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Effect of Replacing Soybean Meal with Cotton Seed Meal With or Without Supplementation of Lysine on Different Biological Traits of *Catla catla*

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ABSTRACT

This study aimed to assess the benefits of incorporating an exogenous enzyme (Lysine) and replacing soybean meal (SBM) with cottonseed meal (CSM), with or without lysine supplementation, in Thalia (*Catla catla*) diets. A total of 225 fish were divided into five groups: Control, 75CSM, 75CSM+Lys, 100CSM, and 100CSM+Lys, with 15 fish per group (Three replicates each). The control group received a CSM-free diet, while CSM replaced SBM in the other groups. Survival rates were unaffected by dietary changes. Growth performance in the 75CSM+Lys group matched the control, but 100% CSM substitution, with or without lysine, negatively impacted growth. Body composition and muscle amino acids were largely unaffected, except for higher arginine and total amino acids in the control. Amylase and lipase activities decreased with CSM replacement, with the highest levels in the control, while protease activity remained same. Replacing SBM with CSM significantly increased blood ALT and AST levels but left ALP levels unchanged. Antioxidant enzyme activities (MDA and CAT) decreased with CSM substitution while SOD remained unchanged. Ratio of villus length/villus width of 75CSM+Lys group was same with respect to control group but other CSM groups showed significant decrease, similarly Tunica muscularis significantly decreased in all dietary groups as compared to control. . In conclusion CSM can be substituted with SBM up to 75% with the supplementation of lysine to balance its level without affecting the growth performance, proximate composition, digestive enzymes activity, antioxidant status and gut morphology of *C. catla.*

INTRODUCTION

Fisheries and aquaculture are important pillars of agriculture in Pakistan and play a vital role in food security. However, there is a dire need to do much more for efficient fish farming. Feed is one of the vital components of the aquaculture industry and accounts for > 70% of the total cost of production (Naseem et al., 2021). Therefore, the profit margin could be increased by using cheaper but good-quality feed ingredients. Protein is the most expensive nutrient used in feed formulation of aquaculture, and its availability becomes limited with the passage of time (Hafez and Attia, 2020). The best protein source of plant origin is soybean meal (SBM) (Saleh *et al.*, 2021), but Pakistan is not self-sufficient in its production, and SBM is being imported from other countries. SBM is used in the feed formulation of fish up to 50% and higher inclusion levels (> 50%) might become the reason for damage to the intestinal mucosa and its inflammation (Yue and Zhou, 2008). In addition, increasing prices of SBM demand the search for and use of alternative plant protein sources for use in feed formulation.

Cotton seed meal (CSM) could be a suitable alternative to SBM because of its higher protein content (23-53%) which varies with the oil extraction process (Yue and Zhou, 2008), and lower cost. In addition, cotton seed is being produced by Pakistan at higher levels. CSM has already been used in the feed formulation of other

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livestock animals for many years. In addition, CSM can be safely used in many fish species, including tilapia (Yue and Zhou, 2008) and sunshine bass (Rawles and Gatlin, 2000). However, these studies showed that CSM could be used at lower inclusion levels, which might be due to an imbalance of amino acids, lower digestibility, or the presence of antinutritional factors (Li and Robinson, 2006). Gossypol is an important antinutritional factor present in CSM that negatively influences the growth, reproduction, and immunity of animals (Yue and Zhou, 2008).

The imbalanced amino acid profile could be managed with the supplementation of lysine in diets when CSM is being used in fish diet formulation. Previous studies also suggested that the addition of lysine with CSM had no negative impact on growth performance in catfish (Robinson, 1991). However, another study stated that supplemented lysine might be biologically unavailable to fish when CSM with higher gossypol levels is used in the diet (Liu *et al*., 2009). Similarly, in Cyprinid fish species, supplemental lysine is not an efficient source of protein (Leng and Wang, 2005). The literature is deficient in the use of lysine with CSM in place of SBM in the *Catla catla* diet formulation. Therefore, this experiment is designed to evaluate the impact of alternating SBM with CSM with or without the addition of lysine on growth performance and other biological parameters such as antioxidant activities, blood biochemical indices, intestinal enzyme activity, muscle amino acid profile, of *C. catla.*

MATERIALS AND METHODS

Experimental design and management conditions

In this experiment, two hundred and twenty-five Thalia fish (*Catla catla*) with an average body weight of 42.97±0.68 g were used for 90 days. Fish were randomly divided into five groups (Control, 75CSM, 75CSM+Lys, 100CSM and 100CSM+Lys) with three replicates under each group and 15 fish per group. Fish in the control group were fed a basal diet in which SBM was used. Diets of groups 75CSM and 75CSM+Lys were prepared by replacing 75% SBM with CSM, without, and with supplementation of lysine, respectively. Similarly, diets of groups 100CSM and 100CSM+Lys were prepared by replacing 100% SBM with CSM, without and with supplementation of lysine, respectively. Supplementation of lysine was performed to balance it with the control diet, as shown in Table I. Happas installed in the earthen pond were used as experimental units, and each happa was assigned to a replicate. Diets were prepared exactly before the start of the feeding trial using a Qidong extruder and were stored in sealed plastic bags after drying in a hot air oven. Feeding was performed two times per day at a level of 3% of their body weight. All environmental conditions were kept constant during the experiment, and pH, temperature and dissolved oxygen were maintained at 24.9-28.7 °C, 7.4-8.6 and 5.8-7.3 mg/L, respectively.

Table I. Ingredient and nutrient composition of experimental diets.

Ingredients	Cont	Treatments			
		75 CSM	75CSM +Lys	100 CSM	100CSM +Lys
Maize grain	20	20	20	20	20
Soybean meal	25	6.25	6.25	0	0
Cottonseed meal	0	18.75	18.75	25	25
Fish meal	10	10	10	10	10
Corn gluten 60%	7	8.5	8.5	9	9
Rice polish	16	16	15.76	16	15.51
Wheat flour	15	13.5	13.5	13	13
Fish oil	5	5	5	5	5
Lysine sulphate	-	-	0.23	-	0.49
Mineral mixture#	1	1	1	1	1
Vitamin premix*	1	1	1	1	1
Total	100	100	100	100	100
Nutrient's composition					
Crude protein%	25.11	25.06	25.14	25.12	25.20
EE%	9.43	9.78	9.78	10.12	10.12
Crude fibre%	3.17	4.73	4.73	6.29	6.29
Ash%	5.53	5.64	5.64	5.76	5.76
Metabolizable energy (kcal/kg)	3131	3324	3324	3517	3517
Lysine%	1.36	1.23	1.36	1.1	1.26
Methionine%	0.51	0.5	0.5	0.5	0.5

Each kg of the mineral mixture contains: KH_2PO_4 479 mg/g, $MgSO_4.7H_2O$ 153 mg/g, $CoCl.6H_2O$ 0.0816 mg/g, NaCl 51 mg/g, $AlCl_3.6H_2O$ 0.255 mg/g $CuSO_4.5H_2O$ 210.67 mg/g, $FeSo_4$. H_2O 100.67 mg/g, $MnSo_4.5HO$ 116.67 mg/g, $ZnSO_4.7H_2O$ 121.33 mg/g $CaCO_3$ 316 mg/g and Cellulose 65 mg/g.

*Each kg vitamin premix contains the following: Vitamin A 15 M.I. U, Vitamin D3 3 M.I. U, Nicotinic acid 25000 mg, Vitamin B1 5000 mg, Vitamin E 6000 IU, Vitamin B2 6000 mg, Vitamin K3 4000 mg, Vitamin B6 4000 mg, Folic acid 750 mg, Vitamin B12 9000 mg, Vitamin C 15000 mg, Calcium pentothenate 10000 mg. CSM, cotton seed meal; EE, ethanol extract; Lys, lysime.

Cont: without CSM; 75CSM: 75% SBM replaced with CSM; 75CSM+Lys: 75% SBM replaced with CSM supplemented with lysine; 100CSM: 100% SBM replaced with CSM; 100CSM+Lys: 100% SBM replaced with CSM supplemented with lysine.

Growth performance

At the start of the experiment, body weight and other growth-related measurements were performed and recorded, followed by fortnightly bases, and at the

termination of the experiment. To determine the feed intake, a weighed amount of feed was offered, and leftovers were collected and weighed. The following equations were used to compute the specific growth rate (SGR), body weight gain (BWG), and feed conversion ratio (FCR).

Body weight gain (g) = Final body weight (g) – Initial body weight (g)

SGR = [Final weight gain – Initial weight)/days of growth trial] × 100

FCR = total dry feed intake (g)/wet weight gain (g)

Sample collection

At the termination of the experiment, fish were numbed by using MS 222 (Zheng *et al*., 2012) after being starved for 24 h. Fish were slaughtered for sampling regarding biological indices, amino acid profile and digestive enzyme activities, proximate analysis of whole body and blood sampling. A set of five fish per replicate were selected for sampling of each type of parameter. For proximate analysis, whole fish were oven dried at 105 °C until constant weight. Blood sampling was performed to obtain serum for biochemical parameters and antioxidant enzyme activities.

Biological indices

The hepatosomatic index (HSI) and viscerosomatic index (VSI) were calculated using the following equations:

$$HSI = \frac{wet\ weight\ of\ sample\ (g)}{wet\ weight\ of\ fish\ (g)} \times 100$$

$$VSI = \frac{Viscera\ weight\ (g)}{wet\ weight\ of\ fish\ (g)} \times 100$$

For calculation of HSI, the weight of liver samples was recorded and used, while for calculation of VSI, visceral weight was used.

Whole body proximate composition

Whole fish were dehydrated and stored for whole body composition analysis. Proximate analysis was performed by following AOAC (2006). Fish samples were dehydrated at 105°C for determination of dry matter (DM) contents. Kjeldhal's method was employed for the determination of crude protein (CP), and CP was calculated from the nitrogen contents of the samples (CP = N × 6.25). A Soxhlet apparatus was used for ether extract (EE) determination, while total ash was determined by using a muffle furnace (660°C).

Serum biochemistry

For serum biochemical analysis, blood samples were centrifuged to obtain serum, which was examined for alanine aminotransferase (ALT), alkaline phosphatase activity (ALP), and aspartate aminotransferase (AST) using appropriate kits. The ALP kit works on the principle of hydrolysing p-nitro-phenyl phosphate conversion to nitrophenolate. Absorbance was recorded at 405 nm. In this case, the activities of the enzyme correlated with its hydrolysis rate. The AST activities were evaluated by determining the rate of NADH oxidation, which was proportional to the decrease in absorbance at 340 nm with the passage of time.

Amino acid profile

For amino acid profiling of muscle samples, the method of Huang *et al*. (2018) was followed. In this analysis, muscle samples were hydrolysed at 110 °C for 22 hours in 6 N HCl solution in a sealed tube. An automatic amino acid analyser (Hitachi, 835-50 Japan) was used for amino acid profiling after the hydrolysis of samples.

Gut histology

For analysis of gut histology, suitable samples were gathered from dissected fish and stored in 10% formalin solution. The method of Chen *et al*. (2012) was followed for histological analysis. Slides were stained by hematoxylin and eosin (H & E) staining and examined under a microscope (Nikon Corporation, Kanagawa, Japan) using Image-Pro Plus 6.3 software (Media Cybernetics, Inc).

Statistical analysis

Data are presented as the mean ± SEM. Data collected from each parameter were arranged and examined by one-way ANOVA, and significant differences were determined. Tukey's test was performed using SPSS (Version 16; SPSS Inc., Chicago, IL, USA), and the level of significance was set at $P<0.05$.

RESULTS

Growth performance

The results related to growth performance are shown in Table II. In this experiment, the endurance rate was not impacted by dietary treatments and remained 100% in all groups. The initial body weight of the fish ranged from 42.11 to 42.95 g with no significant difference between the groups. Dietary treatments had a significant effect on all parameters of growth performance. The results presented below clearly depict that the final weight (108.33±0.88 and 108±1.53), weight gain (65.37±1.01 and 65.72±1.44), feed intake (83±0.58 and 65.72±1.44), feed efficiency (0.79±0.01 and 0.80±0.01), and specific growth rate (1.03±0.02 and 1.04±0.02) of the control group and 75 CSM + Lys group, respectively, were comparable to the highest values but significantly different from those of the other groups. However, the viscerosomatic index and hepatosomatic index were not affected ($P>0.05$) by dietary treatments.

Table II. Effect of soyabean meal (SBM) replacement with cotton seed meal (CSM), with or without added lysine on growth performance in *C. catla.*

Parameters	Cont	Treatments				P value
		75CSM	75CSM+Lys	100CSM	100CSM+Lys	
Initial weight (g)	42.97±0.68	42.95±0.35	42.28±0.34	42.56±0.35	42.11±0.90	0.766
Final weight (g)	108.33±0.88^{a}	94.00±1.15^{b}	108±1.53^{a}	88.33±1.20^{c}	91.67±0.33^{bc}	0.001
Weight gain (g)	65.37±1.01^{a}	51.05±1.47^{b}	65.72±1.44^{a}	45.77±0.99^{b}	49.55±0.79^{b}	0.001
PWG (%)	152.24±4.15^{a}	118.94±4.37^{b}	155.47±3.45^{a}	107.532.06^{b}	117.85±4.24^{b}	0.001
Feed Intake (g)	83.00±0.58^{a}	80.33±0.33^{ab}	82.33±0.67^{ab}	75.00±0.58^{c}	79.67±0.67^{b}	0.001
FI%	0.71±0.01^{b}	0.88±0.03^{a}	0.70±0.01^{b}	0.91±0.01^{a}	0.89±0.02^{a}	0.001
Feed efficiency	0.79±0.01^{a}	0.64±0.02^{b}	0.80±0.01^{a}	0.61±0.01^{b}	0.62±0.01^{b}	0.001
FCR	1.27±0.01^{b}	1.58±0.05^{a}	1.25±0.02^{b}	1.64±0.03^{a}	1.61±0.04^{a}	0.001
Specific growth rate	1.03±0.02^{a}	0.87±0.02^{b}	1.04±0.02^{a}	0.81±0.01^{b}	0.86±0.02^{b}	0.001
Viscerosomatic index	8.06±0.59	8.55±0.07	8.22±0.46	8.25±0.13	8.26±0.26	0.910
Hepatosomatic index	1.43±0.04	1.54±0.08	1.53±0.05	1.68±0.04	1.60±0.08	0.133

Mean values with dissimilar superscripts (a-c) in the same row are significantly different (P<0.05). For details of control and treatment group, see Table I. FCR, feed coversion ration; F1, feed intake; BWG, body weight gain.

Table III. Effect of replacing SBM with CSM, with or without added lysine on body composition in *C. catla*.

Parameters (%)	Cont	Treatments				P value
		75CSM	75CSM+Lys	100CSM	100CSM+Lys	
Dry matter	79.92±0.58	78.30±0.27	79.45±0.26	79.62±0.17	79.13±1.23	0.471
Crude protein	16.68±0.18	16.38±0.26	15.77±0.01	16.31±0.29	16.31±0.02	0.080
Ether extract	26.79±0.29	26.43±0.40	26.51±0.31	26.86±0.33	26.41±0.18	0.764
Total ash	32.39±0.32	33.28±0.16	32.99±0.72	33.36±0.72	33.34±0.64	0.717

For details of control and treatment group, see Table I.

Whole body proximate composition

The body composition of *C. catla* was not significantly impacted by dietary treatments (Table III). Numerically, the highest dry matter content was observed in the control (79.92%), and the lowest dry matter content was observed in 75CSM (78.3 0%). Crude protein contents varied from 15.77% (75CSM+Lys) to 16.68% (Cont). The numerically highest values of ether extract (26.86%) and total ash (33.36%) contents were observed in 100CSM.

Muscle amino acid profile

Table IV shows the results regarding the amino acid profile of muscle samples of *C. catla*. Statistical analysis revealed that the replacement of SBM with CSM had no significant impact (P>0.05) on the levels of amino acids except the concentrations of arginine and total amino acids (TAAs). Significantly (P<0.05) higher concentrations of arginine (76.51 g/kg) were observed in the control group. The remaining treatments showed no significance when compared with each other. Similarly, the highest (P<0.05) concentration of TAA was also observed in the control group (820.21 g/kg), and the lowest was observed in the 100CSM+Lys group (817.14 g/kg).

Intestinal digestive enzyme activities

Results regarding the effect of replacing SBM with CSM with or without lysine on digestive enzyme activities in *C. catla* are given in Table V. The results showed significantly (P<0.05) the highest activities of amylase (91.88 U/g) and lipase (34.56 U/g) in the control. Performances of amylase and lipase were decreased by using CSM as a substituent for SBM. The minimum activities of both amylase and lipase were found in both 100 CSM and 100 CSM + Lys. The activities of protease in the control, 75CSM, and 75CSM+Lys groups were statistically similar and higher than those in the 100 CSM and 100 CSM + Lys groups.

Table IV. Effect of replacing SBM with CSM with or without added lysine on muscle amino acid profile in *C. catla*.

Parameters (g/kg)	Cont	Treatments				P value
		75CSM	75CSM+Lys	100CSM	100CSM+Lys	
Methionine	22.84±0.14	22.65±0.07	22.64±0.09	22.67±0.11	22.49±0.17	0.430
Lysine	64.99±0.10	64.91±0.03	64.83±0.04	64.94±0.03	64.91±0.04	0.400
Threonine	30.58±0.12	30.48±0.03	30.67±0.09	30.48±0.03	30.50±0.06	0.389
Leucine	59.88±0.09	59.75±0.03	59.96±0.12	59.82±0.03	59.89±0.06	0.439
Arginine	76.51±0.06^{a}	76.32±0.21^{ab}	75.99±0.02^{b}	76.02±0.02^{b}	76.01±0.03^{b}	0.013
Valine	35.89±0.14	35.68±0.13	35.58±0.03	35.68±0.13	35.60±0.01	0.357
Isoleucine	33.53±0.04	33.51±0.04	33.76±0.12	33.70±0.09	33.65±0.09	0.217
Histidine	15.48±0.09	15.40±0.07	15.41±0.03	15.39±0.03	15.41±0.02	0.781
Aspartic acid	81.36±0.08	81.05±0.01	81.09±0.11	81.12±0.09	81.13±0.09	0.159
Phenylalanine	32.88±0.01	32.77±0.11	32.63±0.10	32.53±0.06	32.65±0.07	0.062
Serine	29.58±0.09	29.53±0.01	29.62±0.10	29.50±0.03	29.68±0.08	0.470
Glycine	64.73±0.20	64.92±0.02	64.88±0.01	64.90±0.03	64.83±0.04	0.616
Alanine	53.69±0.05	53.75±0.01	53.60±0.04	53.75±0.20	53.75±0.01	0.755
Glutamic acid	133.92±0.20	133.56±0.01	133.70±0.14	133.73±0.13	133.51±0.04	0.240
Proline	56.95±0.09	56.84±0.04	56.83±0.03	56.85±0.02	56.85±0.04	0.491
Tyrosine	26.83±0.12	26.53±0.04	26.56±0.03	26.56±0.04	26.66±0.14	0.168
Total amino acid	820.21±0.1^{a}	817.23±0.32^{b}	817.71±0.24^{b}	817.88±0.29^{b}	817.14±0.06^{b}	0.001

For details of control and treatment group, see Table I.

Table V. Effect of replacing SBM with CSM with or without added lysine on intestinal digestive enzyme activity, blood biochemical indices and antioxidant enzyme activity in *C. catla*.

Parameters	Cont	Treatments				P-value
		75CSM	75CSM+Lys	100CSM	100CSM+Lys	
Intestinal digestive enzymes						
Amylase (U/g)	91.88±0.17a	84.24±1.25b	84.02±0.68b	74.56±0.75c	74.90±0.44c	0.001
Protease (U/mg)	56.44±0.18a	54.56±0.44a	54.71±0.58a	47.68±0.52b	47.88±0.40b	0.001
Lipase (U/g)	34.56±0.62a	30.91±0.40ab	30.01±0.13b	22.05±1.68c	21.78±0.27c	0.001
Blood biochemical indices						
ALT (U/L)	253.71±2.85a	331.77±2.27c	303.61±0.45b	414.42±2.88d	399.42±0.60d	0.001
AST (U/L)	43.26±1.35a	65.07±0.62c	59.82±0.58b	82.78±1.68e	77.27±0.64d	0.001
ALP (U/L)	75.15±0.34	76.50±0.67	76.50±0.34	76.84±1.01	76.16±0.34	0.383
Antioxidant enzyme activity						
MDA (µmol/mg protein)	4.57±0.04^{a}	3.71±0.14^{b}	3.89±0.10^{b}	3.52±0.13^{b}	3.70±0.02^{b}	0.001
SOD (U/mg protein)	15.18±0.24	14.71±0.21	14.98±0.19	14.51±0.16	14.67±0.07	0.159
CAT (U/mL)	4.64±0.03^{a}	3.76±0.09^{b}	3.96±0.09^{b}	3.54±0.18^{b}	3.69±0.07^{b}	0.001

For details of control and treatment group, see Table I.

Blood biochemical indices

The levels of ALT, AST and ALP were analysed to assess the impact of alternating SBM with CSM on blood biochemical indices (Table V). Statistical analysis showed that the values of ALT and AST were significantly ($P<0.05$) increased by alternating SBM with CSM, while the values of ALP remained unaffected ($P>0.05$). The highest value of ALT was observed in both the 100 CSM (414.42±2.88)

Table VI. Effect of replacing SBM with CSM with or without added lysine on intestinal morphology in *C. catla*.

Parameters	Cont	Treatments				P-value
		75CSM	75CSM+Lys	100CSM	100CSM+Lys	
VH/VW*	9.28±0.18^{a}	6.80±0.12^{b}	9.43±0.07^{a}	5.39±0.33^{c}	5.55±0.07^{c}	0.001
Tunica muscularis ^	112.89±3.85^{a}	52.88±0.10^{bc}	53.22±0.34^{b}	44.33±1.75^{c}	45.55±0.40^{bc}	0.001

* Villus height (µm) /villus width (µm). ^ Thickness of intestinal tunica muscularis (µm). Mean values with dissimilar superscripts (a-c) in a same row different statistically (P<0.05). For details of control and treatment group, see Table I.

and 100 CSM + Lys (399.42±0.60) groups, while the lowest level of ALT was observed in the control group (253.71±2.85). Similarly, the maximum level of AST was found in the 100 CSM group (82.78±1.68), but the lowest value was noticed in the control group (43.26±1.35).

Antioxidant enzyme activities

The results regarding the effect of replacing SBM with CSM on the antioxidant enzyme activities in *C. catla* are presented in Table V. The activities of MDA and CAT were significantly affected (P<0.05), while SOD remained unaffected. The highest activities of MDA (4.57 µmol/mg protein) and CAT (4.64 U/mL) were observed in the control group, while in the other groups, their values were statistically similar.

Intestinal morphology

The results clearly depict that SBM replacement with CSM significantly affected (P<0.05) the villus height to villus width ratio (VH/VW) and thickness of the intestinal tunica muscularis, as presented in Table VI. There was no significant difference observed between the control and 75CSM+Lys groups for VH/VW; however, it was higher than that of the other groups. The minimum VH/VW was observed in the group with 100 CSM replacements with or without lysine (100 CSM and 100 CSM + Lys). On the other hand, the highest thickness of the intestinal tunica muscularis was observed in the control group, and the lowest was observed in the 100CSM group. Treatment with 75 CSM and 100 CSM+Lys showed nonsignificant outputs when compared with 75 CSM+Lys and 100 CSM but revealed significance when compared with the control group.

DISCUSSION

This experiment was designed to assess the response of *Catla* against dietary alternation of SBM with CSM with and without lysine supplementation. CSM has already been used in the feed formulation of different fish species, such as black sea bass (Anderson *et al*., 2016), grass carp (Liu *et al*., 2016), and blunt snout bream (Zhou et al., 2017). The response of fish against dietary inclusion levels of CSM depends upon different factors, such as the processing method of CSM, the source of raw material and fish species (Liu *et al*., 2020). The current experiment stated that SBM could be alternated with CSM up to 75% with supplementation of lysine in the diet of *C. catla.* Growth performance-related parameters were not affected by replacing 75% SBM with CSM with added lysine, but without lysine, growth performance was negatively affected. Replacement of SBM with CSM, with or without lysine beyond 75%, was not suitable for the growth of *C. catla.* However, body composition and muscle amino acid levels were not disturbed by dietary treatments. The results suggested that lysine deficiency of CSM could be accomplished if CSM is used up to 75% in the diet of *C. catla,* while higher inclusion levels with or without lysine could not support the growth in *C. catla*, which might be due to different factors, including the processing method of CSM, type and development stage of cotton seed plant and species or growth period of fish (Wang *et al*., 2020). At higher inclusion levels (> 75%) of CSM, the concentration of gossypol might be the reason to bind free lysine and reduce its bioavailability (Hu et al., 2015), which negatively affects the growth rate in *C. catla,* as stated in juvenile rainbow trout (Cheng and Hardy, 2002). Other reasons for the reduced growth performance at 100% replacement of SBM with CSM might be the lower bioavailability of essential amino acids and the different amino acid profiles of SBM and CSM (Dias *et al*., 2005). Previous studies also stated that SBM could not be fully alternated with CSM in the diet of different species of fish (Yue and Zhou, 2008). Lim (1996) observed no significant impact on the growth performance in *Litopenaeus vannamei* by dietary inclusion of CSM up to 26.5%. Similarly, Jiang *et al*. (2012) stated that the Chinese mitten crab, *Eriocheir sinensis*, responds similarly to equal dietary inclusion levels of SBM and CSM as an alternative to fish meal. On the other hand, Robinson (1991) stated that SBM could be fully alternated with CSM, with sufficient supplementation of lysine in the diet of channel catfish.

In this experiment, digestive enzyme activities were significantly affected by replacing SBM with

CSM. The highest amylase activity was observed in the control, which was significantly decreased by increasing dietary inclusion levels of CSM; however, no significant difference was noticed for supplementation of lysine at the same inclusion levels of CSM. Similarly, a response was observed for lipase, and the only difference from amylase activity was that its activity at the 75% CSM inclusion level was parallel to that of the control. However, protease activity was not disturbed by alternation of SBM with CSM up to 75%, and further alternation of SBM with CSM significantly reduced the protease activities in *C. catla*. A similar reaction in terms of digestive enzyme performance was observed in black carp with dietary replacement of SBM with CSM (Huang *et al*., 2012). The results of the current study are also in line with Hu et al. (2015), who stated that the activities of amylase and lipase were significantly reduced by dietary substitution of SBM with CSM. Several factors affect the activities of digestive enzymes, such as antinutritional factors present in two different sources of protein, the level of fibre and the presence of a high level of free gossypol in CSM, which could be the reason for the lower enzymatic activities of digestive enzymes (Guimaraes *et al*., 2008).

The liver is the major metabolic gland that protects the body from many toxic compounds. Liver health status is evaluated by analysing the activities of some indicators in blood, including ALT, AST, and ALP (Pan *et al*., 2003). An increase in the levels of these indicators in response to any stimuli showed a negative effect. In the present study, the ALP concentration was not changed, but the concentrations of other parameters were improved by alternating SBM with CSM in the diet of *C. catla.* Therefore, higher inclusion levels of CSM might have a negative effect on liver health in *C. catla.*

The activities of antioxidant enzymes are one of the vital indicators for the physical health of animals (Johnson, 2002). The literature suggests that fish respond to changes in feed ingredients in terms of antioxidant enzyme activities (Tovar-Ramirez *et al*., 2010). SOD and CAT are considered to be the first line of the antioxidant defense system against reactive oxygen species produced in the organism (Yin *et al*., 2018). The present results suggested that the performances of MDA and CAT were retarded by using CSM in the diet of *C. catla* regardless of its inclusion levels, while SOD activities were not affected, and the present results are supported by previous results for MDA activities (Wang *et al*., 2020). Decreased antioxidant activities might be due to the existence of gossypol in CSM, which is involved in the production of reactive oxygen species. The results suggested that CSM slowed the antioxidant potential of *Catla.* Reduced antioxidant potential also decreases the immune status of aquatic animals (Liu et al., 2009).

Gut health plays a vital role in the production performance of animals, as the intestine is the main site of nutrient absorption. The present results suggested that the villus height to villus width ratio (VH/VW) was not affected by replacing SBM with CSM up to 75% with lysine supplementation, but a higher inclusion level of CSM with or without lysine negatively affected the VH/VW. On the other hand, the thickness of the intestinal tunica muscularis was reduced by replacing SBM with CSM. The negative impact of alternating SBM with CSM might be due to the higher fibre contents in CSM than in SBM. The negative impact of alternating SBM with CSM on gut health might be the reason for the reduced growth performance in *C. catla* at higher replacement levels.

CONCLUSION

To summarize, it can be concluded that *Catla* can tolerate up to a 75% replacement of SBM (soybean meal) with CSM (cottonseed meal) when supplemented with lysine without affecting growth performance and the overall proximate composition of the fish's body. However, it is important to note that substituting higher levels of SBM with CSM, regardless of lysine supplementation, had detrimental effects on antioxidant enzyme activities, growth performance, and various other parameters in *C. catla*.

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Ethical statement

This study was performed after approval from the Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Anderson, A.D., Alam, M.S., Watanabe, W.O., Carroll, P.M., Wedegaertner, T.C. and Dowd, M.K., 2016.

Full replacement of menhaden fish meal protein by low-gossypol cottonseed flour protein in the diet of juvenile black sea bass *Centropristis striata*. *Aquaculture*, **464**: 618-628. https://doi.org/10.1016/j.aquaculture.2016.08.006

AOAC, 2006. *Official methods of analysis.* Association of Official Analytical Chemists, international. 18th edn. Gaithersburgs, MD.

Chen, Q.L., Luo, Z., Zheng, J.L., Li, X.D., Liu, C.X., Zhao, Y.H. and Gong, Y., 2012. Protective effects of calcium on copper toxicity in *Pelteobagrus fulvidraco*: Copper accumulation, enzymatic activities, histology. *Ecotoxicol. Environ. Saf.*, **76**: 126-134. https://doi.org/10.1016/j.ecoenv.2011.10.007

Cheng, Z.J. and Hardy, R.W., 2002. Apparent digestibility coefficients and nutritional value of cottonseed meal for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **212**: 361-372. https://doi.org/10.1016/S0044-8486(02)00260-0

Dias, J., Alvarez, M.J., Arzel, J., Corraze, G., Diez, A., Bautista, J.M. and Kaushik, S.J., 2005. Dietary protein source affects lipid metabolism in the European seabass (*Dicentrarchus labrax*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, **142**: 19-31. https://doi.org/10.1016/j.cbpb.2005.07.005

Guimarães, I.G., Pezzato, L.E. and Barros, M.M., 2008. Amino acid availability and protein digestibility of several protein sources for Nile tilapia, *Oreochromis niloticus*. *Aquacult. Nutr.*, **14**: 396-404. https://doi.org/10.1111/j.1365-2095.2007.00540.x

Hafez, H.M. and Attia, Y.A., 2020. Challenges to the poultry industry: Current perspectives and strategic future after the COVID-19 outbreak. *Front. Vet. Sci.*, **7**: 516. https://doi.org/10.3389/fvets.2020.00516

he Liu, X., dan Ye, J., Wang, K., Yang, W. and Zhou, L., 2012. *Partial replacement of fish meal with peanut meal in practical diets for the Pacific white shrimp,* Litopenaeus vannamei. https://doi.org/10.1111/j.1365-2109.2011.02883.x

Hu, Y., Huang, Y., Feng, F., Zhong, L., Ai, Q., Xiao, T. and Wen, H., 2015. Effect of soybean meal replacement by cottonseed meal on growth, feed utilization and some blood physiological/biochemical indices of juvenile black carp, *Mylopharyngodon piceus. Aquacult. Res.*, **46**: 2490-2500. https://doi.org/10.1111/are.12409

Huang, Y., Hu, Y., Xiao, T.Y., Huan, Z.L., Wen, H., Feng, F.X. and Yu, J.B., 2012. Influence of dietary canola meal levels on growth and biochemical indices in juvenile *Mylopharvngodon piceus*. *Acta Hydrobiol. Sin.*, **36**: 41-48.

Huang, Y.J., Zhang, N.N., Fan, W.J., Cui, Y.Y., Limbu, S.M., Qiao, F., Zhao, Y.L., Chen, L.Q., Du, Z.Y. and Li, D.L., 2018. Soybean and cottonseed meals are good candidates for fishmeal replacement in the diet of juvenile *Macrobrachium nipponense*. *Aquacult. Int.*, **26**: 309-324. https://doi.org/10.1007/s10499-017-0215-1

Jiang, H., Liqiao, C.H.E.N., Erchao, L.I., Jiang, X. and Shengming, S.U.N., 2012. Partial or total replacement of soybean meal by cottonseed meal in practical diets for Chinese mitten crab, *Eriocheir sinensis*: Effects on oxygen consumption, ammonia excretion, O: N ratio and amino transferases activities. *Turk. J. Fish. Aquat.*, **12**. https://doi.org/10.46989/001c.20632

Johnson, P., 2002. Antioxidant enzyme expression in health and disease: Effects of exercise and hypertension. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **133**: 493-505. https://doi.org/10.1016/S1532-0456(02)00120-5

Leng, X.J. and Wang, G., 2005. Effect of feeding frequency on feeding *allogynogenetic crucian* carp with diets added with crystalline amino acids. *Feed Res.* (China), **12**: 50-52.

Li, M.H. and Robinson, E.H., 2006. Use of cottonseed meal in aquatic animal diets: A review. *N. Am. J. Aquacult.*, **68**: 14-22. https://doi.org/10.1577/A05-028.1

Lim, C., 1996. Substitution of cottonseed meal for marine animal protein in diets for *Penaeus vannamei*. *J. World. Aquacult. Soc.*, **27**: 402-409. https://doi.org/10.1111/j.1749-7345.1996.tb00624.x

Liu, H., Dong, X., Tan, B., Du, T., Zhang, S., Yang, Y., Chi, S., Yang, Q. and Liu, H., 2020. Effects of fish meal replacement by low-gossypol cottonseed meal on growth performance, digestive enzyme activity, intestine histology and inflammatory gene expression of silver sillago (*Sillago sihama Forsskál*) (1775). *Aquacult. Nutr.*, **26**: 1724-1735. https://doi.org/10.1111/anu.13123

Liu, H., Yan, Q., Han, D., Jin, J., Zhu, X., Yang, Y. and Xie, S., 2016. Effect of dietary cottonseed meal on growth performance, physiological response, and gossypol accumulation in pre-adult grass carp, *Ctenopharyngodon idellus*. *Chinese J. Oceanol. Limnol.*, **34**: 992-1003. https://doi.org/10.1007/s00343-016-4115-4

Liu, X., Wang, Y. and Wang, J., 2009. Effects of replacing fish meal with soybean meal, rapeseed meal or cottonseed meal on feeding, growth and feed utilization of sutchi catfish (*Pangasius*

sutchi). *J. Fish. China*, **33**: 479-487.

Naseem, S., Bhat, S.U., Gani, A. and Bhat, F.A., 2021. Perspectives on utilization of macrophytes as feed ingredient for fish in future aquaculture. *Rev. Aquacult.*, **13**: 282-300. https://doi.org/10.1111/raq.12475

Pan, C.H., Chien, Y.H. and Hunter, B., 2003. The resistance to ammonia stress of Penaeus monodon Fabricius juvenile fed diets supplemented with astaxanthin. *J. Exp. Mar. Biol. Ecol.*, **297**: 107-118. https://doi.org/10.1016/j.jembe.2003.07.002

Rawles, S.D. and Gatlin III, D.M., 2000. Nutrient digestibility of common feedstuffs in extruded diets for sunshine bass *Morone chrysops*♀× *M. saxatilis*♂. *J. World Aquacult. Soc.*, **31**: 570-579. https://doi.org/10.1111/j.1749-7345.2000.tb00906.x

Robinson, E.H., 1991. Improvement of cottonseed meal protein with supplemental lysine in feeds for channel catfish. *J. appl. Aquacult.*, **1**: 1-14. https://doi.org/10.1300/J028v01n02_01

Saleh, A.A., El-Awady, A., Amber, K., Eid, Y.Z., Alzawqari, M.H., Selim, S., Soliman, M.M. and Shukry, M., 2021. Effects of sunflower meal supplementation as a complementary protein source in the laying hen's diet on productive performance, egg quality, and nutrient digestibility. *Sustainability*, **13**: 3557. https://doi.org/10.3390/su13063557

Tovar-Ramírez, D., Mazurais, D., Gatesoupe, J.F., Quazuguel, P., Cahu, C.L. and Zambonino-Infante, J.L., 2010. Dietary probiotic live yeast modulates antioxidant enzyme activities and gene expression of sea bass (*Dicentrarchus labrax*) larvae. *Aquaculture*, **300**: 142-147. https://doi.org/10.1016/j.aquaculture.2009.12.015

Wang, X.X., Yuan, Y., Li, C.C., Zhou, F., Jin, M., Sun, P., Zhu, T.T., Ding, X.Y. and Zhou, Q.C., 2020. Partial substitution of fish meal with soy protein concentrates in commercial diets for juvenile swimming crab, *Portunus trituberculatus*. *Anim. Feed Sci. Technol.,* **259**: 114290. https://doi.org/10.1016/j.anifeedsci.2019.114290

Yin, B., Liu, H., Tan, B., Dong, X., Chi, S., Yang, Q., Zhang, S. and Chen, L., 2018. Cottonseed protein concentrate (CPC) suppresses immune function in different intestinal segments of hybrid grouper♀ *Epinephelus fuscoguttatus*×♂ *Epinephelus lanceolatu* via TLR-2/MyD88 signalling pathways. *Fish Shellfish Immunol.*, **81**: 318-328. https://doi.org/10.1016/j.fsi.2018.07.038

Yin-Huan, Z., Hai-Li, H., Tao, D. and Yang, H., 2017. Study on adaptability of juvenile of *Sillago sihama* to salinity. *Fish. Res.*, **39**: 22.

Yue, Y.R. and Zhou, Q.C., 2008. Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indexes for juvenile hybrid tilapia, *Oreochromis niloticus*× *O. aureus*. *Aquaculture*, **284**: 185-189. https://doi.org/10.1016/j.aquaculture.2008.07.030

Zheng, Q., Wen, X., Han, C., Li, H. and Xie, X., 2012. Effect of replacing soybean meal with cottonseed meal on growth, hematology, antioxidant enzymes activity and expression for juvenile grass carp, *Ctenopharyngodon idellus*. *Fish Physiol. Biochem.*, **38**: 1059-1069. https://doi.org/10.1007/s10695-011-9590-0

Zhou, Q.L., Habte-Tsion, H.M., Ge, X., Liu, B., Xie, J., Ren, M., Miao, L. and Pan, L., 2017. Growth performance and TOR pathway gene expression of juvenile blunt snout bream, *Megalobrama amblycephala,* fed with diets replacing fish meal with cottonseed meal. *Aquacult. Res.,* **48**: 3693-3704.