The Inclusion of Sorghum in the Diet of Farmed Common Carp *Cyprinus carpio* L.

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Abstract
The present study investigated the effects of sorghum inclusion in carp (Cyprinus Carpio) diets on growth, blood parameters, meat composition, microbiota in carp. Experimental diets with varying sorghum contents were fed to carp with an initial body weight of 50 g. The diets were tested for the presence sorghum in the diet. In particular at 30% sorghum completely replaced corn and barley. Inclusion of sorghum reduced final body weight and growth, especially in the R4 group with 30% sorghum. However, other physiological parameters such as feed requirement, specific growth rate and organ index were not affected. Carp meat protein content and salt increased with increasing sorghum inclusion rate. In addition, there was a positive correlation between sorghum concentration and malondialdehyde levels and a negative correlation with glutathione levels, indicating a breakdown of antioxidant defence mechanisms and an increase in oxidative stress.

Keywords: common carp, diets, growth, sorghum.

1. Introduction

Aquaculture plays a significant part in assembly the worldwide request for nourishment and sustenance, being the speediest developing nourishment industry segment [1].
Be that as it may, the segment faces critical challenges, especially related to bolster. The challenge with respect to nourishing stems from the rising costs and shortage of conventional fixings like fish meal and fish oil, requiring the investigation of economical options, in this setting recognizing and coordination elective fixings into aqua feed definitions gets to be progressively pivotal. This not as it were addresses financial concerns but too adjusts with supportability objectives by decreasing dependence on limited marine assets [1].

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Sorghum has a chemical composition that is comparable to other grains, sorghum is advantageous because it can be grown in areas with low rainfall. The United States is the top producer of sorghum, accounting for about 15% of the total production. The primary benefits of using sorghum in fish feed are its high levels of antioxidants, nutrients. Sustainability, and large production worldwide.
Nevertheless, difficulties in using sorghum consist of its lower protein content, decreased starch digestibility, the existence of antinutritional factors, deficiencies in lysine and threonine, and limited research and its use in fish feeding.
According to Eurostat, the common carp is the most crucial species farmed in Romania and is regarded as a traditional species in several European countries, in Romania, carp farming is usually done in semi-intensive systems, which use earthen ponds and include cereals and oilseeds in the fish feed [2].
The primary concerns with using plant-based ingredients are the existence of antinutrients and

their poor digestibility, in this situation, it was shown that sorghum can be digested by carp just about as well as rye when it comes to fats, and slightly less well for protein [3].

Due to the traditional nature of carp farming, farms that do this also help maintain these practices and contribute to the creation of local ecosystem services. The importance of using sustainable fish food for raising carp is that it helps to support environmentally friendly fish farming, which is important for protecting the services provided by the local ecosystem [4]. In animal agriculture, sorghum is frequently used and typically substitutes corn. A study showed that it's possible to use sorghum instead of corn in ration of up to 55% without any bad effects on growth of health. This research is focused on evaluating if sorghum can be a main ingredient in the diet of common carp. Specifically, the study will determine the best levels to include sorghum and look into how it affects growth, blood parameters, oxidative stress, intestinal microbiota and meat quality [5, 6].

2. Materials and methods

In 2024, at the research site, we carried out natural breeding in a pond using carp parents from the station, 100 carp fingerlings were chosen from a group of individuals, each weighing 50 grams at the start. The fish were moved to the station's circulating aquaculture system and adjusted to the new environment before the experiment began. The fish were distributed in 10 cylindrical fiberglass tanks with a volume of 0.75 m^3. Each water tank housed 10 fish. The feeding experiment lasted for four weeks, starting in August and ending in September. Fish were randomly selected. The water temperature began at 25.5°C and went down to 21.5°C by the end of the trial. Every day, we measured the amount of oxygen and the temperature. We also measured the levels of pH, ammonia, nitrates, nitrites, and phosphorus once a week. Water quality was evaluated using a Spectrophotometer. The specific details of the water are shown in Table 1. Analysis of the proximate composition of ingredients and diets was performed with a DA 7250 NIR Analyser (Perten Instruments, Hagersten, Sweden) (Table 2). Four different diets were formulated, with the amount of sorghum changing as follows: control 0%, R1-10%, R2-20%, R3-30%. The regular diet included sunflower seeds 22%, fresh peas 22%, corn 10%, barley 15%, fish meal 22%, and sunflower oil 3%, and did not contain sorghum (Table 3). The ingredients used were specially formulated for carp diets and came from Romania. The diets were created using extrusion, crushing, blending, and forming into pellets.

Table 1. Water purity in the RAS during the fish test with experimental sorghum meals

Parameter	Units	Week 1	Week 2	Week 3	Week 4
Temperature	°C	25.5	22.8	25.00	21.5
pH	Units	8.20	7.82	7.34	7.88
Dissolved oxygen	mg/L	8.10	8.10	8.15	8.20
(NO_3^-)	mg/L	0.08	0.14	0.13	0.05
(NO_2^-)	mg/L	0.09	0.17	0.06	0.03
(NH_3^+)	mg/L	0.11	0.21	0.14	0.25
(NH_4^+)	mg/L	11.01	12.21	10.24	10.35
(PO_4^{3-})	mg/L	8.25	8.03	7.78	7.98
Hardness total	°G	95.00	80.00	98.00	97.00
(Ca_2^+)	mg/L	62.00	53.00	57.00	60.00
(Mg_2^+)	mg/L	0.00	0.00	0.00	0.00
(Fe)	mg/L	119.00	115.00	120.00	112.00
(SO_4^{2-})	mg/L	0.11	0.05	0.12	0.20

A small amount of fish meal (20%), was utilized, producing a diet with 20% protein, similar to the diets used by carp farmers in natural ponds to keep costs down. The ingredients and diets were analyzed using a DA7250 NIR Analyzer to determine their proximate composition.

The fish were placed in 10 round fiberglass tanks with a capacity of 0.75 cubic meters, each water container contained 10 fish. At the start of the experiment, the fish were checked for their weight and size.

Table 2. Nutritional makeup of the elements utilized in trial sorghum meals for carp

Ingredient	Moisture	Fat	Protein	Ash	Sugar	Fiber	Calcium	Starch
Sorghum	14.02	3.78	15.24	13.16	2.22	4.32	1.20	52.12
Sunflower meal	10.34	1.56	40.98	7.23	-	20.34	-	-
Peas	11.60	-	23.13	-	-	-	-	-
Corn	12.57	4.01	8.16	-	-	-	-	60.12
Barley	11.54	-	12.35	-	-	5.37	-	49.96
DDGS	10.03	-	25.96	4.23	-	10.18	-	4.16
Fish meal	10.54	7.90	37.45	22.35	-	-	-	-

Table 3. Ingredients and composition of experimental diets for carp

Ingredient	R0 (0%)	R1 (10%)	R2 (20%)	R3 (30%)
Sorghum	-	12	22	25
Sunflower meal	22	22	22	24
Peas	22	22	22	19
Corn	12	10	9	-
Barley	10	6	-	-
DDGS	12	12	12	7
Fish meal	4	4	2	-
Sunflower oil	3	2	2	-

The experimental diets were fed manually three times daily. The daily amount of food given to the fish was 3%, decided according to the temperature of the water and the weight of the fish. To find out the growth factors, the fish were measured (in cm) and weighed (in g) three times during the specified growth time.

Growth performance and meat composition, were statistically analyzed using analysis of variance (ANOVA), followed by the Tukey test ($p<0.05$) with SPSS software version 21 (IBM Corp, Armonk, NY, USA). Results are shown as means with standard error of the mean (S.E.M.). Chemical results (Oxidative status) were examined using a two-way ANOVA followed by Tukey's multiple comparisons tests with GraphPad Prism software v9.3.1 (La Jolla, CA, USA). Differences were considered important when $p<0.05$, and the values are reported as means ± S.E.M.

3. Results and discussion

The following growth results were found for carp.

The findings show a noticeable pattern in how well common carp grow when their diets include different amounts of sorghum. When the amount of sorghum went up from R0 to R3, there was a clear drop in both the final body weight and weight gain. In particular, carp that were fed the R3 diet with 30% sorghum showed the lowest values for final weight and weight gain.

Nevertheless, there were no noticeable variations in factors such as feed conversion ratio, specific growth rate, condition factor, protein efficiency ratio, and lipid efficiency ratio. These findings show that adding sorghum had a negative effect on the growth of common carp in terms of their final weight and weight gain. However, it did not have a significant impact on the other production traits that were measured in this research. Furthermore, there were no notable differences observed between the control group R0 and the group with the lowest sorghum inclusion R1. This indicates that adding a small amount of sorghum 10% to the diet did not significantly change the common carp's growth compared to the control group.

Table 4 summarizes the chemical makeup of the flesh of common carp that were fed experimental diets containing sorghum. Significant differences were noted in protein and salt levels among the test groups. Protein levels rose as more sorghum was used, peaking at 18.00% in the R2 group. On the other hand, the amount of salt varied significantly between the groups, reaching its highest point in the R2 group at 3.80%. The moisture, ash, fat, and collagen levels did not differ significantly among the experimental groups. These findings indicate that adding sorghum mainly affects the protein and salt levels in common carp meat, which could affect its nutritional composition [7].

This suggests that dietary changes could affect protein levels in fish.

Table 4. Chemical composition of carp meat when given sorghum diets in an experiment

Parameter (%)	R0 (0%)	R1 (10%)	R2 (20%)	R3 (30%)
Protein	14.76±0.31	15.95±0.24	18.00±0.25	14.87±0.16
Fat	5.92±0.53^{ns}	5.34±0.58	6.72±0.24^{ns}	6.03±1.34^{ns}
Moisture	68.23±0.51^{ns}	69.72±0.33^{ns}	67.97±0.88^{ns}	68.91±0.59^{ns}
Ash	2.54±0.17^{ns}	2.96±0.2^{ns}	2.96±0.16^{ns}	2.93±0.16^{ns}
Salt	2.20±0.12	3.52±0.43	3.80±0.37	2.50±0.34
Collagen	1.12±0.09^{ns}	0.69±0.15^{ns}	0.62±0.28^{ns}	0.89±0.13^{ns}

Different lowercase letters represent statistically significant differences according to Tukey's test at $p<0.05$
ns= non-significant differences

These results emphasize how dietary changes can affect the makeup of fish meat, which in turn can influence its taste and nutritional benefits. Also, Hussein et al. (2016) [8] found a rise in protein levels in Nile tilapia meat. Sorghum was the ingredient examined in the diet in the study mentioned earlier. During the hematological examination, the blood parameters of the common carp do not show significant differences white blood cell counts remained constant in all groups with no significant differences observed. Figure 1 shows the function of the antioxidant enzymes SOD, CAT and GPX, the differences in enzyme function in common carp are affected by the type of tissue and the amount of sorghum in the diets used in the experiment.

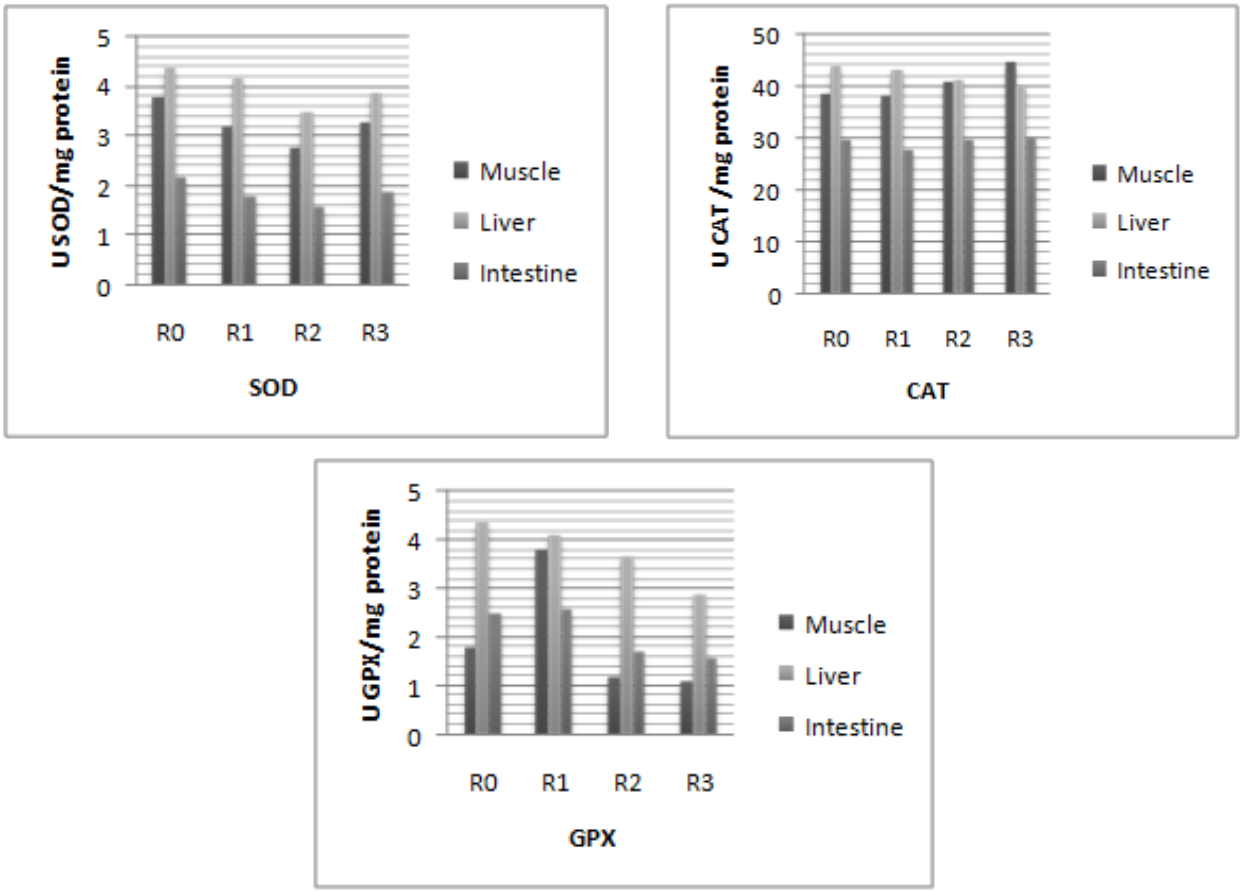

Figure 1. The effect of sorghum on oxidative status in the muscle, liver, and intestinal tissues of *Cyprinus carpio*
The enzymatic parameters consisted of measuring superoxide dismutase (SOD) catalase (CAT), and glutathione peroxidase (GPx), specific activities

As the amount of sorghum increased, there were significant changes in enzyme levels, especially with decreasing trends seen in SOD activity, particularly in the muscle 2.9 USOD/mg protein and intestine 1.6 USOD/mg protein tissue of R2 compared to the control 3.8 USOD/mg protein in

muscle and 2.6 USOD/ mg protein in intestine. Liver tissue also showed a decrease in SOD activity, dropping from 4.4 USOD/mg protein in the control to 3.2 USOD/mg protein in R2.

Nevertheless, the differences were not statistically significant. We noticed that the least amount of activity was found in the intestinal tissue, then in the muscle and liver tissues.

CAT function in the fish liver decreased in R1 compared to the control group. We noticed a rise in CAT activity in muscle tissue in R3 45.2 UCAT/mg protein in comparison to the control 38.4 UCAT/mg protein. In the tissue of the intestines, the differences were not important.

The liver's GPX in the control group was 4.3 UGPX/mg protein. The activity decreased as the sorghum concentration increased, with 3.9 UGPX/mg protein for the R1 variant, 3.3 UGPX/mg protein for the R2, and 2.2 UGPX/mg protein for the group with 30% sorghum. The enzyme function was nearly the same in the samples collected from the intestine tissue, with an average GPX value of 2.6 UGPX/mg protein in carp from the control group and 2.8 UGPX/mg protein in R1.

In the experimental groups that received 20% and 30% sorghum, the activity level reached 1.5 UGPX/mg protein and 0.6 UGPX/mg protein, respectively. The muscle tissue showed a noticeable difference in activity levels, with R1 having the highest average activity at 3.2 UGPX/mg protein. Meanwhile, R2 and R3 had lower enzyme activity compared to both the control group 0.8 UGPX/mg protein in R2 and 0.6 UGPX/ mg protein in R3, compared to 1.4 UGPX/ mg protein in the control and R1.

Information in books and articles demonstrates that sorghum has similar nutritional value to other grains in terms of its chemical makeup, fat, protein, carbohydrates, and polysaccharides, as well as its bioactive components.

Adding sorghum to carp feed at levels of 20% an 30% caused notable decreases in the carp's final body weight and weight gain. Nevertheless, when sorghum was included at a rate of 10%, these factors did not have any significant impact. Additionally, there were no changes in weight gain, feed conversion ratio, and condition factor in any of the variations, even when sorghum was used to replace corn and barley completely at a rate of 30%. These results are backed up by the near composition of sorghum [9].

The basic makeup of fish meat, such as water, fat, minerals, protein, collagen, and salt level, shows how nutritious and appealing it is to consumers. Although the moisture levels were similar across all treatment groups ranging from 68.23% to 69.72%, there were notable disparities in protein levels [10].

The use of sorghum in carp diets had different effects on MDA levels in the examined tissues (Figure 2). In muscle cells, the typical level of MDA in the control group was decreasing in experimental groups R1 and R2. However, in carp fed 30% sorghum, MDA levels were 1.25 times higher than in the control group [11].

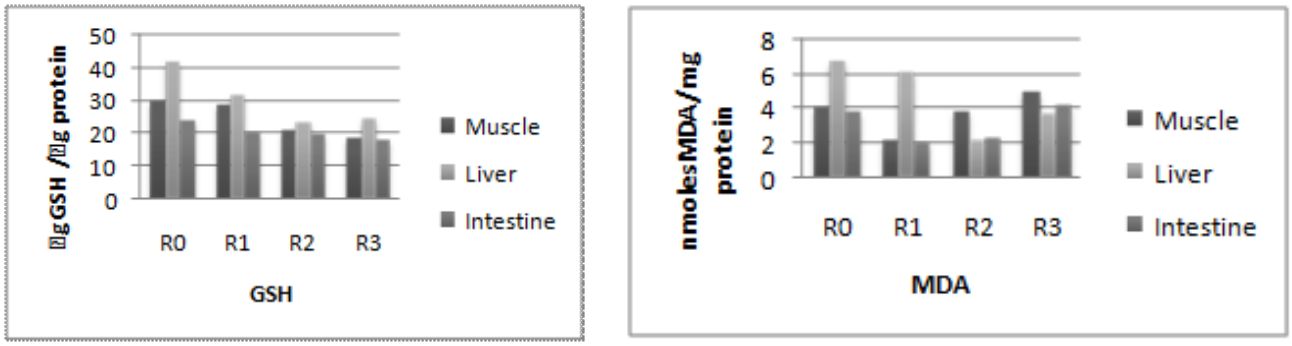

Figure 2. The impact of sorghum on the oxidative tatus found in muscle, liver and intestinal tissues
The non-enzymatic parameters consisted of estimating the levels of glutathione (GSH) and malondialdehyde (MDA)

A similar pattern was observed in intestinal tissue, with the highest MDA levels found in the control group and decreasing in the 10% sorghum concentration group. Liver tissue had the highest MDA levels, with R1 control strains having the highest mean values of 6.1 n mol MDA/ mg protein. Significantly, variant R2 presented the lowest value, at 2.1 n mol MDA/mg protein.

It shows a clear link between the amount of sorghum in carp diets and research on glutathione and malondialdehyde levels in different tissue types [12]. As the amount of sorghum increased, GSH levels decreased significantly, indicating a possible disruption of antioxidant defense systems. At the same time, MDA levels increased, indicating higher levels of oxidative stress [13].
The highest protein content was found in R2 18%, while the lowest was in the control group 14.76%. This suggests that dietary interventions have the potential to affect protein deposition in fish.
Oxidative stress in the unevenness between the creation of reactive oxygen species and the protection of antioxidants, which can change the shape and the operation of mitochondrial and cellular parts [14].

4. Conclusions

This research emphasizes the important effect of adding sorghum to the diet of common carp on different physiological measurements. While sorghum had a positive impact on the protein and salt levels in the fish's flesh, it had a negative effect on their growth performance and indicators of oxidative stress. These results emphasize the need to carefully think about the food makeup in fish diet to improve growth and overall health. Notably, when 10% sorghum was used, there were no major effects on the final body weight and weight gain. This suggests that there may be a threshold at which the negative effects on growth performance can be reduced.

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