حرارة 24 °م لمدة 5 و 10 و 15 دقيقة . تم استبدال عليقة السيطرة الحاوية على كسبة فول الصويا ببذور عباد الشمس بنسب 15 و 30 و 45% للحصول على 40% بروتين كلي، 18 جول / كغم في العليقة. استخدم ثلاث مكررات في معاملات الاسماك باستعمال احواض بلاستيكية بسعة 70 لتر للحوض الواحد وزعت صغار الاسماك عشوائياً بواقع 15 سمكة وبمعدل وزن 4,97 ± 0,42 غم لكل حوض اذ غذيت الاسماك لحد الاشباع . اتبع نظام التباين باتجاه واحد في تحليل النتائج احصائيا. بينت نتائج التجربة بان معاملة الاستبدال 30% بذور عباد الشمس مشابهة الى نتائج عليقة السيطرة في صفات النمو ومكونات الدم والهظم والدم . اظهرت نتائج الفحص النسجي للكبد ارتشاح كبير للدهون في الاسماك المغذات على TSF145 و TSF 345 مع تنكس دهني لخلايا الكبد وتضخم خلايا كوبفر في الاسماك المغذاة على TSF245 و TSF330 .

الكلمات المفتاحية: تغذية، دم، بروتين، احواض الاسماك، عليقة السيطرة

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INTRODUCTION

Aquaculture contribution to total fish production around the globe is increasing every year with global production estimated to be 110.2 million in 2016 (22) This rapid growth of aquaculture in the world is facilitated by proliferation of semi- and intensive culture system which rely majorly on complete feed production (23). The major ingredient in complete feed manufacture is fishmeal but its use is not sustainable owing to its dwindling availability as a result of ever-increasing demand, overfishing of wild stock and prohibitive cost (6; 31; 70-72). Fishmeal has exceptional nutritive profile that are essential, particularly for fish. Fishmeal production is quantitatively and qualitatively variable because it depends on catch gotten from the fishery (60). Soybean meal have successfully been tested and now being used to replace at least 50% of the fishmeal portion in fish feed (67). McGoogan and Gatlin III (47) reported that 90% of the protein from soybean meal could produce comparable weight gain as 100% of protein from fish meal. El-Saidy and Gaber (14) reported the possibility of total replacement of fishmeal with 55% soybean meal supplemented with 0.5% L-lysine in Nile tilapia fingerlings diet. This is because soybean meal has the best amino acid profile of the plant protein sources available, highly palatable and digestible (9). However, owing to its different use as food for man and other livestock, its price is becoming prohibitive in the world especially among fish farmers in the developing countries. Ng and Romano (53) reported that numerous alternatives to soybean meal have to be explored in order to witness cost-effective and sustainable aquaculture growth. Such a veritable plant protein that could be used to replace soybean meal must be less costly, readily available locally and must possess nutrient density that is relatively comparable to soybean meal that would provide greater flexibility to feed millers and farmers to improve cost-effectiveness (18). Sunflower protein has such potential to replace soybean meal. It is widely produced in Nigeria. . Fagbenro et al (20) reported its protein content ranging from 35.98% for sunflower cooked at 100^0C for 30 minutes to 44.29% crude protein for sunflower toasted at 204^0C for 10 minutes. Sunflower seed meal has been tested in the diets of many fishes: Tilapia, *Oreochromis niloticus*, (13; 15; 44; 55; 65); Trout, *Oncorhynchus mykiss*, (50); Silver barb, *Puntius gonionotus*, (48; 49); hybrid striped bass, *Morone chrysops* x *M. saxatilis*, (29); Russian sturgeon, *Acipenser gueldenstaedtii*, (62); Indian major carp, *Cirrhinus cirrhosus* (1); Salmon, *Salmo salar*, (30); Shrimp, *Penaeus schmitti*, (25); Gilthead sea bream, *Sparus aurata*, (4; 43); Abalone *Haliotis midae* (58; 59; 63; 64); European Eel, *Anguilla Anguilla*, (11; 27). Jackson et al (36) reported good growth in tilapia (*Sarotherodon mossambicus*) fed rations containing 35.2% sunflower meal replacing 50% of the fish meal protein. Martinez (46) reported that there was no loss in growth performance and diet utilization efficiency when rainbow trout (*Salmo girdineri*) was fed diet containing 22 and 37.3% sunflower meal though he added L-methionine to that of 37.5% sunflower meal. Tacon et al (69) earlier reported that 36.5% sunflower seed meal had a statistically similar growth with soybean control ration, that supplementation with 0.2% exogenous L-methionine did not yield beneficial effect on fish growth and feed utilization efficiency. Stickney et al (66) found that sunflower protein concentrate can replace 25% fish meal protein in rainbow trout diets. Sanz et al (61)) observed better results when replacing 40% of the animal protein in rainbow trout with sunflower protein supplemented with EAA. However, the antinutrient present in sunflower meal restricted its use as soybean replacer in fish feed. Fagbenro et al (20) reported differently processed sunflower meal to contain significantly reduced content of trypsin inhibitors, lectin, tannin, phytin, saponin and oxalates which was completely eliminated at 30 minutes cooking and 15 minutes toasting at 204^0C. Francis et al (26) reported how inclusion level could affect physiological functioning of fish with respect to antinutrients present in fish feed ingredients that fish generally has compensatory mechanism to cope with antinutrients in feed when it does not exceed the critical limit. The level of destruction of antinutrients in feed ingredients is generally related to moisture conditions, particle size, duration of heating and temperature (37; 41). This study attempts to examine the effect of different toasting time and inclusion level of sunflower protein on the growth, body composition, digestibility, haematological and histopathological condition of the liver of *Clarias gariepinus*

MATERIALS AND METHODS

Diet preparation

Toasted sunflower meal were produced by toasting sunflower seed at 204^{0}C for 5, 10 and 15 minutes and separately milled into powdery form by the use of split-disc grinder. The differently toasted sunflower meal was taken for proximate (5) and amino acid analysis. Based on their proximate composition of differently toasted sunflower seedmeal (Table 1), ten dietary treatments were developed, consisting of a control diets and nine test diets. The control diet was prepared with soybean replacing up to 50% of the fishmeal and other feeding ingredients to produce 40% crude protein diet. The nine test diets were prepared by replacing soybean portion of the control diet with each of the differently toasted sunflower meal at 15, 30 and 45% replacement level. Proximate analysis (5) of the dietary treatments was done in triplicates (Table 2).

Experimental fish and system design

Clarias gariepinus fingerlings (4.97±0.42g average weight) were obtained from Alagbaka fish farm Akure and transported live in an aerated polythene bags to the Fish Nutrition Laboratory of Federal University of Technology, Akure. The fish were acclimated to laboratory condition for 14 days during which they were fed complete imported feed. Triplicate groups of randomly picked fifteen fingerlings in aquaria containing 70-litre water were randomly assigned to each dietary treatment with continuous aeration. Group feeding of fish were done two times a day at 5% body weight on equal proportion in the morning 8:00-9:00hr and evening 17:00-18:00hrs. Batch weighing were conducted every other week and the diet rations were adjusted according to their body weight. Mortality and water quality parameters were monitored routinely during the period of the feeding trial.

Whole-body composition

Six fingerlings at the beginning and six fish from each treatment at the end of the experiment were randomly selected for proximate analysis (5).

Digestibility study

Faecal samples from each tank were pooled together and oven dried for 24 hours at 55^{0}C. The feed and the faecal samples were analyzed for acid insoluble ash (AIA) using the method explained Jimoh et al (2010) and the apparent organic matter (AOMD) and nutrient

Table 1. Proximate (g/100g dry matter) and amino acid (g/100g protein) composition and sunflower seed meal

Proximate	TSF5	TSF10	TSF15
Moisture	9.23 ±0.08	9.36 ± 0.18	9.48 ± 0.06
Crude Protein	42.08 ± 1.68	44.29 ± 2.26	39.76± 1.42
Crude Lipid	13.28 ± 1.09	12.33 ± 0.84	12.05± 2.92
Crude Fibre	14.22 ± 2.2	14.38 ± 0.66	15.37 ± 0.56
Ash	5.99 ± 1.01	6.32 ± 1.31	6.23 ± .45
NFE	15.18± 2.03	13.33 ± 1.20	17.79 ± 4.4
Amino Acid			
Lysine	3.64 ± 0.02	4.32 ± 0.01	5.11 ± 0.03
Histidine	2.24 ± 0.08	2.04 ± 0.01	1.91 ± 0.01
Arginine	8.57 ± 0.03	8.90 ± 0.02	9.05 ± 0.04
Aspartic Acid	8.98 ± 0.04	8.95 ± 0.04	9.09 ± 0.03
Threonine	3.10 ± 0.07	3.66 ± 0.04	3.81 ± 0.03
Serine	3.77 ± 0.01	3.61 ± 0.01	3.66 ± 0.01
Glutamic Acid	14.29 ± 0.01	14.33 ± 0.04	14.47 ± 0.02
Proline	2.66 ± 0.02	2.81 ± 0.01	2.75 ± 0.03
Glycine	3.03 ± 0.02	6.22 ± 0.02	3.27 ± 0.04
Alanine	3.01 ± 0.03	3.11 ± 0.01	3.31 ± 0.03
Cystine	1.45 ± 0.04	1.36 ± 0.01	1.00 ± 0.14
Valine	3.92 ± 0.04	3.93 ± 0.05	3.78 ± 0.01
Methionine	1.27 ± 0.04	1.21 ± 0.01	1.09 ± 0.01
Isoleucine	4.80 ± 0.03	5.81 ± 0.01	5.16 ± 0.01
Leucine	5.06 ± 0.04	5.16 ± 0.02	5.77±0.04
Tyrosine	1.41 ± 0.02	1.57 ± 0.02	1.72 ± 0.02
Phenylalanine	4.36 ± 0.02	3.93 ± 0.04	3.77 ± 0.03

digestibility coefficient (ADC) of the nutrients in each diets estimated using the formula$\text{AOMD}\ (\%) = 100 - \left(100\left(\frac{\%\ \text{AIA in Feed}}{\%\ \text{AIA in Faeces}}\right)\right)$

$$\textbf{ADC}\ (\%) = \mathbf{100} - \left(\mathbf{100}\left(\frac{\%\ \textbf{AIA in Feed}}{\%\ \textbf{AIA in Faeces}} \times \frac{\%\ \textbf{Nutrients in Faeces}}{\%\ \textbf{Nutrients in Feed}}\right)\right)$$

Blood sampling and hematological analysis

At the end of the 56-day feeding trial, the fish were left unfed for 24hours and were euthanized in 100ml/l clove oil. The blood samples of the six fish from each tank were collected and pooled together in EDTA bottle using heparinized syringe Haematological analysis were carried out following the standard procedures explained in Fagbenro et al (21) and Jimoh et al (38)

Hepatosomatic Index (HSI)

The six fish per dietary treatment that have been priorly individually weighed were euthanized in 100ml/l clove oil. The fish were dissected and the livers were removed. The removed livers weight from each dietary treatment were measured to calculate Hepatosomatic index (HIS).

Histological examination

Figure 1 describes the steps followed in conducting histological examination for the excised livers. The interpretation of the digital images of the liver of each dietary treatment taken was conducted at the Department of Veterinary Anatomy, University of Ibadan. Ibadan, Nigeria.

The organ was fixed in phosphate buffered 10% formalin for 24 hours

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It was then dehydrated in graded level of alcohol

↓

The dehydrated organs were cleared in xylene and embedded in paraffin wax

↓

Sectioning into 5µm thickness and staining with haematoxylin and eosin (H&E) then followed

↓

The stained slides were observed and photographed under a light microscope (Olympus BH 2) fitted with photographic attachment (Olympus C35 AD4) camera

Figure 1. flow-chart describing histological examination of the excised liver

RESULTS AND DISCUSSION

Growth Performance and Nutrient Utilization

Growth performance and nutrient utilization of *Clarias gariepinus* fed varying levels toasted sunflower meal based experimental diets is presented in Table 3. There was no significant difference (p>0.05) in the mean initial weight in the experimental fish. The fish responded well to all the dietary treatment given as evidenced in the percentage survival that was very high and did not significantly differ (p>0.05). At the end of the feeding trial, the final weight of fish fed up to 30% replacement level of variously toasted sesame seed meal did not significantly differ (p>0.05) from the fish fed control diets, except the fish fed TSF145, TSF245 and TSF345. The mean weight gain also followed the same trend as observed for mean final weight. The percentage weight gain of fish fed TSF215 was the highest. Specific growth rate of fish fed various dietary treatment did not show any significant difference from the control except those fed TSF145, TSF245 and TSF345. The highest value of feed conversion ratio was recorded in fish fed TSF145. The protein efficiency ratio of the fish fed various dietary treatments followed a reversed trend with the results observed for feed conversion ratio. The protein retention of the fish fed various dietary treatment were not significantly (p>0.05) different from each other.

Table 2. Gross (g/100g) and proximate (g/100g dry matter) composition of experimental diets at varying replacement levels of differently toasted sunflower seed meals

Ingredients	CTR	TSF115	TSF130	TSF145	TSF215	TSF230	TSF245	TSF315	TSF330	TSF345
Fishmeal	27.24	27.24	27.24	27.24	27.24	27.24	27.24	27.24	27.24	27.24
Soybean Meal	46.71	39.71	32.70	25.70	39.71	32.70	25.70	39.71	32.70	25.70
Toasted Sunflower	-	7.13	14.25	21.38	6.78	13.55	20.33	7.55	15.09	22.64
Corn Meal	11.25	11.25	11.25	11.25	11.25	11.25	11.25	11.25	11.25	11.25
Fish Oil	5.09	5.09	5.09	5.09	5.09	5.09	5.09	5.09	5.09	5.09
*Vit/Min Premix	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Starch	4.71	4.58	4.47	4.34	4.93	5.17	5.39	4.16	3.63	3.08
<u>Proximate</u>										
Moisture	9.45±0.19	9.49±0.30	9.41±0.23	9.46±0.31	9.45±0.15	9.51±0.13	9.58±0.09	9.46±0.19	9.54±0.06	9.52±0.24
Crude Protein	40.18±0.03	40.20±0.23	40.25±0.15	40.12±0.11	40.16±0.11	40.10±0.24	40.18±0.08	40.28±0.27	40.28±0.24	40.18±0.23
Crude Lipid	12.17± 0.08	12.17±0.21	12..21±0.11	12.15±0.07	12.28±0.07	12.19±0.14	12.15±0.11	12.23±0.12	12.22±0.03	12.16±0.11
Ash	8.73±1.46	8.41±0.77	8.62±0.46	8.99±0.24	8.61±0.73	8.84±0.71	8.48±1.00	8.35±0.11	8.57±0.98	9.35±0.08
Crude Fibre	6.36 ±0.12[ab]	6.50±0.12[a]	6.14±0.13[b]	6.26±0.09[ab]	6.48±0.17[a]	6.27±0.10[ab]	6.40±0.16[ab]	6.35±0.05[ab]	6.38±0.02[ab]	6.51±0.34[a]
NFE	23.12±1.46	23.24± 0.73	23.37±0.65	23.01±0.51	23.03±0.50	23.09±1.05	23.21±1.02	23.33±0.65	23.01±0.77	22.28±0.21
AIA (%)	0.76±0.06	0.81±0.02	0.78 ±0.03	0.79±0.26	0.77±0.05	0.78±0.02	0.80±0.02	0.81±0.04	0.81±0.01	0.81±0.02
Energy (kcal/100g)	435.37±6.56	436.01±3.04	437.21±1.92	434.48±1.93	435.93±3.09	435.03±2.07	435.58±4.47	437.42±0.30	435.99±4.12	431.86±1.16

*** Specification: each kg contains: Vitamin A , 4,000,000IU; Vitamin B, 800,000IU; Vitamin E, 16,000mg, Vitamin K_3, 800mg; Vitamin B_1, 600mg; Vitamin B_2, 2,000mg; Vitamin B_6, 1,600mg, Vitamin B_{12},8mg; Niacin,16,000mg; Caplan, 4,000mg; Folic Acid, 400mg; Biotin, 40mg; Antioxidant 40,000mg; Chlorine chloride, 120,000mg; Manganese, 32,000mg; Iron 16,000mg; Zinc, 24,000mg; Copper 32,000mg; Iodine 320mg; Cobalt,120mg; Selenium, 800mg manufactured by DSM Nutritional products Europe Limited, Basle, Switzerland**

Row means with no superscripts are not significantly different (p>0.05)

Row means with different superscripts are significantly different (p<0.05)

Comparable growth performance that was not statistically different from control was observed in this study for *Clarias gariepinus* fed diets containing differently toasted sunflower meal replacing up to 30% of soybean meal. At higher replacement level of 45% of the differently toasted sunflower meal, there was significant reduction in growth of *Clarias gariepinus* plausibly attributed to incomplete detoxification of anti-nutrients in sunflower seedmeal by toasting, or increased deficiencies of essential amin acids. This result corroborates the finding of Olvera-Novoa et al (55) who observed growth depletion with diets containing higher sunflower seed meal percentage and attributed it to increasing phenylalanine and methionine deficiencies and high non-digestible fibre content. Hertrampf and Piedad-Pascual (35) reported higher contents of crude fibre in sunflower meal reduced its utilization as dietary ingredients in aquaculture. González-Pérez and Vereijken (32) further explains that the phenolic compound found in sunflower meal such as caffeic acid and chlorogenic acid has the capacity to reduce protein solubility. Eusebio et al (17)and Zhou et al (75)attributed the lowered digestibility recorded at higher inclusion of plant protein to imbalanced amino acid; increased antinutrient and cellulose content as the plant protein increases. Köprücü and Sertel (40) also recorded lower growth in carp, *Ctenopharyngodon Idella*, fed sunflower meal when compared to soybean meal fed control group. Earlier work by Jackson et al (36); Tacon et al (69); Stickney et al (66) and Sintayehu et al (65) also reported lower growth performance of fish at higher inclusion of sunflower seedmeal. Francis et al (26) reported that fish generally has compensatory mechanism to cope with antinutrients in feed when it does not exceed the critical limit which practically suggests that at lower inclusion level of sunflower seedmeal, the critical level of the different anti-nutrients in them has not been exceeded.

Whole body composition

Table 4 revealed the whole-body composition of fish at the beginning and at the end of the experiment. There was significant difference ($p<0.05$) between the initial and final body composition of fish used during the experiments with respect to moisture crude protein, crude lipid and ash content. Among the fish fed the experimental diets, the highest value of crude protein was recorded in fish fed CTR which was not significantly ($p>0.05$) fish fed other dietary treatments except those fed TSF145, TSF245 and TSF345. Carcass lipid of fish fed the experimental diets did not show any significant variation ($p>0.05$). Carcass ash in fish fed the TSF345, TSF245 and TSF230 did not show any significant difference ($p>0.05$) from each other. No significant difference was found in carcass crude protein and crude lipid among the control group and sunflower fed groups up to 30% replacement level following same result trends in the growth studies. Although no significant variations were recorded in the protein retention among the different dietary treatment groups, there was marked differences in carcass protein and lipid. at higher replacement level of 45%. The reduction in carcass lipid at higher replacement level might not be unconnected with the phenolic compound, chlorogenic acid present in sunflower, an anti-oxidant that has the capacity to reduce carcass lipid (68). Hassaan et al (34) observed same trends of results on growth and carcass composition of *Oreochromis niloticus* fed fermented sunflower meal despite the fermentation process higher inclusion could

Table 3. Growth performance and nutrients utilization of *Clarias gariepinus* fed toasted sunflower meal-based diets

Parameters	Experimental Diets									
	CTR	TSF115	TSF130	TSF145	TSF215	TSF230	TSF245	TSF315	TSF330	TSF345
Initial Weight	5.24±0.37	5.49±0.16	5.04±0.49	4.67±0.00	4.62±0.07	4.79±0.47	4.81±0.47	5.08±0.07	4.90±0.55	5.07 ±0.65
Final Weight	15.57±0.41^{a}	15.79±0.14^{a}	15.23±0.56^{a}	13.90±0.04^{b}	15.62±0.41^{a}	15.23±0.19^{a}	13.46±0.26^{b}	15.42±0.73^{a}	15.14±0.55^{a}	13.39±0.32^{b}
[1]Mean Weight Gain	10.33±0.04^{a}	10.31±0.02^{a}	10.19±0.06^{a}	9.23±0.04^{b}	11.00±0.34^{a}	10.45±0.28^{a}	8.65±0.73^{b}	10.34±0.01^{a}	10.24±0.01^{b}	8.32±0.98^{a}
[2]% Weight Gain	197.90±13.49^{ab}	187.96±5.96^{ab}	203.20±18.69^{ab}	197.65±0.91^{ab}	238.07±3.70^{a}	219.65±27.51^{ab}	181.31±32.73^{ab}	205.61±29.90^{ab}	210.59±23.28^{ab}	166.70±40.64^{b}
[3]SGR	2.72±0.03^{a}	2.73±0.01^{a}	2.69±0.04^{a}	2.60±0.00^{b}	2.72±0.03^{a}	2.70±0.01^{a}	2.57±0.02^{b}	2.70±0.04^{a}	2.69±0.03^{a}	2.57±0.03^{ab}
[4]FCR	1.22±0.03^{c}	1.24±0.02^{bc}	1.26±0.02^{bc}	1.30±0.01^{a}	1.25±0.00^{bc}	1.24±0.01^{bc}	1.26±0.0^{bc}	1.24±0.01^{bc}	1.25±0.02^{bc}	1.28±0.02 ^{ab}
[6]PER	2.05±0.04^{a}	2.01±0.03^{ab}	1.99±0.03^{ab}	1.92±0.02^{c}	2.00±0.00^{ab}	2.02±0.01^{ab}	1.99±0.02^{abc}	2.02±0.02^{ab}	2.00±0.02^{ab}	1.96±0.03^{bc}
[7]Protein retention	49.53±2.54	49.65±4.74	46.87±2.29	47.61±2.97	46.44±1.27	46.04±1.90	50.91±4.20	49.88±2.34	47.52±0.99	51.24±8.19
[8]Survival Rate (%)	96.67±4.71	96.67±4.71	93.33±0.00	86.67±0.00	96.67±4.71	96.67±4.74	90.00 ±4.71	96.67±4.71	90.00±4.71	86.67± 0.00

Row means with no superscripts are not significantly different (p>0.05)

Row means with different superscripts are significantly different (p<0.05)

[1] Mean weight gain= final mean weight –initial mean weight **[2] Percentage weight gain= [final weight-initial weight /initial weight] X 100**

[3] Specific growth rate= [In final weight-In initial weight] X 100 **[4] Feed conversion ratio=dry weight of feed fed /Weight gain (g)**

[5] Protein efficiency ratio=fish body weight (g)/ Protein fed **[6]Protein retention= [protein gain/protein fed] X 100**

[7] Percentage survival = {(total number of fish- mortality)/total number of fish] X 100

Table 4. Whole body composition of *Clarias gariepinus* fed varying replacement levels of toasted sunflower seedmeal based diets

Parameter	Initial	Experimental Diets									
		CTR	TSF115	TSF130	TSF145	TSF215	TSF230	TSF245	TSF315	TSF330	TSF345
Moisture	76.80±0.34^{c}	72.50±0.08^{bc}	72.59±0.09^{bc}	72.78±0.13^{bc}	71.96±1.64^{c}	72.60±0.15^{bc}	72.74±0.05^{bc}	73.03±0.06^{b}	72.53±0.10^{bc}	72.74±0.02^{bc}	73.09±0.32^{b}
Crude Protein	15.14±0.09^{c}	17.66±0.08^{a}	17.66±0.15^{a}	17.57±0.10^{a}	17.38±0.10^{b}	17.66±0.11^{a}	17.55±0.08^{a}	17.32±0.05^{b}	17.65±0.09^{a}	17.55±0.06^{a}	17.26±0.26^{b}
Crude Lipid	5.04±0.23^{a}	6.25±0.12^{b}	6.21±0.20^{b}	6.18±0.22^{b}	6.36±0.03^{b}	6.26±0.09^{b}	6.26±0.13^{b}	6.39±0.03^{b}	6.25±0.11^{b}	6.25±0.11^{b}	6.42±0.11^{b}
Ash	3.03±0.18^{b}	3.58±0.09^{ab}	3.55±0.14^{ab}	3.47±0.14^{ab}	4.30±1.68^{a}	3.47±0.05^{ab}	3.44±0.09^{ab}	3.26±0.04^{b}	3.56±0.12^{ab}	3.45±0.06^{ab}	3.23±0.04^{b}

Row means with no superscripts are not significantly different (p>0.05)

Row means with different superscripts are significantly different (p<0.05)

not avail better growth performance. This result is in tandem with earlier finding by Zhou et al (75) on sea bream, *Acanthopagrus schlegelii*, juveniles fed fermented soybean meal in diets and by Lim et al (42) on Korean rockfish, *Sebastes schlegeli* fed dehulled soybean meal. Other researchers who reported decrease in carcass protein and lipid with increasing plant protein are Jimoh et al (39); Hassaan et al (33) and Saha and Ghosh (57).

Apparent digestibility coefficients of the nutrients in each diet

Table 5 showed the proximate composition of faecal samples of *Clarias gariepinus* fed varying levels of toasted sunflower meal-based diets. There was reduction in the nutrient contents of the faecal samples tested when compared with the nutrient contents of the feed. The highest faecal protein in fish fed TSF345 while the least faecal protein was recorded in fish fed CTR. There were no significant differences ($p>0.05$) in the faecal protein of the fish fed TSF145, TSF245 and TSF345. However, fish exposed to other test diets apart from the above did not any significant variation ($p>0.05$) from the fish fed CTR. The highest faecal lipid was observed in fish fed TSF230 while the least faecal lipid was recorded in fish fed TSF315. There was no significant difference ($p>0.05$) in faecal lipid of fish fed control diets and fish fed all other test diets except the fish fed TSF230. Faecal energy of fish fed CTR were not significantly different ($p>0.05$) from the fish fed other dietary treatment. The faecal AIA of fish fed TSF345 recorded the least value; the fish fed TSF315 recorded the highest value. There was no significant variation ($p>0.05$) in the AIA of fish fed various dietary treatment. Table 6 presents apparent digestibility coefficient of nutrients in toasted sunflower meal-based diets fed to *Clarias gariepinus* fingerlings. The fish fed various dietary treatment showed significant difference in ($p<0.05$) in their nutrient digestibility's values. CTR had the highest apparent organic matter digestibility (AOMD) which was not significantly different from AOMD of fish fed TSF115, TSF130, TSF215, TSF230, TSF315 and TSF330 while TSF345 had the least AOMD. Same trend as above was observed for apparent protein digestibility (APD) coefficient. There was no significant difference ($p>0.05$) in the APD of the fish fed CTR and fish fed TSF115, TSF130, TSF215, TSF230, TSF315 and TSF330. Fish fed TSF315 had the highest apparent lipid digestibility (ALD) coefficient which was not significantly different ($p>0.05$) from fish fed diet CTR, TSF145 and TSF215. The lowest ALD coefficient was recorded in fish fed TSF345 which was not significantly different ($p>0.05$) from fish fed TSF230, TSF245, TSF130, TSF115, TSF330 and TSF215. Similarly, the highest apparent energy digestibility (AED) coefficient was found in fish fed TSF315 while the lowest AED coefficient was found in fish fed 345. There was no significant difference ($p>0.05$) in the AED coefficient among the fish fed TSF315, CTR, TSF215, TSF130, TSF230 and TSF115. Hassaan et al (34) reported that higher inclusion of fermented sunflower seedmeal reduced protein and lipid digestibility in tandem with what was observed in this study. Eusebio et al (17)and Zhou et al (75)attributed the lowered digestibility recorded at higher inclusion of plant protein to imbalanced amino acid.

Haematological profile

The summary of comparison of haematological parameters of *Clarias gariepinus* fed toasted sunflower meal-based diets replacing soybean meal at different levels is presented in Table 7. There was no significant difference ($p>0.05$) in the primary haematological parameters (Hb, PCV, RBC, WBC) of the fish fed CTR and those fed differently toasted sunflower meal up to 30% replacement level. However, significant variations ($p<0.05$) existed between these fish and the fish fed 45% of the differently toasted sunflower seedmeal.

Table 5. Proximate composition (g/100g dry matter) of faecal samples of *Clarias gariepinus* fed varying replacement levels of toasted sunflower seedmeal based diets

	CTR	TSF115	TSF130	TSF145	TSF215	TSF230	TSF245	TSF315	TSF330	TSF345
Moisture	9.49 ±0.44	9.24±0.74	8.45±0.37	8.83±0.85	9.43±0.44	9.19±1.01	9.10 ±0.72	9.56 ±0.52	8.81±0.66	8.90±0.63
Crude Protein	14.13±1.51^{a}	14.36±1.04^{a}	14.78±1.33^{a}	19.81±0.71^{b}	14.30±1.15^{a}	15.04±1.32^{a}	19.96±0.84^{b}	14.37±0.24^{a}	14.81±1.07^{a}	19.93±2.10^{b}
Crude Lipid	6.19±0.42^{bc}	6.83±0.70^{abc}	7.10±0.54^{ab}	6.15±0.99^{bc}	6.95±0.14^{abc}	7.73±0.33^{a}	6.87±0.30^{abc}	5.97±0.85^{c}	6.63±0.16^{bc}	6.87±0.32^{abc}
Ash	8.27±1.30^{ab}	6.58±2.16^{b}	8.32±0.49^{ab}	8.66±0.34^{a}	8.59±0.65^{a}	9.37±0.64^{a}	8.47±1.13^{ab}	8.87±0.61^{a}	8.67±1.23^{a}	9.51±0.45^{a}
Crude Fibre	13.46±0.14^{a}	13.07±0.38^{ab}	12.67±0.46^{ab}	12.26±0.75^{b}	13.38±0.78^{ab}	12.95±0.22^{ab}	12.75±0.79^{ab}	13.67±0.81^{a}	12.75±0.15^{ab}	12.54±0.71^{ab}
NFE	48.47±0.84^{ab}	49.93±0.40^{a}	48.67±1.45^{ab}	44.29±1.53^{cd}	47.35±1.73^{ab}	45.72±1.06^{bc}	42.85±2.55^{cd}	47.56±1.87^{ab}	48.33±1.72^{ab}	42.16±2.45^{d}
AIA (%)	3.36±1.73	3.44±0.26^{a}	3.42±0.21^{a}	3.22±0.09^{ab}	3.37±0.31^{ab}	3.37±0.09^{ab}	3.27±0.05^{ab}	3.54±0.19^{a}	3.32±0.09^{ab}	3.04±0.03^{b}
Energy (kcal/100g)	336.67±7.1^{bc}	349.98±11.19^{ab}	349.80±4.39^{ab}	350.93±7.02^{a}	340.24±5.46^{abc}	345.05±7.61^{abc}	352.79±7.53^{a}	332.17±3.29^{c}	344.08±7.48^{abc}	349.73±54.21^{ab}

Row means with different superscripts are significantly different ($p<0.05$)
NFE: Nitrogen free Extract AIA: Acid insoluble Ash

Table 6. Apparent digestibility coefficient of nutrients in toasted sunflower meal-based diets fed to *Clarias gariepinus*

	CTR	TSF115	TSF130	TSF145	TSF215	TSF230	TSF245	TSF315	TSF330	TSF345
AOMD	77.49±0.52^{a}	76.41±1.18^{ab}	77.19±0.98^{ab}	75.42±1.19^{b}	77.05±1.73^{ab}	76.83±0.75^{an}	75.38±0.57^{b}	77.23±0.79^{ab}	75.58±0.83^{ab}	73.36±0.96^{c}
APD	92.09±0.80^{a}	91.56±0.91^{a}	91.21±0.89^{a}	87.87±0.70^{b}	91.82±1.04^{a}	91.32±0.50^{a}	87.77±0.37^{b}	91.87±0.45^{a}	91.02±0.65^{a}	86.80±1.31^{b}
ALD	88.54±0.97^{ab}	86.74±1.82^{bcd}	86.72±1.44^{bcd}	87.62±1.58^{abc}	87.02±0.76^{abcd}	85.23±0.22^{d}	86.07±0.72^{cd}	88.93±1.15^{a}	86.75±0.55^{bcd}	84.97±0.51^{d}
AED	82.59±0.49^{ab}	81.04±1.62^{abc}	81.75±1.00^{abc}	80.15±0.89^{cd}	82.08±1.60^{ab}	81.63±0.27^{abc}	80.06±0.67^{cd}	82.71±0.67^{ab}	80.73±0.85^{bc}	78.43±0.93^{d}
AAD	78.65±1.33^{ab}	81.65±5.41^{a}	77.99±0.38^{b}	76.33±1.11^{bc}	77.13±0.98^{b}	75.42±0.41^{bc}	75.41±1.91^{bc}	75.85±0.57^{bc}	75.36±0.21^{bc}	72.95±0.58^{c}
AF D	52.34±1.52^{a}	52.59±2.13^{a}	52.96±1.02^{a}	51.96±1.58^{a}	52.71±2.13^{a}	52.12±1.24^{a}	51.03±1.25^{a}	51.01±1.55^{a}	51.18±2.08^{a}	48.71±0.81^{b}
ACD	52.71±2.60	49.20±4.52	52.50±2.23	52.64±3.73	52.77±4.59	54.06±3.08	54.54±1.84	53.57±2.15	48.71±1.87	49.57±3.90

Row means with different superscripts are significantly different ($p<0.05$
Row means with no superscripts are not significantly different ($p>0.05$)

AOMD	Apparent Organic Matter Digestibility	AAD	Apparent Ash Digestibility
APD	Apparent Protein Digestibility	AF D	Apparent Fibre Digestibility
ALD	Apparent Lipid Digestibility	ACD	Apparent Carbohydrate Digestibility
AED	Apparent Energy Digestibility		

Table 7. Haematological profile of blood of *Clarias gariepinus* fed toasted sunflower meal based diets

	CTR	TSF115	TSF130	TSF145	TSF215	TSF230	TSF245	TSF315	TSF330	TSF345
Hb	10.20±0.10^{a}	10.05±0.05$^{a.}$	10.01±0.3^{a}	9.39±0.21^{b}	10.07±0.01^{a}	9.82± 0.33^{a}	9.41±0.32^{b}	10.06±0.04^{a}	10.01±0.05^{a}	9.37 ± 0.21^{b}
PCV	31.66±0.24^{a}	29.21±0.19^{b}	29.03±0.23^{bc}	26.15±0.91^{d}	29.26±0.06^{b}	28.02±0.18^{c}	26.12±0.81^{d}	29.23±0.09^{b}	29.06 ± 0.26^{bc}	25.78±0.79^{d}
WBC	7150±70.71^{c}	7200±70.71^{abc}	7300±70.71^{abc}	7400±70.71^{a}	7175±35.36^{abc}	7225±10.61^{abc}	7385±91.92^{ab}	7200±11.31^{abc}	7315±91.92^{abc}	7340±127.27^{abc}
RBC	3.37 ± 0.04^{a}	3.30±0.01^{a}	3.27±0.10^{a}	3.07±0.08^{b}	3.28±0.10^{a}	3.25±0.03^{a}	3.01±0.04^{b}	3.28±0.13^{a}	3.24±0.04^{a}	3.02±0.04^{b}
MCHC	32.22±0.07^{a}	34.40±0.05^{b}	34.48±0.17^{b}	35.89±0.46^{c}	34.40±0.10^{b}	35.05±0.93^{b}	36.01±0.10^{c}	34.42±0.25^{a}	34.44±0.13^{a}	36.34±0.31^{b}
MCV	94.09±0.03^{a}	88.50±0.20^{ab}	88.78±1.07^{ab}	85.38±5.12^{b}	89.23±2.50^{ab}	86.22±1.32^{b}	86.90±1.69^{b}	89.18±0.25^{a}	89.82±0.18^{a}	85.38±3.80^{b}
MCH	30.31± 0.03	30.44±0.01	30.62±0.22	30.64±1.44	30.70±0.95	30.22±1.26	31.30±0.69	30.70±1.32	30.93±0.18	31.02±1.12
ESR	3.73±0.03^{a}	3.76±0.04^{ab}	3.82±0.01^{bc}	3.94±0.02^{e}	3.76±0.01^{ab}	3.83±0.04^{bc}	3.92±0.01^{de}	3.76±0.05^{ab}	3.86±0.05^{cd}	3.99±0.03^{e}
HSI	1.80±0.01^{ab}	1.79±0.02^{ab}	1.76±0.01^{bc}	1.73±0.01^{c}	1.82±0.04^{a}	1.76±0.03^{bc}	1.72±0.00^{c}	1.83±0.01^{a}	1.79±0.01^{ab}	1.72±0.04^{c}

***Row means with different superscripts are significantly different ($p<0.05$), Row means with no superscripts are not significantly different ($p>0.05$)*, Hb: Haemoglobin content (gm/100ml) PCV: Packed Cell Volume (%)WBC: White Blood Cell Count ($10^4 mm^3$),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),ESR: Er.ythrocyte Sedimentation Rate (mm/hr).**

MCV followed similar result trend as recorded above. However, reverse trend of results was recorded for MCHC; The blood of fish fed CTR was the lowest which is significantly different ($p<0.05$) from the MCHC of the blood of the fish fed other experimental diets. No significant variation ($p>0.05$) existed in the MCHC of fish fed TSF115, TSF215, TSF315, TSF330, TSF130 and TSF230. The MCHC of the blood of fish fed TSF145, TSF 245 and TSF345 also showed no significant variation ($p>0.05$). Fish fed TSF345 had the highest MCHC. There was no significant variation ($p>0.05$) in the mean cell haemoglobin (MCH) among fish fed various experimental diets. The erythrocyte sedimentation rate (ESR) of fish fed CTR was the lowest which was not significantly different ($p>0.05$) from the ESR of the blood of fish fed TSF115, TSF215 and TSF315. So also, no significant variation existed in the ESR of the blood of fish fed TSF145, TSF245 and TSF345. The Hepatosomatic index (HSI) of fish fed TSF315 had the highest value while the lowest value of HSI was recorded in fish fed TSF245. There was no significant variation in the HSI of fish fed CTR, TSF215, TSF315 TSF115 and TSF330. Primary haematological parameters(Hb, PCV, RBC, WBC) reveal health status of animal (7). Feist and Longshaw (24) reported that blood profile can be altered by diet composition. The non-significant difference in the primary haematological parameters recorded among the fish fed soybean-based control diet and those fed differently toasted sunflower meal up to 30% replacement level in this study further elucidate that the physiological functioning of fish is not hampered by toasted sunflowers up to 30% replacement. The blood profile and health status of the fish is not compromised by toasted sunflower based diets. This results trend of reduced primary haematological parameters with increasing plant protein was also reported by Blom et al (10) and Rinchard et al (56) plausibly due to higher contents of anti-nutrients, poor quality amino acid contents and higher fibre content of the diet (75). The blood profile of *Clarias gariepinus* fell within the range of normal haematology of a healthy fish (19; 51; 54). By contrast, other researchers reported increase in haematological parameters include but not limited to Barros et al (8) for channel catfish fed cotton seedmeal replacing soybean meal; El-Saidy and Gaber (16) for Nile Tilapia fed the same seedmeal; Akintayo et al (3) who fed the toasted sunflower seed meal to *Clarias gariepinus*, (38) who fed watermelon seedmeal to Nile tilapia; Yue and Zhou (74) who fed cotton seed meal to juvenile hybrid Tilapia.

Histology of the liver

Photomicrographs (5µm sectioned, H&E stained, x100 Magnification) of liver of *Clarias gariepinus* fed varying levels of differently toasted sunflower based diet is presented in Figure 2. No visible lesions were seen in the liver of fish fed diet CTR, TSF115, TSF130, TSF215, TSF230, TSM315. Severe hepatic necrosis, with massive fatty infiltration was observed in the liver of TSF145-fed fish. Diffuse vacuolar degeneration of hepatocytes and kupffer cell hyperplasia were observed in fish fed TSF245. Diffuse vacuolar degeneration of hepatocytes was observed in fish fed TSF330 while there was severe hepatic necrosis, with massive fatty infiltration TSF345. The pathological condition of severe hepatic necrosis, with massive fatty infiltration recorded in the liver of fish fed TSF145 and TSF 345 in this study revealed a degraded liver as classified by Martínez-Llorens et al (45) to be grade 3 liver with necrosis, pyknotic nuclei, hyaline cytoplasm and/or large sinusoid spaces. Diffuse vacuolar degeneration of hepatocytes and kupffer cell hyperplasia observed in the liver of fish fed TSF245 and TSF330 depicted a grade 2 liver associated with an intermediate condition of the liver possessing displaced nuclei. The non-visible lesion recorded among the liver of fish fed control diets and those fed differently toasted up to 30% replacement level for 5- and 10-minute toasted sunflower and 15% replacement level for 15-minute toasted sunflower aptly categorized as grade 1 liver described as normally shaped and sized hepatocytes with uncongested vessels and normal sinusoid organization based on semi-quantitative assessment criteria of Martínez-Llorens et al (45). The pathological condition of the liver of fish fed higher content of toasted sunflower meal could be due to incomplete destruction of anti-nutrients in the seedmeal which increased with increasing sunflower meal in the diet (2; 12; 18). Nero et al (52) reported that liver being vulnerable to destruction owing to its function as xenobiotic compounds detoxifier. The massive fatty infiltration of the hepatocytes is dietary lipid induced (28; 73).

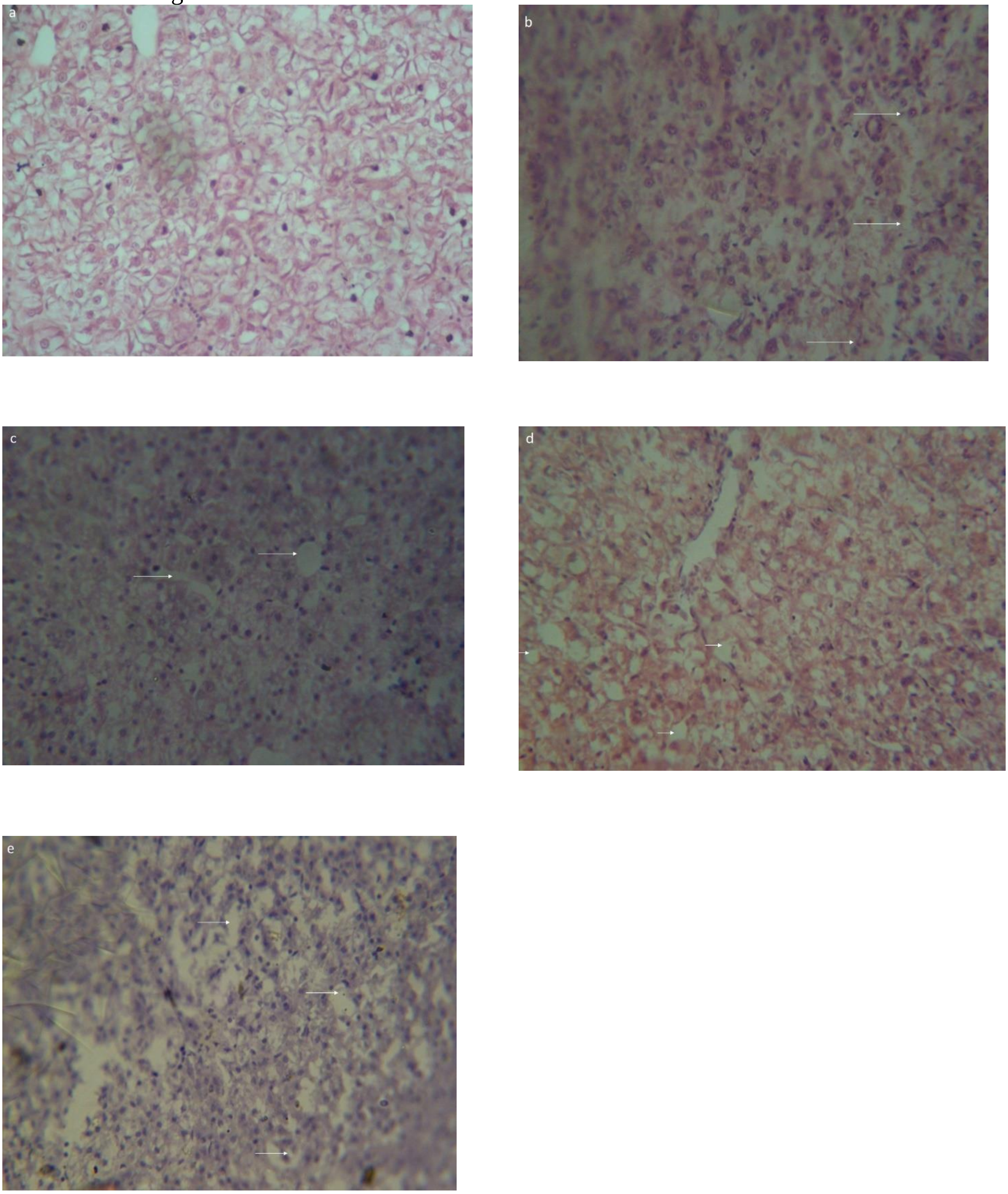

Figure 2. Photomicrographs (5µm sectioned, H&E stained, x100 Magnification) of liver of *Clarias gariepinus* fed diet (a) CTR: No visible lesions seen; (b) TSF145: There is severe hepatic necrosis, with massive fatty infiltration. (c) TSF245: Diffuse vacuolar degeneration of hepatocytes, kupffer cell hyperplasia; (d) TSF330: There is diffuse vacuolar degeneration of hepatocytes; (e) TSF345: There is severe hepatic necrosis, with massive fatty infiltration

CONCLUSION

It is evident from the results of this study that dietary replacement of soybean meal by differently toasted sunflower seedmeal in *Clarias gariepinus* diet can be done up to 30% level using survival, growth, carcass composition, digestibility, haematology and histology of the liver as indices. The physiological condition was comparable with soybean-based control diet. At higher replacement level of 45% for the differently toasted sunflower meal, the fish physiological functioning was becoming compromised though survival was not affected, there was reduced growth and changes to the blood and histopathological parameters of the liver.

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