

Total replacement of soybean meal by rice distilled dried grain soluble (DDGS) with lysine supplementation: effect on growth, nucleic acid content, digestive enzyme activity, and health status in Carp (*Cirrhinus mrigala*)

Aditi Banik¹, Shivendra Kumar^{1,*}, Prem Prakash Srivastava¹, Pankaj Kishore², Sujit Kumar Nayak³, Pravesh Kumar¹, Abhishek Kumar^{1,4} and Maneesh Kumar Dubey¹

¹ Department of Aquaculture, College of Fisheries (Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar), Dholi, Muzaffarpur 843121, Bihar, India

² Quality Assurance and Management Division, ICAR-Central Institute of Fisheries Technology, Cochin 682029, Kerala, India

³ Department of Aquatic Animal Health Management, College of Fisheries (Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar), Dholi, Muzaffarpur 843121, Bihar, India

⁴ College of Fisheries (Bihar Animal Sciences University, Patna, Bihar), Kishanganj, Bihar, India

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Abstract – Objective: Present study was conducted to evaluate the suitability of Rice DDGS as a protein source and its influence, with or without lysine supplementation, on nutrient utilization, growth and health status of *Cirrhinus mrigala* juveniles. **Methods:** Juvenile of *Cirrhinus mrigala* (14.00 ± 0.10 g) were fed *ad-libitum* for 45 days with twelve iso-proteinous and iso-lipidic practical diets containing 0, 6, 12, 18, 24, 30% of rice DDGS replacing up to total dietary soybean meal (SBM) with and without lysine supplementation (0.1%). **Result:** At the end of the feeding trial, treatment fed with a diet having 18% or more rice DDGS recorded a significant decrease in growth performance in juveniles. However, lysine (0.1%) supplementation in the diet showed an improved growth performance of juveniles as compared to the non-supplemented counterpart diet containing at least 30% rice DDGS. Fish fed diet D₃₀ had the lowest RNA/muscle mass and RNA/DNA ratio. The digestive enzyme activities were significantly higher in the lysine-supplemented group compared to the non-supplemented group. The haemato-immunological parameters tended to increase with an increasing level of rice DDGS up to 12% but the differences beyond this were not significant with the control. **Conclusion:** Considering all the factors, a negative trend was observed and signalled that more than 12% inclusion of DDGS in practical diets without supplementation of lysine recorded negative physiological effects, however, supplementation of lysine nullifies the negative consequence of total substitution of SBM by rice DDGS. Overall, the present study gave a demonstration of the high potential of rice DDGS as a substitute for the soybean meal forages for *C. mrigala*, and the incorporation of rice DDGS can be augmented to at least 30% (highest level assessed) if lysine is added.

Keywords: Indian major carp / digestive enzyme / nucleic acid / haematology / serum protein

1 Introduction

In recent years, the growing demand for ethanol as a fuel additive and the push to reduce reliance on fossil fuels have significantly increased the volume of grains utilized for ethanol production (Iram et al., 2020). The dry-grinding process, which is the primary method for ethanol production, generates distillers dried grains with solubles (DDGS) as a key byproduct

(Dennis et al., 2024). As with ethanol, DDGS has rapidly become a valuable commodity, with global production reaching 44 million tons in 2021–2022. This surge is largely due to the increasing awareness of ethanol's role in reducing greenhouse gas emissions and its potential to alleviate the dependence on petroleum (Shurson et al., 2008). DDGS has found a growing niche in animal feed, particularly in aquaculture. It is a cost-effective alternative to traditional protein sources, such as soybean meal (SBM), because it contains a higher crude protein content (30–45%) and lacks the anti-nutritional factors found in many plant-based protein

*Corresponding author: shivdholi@rediffmail.com

sources, making it a viable ingredient for fish diets (Lim et al., 2007). Despite its advantages, DDGS is not without challenges. The nutritional composition of DDGS can vary significantly depending on the grain used and the ethanol production process, which can affect its consistency and digestibility. This variability, along with concerns over the potential impact on the texture and quality of fish muscle, has limited its widespread adoption. Rice DDGS has efficiently been made use of in chicken diets (Dinani et al., 2019), and research on its potential use in fish has only been done on a few fish species (Bae et al., 2015; Choi et al., 2014), with an emphasis on the fish's performance on growth, feed utilization, and biochemical composition of whole-body fish carcass. Moreover, research on DDGS in fish diets has generally shown positive results, with studies indicating that up to 40% of maize DDGS and 30% of wheat DDGS can be incorporated into fish diets without compromising growth performance (Lim et al., 2007; Sándor et al., 2021). However, rice DDGS, a less studied variant, presents unique challenges and may differ in its nutritional profile, which could influence the growth and quality of fish. Some studies suggest that while DDGS can support fish growth, it may lead to decrease cooked muscle texture (Azm et al., 2021) and increased fat deposition in certain species. This could impact marketability, as fish with poor texture may be less desirable for consumers. Additionally, DDGS may require lysine supplementation to ensure optimal growth, as some variants of DDGS lack essential amino acids, particularly lysine, which is critical for proper fish development.

Despite these potential drawbacks, the positive aspects of using DDGS—such as its high protein content, cost-effectiveness, and sustainability—outweigh the potential negatives. When properly formulated and balanced with the necessary nutrients, DDGS can be an excellent feed ingredient, supporting the growth and health of fish species like the Indian major carps. Indian major carp fish, *Cirrhinus mrigala* (mrigal) is mostly illiophagous, feeding on rotting plants on the floor, although it can also switch to filter-feeding. The Indus and Ganges River systems are naturally home to *C. mrigala*, but aquaculture has effectively spread it to other nations in Asia (particularly India) and Europe. The species' economic worth has significantly expanded because of its high aquaculture potential and growing consumer demand. Mrigal plays a crucial role in a polyculture system where it is grown with other big Indian carp and efficiently fills the bottom niche. Aquaculture is reported to generate 3–5 tonnes of *C. mrigala* yearly per ha, making up 1.6% of the world's fish production (FAO, 2022). This species thus represents a sizable group of freshwater fish that are prized commercially. It is widely liked in Indian markets as well because of its ease of growing, flavor, and size.

DDGS has been used in several studies to look into replacing fish meal or soybean in fish diets (Lim et al., 2007; Sándor et al., 2021). Depending on the fish species, the proximate composition of the basal diet, the ingredient replaced, and the addition of deficient nutrients, especially lysine, the results of these studies indicate that up to 40% of maize DDGS and 30% of wheat DDGS could be included in fish diets without negatively affecting the overall growth performance. On the other hand, more information is needed on the usage of rice DDGS in *C. mrigala* diets. Therefore, the goal of this finding was to ascertain the impact of nutritional inclusion of rice DDGS with or without lysine supplementation on the fish's ability to use nutrients and grow.

2 Material and methods

2.1 Declaration of ethics

The experimental fish were handled and sampled after the feeding trial in accordance with the recommendations of Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India.

2.2 Experimental animal

Healthy juveniles of the freshwater teleost fish species *Cirrhinus mrigala*, with an average weight of 14 ± 0.1 g, were sourced from the Fish hatchery Facility at the College of Fisheries in Dholi, Muzaffarpur, Bihar. These fish were maintained in aerated freshwater for a period of 15 days within 150 L FRP tanks to facilitate acclimatization. Before the commencement of the trial, all fish were fed a commercial meal with 30% crude protein twice a day during the acclimatization period. Faeces and uneaten feed were routinely siphoned off, and water renewal was carried out every day, to reduced stress, constant aeration was maintained throughout the acclimatization period.

2.3 Diets

Twelve iso-proteinaceous (30% CP) and iso-lipidic (6%) diets were formulated. A control diet was prepared without lysine (C) and with lysine (CL). The control diet covered 30% of locally available soybean meal without any inclusion of rice DDGS (0%). Ten test diets were formulated with increasing levels of rice DDGS at 6%, 12%, 18%, 24%, and 30%, replacing 20%, 40%, 60%, 80%, and 100% of soybean meal, respectively. These test diets were prepared without lysine supplementation (D6–D30) and with lysine supplementation (D6L–D30L) at 0.1%. The diets were designed to ensure partial dietary intake comparable to the control diet. Complete replacements for a blend of soybean meal (SBM) and rice DDGS on an equal protein basis were also prepared (Tab. 1, Fig. 1). The control or reference diet was designed to provide vital nutrients at levels that satiated Mrigal's established nutritional needs. Diets were made into 2-mm diameter pellets, spread out, and sun-dried. After drying, the manufactured feed pellets were placed in re-sealable polythene pouches and stored at 4 °C until needed.

2.4 Experimental strategy

A growth trial was undertaken to assess the rice DDGS as a replacement for soybean meal at fortnightly intervals during the experimental period. Before stocking, the *Cirrhinus mrigala* juveniles had a 2-week acclimation period to the control diet. Seven hundred twenty *Cirrhinus mrigala* juveniles with an average initial weight of 14 ± 0.10 g (Mean $\pm$ S.E.) were arbitrarily disseminated in thirty-six separate experimental tanks (150 L) (each treatment in triplicates), according to a completely randomized design. Throughout the 45-day growth trial, water exchange was carried out at 50% on alternate days to provide the best possible water quality. In the course of this experiment, the

Table 1. Experimental diet formulation in *Cirrhinus mrigala* fingerlings (g/100 g).

Ingredients (g/100g)	Experimental diets											
	C	D ₆	D ₁₂	D ₁₈	D ₂₄	D ₃₀	CL	D ₆ L	D ₁₂ L	D ₁₈ L	D ₂₄ L	D ₃₀ L
Soybean meal	30	24	18	12	6	0	30	24	18	12	6	0
Rice DDGS ¹	0	6	12	18	24	30	0	6	12	18	24	30
Mustard oil cake	24	24	24	24	24	24	24	24	24	24	24	24
DORB ²	19	19	19	19	19	19	19	19	19	19	19	19
Wheat	8.1	8.1	8.1	8.1	8.1	8.1	8	8	8	8	8	8
Meat and Bone meal	11	11	11	11	11	11	11	11	11	11	11	11
Sunflower oil +	6	6	6	6	6	6	6	6	6	6	6	6
Cod liver oil (2:1)												
Vitamin-Mineral mix ³	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Lysine ⁴	0	0	0	0	0	0	0.1	0.1	0.1	0.1	0.1	0.1
Proximate composition (g/100g)												
Crude protein (%)	29.7	29.46	29.22	28.98	28.74	28.5	29.75	29.62	29.4	29.05	28.84	28.63
Crude lipid (%)	8.04	8.1	8.16	8.22	8.28	8.34	8.12	8.05	8.19	8.25	8.22	8.28
Ash (%)	8.23	8.35	8.45	8.41	8.36	8.76	8.11	8.26	8.51	8.52	8.47	8.62
Total carbohydrate (%)	45.88	45.59	46.07	46.14	46.47	46	45.78	45.7	45.62	45.79	45.95	46.01
Energy* (Kcal/100g)	374.7	373.1	374.6	374.5	375.4	373.1	375.2	373.7	373.9	373.6	373.1	373.1

¹ DDGS: Distillers Dried Grains with Soluble.² DORB: De-oiled Rice Bran.³ Vitamin: mineral mixture (Agrimin forte) composition (quantity per kg): Vitamin A 7,00,000 IU; Vitamin D3 70,000 IU; Vitamin B2 2,000 mg; Vitamin E 250 mg; Mn 1500 mg; I 325 mg; Fe 1500 mg; Zn 9600 mg; Cu 1200 mg; Co 150 mg; Mg 6000 mg; K 100 mg; Na 5.9 mg; S 0.72% Ca 25.5%; P 12.75%; DL- Methionine 1000 mg.⁴ Lysine: L-Lysine monohydrochloride extra pure CHR, 99%, Sisco Research Laboratories Pvt. Ltd., Maharashtra, India.

* Calculated gross energy (Kcal/100 g)=[Crude protein (%) × 4] + [Crude lipid (%) × 9] + [Total carbohydrate (%) × 4].

treatment groups were provided with either of the twelve isoproteinous (30%) and isolipidic (6%) diets. Fish were fed *ad-libitum* for twice a day (10 a.m. and 4 p.m.) at a rate of 3% of biomass in all tanks. The water quality parameters like temperature (27 °C), pH (7–8), dissolved oxygen (6.1 mg/l), and hardness (230 mg/l) were measured daily and maintained within an optimum range throughout the growth trial.

2.5 Proximate composition of rice DDGS, experimental diet, and fish

At the end of the trial, eight fish from each treatment were randomly sampled, pooled, and stored at –20 °C for the determination of whole-body composition (after being bled) (AOAC 1995). Triplicates of each sample were used for the analysis. The proximate analysis of rice DDGS, experimental diets, and fish samples was conducted in accordance with the standard procedures outlined by AOAC (1995). Through six hours of oven drying at 105 °C, moisture was detected. After acid digestion, crude protein ($N \times 6.25$) was quantified using the Kjeldahl technique with a Kjeltex machine. Using the Soxhlet System HT, the ether extraction technique was used to analyze crude fat. Crude ash was calculated by putting a known weight of the sample in a silica crucible and heating it to 600 °C for 6 h in a muffle furnace (Hasting, 1969). The total carbohydrate (TC) was calculated by subtracting the percentage of other nutrients from 100 (Hasting, 1969). TC (%) = Dry matter – [Crude protein (%) + Lipid content (%) + Ash (%)]. The proximate composition of rice DDGS and that of

experimental diets used for the study ($n=3$) is shown in Tables 1 and 2 respectively.

2.6 Growth

Fish in each tank were group-weighted and tallied at biweekly and monthly intervals—specifically on days 15, 30, and 45—to assess growth performance. Before the start of the experiment, 20 fish were from the initial stock, and as soon as the experiment was over, 8 fish from each treatment were randomly sampled, pooled, and stored at –20 °C for the determination of whole-body composition (after being bled) (AOAC 1995). Triplicates of each sample were used for the analysis.

The following growth parameters were calculated:

Weight gain (%) = $[\text{Final body weight} - \text{Initial body weight}] \times 100 / \text{Initial body weight of fishes}$

Specific growth rate (%/day) = $[\ln \text{Final weight} - \ln \text{Initial weight}] \times 100 / \text{duration of days}$

Feed conversion ratio (FCR) = Dry weight of feed given to fish / Wet weight gain by fish

Protein Efficiency ratio (PER) = Net wet weight gain by fish / Protein intake

2.7 Quantitative estimation of DNA, RNA, and RNA-DNA ratio

Five fish from each triplicate group were randomly selected at the end of the experiment and were anaesthetized with clove oil (50 $\mu\text{L L}^{-1}$) prior to dissection, and the muscular

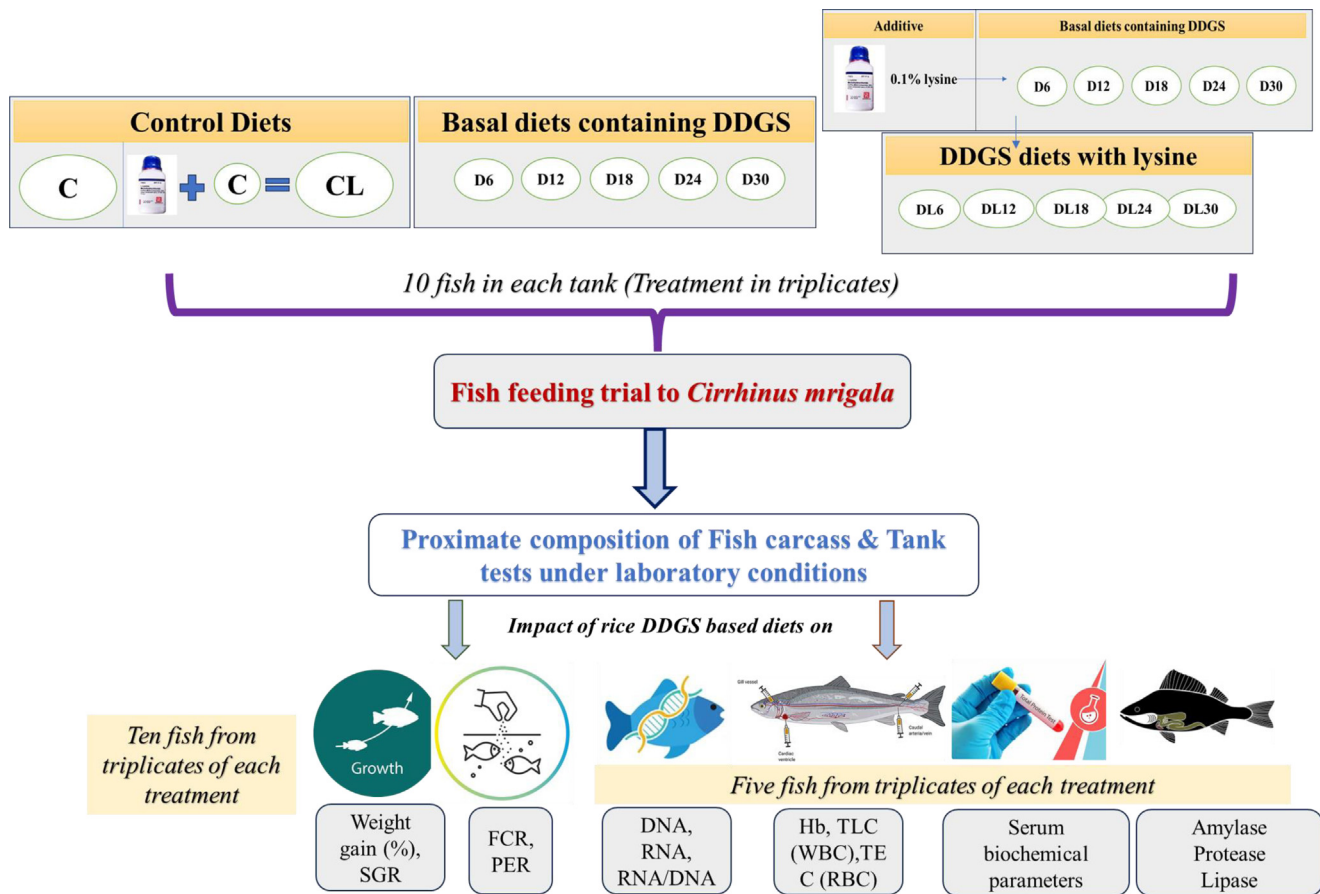


Fig. 1. Experimental design.

Table 2. Proximate composition of rice distillers dried grains with soluble (% Dry matter basis).

Parameters	Rice DDGS (%)
Moisture	8.65 ± 0.76
Crude protein	38.0 ± 7.63
Crude lipid	2.15 ± 1.68
Crude ash	10.04 ± 0.58
Total carbohydrate	49.81 ± 0.85

Data is expressed as mean ± SE, $n = 3$.

tissues were quickly and carefully removed and weighed. With the help of a Probe sonicator from IGENE LABSERVE, New Delhi, India, the tissue homogenate was prepared in a cold 0.25 M sucrose solution. Pentose analysis was used to determine the amount of nucleic acid in tissue using [Schneider's \(1945\)](#) approach by use of Microprocessor UV-VIS Double beam Spectrophotometer (IG28DS) and was calculated as follows:

The subsequent equation illustrates the DNA content of the nucleic acid extract:

$$\mu\text{g DNA/ mL} = \text{OD at 600 nm} / 0.019$$

The equation below represents the RNA composition of the nucleic acid extract:

$$\mu\text{g RNA/mL} = [(\text{OD at 660nm} + 0.0081) - (\mu\text{g DNA} / \text{mL} \times 0.013)] / 0.116$$

2.8 Digestive enzyme assays

Five fish from each triplicate group were arbitrarily selected towards the conclusion of the experiment and forfeited for assay of digestive enzymes. Fish were sedated (50 $\mu\text{L/L}$ clove oil) prior to dissection, and the gut was quickly and carefully removed and weighed. With the help of a Probe sonicator (IGENE LABSERVE, New Delhi, India), 5% of the tissue was sonicated in an ice-cold 0.25M sucrose solution. At -4°C , sonicated samples were centrifuged for 10 min at 6000 rpm. The supernatant was transferred to a separate Eppendorf tube and stored in a deep freezer at -20°C for the enzyme assay using Microprocessor UV-VIS Double beam Spectrophotometer (IG28DS). Protease activity in sonicated tissue slurry was measured using the [Drapeau \(1974\)](#) approach, which involved the breakdown of casein. The amylase activity was measured following the dinitro salicylic acid technique ([Rick and Stegbauer, 1974](#)), using a substrate of 2% (w/v) starch solution. The lipase activity was assayed based on the conventional method ([Cherry and Crandall, 1932](#)). The milliequivalent of alkali consumed was used to calculate the enzyme unit/mg protein activity.

Table 3. Effect of feeding experimental diet on proximate of *Cirrhinus mrigala* fingerlings at the end of the experiment (g/100 g).

Treatments	Moisture	Crude protein	Crude lipid	Ash
C	72.32 ^{ab} ±4.42	49.24 ^c ±2.14	22.54 ^c ±1.10	14.23 ^a ±0.52
D ₆	70.25 ^b ±3.52	51.42 ^{ab} ±4.52	23.62 ^{ab} ±0.94	12.52 ^b ±0.72
D ₁₂	70.04 ^b ±4.22	51.16 ^{ab} ±2.33	23.94 ^{ab} ±1.55	12.66 ^b ±0.18
D ₁₈	72.42 ^{ab} ±3.55	49.35 ^c ±3.72	22.45 ^c ±1.52	13.14 ^{ab} ±0.52
D ₂₄	73.11 ^a ±3.47	48.19 ^d ±3.17	22.06 ^{cd} ±1.32	14.08 ^a ±0.61
D ₃₀	73.8 ^a ±4.25	46.55 ^e ±2.54	21.57 ^d ±0.85	14.38 ^a ±0.48
CL	69.5 ^c ±2.37	52.31 ^{ab} ±3.12	24.08 ^{ab} ±1.05	12.11 ^b ±0.34
D ₆ L	68.14 ^d ±3.17	53.71 ^a ±3.18	24.82 ^a ±2.07	11.5 ^c ±0.61
D ₁₂ L	68.31 ^d ±3.67	52.87 ^a ±3.45	24.41 ^a ±1.64	11.82 ^c ±0.37
D ₁₈ L	68.92 ^{cd} ±5.42	51.72 ^{ab} ±4.23	23.89 ^{ab} ±0.82	12.08 ^{bc} ±0.26
D ₂₄ L	69.02 ^{cd} ±3.61	51.64 ^{ab} ±4.11	24.15 ^{ab} ±2.11	12.27 ^{bc} ±0.22
D ₃₀ L	69.07 ^{cd} ±4.72	51.37 ^{ab} ±3.22	23.11 ^{ab} ±1.72	12.41 ^{bc} ±0.55
Variation source				
Two way ANOVA				
Level of rice DDGS	$P < 0.05$	$P < 0.05$	NS	$P < 0.05$
With or without lysine	$P < 0.05$	$P < 0.05$	$P < 0.05$	NS
Interaction	NS	NS	NS	NS

Data are expressed as mean ± S.E. where $n = 3$.

Mean values in the same column with different superscripts vary significantly ($P < 0.05$).

2.9 Haematological and haemato-biochemical parameters

Five fish from triplicates of each treatment were sedated with 50 µL/L clove oil. The caudal vein of a fish was ruptured to collect blood using a medical syringe and with the use of 0.1N EDTA solution, the blood sample was straight away shifted to a centrifuge tube of volume 2 ml. The total number of RBCs was counted using an improved Neubauer hemocytometer (Shah and Altindag, 2004). Counting of WBCs was done at 10× microscope focus and measured in the four corners of a 1 square millimeter central ruled area on both sides of the hemocytometer's counting chambers. The cyanomethemoglobin method was used to estimate blood hemoglobin (Blaxhall and Daisley, 1973). The ERBA kit was used to estimate serum total protein using the biuret method (Reinhold, 1953). Serum albumin estimation was done by the binding method of bromocresol green (Dumas et al., 1971) by using the ERBA Kit. The analysis of serum globulin levels involved the subtraction of albumin values from total protein. Bovine serum albumin (BSA) served as the standard for this analysis.

2.10 Statistical analysis

The primary consequence was examined with a two-way analysis of variance (ANOVA) with dietary treatment inclusion level of rice DDGS (0, 6, 12, 18, 24 & 30 %) and lysine supplementation (with or without) as two fixed factors. One-way ANOVA was used to analyze differences in the mean values of all parameters using IBM SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The variations in the above variables were scrutinized using Duncan's multiple range test ($P < 0.05$).

3 Results

3.1 Proximate composition of whole-body fish

The effect of different inclusion levels of dietary rice DDGS on the whole-body composition of *C. mrigala* is presented in Table 3. Fish fed DDGS diets with an inclusion level up to 12% exhibited significantly ($P < 0.05$) higher levels of crude protein and crude lipid content, however, an increase from 18% and above noted a significant ($P < 0.05$) decrease in comparison to the control diet, recording the lowest in fish fed 30% DDGS diets. There were no significant differences ($P > 0.05$) in crude protein and lipid content among the juveniles fed with either 6% or 12% inclusion of rice DDGS. Irrespective of the inclusion level of rice DDGS, supplementation of lysine significantly ($P < 0.05$) increased the levels of crude protein and lipid content in juveniles (Tab. 3).

3.2 Growth and nutrient utilization parameters

No mortality was recorded during the feeding period for fish in all experimental groups. The percentages of weight gain and the specific growth rate (SGR) following the administration of diets containing varying concentrations of rice DDGS, with or without lysine supplementation, are detailed in Table 4. The growth performance in the current study was significantly influenced by the level of rice DDGS in the diet ($P < 0.05$). Weight gain percentages and SGR did not differ significantly among fish fed diets with 0%, 6%, and 12% rice DDGS inclusion, but decreased in those fed diets containing 18% or more rice DDGS. Fish fed with the D30 diet (30% rice DDGS) exhibited the lowest weight gain (%) and SGR compared to all other treatments. Regardless of the level of rice DDGS in the diet, juvenile fish fed with lysine-supplemented diets displayed significantly higher growth performance ($P < 0.05$) than those fed diets without lysine supplementation (Tab. 4). The

Table 4. Growth performance of *Cirrhinus mrigala* juveniles fed with different experimental diet.

Treatments	Weight gain (%)	SGR (%/day)	FCR	PER
C	117.85 ^a ± 5.14	1.73 ^a ± 0.07	1.09 ^c ± 0.06	3.05 ^a ± 0.18
D ₆	117.80 ^a ± 4.71	1.72 ^a ± 0.1	1.06 ^c ± 0.03	3.15 ^a ± 0.19
D ₁₂	105.35 ^{ab} ± 2.35	1.59 ^{ab} ± 0.05	1.20 ^b ± 0.06	2.78 ^{ab} ± 0.13
D ₁₈	94.64 ^{bc} ± 2.35	1.47 ^c ± 0.06	1.30 ^a ± 0.06	2.56 ^b ± 0.12
D ₂₄	78.09 ^{cd} ± 4.76	1.27 ^{cd} ± 0.18	1.45 ^a ± 0.07	2.44 ^b ± 0.12
D ₃₀	58.57 ^d ± 1.42	1.02 ^d ± 0.02	1.38 ^a ± 0.01	2.43 ^b ± 0.15
CL	103.45 ^{ab} ± 1.11	1.57 ^{ab} ± 0.01	1.08 ^c ± 0.01	3.06 ^a ± 0.03
D ₆ L	100.11 ^{ab} ± 2.45	1.54 ^{ab} ± 0.38	1.13 ^c ± 0.09	2.98 ^a ± 0.24
D ₁₂ L	103.33 ^{ab} ± 3.33	1.57 ^{ab} ± 0.36	1.09 ^c ± 0.04	3.05 ^a ± 0.11
D ₁₈ L	103.45 ^{ab} ± 1.11	1.57 ^{ab} ± 0.001	1.05 ^c ± 0.04	3.14 ^a ± 0.01
D ₂₄ L	105.35 ^{ab} ± 1.78	1.59 ^{ab} ± 0.019	1.10 ^c ± 0.02	3.01 ^a ± 0.07
D ₃₀ L	122.61 ^a ± 2.95	1.77 ^a ± 0.059	1.01 ^c ± 0.01	3.29 ^a ± 0.04
Variation source	Two way ANOVA			
Level of rice DDGS	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
With or without lysine	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Interaction	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

Data are expressed as mean ± S.E. where $n = 3$.

Mean values in the same column with different superscripts vary significantly ($P < 0.05$).

combined influence of rice DDGS concentration in the diet and lysine supplementation significantly impacted the weight gain (%) and SGR of *C. mrigala* juveniles (Tab. 4).

When rice DDGS replaced 12% or more soybean meal, FCR and PER were lower and higher respectively, compared with other groups, however, no significant differences ($P > 0.05$) were observed at or up to 6% substitution level (Tab. 4). Treatment group fed with 6% rice DDGS in place of soybean meal registered significantly highest ($P < 0.05$) PER and lowest ($P < 0.05$) FCR (Fig. 2). Irrespective of dietary rice DDGS level, FCR and PER significantly ($P < 0.05$) decreased and increased, respectively, with the supplementation of dietary lysine (Tab. 4). There was significant ($P < 0.05$) interaction between dietary rice DDGS level and lysine supplementation on FCR and PER (Tab. 4).

3.3 Nucleic acid content in muscle

The DNA/muscle mass (wet weight), muscle protein / DNA, RNA/muscle mass, and RNA/DNA ratio of *C. mrigala* juveniles fed different test diets and the interaction effect of the level of rice DDGS and lysine supplementation are synopsized in Table 5. No marked changes ($P > 0.05$) in the DNA/muscle mass ratio were recorded in the fish receiving different dietary treatments followed by the supplementation of lysine and their interaction.

The muscle protein/DNA ratio in juveniles was significantly affected ($P < 0.05$) by the changes in the inclusion level of rice DDGS in the diets (Tab. 5). The muscle protein/DNA showed a progressive trend as the dietary inclusion level was elevated up to 12% and subsequently reduced, exhibiting similar ratio ($P > 0.05$) as observed in fish fed D₁₈-D₃₀ groups, in comparison to the control group (Tab. 5). Irrespective of the level of rice DDGS, the muscle protein/DNA ratio was significantly higher ($P < 0.05$) in lysine supplemented group.

The RNA/muscle mass ratio significantly decreased ($P < 0.05$) in varying level of rice DDGS fed groups, however, juveniles recorded higher ($P < 0.05$) RNA/muscle mass ratio when lysine is added to the varying DDGS diets as compared to those of the control group. The RNA/DNA ratio was not affected ($P > 0.05$) as a result of increase in the DDGS inclusion up to 18%; however, further increasing the level with either 24% or 30% in fish recorded a significant decrease ($P < 0.05$) in the ratio as compared to the control group. Irrespective of the level of DDGS, RNA/muscle mass ratio and RNA/DNA ratio in the fish fed DDGS-based diets without lysine were significantly ($P < 0.05$) lower than those in the lysine-supplemented groups (Tab. 5). Also, a significant ($P < 0.05$) interaction was found between the level of DDGS and lysine supplementation on muscle protein/DNA, RNA/ muscle mass, and RNA/DNA ratio (Tab. 5).

3.4 Digestive enzyme activity

The data on activity of digestive enzymes in the intestine of *Cirrhinus mrigala* is summarized in Table 6. The intestinal amylase activity of control group (C) was found similar ($P > 0.05$) with 6 and 12% rice DDGS fed group, however significantly decreased ($P < 0.05$) activity was recorded in treatment fed with 18% or more rice DDGS (Tab. 6). Fish fed DDGS based diets up to an inclusion level of 12% had similar ($P > 0.05$) protease activity, however, a discernible decline in protease activity ($P < 0.05$) was observed when the inclusion level was increased to 18% or higher (specifically, at 24% and 30%), in comparison to the control group. The intestinal lipase activity showed an increasing pattern as the dietary inclusion level was raised up to 12% and thereafter levelled off with similar lipase activity ($P > 0.05$) observed in fish fed with 18% or more dietary rice DDGS (Tab. 6). The lysine addition to the DDGS diet promoted higher activities of digestive enzymes compared to the non-supplemented group (Tab. 6). Also, a

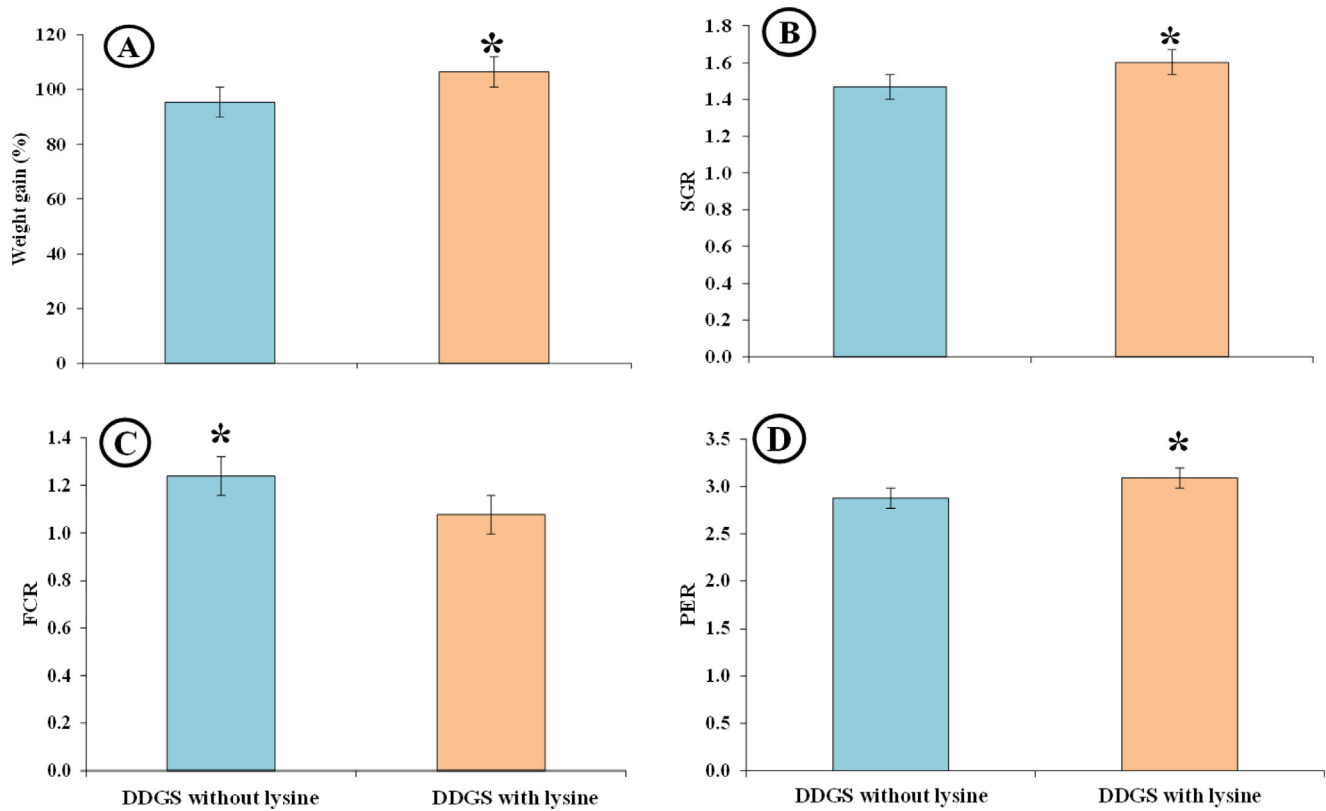


Fig. 2. (A–D) Growth performance of *C. mrigala* (Mean $\pm$ Standard error) fed rice DDGS based diets without and with lysine for 45 days. Asterisk (*) indicates a significant difference ($P < 0.05$) between lysine and non-lysine supplemented DDGS diet.

Table 5. Nucleic acid (DNA and RNA) contents in muscle of *C. mrigala* juveniles fed with different experimental diets.

Treatments	DNA/muscle mass	Muscle protein/DNA	RNA/muscle mass	RNA/DNA
C	13.68 $\pm$ 0.86	2.39 ^d $\pm$ 0.15	46.9 ^d $\pm$ 0.73	3.45 ^{bc} $\pm$ 0.20
D₆	11.86 $\pm$ 0.92	3.56 ^a $\pm$ 0.27	40.6 ^e $\pm$ 0.80	3.47 ^{bc} $\pm$ 0.31
D₁₂	13.47 $\pm$ 1.23	2.93 ^{abcd} $\pm$ 0.28	37.4 ^{ef} $\pm$ 1.51	2.83 ^{cd} $\pm$ 0.32
D₁₈	12.81 $\pm$ 0.91	2.48 ^d $\pm$ 0.16	35.5 ^f $\pm$ 0.85	2.80 ^{cd} $\pm$ 0.21
D₂₄	12.75 $\pm$ 0.39	2.54 ^{cd} $\pm$ 0.07	29.2 ^g $\pm$ 1.40	2.28 ^d $\pm$ 0.06
D₃₀	12.04 $\pm$ 0.90	2.54 ^{cd} $\pm$ 0.19	25.0 ^h $\pm$ 0.40	2.10 ^d $\pm$ 0.18
CL	12.63 $\pm$ 1.05	3.29 ^{ab} $\pm$ 0.26	57.7 ^a $\pm$ 1.13	4.62 ^a $\pm$ 0.34
D₆L	13.68 $\pm$ 0.95	3.19 ^{ab} $\pm$ 0.21	51.8 ^c $\pm$ 0.34	3.82 ^{ab} $\pm$ 0.24
D₁₂L	13.68 $\pm$ 0.96	3.12 ^{abc} $\pm$ 0.22	52.9 ^{bc} $\pm$ 0.51	3.91 ^{ab} $\pm$ 0.29
D₁₈L	12.63 $\pm$ 0.05	3.23 ^{ab} $\pm$ 0.01	53.6 ^{bc} $\pm$ 1.45	4.24 ^{ab} $\pm$ 0.11
D₂₄L	13.68 $\pm$ 0.24	3.01 ^{abcd} $\pm$ 0.06	54.2 ^{abc} $\pm$ 1.71	3.96 ^{ab} $\pm$ 0.14
D₃₀L	14.12 $\pm$ 1.01	2.86 ^{bcd} $\pm$ 0.21	55.7 ^{ab} $\pm$ 1.83	3.99 ^{ab} $\pm$ 0.36
Variation source				
Two way ANOVA				
Level of rice DDGS	NS	$P < 0.05$	$P < 0.05$	$P < 0.05$
With or without lysine	NS	$P < 0.05$	$P < 0.05$	$P < 0.05$
Interaction	NS	$P < 0.05$	$P < 0.05$	$P < 0.05$

Data are expressed as mean $\pm$ S.E. where $n = 3$.

Mean values in the same column with different superscripts vary significantly ($P < 0.05$).

Unit: DNA/Muscle mass = $\mu\text{g/g}$; Protein/DNA = $\text{mg}/\mu\text{g}$; RNA/Muscle = $\mu\text{g/g}$; RNA/DNA = $\mu\text{g}/\mu\text{g}$.

Table 6. Digestive enzyme activities (U/mg protein) in the intestine of *C. mrigala* juveniles fed with different experimental diet at the end of the experiment.

Treatments	Amylase	Protease	Lipase
C	9.59 ^{bc} ± 0.19	207.71 ^c ± 1.92	5.24 ^{cd} ± 0.07
D ₆	10.43 ^{ab} ± 0.19	198.06 ^c ± 1.86	6.64 ^a ± 0.32
D ₁₂	10.07 ^{ab} ± 0.14	181.24 ^{cd} ± 1.53	6.58 ^{ab} ± 0.26
D ₁₈	8.31 ^{cd} ± 0.44	177.94 ^d ± 1.69	5.55 ^{bc} ± 0.38
D ₂₄	8.16 ^d ± 0.29	175.37 ^d ± 1.31	5.36 ^{cd} ± 0.07
D ₃₀	6.86 ^e ± 0.05	163.71 ^d ± 1.23	4.40 ^d ± 0.06
CL	11.06 ^{ab} ± 0.50	247.99 ^b ± 1.38	6.53 ^{ab} ± 0.42
D ₆ L	11.18 ^a ± 0.60	265.88 ^b ± 1.88	6.83 ^a ± 0.23
D ₁₂ L	10.09 ^{ab} ± 0.58	251.97 ^b ± 1.84	6.49 ^{ab} ± 0.33
D ₁₈ L	9.96 ^{cd} ± 0.36	260.85 ^b ± 1.24	6.51 ^{ab} ± 0.69
D ₂₄ L	9.73 ^{ab} ± 0.35	278.25 ^{ab} ± 1.2	6.24 ^{abc} ± 0.09
D ₃₀ L	9.63 ^{bc} ± 0.84	281.25 ^{ab} ± 1.01	6.24 ^{abc} ± 0.24
Variation Source	Two Way ANOVA		
Level of rice DDGS	$P < 0.05$	$P < 0.05$	$P < 0.05$
With or without lysine	$P < 0.05$	$P < 0.05$	$P < 0.05$
Interaction	$P < 0.05$	$P < 0.05$	$P < 0.05$

Data are expressed as mean ± S.E. where $n = 3$.

Mean values in the same column with different superscripts vary significantly ($P < 0.05$).

significant ($P < 0.05$) interaction was recorded between the level of DDGS and lysine supplementation on the digestive enzyme activity (Tab. 6).

3.5 Haematological parameters

Dietary treatments recorded significant ($P > 0.05$) outcomes on haematological parameters viz. Haemoglobin (Hb), Total Erythrocyte Count (TEC), and Total Leukocyte Count (TLC) as shown in Table 7. The Haemoglobin content of *Cirrhinus mrigala* juveniles was not affected by dietary rice DDGS supplementation up to 12%, however, it tended to decrease in fish fed 18-30% rice DDGS diets (D₁₈-D₃₀), recording the lowest in D₃₀ (30% rice DDGS included treatment group). TEC of *C. mrigala* juveniles showed an increasing pattern as the dietary rice DDGS level increased up to 12% and thereafter levelled off with similar TEC ($P > 0.05$) observed in fish fed 18-30% rice DDGS (D₁₈-D₃₀) to that of the control group. In addition, the Hb and TEC in the fish fed DDGS based diets with lysine were recorded significantly ($P < 0.05$) better than those in the non-lysine supplemented groups except for TLC that was not affected ($P < 0.05$) by lysine supplementation. Significant ($P < 0.05$) interaction effects were recorded between the level of DDGS and lysine supplementation for all analysed haematological parameters (Tab. 7).

3.6 Serum biochemical parameters

The impact of dietary inclusion of rice DDGS on different serum biochemical parameters is shown in Table 7. The levels of serum total protein and globulin differed significantly ($P < 0.05$) among the treatment groups. No marked changes ($P > 0.05$) in the serum albumin content were recorded in the fish receiving different levels of rice DDGS as well as

supplementation of lysine and their interaction. The levels of serum globulin showed an increasing pattern as the dietary DDGS level increased up to 12% and thereafter levelled off with similar serum globulin content observed in 18 and 24% dietary DDGS included groups as compared to the control, recording the lowest in fish fed D₃₀ (30% inclusion of DDGS) groups. Irrespective of the level of DDGS inclusion, lysine supplementation recorded higher ($P < 0.05$) levels of serum globulin content. The A/G ratio was significantly ($P < 0.05$) lower in the lysine added group. Significant ($P < 0.05$) interaction was observed between the level of rice DDGS and lysine supplementation for all analysed serum biochemical parameters except for serum albumin ($P > 0.05$).

4 Discussion

Studies on the potential of alternative feedstuffs to costly protein diets including fish meal and soybean meal in fish are centred on few plant feedstuffs and plant protein mixtures viz. wheat gluten meal, soy protein concentrate etc. (Wu et al., 2006). However, alternative ingredients that are not a part of the food chain should be considered to ensure environmentally, economically, and energy friendly aquaculture production. One potential substitute component is Dried Distillers' Grains with solubles (DDGS), which may be integrated into aquafeed to serve as a protein source. Henceforth, the present study aimed to evaluate the effects of substituting soybean meal (SBM) with rice DDGS and determine its optimal level that can meet the nutritional requirements of *C. mrigala* juveniles. This information could potentially reduce the excessive reliance on SBM in fish diets.

Dietary inclusion of different ingredients has a direct effect on feed intake and metabolism, including turnover of protein and lipid, and thereafter on the growth of fish. In the present study, it is evident that the crude protein content of fish was

Table 7. Hematological parameters of *C. mrigala* juveniles fed with different experimental diet at the end of the experiment.

Treatments	Hemoglobin content (g/dl)	Total erythrocyte count (10^6 cells/mm ³)	Total leucocyte count (10^9 cells/mm ³)	Total protein (g/dl)	Albumin (A) (g/dl)	Globulin (G) (g/dl)	A/G ratio
C	5.13 ^b ± 0.14	1.35 ^d ± 0.04	15.68 ^{abc} ± 0.22	5.29 ^{ef} ± 0.13	1.30 ± 0.15	3.98 ^{ef} ± 0.04	0.32 ^{abcde} ± 0.04
D₆	5.66 ^b ± 0.26	1.92 ^{abc} ± 0.03	17.97 ^a ± 0.10	6.28 ^{cd} ± 0.17	1.25 ± 0.05	5.03 ^{bc} ± 0.18	0.25 ^{cde} ± 0.01
D₁₂	5.70 ^b ± 0.17	1.89 ^{bc} ± 0.01	17.56 ^{ab} ± 0.73	6.26 ^{cd} ± 0.07	1.49 ± 0.24	4.76 ^{cd} ± 0.31	0.32 ^{abcde} ± 0.06
D₁₈	4.40 ^a ± 0.20	1.41 ^d ± 0.07	16.59 ^{ab} ± 1.61	5.19 ^{ef} ± 0.14	1.44 ± 0.19	3.76 ^{ef} ± 0.29	0.39 ^{ab} ± 0.07
D₂₄	5.10 ^{bc} ± 0.11	1.69 ^a ± 0.07	15.22 ^{bc} ± 0.06	4.84 ^{fg} ± 0.33	1.45 ± 0.05	3.39 ^{fg} ± 0.35	0.44 ^a ± 0.05
D₃₀	4.33 ^d ± 0.20	1.21 ^d ± 0.05	13.97 ^c ± 0.09	4.44 ^{fg} ± 0.20	1.33 ± 0.06	3.11 ^g ± 0.16	0.43 ^{ab} ± 0.01
CL	5.73 ^b ± 0.18	1.95 ^{ab} ± 0.05	15.74 ^{abc} ± 0.15	7.04 ^b ± 0.28	1.35 ± 0.07	5.69 ^b ± 0.32	0.24 ^{de} ± 0.02
D₆L	6.66 ^a ± 0.38	2.02 ^a ± 0.19	17.68 ^{ab} ± 0.62	7.72 ^a ± 0.26	1.24 ± 0.14	6.48 ^a ± 0.16	0.19 ^c ± 0.02
D₁₂L	6.70 ^a ± 0.32	2.02 ^a ± 0.01	17.43 ^{ab} ± 0.37	6.67 ^{bc} ± 0.24	1.51 ± 0.09	5.16 ^{bc} ± 0.17	0.29 ^{bcde} ± 0.01
D₁₈L	5.06 ^{bc} ± 0.08	1.76 ^{bc} ± 0.03	16.52 ^{ab} ± 1.64	5.73 ^{de} ± 0.27	1.40 ± 0.16	4.33 ^{de} ± 0.18	0.32 ^{abcde} ± 0.03
D₂₄L	5.33 ^b ± 0.14	1.72 ^{bc} ± 0.03	15.42 ^{bc} ± 0.12	5.06 ^{efg} ± 0.11	1.32 ± 0.11	3.74 ^{efg} ± 0.03	0.35 ^{abcd} ± 0.02
D₃₀L	5.03 ^{bc} ± 0.09	1.72 ^{bc} ± 0.06	14.04 ^c ± 0.15	5.09 ^{efg} ± 0.17	1.40 ± 0.11	3.69 ^{efg} ± 0.18	0.38 ^{abc} ± 0.04
Two way ANOVA							
Variation source							
Level of rice DDGS	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$
With or without lysine	$P < 0.05$	$P < 0.05$	NS	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$
Interaction	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$

Data are expressed as mean ± S.E. where $n = 3$.Mean values in the same column with different superscripts vary significantly ($P < 0.05$).

significantly affected by dietary rice DDGS inclusion, recording protein conversion and deposition with increase in rice DDGS inclusion level up to 12%. This increased crude protein content at inclusion level up to 12% indicates better utilization of lipid and carbohydrates for energy and spare protein for its growth. However, reduced crude protein at higher inclusion level (18–30% inclusion of rice DDGS) may be due to impaired key amino acid, especially lysine owing to its damage during the drying process (Nuez Ortín and Yu, 2009), considering the fact that essential amino acids support key metabolic functions in fish likely protein formation and energy production (Gupta et al., 2015). Accordingly, in the present study, supplementation of lysine recorded higher crude protein content in fish. Lim et al. (2007) also reported increased whole body crude protein content in Nile tilapia fed with lysine supplemented DDGS diet. The present study found that fish fed higher rice DDGS inclusion levels had lower lipid content, while those with added lysine had higher lipid content. As lysine is the first limiting amino acid in the diet of fish, its optimum availability in the diet influences the deposition of dietary lipids through gluconeogenesis and lipogenesis (Chatterjee et al., 2016).

The growth in fish is a complex process regulated by several parameters including fish size and nutrient in feed, which perform individually or in synergy (Gupta et al., 2015). DDGS has a high concentration of yeast cells with nucleotides, glucan, and oligosaccharides that alter the intestinal morphology of fish, increasing the area available for nutrient absorption and, as a result, improving the use of dietary nutrient (Lim et al., 2009), enhancing nutrient absorption and exerting a favorable impact on nutrient utilization and growth performance. Similarly, in the present study, growth performance was significantly increased with the increase in the inclusion of rice DDGS up to 12% in the diet of *C. mrigala* juveniles. Thus, *Cirrhinus mrigala* can make effective use of plant-based proteins, and their digestive systems are well-suited for breaking down various amino acids, including those from alternative sources like DDGS. The efficiency of nutrient absorption in this species allows for optimal growth even in diets with lower lysine content, as long as the overall protein quality is adequate. However, increase in the inclusion level of rice DDGS from 18% and onwards significantly decreased the growth performance in *C. mrigala* juveniles. Dietary inclusion of rice DDGS may be limited due to lower level of essential amino acid (EAA), relative to soybean meal, being lysine the most limiting EAA (Ozório et al., 2010). Therefore, inadequate essential amino acid, such as lysine, may be the one reason that the fish diet including higher rice DDGS (18% or more) in place of soybean meal causes detrimental effect on growth. Consequently, the results of the present study showed that juveniles fed diet using 18% or more rice DDGS and supplemented with lysine (0.1%) had better growth performance compared to without lysine supplemented diet fed juvenile. Present study also revealed that lysine supplementation (0.1%) can replace soybean meal by rice DDGS from 12% to 30% (highest level used in this study) in the diet of *C. mrigala* juveniles without adversely affecting the growth. Lim et al. (2007) also observed that fish meal replacement by DDGS can be increased from 30 to 40% in tilapia, if the diets were supplemented with lysine. Dietary rice DDGS significantly affected the nutrient utilization in *C. mrigala* juveniles.

With increasing dietary rice DDGS, nutrient utilization decreased, in particular, when replacing soybean meal 12% or more, feed efficiency and protein efficiency were lowered than those of treatment fed with either 0% (Control) or 6% dietary rice DDGS. The decrease in feed efficiency and protein utilization might stem from an imbalanced essential amino acid (EAA) profile, specifically the reduced lysine content in rice distillers dried grains with solubles (DDGS). To address this issue, supplementation of lysine could potentially rectify the situation, particularly in response to the elevated inclusion levels of rice DDGS in the diet of juvenile *C. mrigala*. Peres and Oliva-Teles (2007) also reported that not only total EAA content but also EAA profile might significantly affect the feed and protein utilization efficiency in fish. This corroborates well with the results of some previous studies. It has been reported that improving feed efficiency by supplementing AA is becoming increasingly important for all species, including mammals, poultry, and fish (Peres and Oliva-Teles, 2007). Lysine as an EAA is often limited in plant ingredients used for aquafeeds (Lim et al., 2019). Therefore, lysine supplementation is becoming a pivotal method to improve the effects of non-fishmeal protein source substitution in aquaculture. The reduced protein retention in *C. mrigala* juveniles fed DDGS diets at higher inclusion levels might be also related to differences in muscle influx of free amino acids, decreased rapamycin signalling activities, or amino acid response signalling activities, as previously observed in turbot fed diets including soybean meal replacing FM (Diógenes et al., 2018). Overall, these results indicate that diet using 18% or more rice DDGS is beyond the tolerance of *C. mrigala* juveniles, which will negatively impact on the growth and feed efficiency and that supplementation of lysine (0.1%) can replace total soybean meal (30%) by rice DDGS in the diet of *C. mrigala* juveniles.

Nucleic acid (RNA & DNA) quantitative analysis is a simple and promising approach for assessing fish growth rate and protein deposition. DNA/muscle mass ratio and protein/DNA ratio is an index of cell number and cell size respectively. As a result, the ratio of RNA to DNA is a more precise indicator of metabolic activity than RNA concentration alone, as the amount of RNA molecules present in tissue samples has no effect on this ratio or the size of the cells. DNA serves as the genetic template; its cellular concentrations may be correlated with cell size and are mostly unresponsive to alterations in the external environment. RNA, in contrast, is involved in protein synthesis, which is necessary for growth. Its growth rate, which is influenced in part by the environment, has a significant impact on cellular concentration. As a result, the concentration of DNA may be a reliable predictor of live biomass, and RNA/DNA ratios may be a reliable gauge of fish growth rates. Results of the present study suggest that the muscle protein to DNA ratio in those diets did alter significantly ($P < 0.05$). This can be correlated with growth performance results that lower weight gain (%) and SGR were observed at 18% and further rice DDGS inclusion in the fish diet as compared to the control, yet, supplementation of lysine increased the growth performance of fish fed diet regardless of the inclusion level of DDGS. It reflected the decrease in the cell size with an increase in the inclusion level of DDGS in the diet and further supplementation of lysine leading to muscle fibre hypertrophy. This is consistent with the study of Pelletier et al.

(1995), who recorded that the muscle protein/DNA ratio rose as that of the growth rate. Correspondingly, Stickland et al. (1988) observed an increase in hypertrophy in muscle fibre with the increase in growth of Atlantic salmon. The RNA content in fish has been recommended as a real-time indicator of body growth and nutritional health. Because fish growth is reliant on protein synthesis, the RNA/DNA ratio has shown high sensitivity to feeding and can be used as an index of fish growth. In the present study, nucleic acid content analysis suggests that supplementation of lysine in the diet of *C. mrigala* could improve muscle protein/ DNA and RNA/DNA ratio. This study further demonstrates that *C. mrigala* juvenile recorded faster growth when fed DDGS-based diets with lysine owing to muscular hypertrophy.

Digestive enzymes are the most potential factor facilitating nutrient utilization in the intestinal tract, henceforth mostly used in evaluating the digestive capacity. Fish's ability to metabolize food is affected by the nature and composition of the feed being fed, which greatly stimulates the production and release of digestive enzymes. These enzymes have proven to be a useful tool for identifying specific dietary elements in animals. To optimize the utilization of certain feed elements, fish may regulate their metabolic processes to the dietary substrates through a modulation in enzyme production (Caruso et al., 2009). This highlights the fact that fish have nutritional adaptations in addition to morphological ones. Fernandez et al. (2001) pointed out that the digestive adaptations of various species are more closely correlated with their diets than with their taxonomic classification and changes in the activity of digestive enzymes may be influenced by feeding habits and feed composition. In the present study, it is evident that the digestive enzyme activity in fish was significantly influenced by dietary DDGS level and the fish fed with either 18, 24, or 30% DDGS in the diet reported a significant ($P < 0.05$) decrease in intestinal amylase activity, the lowest being observed in fish fed D₃₀ diet. It is conceivable that Lysine may interact with various amino acids or compounds present in different plant protein sources in fish, consequently reducing its efficiency in utilization. Moreover, the potential antagonism between lysine and arginine, an excessive amount of dietary leucine or alanine, as well as the presence of antinutritional elements, could potentially impact the voluntary intake of feed and the growth of animals. This is evident from studies by Yamamoto et al., (2012), indicating a plausible influence on the efficiency of lysine utilization. Additionally, the current study found that fish fed diets supplemented with lysine had increased amylase activity which was consistent with the positive effects on digestive enzyme activities in the intestine of juvenile Jian carp. This beneficial impact might be attributed to how amino acids stimulate the release of digestive enzymes. Amino acids, available in free form or digestive end products like L-lys, can be absorbed without digestion, directly promoting enzyme production from pancreatic acinar cells through activation of neuropeptides like bombesin and cholecystokinin (Santigosa et al., 2008). In the present study, the activity of proteases decreased in the intestine of juveniles with increasing dietary rice DDGS level further from 18% and these effects were recorded to be improved by supplementing lysine. The current results showed that lysine addition to the DDGS diet promoted higher activities of lipase compared to the non-supplemented group. Higher activity of lipase in a fish

fed supplemented amino acid diet was also witnessed by [Yaghoubi et al., \(2019\)](#). Mannans, glucans, and nucleotides present in yeast, which account for up to 10% of DDGS biomass, may be the cause of the increase in digestive enzyme activities in the intestinal tracts of fish-fed DDGS diets ([Andrews et al., 2009](#)).

Nutritional status has been linked to malformation in blood cells leading to physiological variations. The indicators of fish health, likely haematological measures, are frequently employed to assess the functional environment and husbandry stressors in fish. Fish blood development is significantly influenced by these factors as they closely monitor the toxicity associated with feed ingredients ([Roberts 1978](#)). The increased Total Erythrocyte Count (TEC) and haemoglobin content in the present study has been supported by [Andrews et al., \(2009\)](#), who testified an elevated count in *Labeo rohita* fingerlings fed with extract of yeast-based diets and also found reduced TEC and Hb count if fed with higher inclusion levels. Moreover, due to its function in innate or non-specific immunity, Total Leukocyte Count (TLC) is thought to be a good indication of fish health. According to earlier research, immune-boosting therapies had a disproportionately greater leucocyte count ([Singha et al., 2023](#)). The β -1,3 glucan, which may spot a particular receptor on TLCs, is the cause of the higher TLC count in the DDGS-supplemented groups. The cells perform phagocytosis facilitated by engulfing, killing, and digesting pathogens when glucans bind to the receptor followed by emission of indicator molecules (cytokines) that encourage the production of more TEC or WBCs ([Raa, 1996](#)). This might be the reason for the noticeably greater levels of white blood cells seen in this study up to an inclusion level of 12% rice DDGS in fish fed diets. However, fish fed diets supplemented with either 18 or 24% DDGS recorded lower TLC counts, but were all within the optimum levels compared to the control diet. The decreased index in the higher DDGS-fed groups may be the result of an overconsumption of DDGS-containing glucan by these groups. [Sajeewan et al. \(2009\)](#) reported that higher consumption of a glucan diet led to immunological exhaustion. Furthermore, [Lim et al. \(2007\)](#) found no variations in the haematological parameters of Nile tilapia fed maize DDGS diets with and without added lysine. The variations in species, fish size/age, and/or the degree of lysine deficiency between the findings of the current investigation and that of other studies ([Lim et al., 2007](#)) may explain these discrepancies. Overall, the hematological responses to rice DDGS in fish diets are linked to the dietary lysine content. At lower inclusion levels (below 12%), lysine may not be a limiting factor, but at higher inclusion rates (18%), the deficiency becomes critical, and supplementing with lysine ensures optimal performance.

Total serum protein has been applied as a basic measure to assess the health and nutritional well-being of fish ([Robertson et al., 1994](#)). The higher quantities of blood proteins may be due to improved liver and other protein-generating organ activity, suggesting enhanced fish immune system function. In the present study, significant differences ($P < 0.05$) were found among fish fed diets supplementing different levels of rice DDGS, both with and without lysine supplementation. Albumin and globulin are the two primary protein subgroups that make up total serum proteins. Albumin is the most prevalent plasma protein and a part of the plasma antioxidant activity, however, no significant ($P < 0.05$) variations in albumin levels were recorded in the present

study. Additionally, it acts as a buffer and a supply of amino acids for peripheral tissue reducing the inflammatory response of platelets and neutrophils by inhibiting the production of leukotrienes and actin. The blood globulin level in *C. mrigala*, however, was altered by the dietary supplementation with the inclusion of rice DDGS. Serum globulin consists of several components of which three groups are recognized as α , β , and γ globulin. The fraction of γ globulin contains nearly all of the immunoglobulins found in blood, which are well known for their important role in immunological response. Furthermore, Distiller's dried grains with solubles are known to possess significant quantities of yeast cells. This yeast component is abundant in β -glucans, which have demonstrated the ability to enhance immune reactions and bolster the host's defense mechanisms against microbial pathogens in various organisms, such as humans, animals, and even fish, as indicated by studies conducted by [Chen and Ainsworth \(1992\)](#). Also, rice DDGS contains several bioactive compounds, such as peptides, carbohydrates, and antioxidants ([Skendi et al., 2020](#)), which can positively influence the immune system. For instance, polysaccharides in DDGS can stimulate the immune system by enhancing the activity of macrophages and other immune cells and antioxidants in DDGS, such as phenolic compounds, may play a role in mitigating oxidative stress, thus supporting immune responses ([Rudrapal et al., 2022](#)). These compounds could have immunostimulatory effects that help to offset the negative impacts of lysine deficiency, as seen in various studies with other plant-based protein sources ([Gatlin et al., 2007](#)). However, total serum protein and globulin levels of fish fed diets supplemented with higher inclusion levels viz. 18, 24, and 30% DDGS recorded a linear decrease in the count compared to the control diet. According to reports, consuming high dosages of yeast can inhibit the immune system. This is probably because too many glucan receptors are present in phagocytic cells, which prevents them from effectively phagocytosing germs in fish ([Couso et al., 2003](#)). Comparable findings on the way to that of the current study were reported in juvenile flounder fed different dietary inclusion levels of DDGS ([Mostafizur et al., 2015](#)). Additional research assessing the impact of yeast and its components (including α -glucans, nucleic acids, and mannan oligosaccharides) as nutritional additives on immune system function and disease resistance of diverse fish species gave up unpredictable outcomes which may be due to disparities in concentration and source of the products, feeding duration, culture management, age/size and species. Conversely, more study is required to assess the potential enhancing impact of dietary rice DDGS and its sub-ingredients on the function of the immune system and fish pathogen resistance.

5 Conclusion

According to the outcomes of the current study, dietary rice DDGS may substitute soybean meal (SBM) in the diet of *C. mrigala* at levels as high as 12% of the entire diet without impairing overall growth performance or fish health. Nevertheless, substituting large concentrations of DDGS for SBM in fish diets may hinder fish growth. In contrast, supplementation of lysine in the DDGS diet may completely replace Soybean meal without a negative impact on the overall growth performance, and health status of *Cirrhinus mrigala*.

Distillers' dried grains with solubles (DDGS) are excellent protein additions to aquafeed, as their nutritional makeup depends on factors like grain content, animal species, and other components. Additionally, when amino acids like lysine are added to the aquafeed, larger concentrations of DDGS might be employed to meet the nutritional requirements of different aquatic species, such as fish. Aside from that, DDGS enhances aquatic animal development performance and strengthens the immune system. Beforehand, as a newer feed ingredient for fish feed, future studies should be taken to evaluate the long-term impact of high DDGS content in aquaculture feed and its impact on fish health and in turn on the environment.

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Conflicts of interest

The authors declare no conflict of interest.

Data availability statement

The data supporting the conclusions of this investigation are accessible from the corresponding author on a justifiable request.

Author contribution statement

Aditi Banik: Research, Laboratory analysis, Data collection, statistical analysis, writing an original draft, and editing. Shivendra Kumar: Conceptualization, Experimental design, statistical analysis, original manuscript writing, and supervising. Praveesh Kumar Rathor: Supervising, Software and reviewing the manuscript. Sujit Kumar Nayak: Biochemical analysis and Supervising. Maneesh Kumar Dubey: Validation, review, and manuscript editing. P. P. Srivastava: Review and manuscript editing. Pankaj Kishore: Laboratory analysis and supervising. Abhishek Kumar: Laboratory analysis and supervising.

Ethical approval

Ethical committee name: College of Fisheries, Dr Rajendra Prasad Central Agricultural University.

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