

Cooked sesame meal in the diet of African catfish *Clarias gariepinus* (Burchell 1822): Effects on haematology, liver and kidney histology

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وجبة السمسم المطبوخة في النظام الغذائي لسماك السلور الأفريقي:

Clarias gariepinus (Burchell 1822) التأثيرات على أمراض الدم والكبد وأنسجة الكلى

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ABSTRACT. Haematological and histological alterations are strong pointers to the health status of farmed fish, farm animals and even humans. These provide dependable information on metabolic disorders and deficiency. The haematological and histological changes in African catfish *Clarias gariepinus* (Burchell 1822) fingerlings fed cooked sesame (*Sesamum indicum*) seed meal was examined in a 56-day feeding trial. Three batches of sesame seeds were cooked for 10, 20 or 30 min, dried, milled, and mechanically defatted using a screw press. Cooked seed meals (CSM) were substituted for defatted soybean meal in the diets of African catfish at 15, 30, and 45 per cent. The highest haemoglobin content (Hb) was found in catfish fed the control diet (CTR), while the lowest Hb levels were found in catfish fed the CSM345 diet. The haemoglobin content of catfish fed different dietary treatments varied significantly ($P < 0.05$). Hb values of catfish fed CTR diet and test diets CSM115, CSM215, CSM315, and CSM130 were not significantly different ($P > 0.05$). Other haematological parameters of the fish fed various dietary treatments showed a similar trend as RBC. Dietary replacement of soybean meal by differently cooked sesame seed meal in *Clarias gariepinus* diet did not affect haematology of the fish at lower inclusion levels for the different cooking times employed in the study (15% and 30% inclusion level for 10 min cooked sesame-based diets; 15% inclusion level for 20 and 30 min cooked sesame-based diets). Although, there was marked vacuolation of hepatocytes in catfish subjected to various dietary treatments, however these did not relate to dose-dependent dietary treatments.

KEYWORDS: Haematology, Histology, Sesame, Packed Cell volume, *Clarias gariepinus*.

المستخلص: تعد التغيرات الدموية والنسجية من المؤشرات القوية على الحالة الصحية للأسماك المستزرعة وحيوانات المزرعة وحتى البشر. توفر هذه المؤشرات معلومات يمكن الاعتماد عليها حول اضطرابات التمثيل الغذائي ونقص. تم فحص التغيرات الدموية والنسجية في إصبعيات السلور الأفريقي التي تتغذى على وجبة بذور السمسم المطبوخ (*Sesamum indicum*) في تجربة تغذية مدتها ٥٦ يوماً. حيث تم طهي ثلاث دفعات من بذور السمسم لمدة ١٠ أو ٢٠ أو ٣٠ دقيقة، وتجفيفها وطحنها وإزالة الدهن ميكانيكياً باستخدام مكبس لولي. تم استبدال وجبات البذور المطبوخة (CSM) بدقيق فول الصويا منزوع الدهن في وجبات سمك السلور الأفريقي بنسبة ١٥ و ٣٠ و ٤٥ في المائة. تم العثور على أعلى محتوى من الهيموجلوبين (Hb) في سمك السلور الذي تم تغذيته على النظام الغذائي (CTR)، بينما تم العثور على أدنى مستويات الهيموجلوبين في سمك السلور الذي تم تغذيته على النظام الغذائي CSM345. أظهرت النتائج عن وجود اختلاف واضح و متباين ($P > 0.05$) في محتوى الهيموجلوبين في سمك السلور الذي تم تغذيته على معالجات غذائية مختلفة. وعلى النقيض لم تكن قيم الهيموجلوبين في الأسماك التي تغذت على نظام CTR والوجبات الغذائية الاختبارية CSM115 و CSM215 و CSM315 و CSM130 مختلفة بشكل كبير ($P < 0.05$). أظهرت معاملات الدم الأخرى للأسماك التي تتغذى على معالجات غذائية مختلفة اتجاهًا مشابهًا لكرات الدم الحمراء. لم يؤثر الاستبدال الغذائي لوجبة فول الصويا عن طريق وجبة بذور سمسم مطبوخة بشكل مختلف في نظام *Clarias gariepinus* الغذائي على الدم في الأسماك عند مستوى استبدال أقل (أي وقت طهي مختلف مستخدم في الدراسة؛ ١٥% و ٣٠% مستوى استبدال لمدة ١٠ دقائق مطبوخ على أساس التغذية المعتمدة على السمسم؛ ١٥% مستوى استبدال لمدة ٢٠ و ٣٠ دقيقة في تغذية السمسم المطبوخة). على الرغم من وجود فجوة ملحوظة في خلايا الكبد في سمك السلور الخاضع للعلاجات الغذائية المختلفة، إلا أن هذه لم تكن لها علاقة بالعلاجات الغذائية المعتمدة على الجرعة.

الكلمات المفتاحية: أمراض الدم، علم الأنسجة، السمسم، حجم الخلايا المعبأة، *Clarias gariepinus*

Introduction

Plant and animal products, especially by-products of processing plants, make up the majority of traditional fish feed ingredients. These items are also consumed by humans (Socas-Rodríguez et al.,

2021). Soybean meal, which is the best plant protein feedstuff used in fish feeds, could potentially replace a large portion of fish meal (Hodar et al., 2020). However, ever-increasing demand for human consumption and other animal feed industries make it difficult to use soybean protein sources for fish feed sustainably (Jimoh et al., 2020c; Siddhuraju & Becker, 2001; Arru et al., 2019). This has lowered the viability of the fish farming industry in many developing countries, necessitating the quest for alternative protein sources for the development of low-cost feeds that can substitute these conven-

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tional feedstuffs. In addition, the high phytate content of soybean meal demands supplementing with inorganic phosphorus sources in monogastric animals. Excessive dietary phosphorus is excreted into the environment, where it forms pollution (Hussain et al., 2021). Phytate has an adverse effect on fish development and body composition. In fish, phytate forms a phytate-phosphorus combination with phosphorus, leaving it unavailable to the fish. In addition, phytate forms complexes with cations such as calcium, iron, copper, magnesium, and others, lowering mineral bioavailability. Phytate binds to trypsin and prevents the protein from being digested (Kumar et al., 2012). Furthermore, soybeans are deficient in sulphur-containing amino acids, particularly methionine and lysine (Jannathulla et al., 2019). Other legumes are less costly protein sources that have the potential to reduce the cost of fish feed when used as supplementary ingredients to fulfil the nutritional needs of fish (Dorothy et al., 2018 ; Pelletier et al., 2018). Thus, the quest for less expensive and more reliable protein sources becomes a priority. The majority of the studies focused on underutilized plant proteins in fish diets. The reported studies on the use of other oilseed residues in warm water fish feeding, such as sesame meal, is minimal (Davies et al., 2000 ; Jimoh, 2020a ; Jimoh, 2020b).

Sesame seed (*Sesamum indicum*) is one of the world's most valuable annual oil crops. They contained nutrients that are comparable to soybean meal and other conventional legumes quantitatively and qualitatively (Hossain and Jauncey, 1990 ; Jimoh et al., 2011 ; Vera et al., 2020). Their dietary protein sources in the fish diet is well established for *Clarias gariepinus* (Jimoh & Aroyehun, 2011 ; Fagbenro et al., 2010 ; Jimoh, 2021) and common carp (Hossain & Jauncey, 1989a ; Hossain & Jauncey, 1989b ; Hossain & Jauncey, 1990). The protein-rich meal left-over from oil extraction can be used as feed ingredients in aquafeed manufacture (Jimoh, 2021). Furthermore, sesame seed cake is considered to be high in methionine and tryptophan (Saleh, 2020; Jimoh et al., 2014); amino acids that are missing in most plant protein feedstuffs. Its use in fish diets has received little attention. Sesame is palatable and has a high digestibility coefficient and its apparent nutrient digestibility coefficients in *Clarias gariepinus* fed various timed wet-heat-treated sesame seedmeal based were comparable to those reported for fish fed with control diets (Jimoh, 2021; Jimoh et al., 2014). Since they provide dependable information on metabolic disorders and deficiency, haematological and histological alterations are strong pointers (Bahmani et al., 2001; Ferreira et al., 2007). In the light of this consideration, an attempt was made to investigate the effect of feeding diets containing cooked sesame (*Sesamum indicum*) seed meal and their effects on the haematology and histology of the liver and kidney of African catfish *Clarias gariepinus* (Burchell 1822) fingerlings.

Materials and Methods

Sesame seeds were collected from a farm in Kebbi, Nigeria and other feedstuffs were purchased from commercial sources in Nigeria. They were milled separately, and screened to fine particle size (250 µm). Three batches of sesame seeds were dried, milled in a hammer mill, and mechanically defatted using a locally made screw press. After that, the triplicate samples of the cake's proximate composition were determined (AOAC, 2010). The gross energy content of the samples was determined using the physiological values of 5.61 kcal/g protein, 9.50 kcal/g lipid, and 4.11 kcal/g carbohydrate (Tacon, 1999).

Based on the composition of protein in the feedstuff, a control diet and nine test diets (40 per cent crude protein, 12 per cent crude lipid, and 18.45 MJ/kg gross energy) were made (Table 1). Soybean meal, which produced 50% of total protein, was included in the control diet (CTR). Each of these differently processed seed meals was used in nine test diets at three different levels of soybean meal replacement: 15, 30, and 45 per cent (Table 2). The feedstuffs were ground, and hot water was added to assist binding before being fed into a Hobart-200T pelleting and mixing machine. It was then created to a homogeneous mass, which was then minced into 0.8 mm (long) 2 mm (diameter) pellets and immediately sun-dried (30-32°C). After drying, the diets were kept frozen in a refrigerator (-4°C) and samples were taken for proximate and amino acid analysis. Three replicated samples were considered.

Before the feeding trial, *Clarias gariepinus* fingerlings were acclimated to experimental conditions for 7 days. Aquaria with 60 litre size rectangular plastic tanks were stocked with 15 catfish fingerlings (3.38±0.015g). For 56 days, each diet was fed to the catfish in triplicate tanks twice a day (at 9.00 am, 4:00 pm) at 5% body weight. The total fish weight in each tank was calculated every two weeks, and the amount of diet was changed to match the new weight.

Haematological Examination of the Fish

After euthanization in 100 ml/l clove oil, fish (n=6) from each replicate were removed for blood analysis. A 2 ml disposable heparinised syringe treated with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant was used to extract 3 ml blood per treatment from a cardiac puncture on the fish. The blood analysis was performed according to the procedure of Jimoh (2020b).

Histological Examination of Catfish

Three fish per treatment were sampled for histological examination at the end of the experiment. The test organisms were euthanized in 100 ml/l clove oil and then dissected kidney and liver were taken out. The organs were fixed in 10% formalin for three days, and then the tissue was dehydrated for three days in graded levels of 50%, 70%, 90%, and 100% alcohol to enable paraffin

Table 1. Proximate Composition (g/100 g Dry Matter) and Essential Amino Acid Profile of feedstuff (g/100 g protein).

| Proximate Composition | CSMS10 | CSMS20 | CSMS30 | Fishmeal | Soybean Meal | Corn Meal |
|-----------------------|--------|--------|--------|----------|--------------|-----------|
| Moisture | 9.1 | 8.97 | 9.28 | 7.59 | 8.92 | 9.21 |
| Crude protein | 40.39 | 38.36 | 35.83 | 69.76 | 42.81 | 8.89 |
| Crude lipid | 11.90 | 12.83 | 12.58 | 8.82 | 18.56 | 1.49 |
| Crude fibre | 5.38 | 6.22 | 5.41 | - | 5.63 | 29.78 |
| Ash | 11.28 | 10.38 | 12.28 | 13.83 | 6.01 | 3.81 |
| NFE | 22.02 | 22.02 | 24.62 | - | 18.07 | 46.82 |
| Amino Acid Profile | | | | * | * | * |
| Lysine | 3.66 | 3.22 | 3.04 | 4.96 | 3.10 | 0.28 |
| Histidine | 2.72 | 2.88 | 3.06 | 1.47 | 1.26 | 0.29 |
| Arginine | 11.68 | 11.72 | 12.01 | 4.41 | 3.41 | 0.48 |
| Threonine | 2.98 | 3.21 | 3.10 | 2.82 | 1.92 | 0.4 |
| Cystine | 1.95 | 2.02 | 1.82 | 0.82 | 0.63 | 0.25 |
| Valine | 4.80 | 4.92 | 5.08 | 3.31 | 2.53 | 0.5 |
| Methionine | 3.42 | 3.47 | 3.71 | 1.84 | 0.72 | 0.19 |
| Isoleucine | 2.32 | 3.76 | 3.91 | 2.98 | 2.92 | 0.39 |
| Leucine | 3.62 | 8.12 | 5.22 | 4.78 | 4.02 | 1.37 |
| Tyrosine | 2.33 | 2.96 | 2.18 | 2.0 | 1.72 | 0.43 |
| Phenylalanine | 3.68 | 3.34 | 4.81 | 2.50 | 2.45 | 0.54 |

*Values obtained from NRC (1993) what is NRC?

CSM 10: Sesame seeds cooked for 10 minutes

CSM 20: Sesame seeds cooked for 20 minutes

CSM 30: Sesame seeds cooked for 30 minutes

wax to penetrate into the tissue. Melted wax was used to embed the organs. Using a rotatory microtome, the tissue was sectioned into thin parts (5-7 µm) and stained for ten min with Harris hematoxylin-eosin (H&E) stain according to the procedures of Jimoh (2020a). Each section was cleared by immersing it in warm water (38°C), and placed it on a clean slide, and heating it at 58°C for 30 min to melt the wax. Sections of the stained slide were analyzed under a light microscope at 400x magnification. The sections were interpreted at the University of Ibadan, Department of Veterinary Anatomy, Nigeria.

Ethics approval

The care and use of laboratory animals were done following standard procedure for animal welfare during transportation, housing and termination. Animal Research: Reporting of In-vivo Experiments (ARRIVE) guidelines were followed (Kilkenny et al., 2010).

Statistical Analysis

Using SPSS 17.0, all data were expressed as mean ± standard deviation and subjected to a one-way analysis of

Table 2. GROSS COMPOSITION (g/100 g DRY MATTER), OF EXPERIMENTAL DIETS AT VARYING REPLACEMENT LEVELS OF DIFFERENTLY COOKED SESAME SEED MEALS

| | CTR | CSM 115 | CSM 130 | CSM 145 | CSM 215 | CSM 230 | CSM 245 | CSM 315 | CSM 330 | CSM 345 |
|-----------------|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 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 |
| Cooked Sesame | - | 7.42 | 14.86 | 22.80 | 7.86 | 15.72 | 23.58 | 8.37 | 16.75 | 25.12 |
| 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 | 4.29 | 3.86 | 2.92 | 3.85 | 3.00 | 2.14 | 3.34 | 1.97 | 0.60 |

* Specification: each kg contains: Vitamin A , 4,000,000IU; Vitamin B, 800,000IU; Vitamin E, 16,000mg, Vitamin K3,800mg; Vitamin B1, 600mg; Vitamin B2, 2,000mg; Vitamin B6, 1,600mg, Vitamin B12,8mg; 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.

Table 3. PROXIMATE COMPOSITION (%) AND GROSS ENERGY (kcal/100 g) OF EXPERIMENTAL DIETS AT VARYING REPLACEMENT LEVELS OF DIFFERENTLY COOKED SESAME SEED MEALS.

| | CTR | CSM 115 | CSM 130 | CSM 145 | CSM 215 | CSM 230 | CSM 245 | CSM 315 | CSM 330 | CSM 345 |
|-----------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Moisture | 9.43±0.35 | 9.55±0.18 | 9.72±0.40 | 9.79±0.06 | 9.36±0.21 | 9.65±0.33 | 9.57±0.16 | 9.46±0.25 | 9.57±0.31 | 9.33±0.15 |
| Protein | 40.53±.22 | 40.61±0.29 | 40.50±0.29 | 40.41±0.34 | 40.69±0.27 | 40.60±0.41 | 40.48±0.27 | 40.29±0.34 | 40.33±0.45 | 40.05±0.80 |
| Lipid | 12.30±0.25 | 12.25±0.10 | 12.21±0.13 | 12.39±0.22 | 12.29±0.28 | 12.39±0.38 | 12.41±0.18 | 12.40±0.15 | 12.52±0.13 | 12.20±0.11 |
| Fibre | 5.25±0.29 | 5.48±0.16 | 5.36±0.17 | 5.38±0.05 | 5.36±0.14 | 5.34±0.35 | 5.39±0.23 | 5.30±0.36 | 5.63±0.11 | 5.36±0.41 |
| Ash | 6.56±0.38 | 6.63±0.47 | 6.50±0.19 | 6.36±0.20 | 6.53±0.34 | 6.38±0.18 | 6.44±0.23 | 6.76±0.07 | 6.46±0.24 | 6.74±0.14 |
| NFE | 25.93±0.57 | 25.47±0.92 | 25.71±0.77 | 25.67±0.50 | 25.77±0.33 | 25.64±1.19 | 25.71±0.32 | 25.78±0.29 | 25.48±0.46 | 26.31±1.05 |
| Energy (kcal/100g) | 450±5 | 448±2 | 448±1 | 449±1 | 450±3 | 450±1 | 450±2 | 449±3 | 449±1 | 448±2 |

NFE: Nitrogen Free Extracts

Table 4. AMINO ACID PROFILE (g/100g Protein) OF EXPERIMENTAL DIETS AT VARYING REPLACEMENT LEVELS OF DIFFERENTLY COOKED SESAME SEED MEALS.

| | CTR | CSM 115 | CSM 130 | CSM 145 | CSM 215 | CSM 230 | CSM 245 | CSM 315 | CSM 330 | CSM 345 | |
|-------------------|------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|
| Lysine | 3.15 | 2.89 | 2.94 | 3.01 | 2.87 | 2.90 | 2.94 | 2.87 | 2.91 | 2.91 | 4.8 |
| Histidine | 1.02 | 1.08 | 1.14 | 1.21 | 1.21 | 1.40 | 1.60 | 1.18 | 1.33 | 1.49 | 1.2 |
| Arginine | 2.85 | 3.28 | 3.70 | 4.18 | 3.31 | 3.78 | 4.24 | 3.37 | 3.89 | 4.40 | 3.6 |
| Threonine | 1.71 | 1.80 | 1.88 | 1.99 | 1.83 | 1.95 | 2.06 | 1.84 | 1.96 | 2.09 | 2.8 |
| Cystine | 0.55 | 0.65 | 0.75 | 0.86 | 0.66 | 0.78 | 0.89 | 0.65 | 0.76 | 0.87 | |
| Valine | 2.14 | 2.32 | 2.50 | 2.70 | 2.35 | 2.56 | 2.77 | 2.39 | 2.64 | 2.88 | 2.4 |
| Methionine/TSA | 0.86 | 1.06 | 1.27 | 1.49 | 1.08 | 1.30 | 1.53 | 1.11 | 1.38 | 1.64 | 2.4 |
| Isoleucine | 2.22 | 2.19 | 2.16 | 2.14 | 2.31 | 2.40 | 2.49 | 2.34 | 2.47 | 2.59 | 2.0 |
| Leucine | 3.33 | 3.32 | 3.31 | 3.31 | 3.69 | 4.05 | 4.40 | 3.49 | 3.65 | 3.80 | 3.5 |
| Tyrosine | 1.40 | 1.45 | 1.50 | 1.57 | 1.51 | 1.62 | 1.73 | 1.50 | 1.52 | 1.58 | |
| Phenylalanine/TAA | 1.89 | 1.99 | 2.10 | 2.21 | 1.98 | 2.07 | 2.16 | 2.12 | 2.35 | 2.58 | 4.0 |

TAA: Total Aromatic Amino acid

TSA: Total Sulphur-containing Amino Acids

variance (ANOVA) test. When ANOVA showed a significant difference ($P < 0.05$) and Duncan's multiple range test was used to determine the significant differences between treatments ($P < 0.05$).

Results

The proximate composition and amino acids of experimental diets are shown in the Tables 3 and 4. There was no significant difference ($p > 0.05$) in the crude protein, crude lipid, and energy contents of the experimental diets, indicating that they were isonitrogenous, isolipidic, and isocaloric. *C. gariepinus*' dietary requirements for protein, energy, and lipids. Throughout the feeding trial, the fish in each dietary treatment successfully fed on the experimental diets.

Haematological Examination

Table 5 indicates the haematological parameters of *C. gariepinus* fed cooked sesame meal-based diets. The fish fed CTR had the highest primary haematological value (Hb, PCV, and RBC), which was significantly different ($P < 0.05$) from the fish fed the other dietary treatments

except for the test diets CSM115, CSM215, CSM315, and CSM130. Between the control and fish-fed test diets, there was no significant difference in white blood cell count or MCHC ($P > 0.05$). The ESR of fish fed with the control diet and those fed with the test diets CSF115, CSF130, CSF215, and CSF315 did not vary significantly ($P > 0.05$).

Discussion

The reduction in haematological parameters observed in *C. gariepinus* fed cooked sesame diets might be connected to the decrease in the nutritional quality of the seedmeal as the cooking time increased (Table 1). This agrees with the report of Jimoh et al. (2020b). The nutritionally deficient diets can cause haemoglobin concentration, haematocrit, and red blood cell count to decrease. Physiologically, haemoglobin is essential for fish survival since it is directly linked to blood's oxygen binding ability. Given that the values are within the usual range reported for African catfish. The reduction observed in this study may not have harmed *C. gariepinus*. Furthermore, the Hb, PCV, RBC, and WBC values recorded in this study were all within the normal range

Table 5. HAEMATOLOGICAL PROFILE OF BLOOD OF *Clarias gariepinus* FED COOKED SESAME MEAL BASED DIETS EXPERIMENTAL DIETS.

| | CTR | CSM 115 | CSM 130 | CSM 145 | CSM 215 | CSM 230 | CSM 245 | CSM 315 | CSM 330 | CSM 345 |
|------|-------------------------|--------------------------|---------------------------|-------------------------|--------------------------|---------------------------|--------------------------|---------------------------|---------------------------|---------------------------|
| Hb | 10.11±0.10 ^a | 10.03±0.08 ^a | 9.75±0.25 ^{ab} | 8.84±0.13 ^c | 10.01±0.06 ^a | 9.55±0.17 ^b | 8.71±0.03 ^c | 10.00±0.04 ^a | 9.55±0.11 ^b | 8.55±0.31 ^c |
| PCV | 30.55±0.17 ^a | 30.14±0.26 ^{ab} | 29.28±0.75 ^{ab} | 26.57±0.35 ^c | 30.05±0.18 ^{ab} | 28.73±0.57 ^b | 26.51±0.26 ^c | 30.05±0.16 ^{ab} | 28.75±0.31 ^b | 26.06±1.47 ^c |
| WBC | 6600±141.42 | 6450±212.13 | 6375±106.07 | 6300±141.42 | 6450±70.71 | 6350±141.42 | 6250±141.42 | 6400±141.42 | 6337±194.45 | 6330±141.42 |
| RBC | 3.30±0.04 ^a | 3.18±0.04 ^{ab} | 3.04±0.08 ^{bc} | 2.74±0.04 ^d | 3.18±0.02 ^{ab} | 2.98±0.06 ^c | 2.70±0.01 ^d | 3.15±0.01 ^{ab} | 2.99±0.06 ^c | 2.69±0.17 ^d |
| MCHC | 33.09±0.14 | 33.27±0.02 | 33.30±0.01 | 33.27±0.05 | 33.29±0.01 | 33.25±0.06 | 32.86±0.22 | 33.27±0.06 | 33.22±0.04 | 32.83±0.66 |
| MCV | 92.72±.48 ^f | 94.92±0.23 ^e | 96.48±0.01 ^{bc} | 97.13±0.01 ^b | 94.64±0.05 ^e | 96.58±0.16 ^{bc} | 98.17±0.46 ^a | 95.38±0.09 ^{de} | 96.16±0.78 ^{cd} | 96.90±0.64 ^{bc} |
| MCH | 30.68±0.03 ^d | 61.58±0.11 ^{bc} | 32.13±0.02 ^{abc} | 32.33±0.05 ^a | 31.51±0.01 ^{bc} | 32.10±0.11 ^{abc} | 32.25±0.06 ^{ab} | 31.73±0.03 ^{abc} | 31.94±0.23 ^{abc} | 31.81±0.85 ^{abc} |
| ESR | 3.52±0.04 ^a | 3.48±0.03 ^a | 3.39±0.09 ^{ab} | 3.07±0.04 ^c | 3.47±0.02 ^a | 3.32±0.06 ^b | 3.02±0.01 ^c | 3.47±0.01 ^a | 3.32±0.04 ^b | 2.97±0.11 ^c |

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

Hb: Haemoglobin content (gm/100 ml)

PCV: Packed Cell Volume (%)

WBC: White Blood Cell Count (10^4mm^3)

RBC: Red Blood Cell Count (10^6mm^3)

MCHC: Mean Corpuscular Haemoglobin Concentration (%)

MCV: Mean Corpuscular Volume (μ^3)

MCH: Mean Corpuscular Haemoglobin (pg)

ESR: Erythrocyte Sedimentation Rate (mm/h)

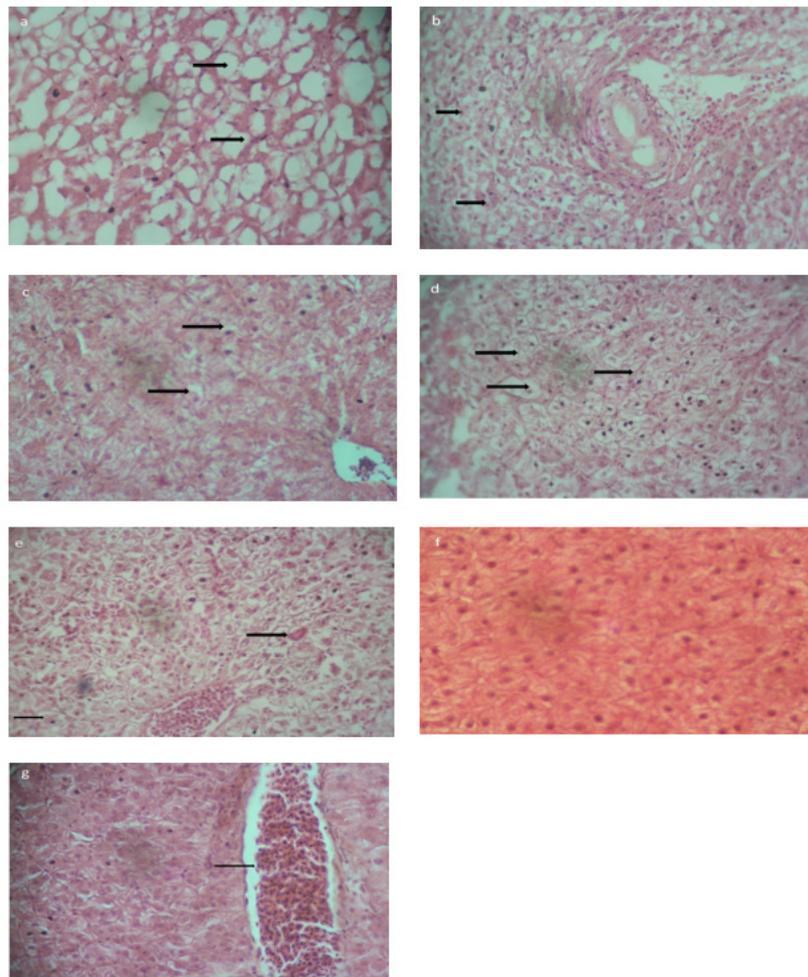


Figure 1. Photomicrographs (H&E stained, 5µm section, x400 magnification) of the liver of *Clarias gariepinus* fed. (a) CTR: There was diffuse vacuolation of the hepatocytes; (b) CSM115: There was very mild, diffuse vacuolation of the hepatocytes; (c) CSM145: There was very mild, diffuse vacuolation of the hepatocytes; (d) CSM215: There very moderate diffuse vacuolation of the hepatocytes; (e) CSM230: There was diffuse vacuolar degeneration of the hepatocytes; (f) no visible lesion was seen in the livers of fish fed CSM130, CSM245, CSM315 and CSM330 (g) CSM345: There was mild central venous and portal congestion

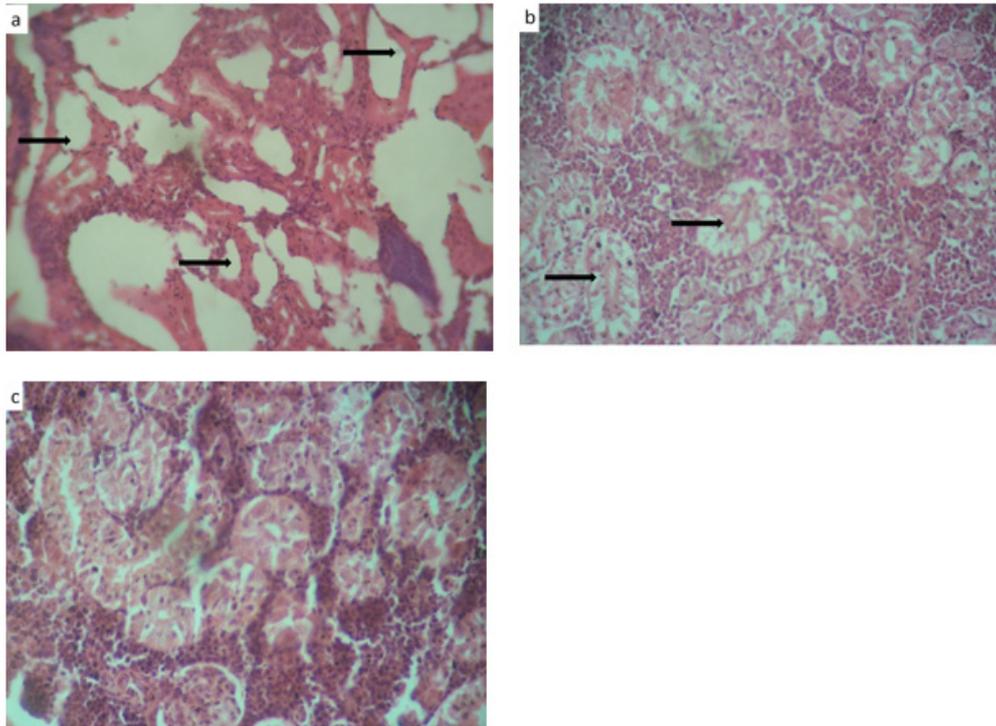


Figure 2. Photomicrographs (H&E stained, 5µm section, x400 magnification) of the kidney of *Clarias gariepinus* fed (a) CTR: The tubular epithelium was completely eroded; (b) CSM230: There was diffuse degeneration of the tubular epithelium; (c) no visible lesion was observed in the kidneys of fish fed CSM115, CSM130, CSM145, CSM215, CSM245, CSM315, CSM330 and CSM345.

of a healthy fish (Jimoh et al., 2020c; Myburgh et al., 2008). The RBC values found in this study were similar to those found in *Clarias lazera* (Zaki et al., 2011), *Clarias gariepinus* (Omitoyin, 2006), *Heteropneustes fossilis* (Khan and Abidi, 2010; Khan and Abidi, 2011). Usually, an erythrocyte count of more than $1 \times 10^6/\text{mm}^3$ is considered high and this indicates that the blood has a high oxygen-carrying capacity. This is typical capability of aerial respiration and high activity (Fagbenro et al., 2013). Haniffa and Mydeen (2011) found a value of Hb for *Channa striatus* was higher than the value found in this study. The Hb values for *hybrid catfish* fed a control diet reported by Osuigwe et al. (2002) for hybrid catfish (*Heterobranchus longifilis* x *C. gariepinus*) were similar to those found in this study. The haematocrit values for fish were also within the usual range of 20-38 per cent (Erondu et al., 1993; Adeyemo et al., 2014).

Increased production of leucocytes in the haematopoietic tissue of the kidney and possibly the spleen is responsible for the rise in WBC as observed in the fish fed with cooked sesame diets (Fazio et al., 2015). Antibodies are produced by lymphocytes, which act as a powerful defence against infection (Hua and Hou, 2020). According to Akinwande et al. (2016), immunity of fish is measured by the number of white blood cells it produces. The increased number of white blood cells seen in this

study indicated that the fish had a high level of disease resistance (Shen et al., 2018) and it may be caused a rise in the rate of haemoglobin destruction or a reduction in its productivity or synthesis (Srivastava and Reddy, 2020). It would be observed that haemoglobin concentration reduced in the fish fed with the test diets as compared to control. Similar observations were recorded by Jimoh et al. (2020c) when *Oreochromis niloticus* was fed with diets containing toasted *Jatropha curcas* seed meal and *Clarias gariepinus* was fed with diets containing *Luffa cylindrica* seed meal (Jimoh et al., 2020b). Other researchers recorded a decrease in haematocrit and haemoglobin with increased ingredient levels (Blom et al., 2001; Jimoh, 2020a; Jimoh, 2020b).

Furthermore, the histology of the kidney and liver of fish fed with the sesame diets appeared unaffected. Similar findings were reported by Mérida et al. (2010) when sunflower meal was used as a partial substitute in the case of juvenile sharp snout sea bream (*Diplodus puntazzo*) diets. Pereira et al. (2002) observed in the case of rainbow trout fed with partial substitution of brassica by-products. Hansen et al. (2006) made a similar observation when gut and liver histology was examined in a cod fed diet containing various inclusion of plant protein. The results of this study showed marked vacuolation of hepatocytes and did not link to dose-dependent

dietary treatment. Olukunle (2011), Jimoh et al. (2020c) and Jimoh et al. (2020b) made the similar observation. Mild to moderate vacuolation of the hepatocytes were recorded in the livers of fish fed cooked *Jatropha*-based diets as compared to the soybean-based control diet (Jimoh et al., 2020a). The high vacuolation of the liver may be due to the organ's high lipid content, which can be traced back to high lipid diets. The existence of numerous and voluminous lipid droplets in hepatocytes is a physiological response to dietary lipid excess or increased lipogenesis (Martins et al., 2007; Valente et al., 2011; Gatta et al., 2011).

Conclusion

Dietary replacement of soybean meal by differently cooked sesame seed meal in *Clarias gariepinus* diet did not affect haematology of the fish at lower inclusion level. The addition of 15% and 30% inclusion level for 10 min cooking time; and 15% inclusion level for 20 and 30 min cooking time had similar profile with control. Although, there was marked vacuolation of hepatocytes in catfish subjected to various dietary treatments, and this was not related to dose-dependent dietary treatments. This indicated that the health of *Clarias gariepinus* fed cooked sesame seed meal diets did not put stress on the fish.

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