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Performance, physiological and immune responses of Nile tilapia *Oreochromis niloticus* fed extruded pellet diets with different binders

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ABSTRACT

The trial was conducted to investigate the effects of inclusion carboxymethyl cellulose (CMC) or calcium lignosulfonate (CLS) on the physical qualities of extruded Nile tilapia diet, growth performance, feed efficiency, physiological and immune response for 70-day. Three identical isonitrogenous and isoenergetic diet were formulated, 0 (control), 4 g CMC and 4 g CLS kg^{-1} diet and fed to tilapia with an average initial body weight (14.20 ± 1.22 g). Inclusion of CLS significantly improved the water stability, durability, bulk density, and sinking speed of the pellets compared to CMC and control diets ($P \leq 0.05$). Compared to the control group, inclusion of 4 g kg^{-1} CLS recorded the highest weight gain (WG, 51.00 g $fish^{-1}$), specific growth rate (SGR, 3.02 %, day $fish^{-1}$) and aveage daily gain (ADG) (0.61 g $fish^{-1}$). The highest activities of amylase (84.102.44 U L^{-1}), lipase (958.3 ± 70.11 L) and trypsin (0.59 ± 0.18 ng ml^{-1}) were detected in fish fed CLS group. The height and width of villi and goblet cell number in both the anterior and posterior intestines were significantly increased in fish fed CLS than other groups. The activities of alanine amino transferase (ALT) and aspertate aminotransferase (AST) levels substantially decreased in the CLS group compared to the control. While CLS supplementation significantly elevated serum total protein, globulin, and albumin levels compared with CMC group and control diet. No significant differences were found in serum lipid profile among fish fed experimental diets. Catalase and superoxide dismutase (SOD) were significanly higher in fish fed diet supplemented with 4 g CLS kg^{-1} compared with others group. Furthermore, immunoglobulin M (IgM) and complement factors (C3, C4), were significantly improved in fish fed diet contained CLS.

1. Introduction

In aquaculture, fish feed is very important because it provides the nutrients that fish need to grow. Although the nutritional quality of feed has received a lot of attention, the physical properties are still needing more attaention, especially in aquatic environments (Sørensen, 2012). Low good durability and water stability of feed may cause monetary losses and environmental issues i.e. lost nutrients as well as uneaten feed are typically released in fish farm effluents (Welker et al., 2018). Moreover, the physical properties of feed affect and/or stimulate the biological response of fish. Setting low water stability of the diet can lead to the presence of oil in the stomach (Aas et al., 2011; Draganovic et al., 2011; Oehme et al., 2014). Some fish species have a lot of special feed property such as the soft texture, special textural properties or low carbohydrate content (Hemre et al., 2002). To ensure optimal growth, raw material quality and nutrient composition in feed formulation must be matched to the demands at a given production stage of the fish.

Pellets are a common form of artificial feeds and their physical properties such as stability, durability and buoyancy are crucial for feed acceptability and effectiveness (Haetami and Abun, 2021). Pellets offer advantages such as high bulk density, improved flow characteristics and reduced dust and particulate matter formation (Thomas and Van der

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Poel, 1996). Producing high-quality pellets is essential to avoid feed waste and higher production costs. Water stability is also important for fish feed pellets to prevent contamination and feed losses during feeding. To produce pellets with the desired properties, appropriate additives should be used (Garcia-Maraver et al., 2013). Binders or adhesives are often added to feed formulations to improve pellet quality by increasing integrity, durability and stability. Binders form strong bridges between feed particles, reducing breakage and improving handling, transportation, and storage (Acar, 1991; Peñaflorida and Golez, 1996; Kaliyan and Morey, 2009; Tumuluru et al., 2016; Attar et al., 2018). Binders are divided into three groups: Natural, modified or and synthetic (Lim and Cuzon, 1994). Natural binders (starch and protein) are feed based components and interact with the feed mix to increase the nutritional quality of feed as well as enhance pellet strength and pelleting durability (the latter, due to heat/moisture induced chemical reactions that change the character of their nature (Lim and Cuzon, 1994; Paolucci et al., 2012). Urea-formaldehyde, Na or Ca bentonite are synthetic binders and thus do not directly provide nutritional value for the feed. By Hindering movement of particles along wall at high surface area granules, Modified binders like carboxymethylcellulose (CMC), lignosulfonate, alginate, agar carrageenan, guar gum gelatin bentonite sepiolite and pectin are also intermediate between more nutrient enriched material and pellet strength and durability because the effectively fill pore spaces between particles and supply adhesive forces to consolidate particles (Lim and Cuzon, 1994; Ouyang et al., 2006; Paolucci et al., 2012; Yalçın et al., 2017).

Binder selection to produce biochar pellets is mostly read by costs and environmental press. In a study by Hu et al. (2015) the binders used were lignin, starch, calcium hydroxide and sodium hydroxide. Starch pellets showed sufficient hydrophobicity, but they had low density and poor mechanical properties as well as high compression strength for the pellets prepared with sodium hydroxide. Moreover, Si et al. (2016) investigated the effect of carboxymethyl cellulose as a binder in pellets from cotton stalks, wheat straw and rape straw. Results showed that the binder could increase density, compressive strength and durability of pellets from cotton stalks and wheat straw while its application lowered pellet quality from rape straw. Similarly, Yahaya and Ibrahim (2012) also fabricated rice husk briquettes with starch as a binder and gum Arabic as a binder observing that starch-based briquette resulted in quickness of boiling water. The acceptability of binders in feed production is hinged on its binding capacity, inclusion level, interference with growth and digestibility, availability and cost. The purpose of the current study is to investigate the influence and benefits of various pellet binders such as carboxymethyl cellulose and calcium lignosulfonate in optimizing pellet quality and ultimately impact Nile tilapia growth, feed utilization, and overall health.

2. Materials and methods

2.1. Ethical approval

All experiments were approved by the authority of NIOF Committee for Institutional Care of Aquatic Organisms and Experimental Animals (NIOF-AQ4-F-25-R-012).

2.2. Diets formulation

Three isonitrogenous and isoenergetic diets were performed and its chemical composition was measured according to AOAC (1995) as shown in Table 1. The control diet without supplementation was compared against two experimental diets enriched with carboxymethyl cellulose (CMC) or calcium lignosulfonate (CLS) at level of 4 g kg^{-1} diet, which were supplied from NUTRIVETmisr Feed Additives Inc., October City, Giza, Egypt.

The dry milled ingredients were carefully combined thoroughly. Then, water (27 % ~ 28 %, wt) was added to all the ingredients with further mixing to the ingredients. Diets were processed through a 2.0 mm diameter die at extrusion temperatures (120 °C) using a Twin-screw extruder FAMSUN (Huasheng Road, Yangzhou City, Jiangsu Province, China). The extruder was operated at a constant throughput of 40 ~ 50 kg/h during the extrusion process. The extrusion parameters of the experimental diets are shown in Table 2. The extruded pellets were dried in the oven at 65 °C. The extruded diets were packed in plastic bags and then stored at 4 °C for the feeding experiment. The chemical composition of diets was estimated as follows; the dry matter was measured. Ash by incineration at 550ºC for 12 h. Crude protein was

Table 1
Formulas, proximate compositions, extruder parameters of experimental diets for Nile tilapia (as-fed basis, %).

Ingredients	Control	Pellets binder (4 g kg^{-1} diet)	
		CMC	CLS
Yellow corn	300	300	300
Soybean meal solvent extracted (44 %)	320	320	320
Fish meal (60 %)	80	80	80
Corn Gluten meal (60 %)	70	70	70
Sunflower meal, Solvent extracted (40 %)	50	50	50
Rice Bran	50	50	50
Wheat Bran	50	46	50
Dicalcium phosphate	13	13	9
Salt	2	2	2
Fish oil	20	20	20
Soybean oil	25	25	25
Vitamin and Mineral premix	20	20	20
Pellets binder	0	4	4
Total	**1000**	**1000**	**1000**
Proximate chemical composition (%)			
Dry matter	87.88	87.94	88.01
Crude protein	30.13	30.21	30.29
Ether extract	4.3	4.54	4.79
Ash	5.15	5.15	5.15
Crude fiber	4.96	5.19	5.42
Nitrogen free extract (NFE)[2]	55.46	54.91	54.35
Gross energy (MJ/kg)	16.94	16.98	17.02
Processing parameters			
Temperature of extruder barrel zone 1 (℃)	107	107	106
Temperature of extruder barrel zone 2 (℃)	110	109	109
Temperature of extruder barrel zone 3 (℃)	120	120	120
Conditioning water (%)	27	27	27
Die diameter (mm)	2	2	2

Vitamin and Mineral premix (per kg of premix): Calcium carbonate as carrier up to 1 kg for zinc,40 g; iron, 20 g; copper, 2.7 g; iodine, 0.34 g; manganese,53 g; selenium, 70 mg and cobalt, 70 mg. Vitamin B1, 700 mg; Vitamin B2, 3500 mg; Vitamin B6, 1000 mg; Vitamin B12, 7 mg; Vitamin A,8000000 IU; Vitamin D3, 2000,000 IU; Vitamin E, 7000 mg; Vitamin K3,1500 mg; biotin, 50 mg; folic acid, 700
mg; nicotinic, 20,000 mg; pantothenic acid,7000 mg.
[2]NFE = 100- (CP% +EE% +CF% +Ash%)].

Table 2
Physical properties of experimental diets.

Parameters	Experimental diets			P value
	Control	CMC¶	CLS#	
Water stability (30 min)	92 ± 0.82[c]	95.13 ± 1.99[b]	99.19 ± 2.04[a]	0.021
Durability	80.16 ± 1.18[c]	84.00 ± 5.00[b]	95.3 ± 1.21[a]	0.032
Bulk density (g L^{-1})	456.39 ± 5.023[c]	506.42 ± 6.015[b]	618.59 ± 3.18[a]	0.022
Sinking speed (cm s^{-1})	3.06 ± 0.02[c]	4.02 ± 0.01[b]	4.96 ± 0.02[a]	0.021

Means followed by different small letters in the same row are significantly different (P < 0.05) by Tukey test.
¶ CMC = Carboxymethyl cellulose.
#CLS = Calcium lignosulfonate.

evaluated using a micro-Kjeldhal method with N% × 6.25 (using a Kjeltech autoanalyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat was determined using a Soxhlet extraction with diethyl ether (40–60ºC).

2.3. Feed physical characteristics

2.3.1. Sinking speed test

The sinking speed test was carried out by measuring the length of time it takes for the feed to move from the surface of water to the bottom. Pellets as many as 5 sticks were inserted into a measuring cup with a height of 20 cm from the surface of the water. The stopwatch was run just when the pellets were dropped on the surface of the water. Sinking speed was the distance divided by the time the pellets through until the base of a measuring cup (Wulansari et al., 2016).

2.3.2. Durability test

Durability is the number of pellets that are returned intact after being stirred mechanically (pneumatic). According to Balazs et al. (1973), durability tests can be formulated as follows:

$$\text{Durability} = \frac{\text{Weight of pellets after rotating}}{\text{Weight of pellets before rotating}} \times 100\%$$

2.3.3. Stability of ration in water

Before dipping to be tested based on immersion time, the sample for each treatment was divided into three equal parts. The soaking time was 30 min, and then after immersion the pellets are removed and dried so that the moisture before soaking was the same as after. Dry weight and stability of the pellets were calculated according to Wulansari et al. (2016) as follows:

$$\text{Water stability} = \frac{\text{Final dry weight after soaking}}{\text{Initial dry weight before soaking}} \times \quad 100\%$$

Notes: Initial sample of Pellets = A gram (30 g) Pellets + aluminum foil = X grams (dry oven 105 °C for 2 h). Aluminum foil was issued and weighed = Y gram Pellet after drying* = Z

2.3.4. Feed bulk density

Bulk density was measured for both fresh and dried feed by placing 5 g of feed pellets in a 50 ml graduated cylinder followed by addition of 30 ml of water. The cylinder was gently shaken to remove air trapped between feed pellets, and the total volume was recorded. If a feed sample was initially buoyant, a foam disk was cut to the diameter of the graduated cylinder and pressed down gently to submerge the floating feed pellets and then the water level was recorded. Feed bulk density (g/ml) was calculated by dividing 5 g with the difference between the total volume measured (water plus feed, in ml) and 30 ml of water (Liu et al., 2021).

2.4. Fish husbandry management

Nile tilapia, *Oreochromis niloticus* monosex, were obtained from the fish farm of the Faculty of Agriculture at Benha University in Egypt. The fish were stocked in two cement ponds (2 × 4 × 1 m) for 15 days after being collected to acclimate to the experimental conditions, during this period fish were fed commercial feed (30 g kg^{-1} protein) at a rate of 4 % of total biomass, divided into two equal meals daily at 09:00 am and 3:00 pm. Following acclimation, nine fiberglass tanks (0.5 m) were randomly filled with 20 uniformly sized fish (14.20 ± 1.22 g). All tanks were supplied with fresh water and housed within an artificially illuminated room 12-h light, 12-h dark (08:00 – 20:00 h) was maintained by using fluorescent ceiling lights. About 20 % of the water volume in each tank was daily replaced by aerated freshwater after removing the accumulated excreta. Fish were manually fed twice daily, six days a week (Hassaan et al., 2019).

Throughout the trial period (70-day), water quality data were recorded. Water quality data was recorded during trial period using various instruments. This included mercury thermometers, an Orion pH meter, and a Jenway 970 Dissolved Oxygen Meter. Standard techniques were used to quantify, nitrate (NO_3, 0.55 ± 0.02 mg L^{-1}), ammonia (NH_4; 0.25 ± 0.04, mg L^{-1}), and nitrite (NO_2, 0.021 ± 0.01 mg L^{-1}) once per week according to APHA (2017).

2.5. Growth and feed indices

At the initial and terminate of the feeding trail, the number of fish in each tank was counted and recorded. All the equations used for estimating the parameters of growth indices and feed utilization efficiency are presented at the footnote of Table 2.

2.6. Digestive enzymes analysis

Four fish from each treatment tank were given gut samples, which were immediately homogenized in 10 volumes (w/v) of ice-cold physiological saline solution. The centrifuged samples were then kept for testing endogenous enzyme activity in the supernatant (Furné et al., 2008). At 540 nm, Bernfeld (1951) method for estimating amylase activity was used and Zamani et al. (2009) method's for determining lipase activity. Trypsin activity and chymotrypsin was measured by using methods of Hummel (1959) and details were found in Hassaan et al. (2019).

2.7. Histological techniques

Following an 70-day feeding trial, the anterior and posterior sections of the intestines of three fish from each treatment group were slaughtered and their tissue examined using histomorphometric analysis. The experimental fish's intestinal tissues were removed and preserved in Bouin's solution for a whole day. The preserved tissues underwent a series of ethanol dehydration grades, xylene cleaning, and paraffin wax (congealing point 58–60 °C) embedding. The tissue slices were inspected using an image analysis program and a light microscope fitted with a full HD microscopic camera (Nikon E600, Tokyo, Japan). Using image analysis software, three fish from each group had their mean villus length (measured from base to top) and width determined. The collected data was used for statistical analysis. The histological measurements were estimated according to Wassef et al. (2016) and Ibrahim et al. (2022).

2.8. Serum biochemical parameters

Blood was obtained from a fish's caudal vein using clean syringes at the termination of the experiment. The blood sample was then centrifuged for 10 min at 3000 rpm after clotting overnight at 4°C. Serum that had not been hemolyzed was collected and kept at 20°C until needed. Standard Kits (Modern Laboratory Kits) were used to determine the serum lipid profile, which included triglycerides, cholesterol, high-density lipoprotein cholesterol (HDL–C), and low-density lipoprotein cholesterol (LDL–C).

2.9. Hepatic antioxidant activity measurements and immune response

Fish (n = 3) livers and muscles from each replicate were weighed, washed and ground in glass homogenizer tubes with ice-cold saline (0.9 ml saline and 0.1 g of liver, pH 7.0), and after that, centrifuged for 10 min at 3000 g. The supernatant was collected and utilized to assess the superoxide dismutase (SOD) activity using Peskin and Winterbourn (2000) method. The catalase (CAT) activity assay was done using a modified Beers and Sizer (1952) method. The amounts of complement component 3 (C3) and complement component 4 (C4) were assessed using the immunoturbidimetric approach (Zhejiang Yilikang Biotech

Co., Ltd). The level of serum total immunoglobulin M (IgM) was determined by an ELISA assay kit (Cusabio, Wuhan, Hubei, China). The test kits were purchased from Shenzhen Mindray Bio-medical Electronics Co., Ltd. By modifying the turbidimetric methods of Parry et al. (1965) and Siwicki (1993), lysozyme activity was assessed.

2.10. Statistical analysis

The data were tested for homogeneity and normality before analysis. All the data were analyzed using the SAS ANOVA procedure (SAS, version 6.03, Soft Inc., Tusla, OK, USA, SAS, 1996). A one-way analysis of variance (One-way ANOVA) was used to determine whether there was significant variation among the treatments followed by post hoc Tukey test ($p < 0.05$) (Zar, 1999) to evaluate significant differences between treatment means.

3. Results

3.1. Physical characteristics of the diets

The physical metrics were showed in Table 3 demonstrate that CLS significantly improved the water stability, durability, bulk density, and sinking speed of the pellets relative to CMC and control diets ($P \leq 0.05$). The CLS group showed the highest water stability and durability (99.19 ± 2.04 % and 95.3 ± 1.21 %) respectively, while the control group recorded the lowest values at 92 ± 0.82 % and 80.16 ± 1.18 %, respectively.

3.2. performance of growth and feed Efficacy

The growth performance and feed utilization metrics showed in Table 3. Growth performance and feed utilization positively affacted by differnt pellets binders. Final body weight (FBW), weight gain (WG), specific growth rate (SGR) and aveage daily gain (ADG) were significantly enhanced in fish that were fed diet supplemented with 4 g k^{-1} diet CLS relative to the other groups diets ($P \leq 0.05$). The feed conversion ratio (FCR) significantly improved in the CLS group (1.21 ± 0.05) compared to the control group (1.76 ± 0.23) and CMC group (1.33 ± 0.38).

Table 3
Growth and feed utilization of Nile tilapia fed extruded diets with different binders.

Parameters	Experimental diets			P value
	Control	CMC[¶]	CLS[#]	
Initial body weight (g $fish^{-1}$)	14.20 ± 1.22	15.10 ± 1.58	15.10 ± 1.54	0.076
Final body weight (g $fish^{-1}$)	51.20 ± 1.08^{c}	63.00 ± 1.02^{b}	66.3 ± 1.11^{a}	0.032
Weight gain (g $fish^{-1}$)	37.00 ± 1.23^{c}	48.50 ± 1.14^{b}	51.00 ± 1.77^{a}	0.021
Specific growth rate (; % day^{-1})	1.83 ± 0.23^{b}	2.04 ± 0.91^{a}	2.09 ± 0.26^{a}	0.032
Average daily gain (g $fish^{-1}$)	0.44 ± 0.02	0.57 ± 0.02^{ab}	0.61 ± 0.01^{a}	0.021
Feed intake (g $fish^{-1}$)	62.00 ± 1.12	63.7 ± 2.17	61.52 ± 1.17	0.073
Feed conversion ratio	1.76 ± 0.23^{a}	1.33 ± 0.38^{b}	1.21 ± 0.05^{c}	0.021
Protein efficiency ratio	1.90 ± 0.08^{c}	2.50 ± 0.28^{b}	2.76 ± 0.8^{a}	0.012

Means followed by different small letters in the same row are significantly different ($P < 0.05$) by Tukey test.
[¶] CMC = Carboxymethyl cellulose.
[#]CLS = Calcium lignosulfonate.

3.3. Activity of digestive enzymes

The activity of digestive enzymes including amylase, lipase and trypsin were positively affacted by CMC and CLS supplemntation (Table 4). The highest activities of amylase (84.102.44 U L^{-1}), lipase (958.3 ± 70.11 L) and trypsin (0.59 ± 0.18 ng ml^{-1}) were detected in CLS group. The lowest activities of digestive enzymes; amaylase, lipase, trypsin were noted in control group

3.4. Intestinal Structure

Table 5 and Figs. 1–3 showed the intestinal morphology at anterior and posterior. The structure of intestinal both of anterior and posterior are positively affacted by CMC and CLS supplementation. The height and width of villi in both the anterior and posterior intestines were significantly increased in fish fed CLS than other groups. Furthermore fish fed diet supplemented with CLS recorded the higher number of goblet cells in the antirior and posterior intestine than other group.

3.5. Serum Biochemical parameters

Table 6 demonstrated the data of serum biochemical analyses of fish fed experimantal diets. Data showed that CMC and CLS inclusion significantly ($P \leq 0.05$) influenced serum biochemical parameters. The activities of alanine amino transferase (ALT) and aspertate aminotransferase (AST) levels substantially decreased in the CLS group compared to the control. While CLS supplementation significantly elevated serum total protein, globulin, and albumin levels compared with CMC group and control diet.

3.6. Serum lipid profile

Table 7 showed the data of serum lipid profile of fish fed experimental diets. Data showed that the inclustion CMC and CLS in fish diets not significantly ($P \geq 0.05$) influenced serum lipid profile.

3.7. Hepatic antioxidant activity and immune response

Antioxidant enzymes like catalase and superoxide dismutase (SOD) (Table 8) were significantly increased in the CLS group suggesting enhancing of oxidative stress control in fish that consumed CLS. Immune Response Markers of immunological response, such as immunoglobulin M (IgM) and complement factors (C3, C4), were significantly raised in fish treated with CLS ($P \leq 0.05$). No significant differnces were found in LZM among fish fed different experimantal diets.

4. Discussion

This research investigated the impact of calcium lignosulfonate (CLS) and carboxymethyl cellulose (CMC) as dietary additions for Nile

Table 4
Growth and feed utilization of Nile tilapia fed extruded diets with different binders.

Parameters	Experimental diets			P value
	Control	CMC[¶]	CLS[#]	
Amylase (U L^{-1})	62.00 ± 1.82^{c}	75.12 ± 1.98^{b}	81.10 ± 2.44^{a}	0.002
Lipase (U L^{-1})	510.20 ± 31.08^{c}	613.00 ± 25.07^{b}	958.3 ± 70.11^{a}	0.021
Trypsin (ng ml^{-1})	0.39 ± 0.023^{c}	0.47 ± 0.015^{b}	0.59 ± 0.18^{a}	0.0024

Means followed by different small letters in the same row are significantly different ($P < 0.05$) by Tukey test
[¶] CMC = Carboxymethyl cellulose
[#]CLS = Calcium lignosulfonate

Table 5
Intestinal structure of Nile tilapia fed extruded diets with different binders.

Parameters	Experimental diets			P value
	Control	CMC[¶]	CLS[#]	
Anterior intestine				
Villi height (mm)	400 ± 5.08^{c}	463.50 ± 3.12^{b}	521.20 ± 3.11^{a}	0.022
Villi width (mm)	37.30 ± 23	48.80 ± 0.96^{b}	52.36 ± 0.47^{a}	0.041
Goblet cells number (No.)	34 ± 0.89^{b}	42.14 ± 0.99^{a}	51.09 ± 0.26^{a}	0.002
Posterior intestine				
Villi height (mm)	263.00 ± 2.12^{c}	363.7 ± 2.17^{b}	401.05 ± 3.88^{a}	0.032
Villi width (mm)	42.76 ± 2.13^{a}	49.03 ± 1.18^{b}	53.21 ± 2.05^{b}	0.024
Goblet cells number (No.)	38.90 ±.88^{c}	51.50 ± 0.98^{b}	52.26 ± 0.78^{a}	0.012

Means followed by different small letters in the same row are significantly different ($P < 0.05$) by Tukey test.
[¶] CMC = Carboxymethyl cellulose.
[#]CLS = Calcium lignosulfonate.

tilapia emphasizing their impacts on feed quality, growth performance, digestive physiology, intestinal health, and immunological response. Prior research has shown that binder additions in aquaculture feeds improve the density and resilience of pelleted feeds, hence decreasing water resistance and dissolving rates (Gui et al., 2018; Palma et al., 2008).

The present data revealed that the CLS group exhibited the greatest feed stability, with a water stability of 99.19 % obtained after 30 min ($P < 0.05$), this outcome was markedly different from the CMC and control groups. Furthermore, CLS had superior results regarding feed durability and density relative to the other groups. The CLS group's feed had a durability rate of 95.3 %. Prior research has shown that CMC improves hardness and water resistance in feeds (Abdollahi et al., 2012). In present investigation, CMC enhanced water stability by 95.13 % relative to the control group, hence augmenting feed resistance to water exposure. Nonetheless, the solubility of feeds in both the CMC and CL groups decreased, enhancing water quality by decreasing organic matter discharge (McMillan et al., 2003).

The results indicate that CMC and CLS impede the solubility of organic compounds, including nitrogen and phosphorus, which may reduce environmental pollution (Wang et al., 2019). In accordance with other studies (van Rooijen et al., 2014; Ćolović et al., 2010), the present study validates the beneficial impacts of CLS and CMC on feed hardness and water stability. The findings suggest that improving the water resistance of pelleted feed might enhance feed consumption by fish in aquatic settings. The denser and more rigid composition of CLS feeds resulted in a slower disintegration rate in water. These results underscore the significance of pellet hardness in modulating the release of organic materials. Lignosulfonate serves as a filler that improves the strength and durability of pellets by occupying voids between particles (Yalçın et al., 2017). Binder additions enhance the quality and durability of pelleted meals while maintaining nutritional integrity. In aquaculture, the delivery of nutrients is crucial for enhancing growth and digestion. Binders equilibrate water intake and nutrient retention, enhancing nutritional accessibility (Argüello-Guevara and Molina-Poveda, 2013). Our findings demonstrate that CLS

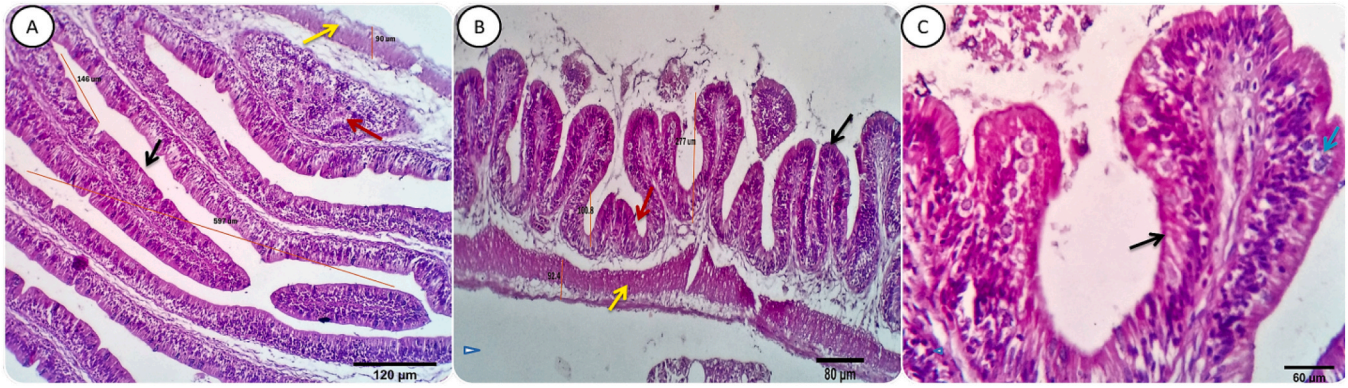

Fig. 1. Photomicrographs from anterior (A) and posterior (B) intestines and mucosal fold (C) of intestinal fish fed the control diet, showing normal histomorphological structures of the villi and intestinal folds; (black arrows), Crypts of both anterior and posterior intestine (red arrows), submucosa (green stars) and muscle layer (yellow arrows). A moderate number of goblet cells are seen (blue arrow).

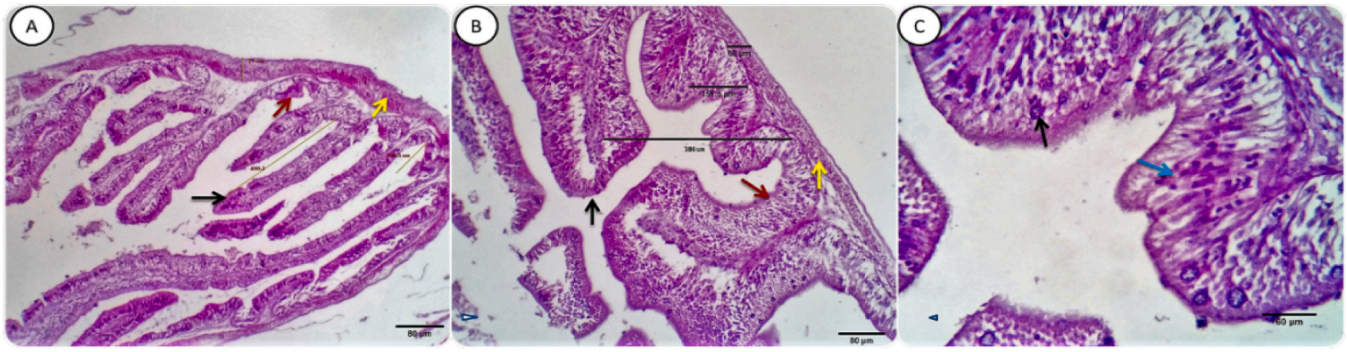

Fig. 2. Photomicrographs from anterior (A) and posterior (B) intestines and mucosal fold (C) of intestinal fish fed 4 g Carboxymethyl cellulose (CMC) kg^{-1}, showing increases in both villous length, folds (black arrows), crypts (red arrows) and muscle thickness (yellow arrows) with a characteristic increase in the number of goblet cells (blue arrows), especially in the posterior intestine.

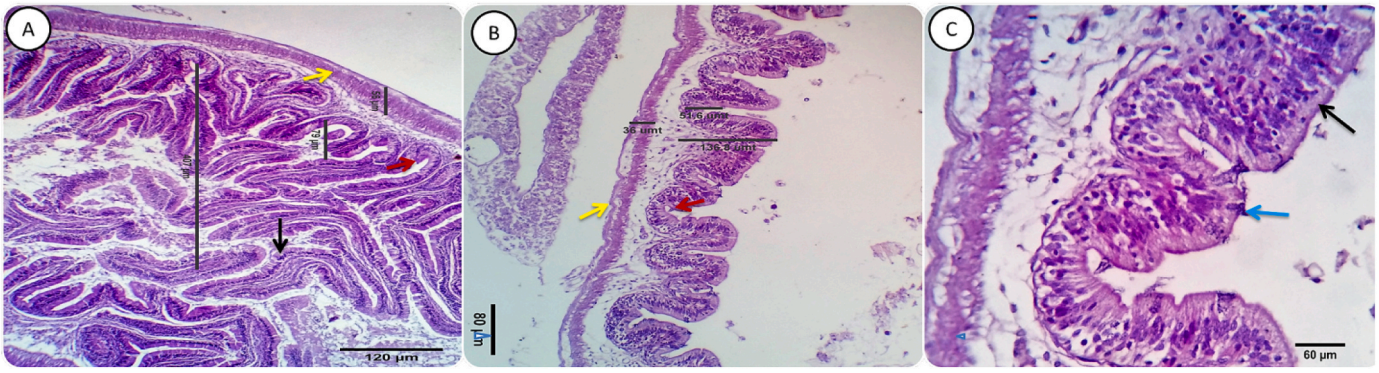

Fig. 3. Photomicrograph from anterior (A) and posterior (B) intestines and mucosal fold (C) of intestinal fish fed 4 g Calcium lignosulfonate (CLS) kg^{-1}, showing mild shortness and slight decreases in the mucosal villous –folds (black arrows), crypts (red arrows) and muscle thickness (yellow arrows), however the goblet cells (blue arrow) and the mucosal and submucosal immune cells are within the normal limits (green star).

Table 6
Serum biochemical parameters of Nile tilapia fed extruded diets with different binders.

Parameters	Experimental diets			P value
	Control	CMC[¶]	CLS[#]	
Alanine aminotransferase (ALT, U L^{-1})	15.00 ± 1.88[a]	12.10 ± 0.89[b]	11.40 ± 0.74[c]	0.032
Aspartate aminotransferase (AST, U L^{-1})	29.00 ± 1.61[c]	21.00 ± 1.02[b]	18.80 ± 0.59[a]	0.024
Total protein (TP, g dl^{-1})	2.13 ± 0.02[c]	2.69 ± 0.01[b]	3.20 ± 0.01[a]	0.027
Globulin (g dl^{-1})	0.92 ± 0.01[c]	1.24 ± 0.02[b]	1.51 ± 0.05[a]	0.004
Albumin (g dl^{-1})	1.21 ± 0.03[c]	1.45 ± 0.04[b]	1.69 ± 0.02[a]	0.013
Uric Acid (mg dl^{-1})	2.21 ± 0.18	2.18 ± 0.57	2.29 ± 0.12	0.074
Creatinine (mg dl^{-1})	0.29 ± 0.01	0.21 ± 0.02	0.23 ± 0.01	0.073

Means followed by different small letters in the same row are significantly different ($P < 0.05$) by Tukey test.
[¶] CMC = Carboxymethyl cellulose.
[#]CLS = Calcium lignosulfonate.

Table 7
Serum lipid profile of Nile tilapia fed extruded diets with different binder.

Parameters	Experimental diets			P value
	Control	CMC[¶]	CLS[#]	
TC* (mg dL^{-1})	166.95 ± 1.07	169.10 ± 1.88	165.40 ± 1.74	0.067
TG[†] (mg dL^{-1})	187.60 ±	178.00 ± 1.02	181.30 ± 3.11	0.063
HDL-C[‡] (mg dL^{-1})	32.27 ± 0.88	33.55 ± 0.98	35.03 ± 0.97	0.084
LDL-C[§] (mg dL^{-1})	100.16 ± 2.17	101.39 ± 2.81	99.98 ± 0.89	0.072

Means followed by different small letters in the same row are significantly different ($P < 0.05$) by Tukey test.
*TC = Total cholesterol; [†]TG = Triglycerides; [‡]HDL-C = High-density lipoprotein cholesterol; [§]LDL-C = Low-density lipoprotein cholesterol; [¶]VLDL-C = Very low-density lipoprotein cholesterol.
[¶] CMC = Carboxymethyl cellulose.
[#]CLS = Calcium lignosulfonate.

supplementation at 4 g kg^{-1} in the diet significantly enhanced physical characteristics metrics in comparison to both CMC and the control group. These results underscore the potential of CLS as a multifunctional feed additive in tilapia farming, affecting both feed characteristics and fish physiology. Gao et al. found that inclusion of CMC in gibel carp

Table 8
Antioxidant response of Nile tilapia fed extruded diets with different binder.

Parameters	Experimental diets			P value
	Control	CMC[¶]	CLS[#]	
Catalase (U ml^{-1})	9.12 ± 1.88[c]	12.10 ± 2.18[b]	15.40 ± 2.74[a]	0.032
Superoxide dismutase (SOD, U ml^{-1})	59 ± 2.08[c]	69.00 ± 1.02[b]	81.30 ± 3.11[a]	0.038
complement component 4 (C4, µg ml^{-1})	92.2 ± 2.83	125.50 ± 2.98[b]	135.23 ± 3.77[a]	0.021
Complement component 3 (C3, µg ml^{-1})	19 ± 0.97[b]	25.39 ± 0.81[a]	31.08 ± 0.86[a]	0.028
Immunoglobin M (IgM, µg ml^{-1})	6.14 ± 0.52[b]	9.59 ± 0.52[ab]	9.91 ± 0.01[a]	0.013
Lysozyme (LZM, U ml^{-1})	6.23 ± 0.12[c]	8.70 ± 0.97[b]	9.52 ± 0.92[a]	0.004

Means followed by different small letters in the same row are significantly different ($P < 0.05$) by Tukey test.
[¶] CMC = Carboxymethyl cellulose.
[#]CLS = Calcium lignosulfonate.

(*Carassius gibelio*) adversely impacted digestive enzyme activity, hence diminishing digestibility. In a similar manner, whereas CMC enhanced growth performance relative to the control group, CLS demonstrated superior efficacy in the present investigation. Furthermore, Gao et al. (2020) revealed that CMC did not significantly ($P \geq 0.05$) affect the growth performance of gibel carp. The binding characteristics and elevated viscosity of CMC may impede digestion and diminish nutritional absorption. Yamamoto and Akiyama (1995) indicated that CMC resulted in reduced growth and protein digestibility in Japanese flounder. Our findings indicated that, whereas CMC improved growth relative to the control group, its efficacy was subpar compared to that of CLS. The denser and more compact structure of CLS increased digestion, hence boosting growth performance.

The specific growth rate (SGR) of fish receiving CL (2.09 ± 0.26 % per day) surpassed that of the CMC group (2.04 ± 0.91 % per day). The inclusion of CLS to enhance digestion and improve nutritional absorption has been previously recorded (Dominy et al., 2004). The unfavorable effects of CMC on feed hardness may hinder digestive enzyme activity, leading to reduced digestibility (Khalaji et al., 2016). Thus, the protein efficiency ratio (PER) of the CMC group (2.50 ± 0.28) was inferior to that of the CLS group (2.76 ± 0.8). The heightened water absorption and viscosity from CMC impeded digestion, thus impacting growth performance. The enzymatic activities of lipase, trypsin, and amylase in the intestines signify digestion efficiency (Kakade et al., 2023). The increased amylase activity seen in fish using CLS feed than other groups indicates that this additives facilitated starch degradation, yielding a more readily available energy source that favorably

influenced development (Lopez-Lopez et al., 2005). Moreover, elevated lipase and trypsin activity signify enhanced digestion of fats and proteins, facilitating development.

The present study found that fish given CMC and CLS diets had increased intestinal villus density, height, and goblet cell count. Yokoyama et al. (2020) discovered the same findings in amberjack (*Seriola dumerili*) administered CMC. Diets with high viscosity may hinder digestion and nutrient absorption in the anterior gut, requiring structural modifications in the posterior segment (Yamamoto and Akiyama, 1995). Our results indicate that posterior intestine growth compensates for insufficient digesting in the front section. These structural modifications correspond with observations in mullet, where an increase in goblet cells and villus height enhanced nutrition absorption (Miegel et al., 2010; Islam et al., 2024). Fish on CMC diets had comparable adaptive intestinal modifications, consistent with the results of Ito et al. (2009), which indicated that high-viscosity diets elevated goblet cell counts in rats. The increased villus height and width seen in the anterior intestines of CLS-fed fish indicate that CLS enhances nutrient absorption, while the effects of CMC were more pronounced in the posterior intestine. These disparities illustrate the adaptive abilities of fish in response to nutritional difficulties, as articulated by Sklan et al. (2004) and Shimeno et al. (1993).

Fish fed with the CLS diet had elevated concentrations of total protein (TP), globulin, and albumin relative to the control and CMC groups ($P \leq 0.001$). CLS may augment protein absorption and utilization by enhancing digestive enzyme activity, as shown by prior study (Neves et al., 2007). Albumin, an indicator of protein nutritional status, was higher in the CLS group, indicating enhanced nutritional status. The reduced protein levels in the CMC group indicate compromised nutrition absorption and digestion. These results correspond with research indicating that high-viscosity diets diminish enzyme function by creating adhesive complexes that obstruct absorption (Nie et al., 2007; Zhang et al., 2021). ALT and AST values, which indicate liver health, were reduced in the CL group relative to the CMC and control groups, suggesting enhanced liver function. The increased ALT and AST values in the CMC group suggest possible liver strain, aligning with research connecting high-viscosity diets to hepatic injury (Cai et al., 2020).

The dietary treatments had little influence on blood lipid profiles, with no significant ($P \geq 0.05$) alterations observed in total cholesterol (TC), triglycerides (TG), HDL-C, or LDL-C ($P \geq 0.05$). This is consistent with previous studies indicating that fish maintain steady cholesterol levels irrespective of dietary cholesterol consumption (Sealey et al., 2001). Although CMC diets somewhat decreased TG levels, the alterations were not statistically significant. This outcome diverges with animal research indicating that dietary fibers diminish fat absorption and lower blood triglyceride levels (Artiss et al., 2006). Comparable results have been seen in fish species like white sea bream and mullet, where fiber-enriched diets did not influence cholesterol levels (Enes et al., 2013; Ramos et al., 2015).

The activities of catalase and superoxide dismutase, indicators of antioxidant capacity, were markedly elevated in fish on the CL diet relative to the CMC and control groups ($P < 0.01$). This augmentation indicates that CL supplementation mitigates oxidative stress. Additionally, immunological markers including C3, C4, and IgM were raised in the CL group, indicating augmented immune activity. In contrast, CMC diets decreased antioxidant enzyme activity, aligning with prior research that suggests CMC compromises gut health and heightens vulnerability to oxidative damage (Yin et al., 2018).

5. Conclusion

In conclusion, this research underscores the significant advantages of CLS and CMC as dietary supplements for Nile tilapia to improve the physical characteristics of the diets and fish health. CLS surpassed CMC and the control group for feed quality, growth, digestion, and immunological response. The enhanced pellet stability, durability, and water resistance of the CLS group not only augmented nutrition retention but also mitigated environmental contamination. CLS supplementation increased growth performance by enhancing nutrient absorption and digestive enzyme activity. Future study must concentrate on optimizing dose and evaluating the long-term impacts of these compounds on diverse aquatic species. The incorporation of CLS into fish meals provides a sustainable method for enhancing production efficiency and promoting environmental conservation in aquaculture.

CRediT authorship contribution statement

El-Haroun Ehab: Data curation, Conceptualization. **Ümit Acar:** Data curation, Conceptualization. **Eman Y. Mohammady:** Data curation, Conceptualization. **Elsayed M. Younis:** Formal analysis, Data curation, Conceptualization. **Mohamed S. Hassaan:** Data curation, Conceptualization. **Simon J. Davies:** Data curation, Conceptualization. **Abdel-Wahab A. Abdel-Warith:** Data curation, Conceptualization.

Ethical approval

All experiments were approved by the authority of NIOF Committee for Institutional Care of Aquatic Organisms and Experimental Animals.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data will be made available on request.

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