

Article

Evaluating the Use of Grape Pomace in *Cyprinus carpio* Nutrition: Effects on Growth, Biochemistry, Meat Quality, Microbiota, and Oxidative Status

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Abstract: This study investigated the effects of incorporating grape pomace (GP) into fish diets on the growth performance, physiological parameters, and biochemical composition of carp (*Cyprinus carpio* L.). A total of 180 carp, with an average initial weight of 65 g, were reared in a recirculating aquaculture system (RAS). They were divided into a control group (C), fed with a diet containing 0% GP, and two experimental groups (V1 and V2), fed with diets containing 5% and 10% GP, respectively. The experiment lasted for eight weeks. Results revealed that most growth parameters were not significantly affected by GP inclusion, except for a notable difference in the hepatosomatic index (HSI), indicating an impact on liver size relative to body weight. The biochemical analysis of carp meat showed significant differences in moisture, protein, fat, collagen, and salt content between the control and experimental groups. Furthermore, the oxidative status assessment indicated that GP supplementation modulates oxidative stress and lipid peroxidation pathways in carp, enhancing their antioxidant defenses and overall health. Microbiological examination of the carp intestinal content showed that GP inclusion in fish diets influenced microbial parameters, particularly affecting the abundance of aerobic germs and Enterobacteriaceae.

Keywords: grape pomace; fish diets; growth performance; physiological parameters; biochemical composition; carp; aquaculture

Key Contribution: This study elucidates the potential of grape pomace (GP) as a sustainable feed ingredient in aquaculture by demonstrating its varied effects on the growth performance, physiological parameters, and biochemical composition of carp. GP supplementation notably influenced the hepatosomatic index, the biochemical composition of carp meat, oxidative stress pathways, and intestinal microbiota, highlighting its role in enhancing antioxidant defenses and influencing fish health.

1. Introduction

In the context of the circular economy, the growing global population, and the increasing production in aquaculture, finding new ingredients or utilizing previously unused



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by-products has become a priority. The evolution of aquaculture production has shown a positive trend in recent years, and it is the sector with the highest growth recorded, reaching a production value of 79,460 tons of cyprinids in Europe in 2021 [1]. Grape pomace is a by-product resulting from wine production and includes pressed grape skins, grape pulp, and seeds [2]. The proportion of grape pomace resulting from wine processing is 20–30% [3,4]. The main advantages of using grape pomace in carp nutrition are its antioxidant properties, content of fatty acids, proteins, fibers, minerals, low cost, and contribution to promoting a circular economy [5–7]. The main disadvantages of using grape pomace in carp nutrition are the presence of antinutritional factors, reduced palatability, and the need for it to be dried before incorporation into feeds [5–7]. Several studies have shown in various animal species that grape pomace may inhibit the development of bacteria, fungi, and protozoa, and it also exhibits anti-viral activity [8–10]. Grape pomace is used in ruminant feeding in combination with other more valuable feeds because it has a higher fiber content and lower digestibility. However, its true value has been recognized more recently as a potential source of valuable bioactive compounds, especially antioxidants such as anthocyanins [2,11]. In order to increase digestibility, studies have used polyethylene glycol to deactivate tannins [12,13]. Another method for increasing digestibility was the use of NaOH, which showed promising results but had the adverse effect of inhibiting microbial activity [14]. For use in animal nutrition, considering its seasonality, grape pomace is stored in the form of silage; otherwise, it is dried [15]. An important aspect of using grape pomace in fish nutrition has been the extraction of bioactive compounds such as polyphenols and anti-oxidants, using them to improve growth parameters, health status, and immunity [16]. The use of grape pomace is significant not only for its antioxidant compounds but also for promoting circular economy practices in sustainable management of agricultural by-products and waste reduction [6,8,17]. In fish, the use of grape pomace has focused on its bioactive compounds and its utilization as additives. Pulgar [5] showed in his study that the use of grape pomace in trout improved growth parameters. In carp, the use of grape pomace at a proportion of 15% had beneficial effects on growth parameters and survival rate [18]. For rainbow trout larvae, it was shown that inclusion of grape pomace at a proportion of 18% did not have negative effects on growth parameters [19]. In the New Zealand abalone species (*Haliotis iris*), a combination of grape residues with insect flour (*Tenebrio molitor*) was used, and it was shown that this combination did not lead to changes in growth parameters but resulted in some changes in the amino acid composition of the meat [20]. For the species *Dicentrarchus labrax*, it was shown that inclusion of grape pomace extracts rich in polyphenols affected the quality of the fillets [21]. In light of the diverse advantages and limitations associated with grape pomace, and recognizing the global quest for sustainable and alternative ingredients in fish nutrition, this study aims to explore the utilization of grape pomace in carp feed formulations, thereby addressing the growing demand for innovative and eco-friendly approaches to enhance carp growth and production.

2. Materials and Methods

2.1. Fish Farming and Management

For this experiment, we conducted directed natural reproduction using the broodstock of *Cyprinus carpio* L. sourced from our research station. The resulting fry were reared for three months in an earthen pond spanning 3000 square meters. Following this period, on September 1st, 180 specimens were selected for further experimentation. The selected fish were transferred to a recirculating aquaculture system (RAS) equipped with fiberglass tanks, each with a volume of 0.35 cubic meters of water. The RAS system was composed of fiberglass basins; supply pipes; a water drainage channel; a drum-type mechanical filter with a 100 micrometer sieve and a capacity of 250 m³ water per hour, which ensures a maximum load of suspended solids of 10 mg/L; a UV filter with 4 UV lamps of 75 W; a biological filter with plastic filter elements with a useful volume of 17 m³. Additionally, aeration was achieved with a 4.3 kw turbo blower, 380 mbar pressure.

Prior to the commencement of the trial, a two-week acclimatization period was provided for the fish. Following this, the fish underwent a two-day fasting period for standardization before the start of the experiment. Subsequently, the fish were individually measured and weighed. The feed trial commenced on September 17th with a water temperature of 21.5 °C and concluded on November 6th, coinciding with a water temperature of 14.6 °C (Table 1). Dissolved oxygen and temperature levels were monitored daily using the Hach HQ30d instrument from the Hach Company, Loveland, CO, USA. Additionally, weekly assessments of pH, ammonia, nitrites, nitrates, phosphorus, and conductivity were performed using the Hanna Iris HI801 Spectrophotometer and Hanna reagent kits from Hanna Instruments, Salaj, Romania, in conjunction with the Hach HQ11d instrument from the Hach Company.

Table 1. Mean Values of the Water Parameters in RAS During Eight Week (W) Experiment.

Parameters	W1	W2	W3	W4	W5	W6	W7	W8
Temperature (°C)	21.50	20.60	18.90	17.40	17.40	16.80	16.40	14.60
Oxygen	8.03	9.15	8.82	9.29	8.74	9.64	9.96	9.80
pH	8.10	8.00	8.00	8.10	8.10	8.10	8.00	8.00
NH ₃ (Amonia)	0.01	0.05	0.02	0.00	0.01	0.02	0.01	0.00
NH ₄ ⁺ (Ammonium)	0.02	0.06	0.02	0.00	0.04	0.03	0.02	0.00
NO ₂ ⁻ (Nitrites)	0.12	0.05	0.01	0.01	0.03	0.14	0.05	0.01
NO ₃ ⁻ (Nitrates)	5.00	27.80	13.00	16.60	10.6	9.00	3.20	2.70
TH (Total Hardness)	8.00	8.10	8.20	7.80	7.60	7.600	7.20	7.40
Ca	96.00	88.00	100.00	82.00	84.00	98.00	102.00	98.00
Mg	60.00	74.00	76.00	78.00	72.00	80.00	80.00	72.00
Fe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SO ₄ ²⁻ (Sulfate)	139.00	126.00	122.00	105.00	110.00	129.00	122.00	128.00
TP (Total Phosphorus)	0.01	0.01	0.02	0.01	0.03	0.02	0.01	0.01

2.2. Grape Pomace Preparation and Composition

The grape pomace was dehydrated, ground, and combined with the base feed in the proportions shown above, and it was obtained from white grapes of various varieties. The biochemical composition of grape pomace (Table 2) was analyzed with the DA 7250 NIR Analyzer (Pertin Instruments, Hagersten, Sweden) to determine the proportions used in the production of the test feeds.

Table 2. Chemical Composition of Grape Pomace.

GP Material	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	p (%)	Fiber (%)	Starch (%)	Sugar (%)	Ca (%)
Dried	9.02	16.33	6.73	10.77	0.74	8.75	18.73	9.72	0.40
Fresh	20.61	18.26	9.34	0.67	1.36	4.71	13.43	4.66	6.09
Bioactive compounds and antioxidant activity									
Antioxidant									
	Polyphenols (mg/g d.w.)	Flavonoids (mg/g d.w.)	activity (DPPH % inhibition)						
Dried	83.07	13.53	81.87						

d.w. dry weight; DPPH 2,2-diphenyl-1-picrylhydrazyl.

2.3. Production of Carp Diets Based on Grape Pomace

The following three dietary treatments were prepared for the experiment: a control group (grape pomace 0%) and two experimental groups, V1 (grape pomace 5%) and V2 (grape pomace 10%). Each treatment was replicated in triplicate with 20 fish per water tank.

The diets were formulated according to the following recipes (Table 3). The control diet consisted of sunflower meal (45%), peas (20%), maize (17%), barley (5.5%), DDGS (10%), and soybean oil (2.5%), with no grape pomace being included.

Table 3. Experimental Diets Recipes with GP for Carp.

Ingredient (%)	Control (GP 0%)	V1 (GP 5%)	V2 (GP 10%)
Sunflower meal	45	45	44.5
Peas	20	20	20
Maize	17	12.3	7.6
Barley	5.5	5	5
DDGS	10	10	10
Soybean oil	2.5	2.7	2.9
Grape pomace	0	5	10
Proximate composition (%)			
Moisture	11.44	11.06	11.08
Protein	22.45	22.16	22.89
Fat	6.24	6.01	6.37
Ash	9.03	9.87	9.50
p	0.89	0.97	0.95
Fiber	8.66	8.41	8.24
Starch	25.37	25.27	25.7
Sugar	4.14	4.66	4.63
Ca	2.74	3.03	3.12

The ingredients were initially extruded, then ground and mixed according to the specified proportions. The mixture was subsequently pelleted in our own feed producing facility. The biochemical composition of feeds (Table 3) was analyzed with the DA 7250 NIR Analyzer (Perten Instruments). The instrument was calibrated for meat (all types), feed ingredients, formulated feeds, and oils. For the analyses, the samples were put on a small rotating tray, the calibration was selected, and the result was ready in six seconds. The sample was mixed and read nine times.

Based on the total weight of the fish and the water temperature, the daily feed rations used were 3%. Feeds were administered three times a day at 9:00 AM, 12:00 PM, and 3:00 PM to ensure consistent feeding practices throughout the experiment duration. The experimental period spanned eight weeks.

2.4. Fish Growth Indices

Weekly, fish length and weight (w) were measured ($n = 15$).

CF—condition factor = $(\text{FBW}/\text{body length}^3) \times 100$;

IBW—initial body weight (g);

FBW—final body weight (g);

WG—weight gain (g) = $\text{FBW (g)} - \text{IBW (g)}$;

FCR—feed conversion ratio (g/g) = $\text{Feed intake (g)}/\text{WG (g)}$;

PER—protein efficiency ratio (g/g) = $\text{WG (g)}/\text{total protein (g)}$;

HSI (hepatosomatic index, %) = $100 \times [\text{final liver weight (g)}/\text{final body weight (g)}]$;

VSI (viscerosomatic index, %) = $100 \times [\text{final visceral weight (g)}/\text{final body weight (g)}]$.

2.5. Body Composition

Proximate body composition parameters were calculated using DA 7250 NIR Analyzer (Perten Instruments).

2.6. Blood Parameters

The fish were anesthetized with clove oil (0.2%), and blood was sampled by heart puncture. The following hematological parameters were evaluated using an Abacus Vet 5 analyzer

(courtesy of Nova Group, Budapest, Hungary): WBC—white blood cells, LYM—lymphocytes, MON—monocytes, NEU—neutrophils, EOS—eosinophils, BAS—basophils, RBC—red blood cells, HGB—hemoglobin, HCT—hematocrit, MCV—mean corpuscular volume, PLT—platelets, MPV—mean platelet volume, PCT—plateletcrit.

2.7. Biochemical Parameters Assay

The tissue samples were homogenized in an ice-cold potassium phosphate-buffered solution 0.1 M, KCl 1.15%, pH 7.4 in a ratio of 1:10 (*w/v*). The homogenates were centrifuged (20 min at 3000 rpm and 4 °C) and the supernatants were further used to measure the biochemical parameters. The biochemical tests consisted of the determination of superoxide dismutase (SOD) by the nitro blue tetrazolium method, catalase activity (CAT) by the Sinha method, glutathione peroxidase (GPx) by the spectrophotometric method with 5,5'-dithiobis-2-nitrobenzoic acid, the content of reduced glutathione (GSH), and the malon-dialdehyde concentration (MDA) with 2-thiobarbituric acid, according to the methods described in Brinza et al. [22]. The SOD, CAT, and GPX activities and the levels of GSH and MDA were normalized to the total content of soluble proteins measured by the Bradford method [23].

2.8. Microbiological Exam

To conduct the microbiological examination, 1 g of intestinal content was meticulously sampled from carp under laboratory conditions to ensure optimal sterility during the sampling process. Subsequently, three fish from each experimental batch underwent analysis. The bacterial groups under investigation included the total number of aerobic germs (TNG-A/g intestinal content), total number of anaerobic germs (TNG-AN/g intestinal content), total number of sulphite-reducing clostridia (TNSRC/g intestinal content), and total number of *Enterobacteriaceae* (TNE/g intestinal content). Mean and logarithmic values (Lg10) were calculated for each experimental group, and the quantitative values were expressed in CFU/g (colony forming units/g).

2.9. Statistical Analysis

The data were statistically processed by ANOVA followed by a Tukey Test ($p < 0.05$) using the SPSS software version 21 (IBM Corp, Armonk, NY, USA). The results were reported as means $\pm$ standard errors.

3. Results

3.1. Effect of Grape Pomace Inclusion in Carp Feed on Growth Parameters and Physiological Responses

The growth parameters and physiological responses were analyzed across treatments incorporating grape pomace (GP), as detailed in Table 4. Overall, no significant differences were observed in most parameters, including CF, IBW, FBW, WG, PER, FCR, and VSI ($p > 0.05$). However, there was a notable discrepancy in hepatosomatic index (HSI) among treatments, with a significant difference being detected ($p = 0.032$). Specifically, HSI values varied between 0.5 for the control group, 0.7 for V1, and 0.4 for V2, indicating a distinct impact of the treatments on liver size relative to body weight.

Table 4. Effect of Grape Pomace Supplementation on Growth and Physiological Parameters in Carp.

Parameter	Control (GP 0%)	V1 (GP 5%)	V2 (GP 10%)	Anova (<i>p</i> -Value)
CF	2.15 $\pm$ 0.17	2.03 $\pm$ 0.13	2.04 $\pm$ 0.08	0.776
IBW (g)	63.91 $\pm$ 2.58	68.60 $\pm$ 2.39	65.60 $\pm$ 2.69	0.426
FBW (g)	106.20 $\pm$ 5.01	115.40 $\pm$ 5.12	106.06 $\pm$ 5.21	0.341
WG (g)	42.30 $\pm$ 2.39	46.80 $\pm$ 4.08	40.46 $\pm$ 1.62	0.397
PER (g/g)	0.50 $\pm$ 0.03	0.45 $\pm$ 0.04	0.52 $\pm$ 0.02	0.394

Table 4. *Cont.*

Parameter	Control (GP 0%)	V1 (GP 5%)	V2 (GP 10%)	Anova (<i>p</i> -Value)
FCR (g/g)	2.41 ± 0.89	2.54 ± 1.05	2.54 ± 1.06	0.994
HIS (%)	0.55 ± 0.10 ab	0.73 ± 0.05 a	0.40 ± 0.02 b	0.032
VSI (%)	16.52 ± 1.61	14.96 ± 2.19	14.06 ± 0.18	0.565

Different lowercase letters represent statistically significant differences according to Tukey's test at $p < 0.05$.

3.2. Biochemical Composition of Carp Meat

The proximate composition of carp meat varied across diets supplemented with grape pomace (Table 5). Diet C, serving as the control with no grape pomace (GP) inclusion, exhibited the highest moisture (72.3%) and salt content (2.83%). Diet V2, with the highest GP inclusion (10%), showed the highest protein (14.5%) and ash (2.6%) levels. Diet V1, with a lower GP inclusion (5%), displayed the highest fat content (13.23%), while Diet V2 had the lowest collagen content (0.2%). These findings highlight the influence of grape pomace supplementation on the composition of carp meat.

Table 5. Biochemical Composition of Carp Meat (%).

Diets	Moisture	Protein	Fat	Ash	Collagen	Salt
Control (GP 0%)	72.3 ± 0.31 a	13.8 ± 0 b	6.97 ± 0.17 c	1.53 ± 0.03 b	0.8 ± 0.12 b	2.83 ± 0.18 ns
V1 (GP 5%)	69.4 ± 0.17 b	12.83 ± 0.15 c	13.23 ± 0.2 a	2.1 ± 0.15 ab	1.27 ± 0.03 a	2.43 ± 0.15 ns
V2 (GP 10%)	72.27 ± 0.35 a	14.5 ± 0.23 a	8.13 ± 0.17 b	2.6 ± 0.17 a	0.2 ± 0.06 c	2.57 ± 0.28 ns

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

3.3. Hematological Profile

Table 6 presents the hematological profile of carp subjected to different dietary treatments involving grape pomace supplementation. The results indicate varied effects of grape pomace supplementation on the hematological parameters of carp. While some parameters, such as WBC, LYM, NEU, EOS, BAS, RBC, HGB, HCT, MCV, PLT, and PCT, did not show significant differences across the groups, MON and MPV exhibited notable variations. Specifically, MON levels were significantly elevated in the V1 group compared to the control, indicating a potential influence of grape pomace on the monocyte count. Additionally, MPV values displayed a significant increase in both V1 and V2 groups compared to the control, suggesting alterations in platelet size, possibly due to dietary factors. These findings suggest that incorporating grape pomace into fish diets may impact certain hematological parameters and have potential implications for fish health and physiology.

Table 6. Hematological parameter.

Parameter	Control (GP 0%)	V1 (GP 5%)	V2 (GP 10%)	Anova (<i>p</i> -Value)
WBC ($\times 10^9$ /L)	51.95 ± 3.18	49.67 ± 1.7	47.03 ± 3.28	0.492
LYM ($\times 10^9$ /L)	38.02 ± 5.87	18.43 ± 6.97	34.00 ± 2.57	0.072
MON ($\times 10^9$ /L)	0.23 ± 0.03 b	1.22 ± 0.12 a	0.88 ± 0.13 a	0.00
NEU ($\times 10^9$ /L)	3.72 ± 0.55	3.25 ± 0.46	3.41 ± 0.32	0.769
EOS ($\times 10^9$ /L)	0.65 ± 0.12	0.64 ± 0.04	0.59 ± 0.03	0.829
BAS ($\times 10^9$ /L)	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.188
RBC ($\times 10^{12}$ /L)	1.65 ± 0.10	1.67 ± 0.19	1.71 ± 0.06	0.945
HGB (g/dL)	8.8 ± 0.42	9.53 ± 0.14	9.18 ± 0.34	0.327
HCT (%)	27.33 ± 1.00	28.74 ± 3.96	29.15 ± 0.99	0.858

Table 6. Cont.

Parameter	Control (GP 0%)	V1 (GP 5%)	V2 (GP 10%)	Anova (<i>p</i> -Value)
MCV (fL)	166.00 ± 4.78	171.25 ± 5.45	170.75 ± 4.66	0.721
PLT (×10 ⁹ /L)	42.00 ± 3.49	43.75 ± 4.27	35.5 ± 8.37	0.588
MPV (fL)	9.58 ± 0.14 b	10.95 ± 0.22 a	11.15 ± 0.28 a	0.001
PCT (%)	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.01	0.555

White blood cell count (WBC), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), eosinophils (EOS), basophils (BAS), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), platelet count (PLT), mean platelet volume (MPV), and plateletcrit (PCT). Different lowercase letters represent statistically significant differences according to Tukey's test at $p < 0.05$. 'ns' denotes non-significant differences.

3.4. Oxidative Status

Figure 1 presents the results of the oxidative status assessment in carp specimens under different dietary conditions supplemented with grape pomace. The observed patterns suggest that grape pomace supplementation may modulate oxidative stress and lipid peroxidation pathways in carp, potentially enhancing their antioxidant defenses and overall health.

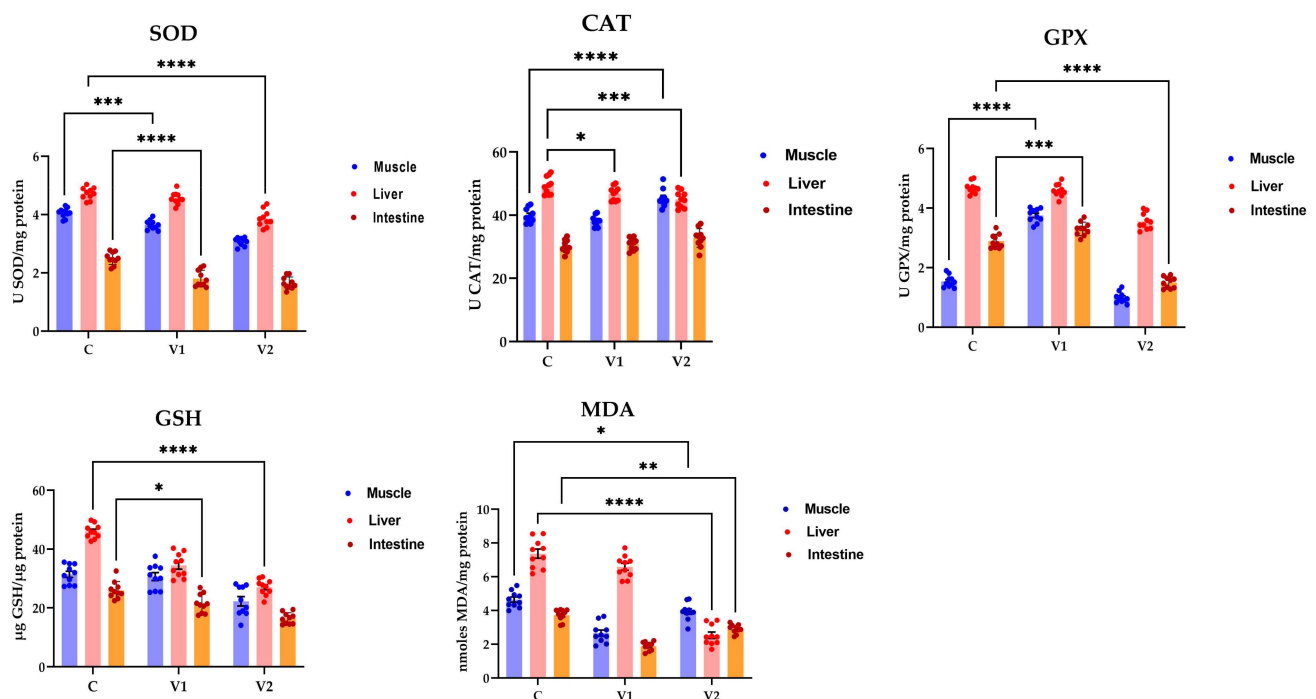


Figure 1. Grape Pomace influence on the oxidative status determined in muscle, liver, and intestine tissues of *Cyprinus carpio*. The enzymatic parameters consisted of measuring SOD, CAT, and GPx-specific activities, while the non-enzymatic parameters consisted of estimating the levels of GSH and MDA. The values are expressed as means ± S.E.M. ($n = 12$). Two-way ANOVA analysis revealed overall significant differences between the experimental groups. For the Tukey multiple comparisons analysis—**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

The fluctuation in activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), across different tissues highlights the dynamic nature of antioxidant defense mechanisms in carp. For instance, in the liver tissue, SOD activity was highest in the control group (4.723 ± 0.195 USOD/mg protein), while, in the experimental group V1, SOD activity decreased to 4.596 ± 0.202 USOD/mg protein, and it further decreased to 3.551 ± 0.258 USOD/mg protein in group V2. Similarly, CAT activity showed variations across tissues, with maximum activity observed in the

experimental group V2 for muscle tissue (45.341 ± 2.631 UCAT/mg protein) and intestines (32.769 ± 2.808 UCAT/mg protein).

The changes in glutathione (GSH) levels and malondialdehyde (MDA) concentrations reflect alterations in antioxidant capacity and lipid peroxidation, respectively. Notably, in the liver tissue, the control group exhibited higher GSH concentration (4.678 ± 0.183 UGPx/mg protein) compared to the experimental groups V1 (4.596 ± 0.202 UGPx/mg protein) and V2 (3.551 ± 0.258 UGPx/mg protein). Conversely, in muscle tissue, the GSH concentration was highest in experimental group V1 (34.455 ± 3.819 μ g GSH/ μ g protein), indicating tissue-specific responses to grape pomace supplementation. Similarly, the concentration of MDA, a marker of lipid peroxidation, showed variations across tissues and experimental groups, with significant decreases being observed in the experimental group V1 compared to the control group, particularly in muscle tissue (2.651 ± 0.559 nmoles MDA/mg protein).

3.5. Microbiological Exam

Figure 2 presents the results of the microbiological examination conducted on the intestinal content of carp specimens under different diets with grape pomace. The total number of aerobic germs (TNG-A) and the total number of Enterobacteriaceae (TN-E) showed significant differences between the control group and the experimental groups V1 and V2 ($p < 0.05$). In contrast, the total number of anaerobic germs (TNG-AN) and the total number of sulphite-reducing clostridia (TN-SRC) did not exhibit significant differences between the experimental groups ($p > 0.05$). These results suggest that the inclusion of grape pomace in the fish diets may have an impact on certain microbial parameters within the intestinal content of carp specimens, particularly affecting the abundance of aerobic germs and Enterobacteriaceae.

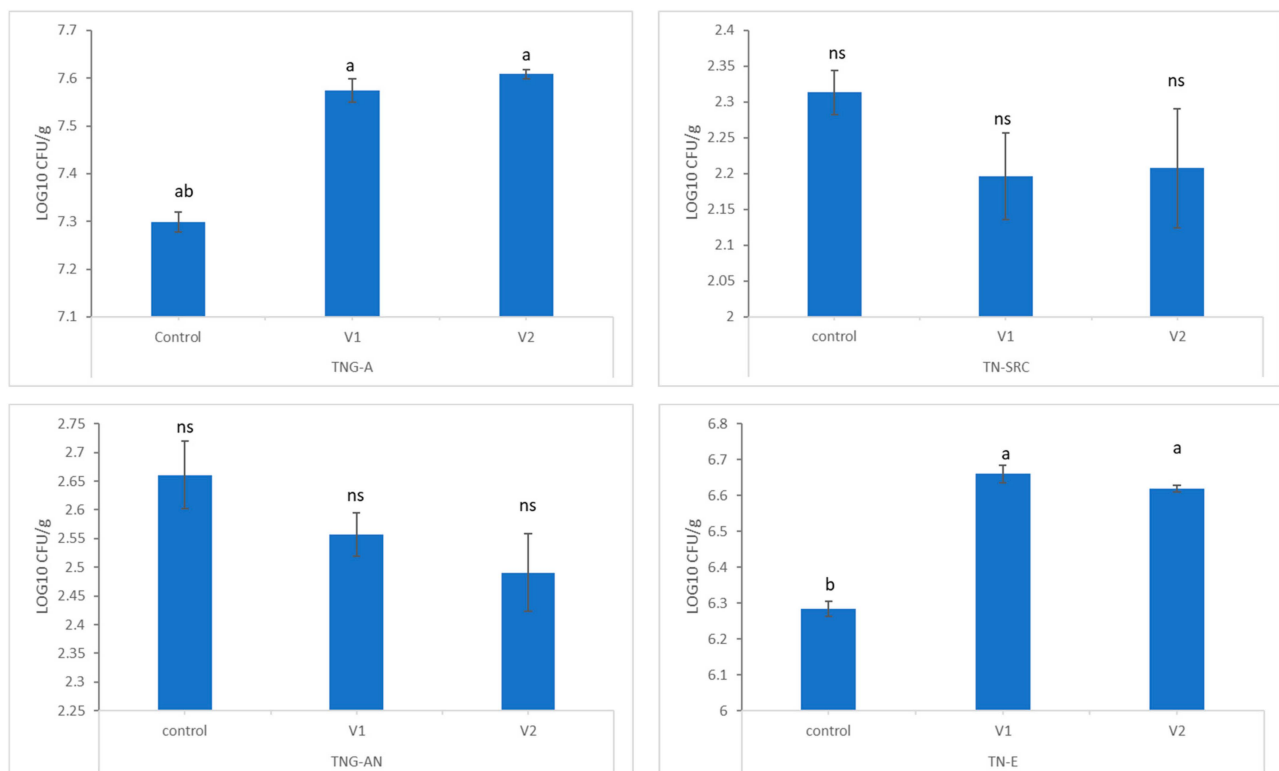


Figure 2. Microbiological examination of carp intestinal content under varied grape pomace diets. TNG-A (total number of aerobic germs), TNG-AN (total number of anaerobic germs), TNSRC (total number of sulphite-reducing clostridia), and TNE (total number of Enterobacteriaceae). Different lowercase letters represent statistically significant differences according to Tukey's test at $p < 0.05$. 'ns' denotes non-significant differences.

4. Discussion

The incorporation of grape pomace (GP) into carp diets aimed to explore its potential as a sustainable feed ingredient. While most growth parameters remained unaffected by GP inclusion, the significant difference in the hepatosomatic index (HSI) indicates a notable impact on liver size relative to body weight, similar to results obtained by Mahmoodi et al. [18]. This alteration in HSI suggests a metabolic response to GP inclusion, potentially related to nutrient utilization or detoxification processes [18]. The lack of significant differences in other growth parameters such as condition factor (CF), final body weight (FBW), or weight gain (WG) suggests that GP inclusion did not compromise overall growth performance even at the highest concentration of 10%. Moreover, the rich content of GP in protein and bioactive compounds [5–7] can have a stimulating effect. For instance, Mahmoodi et al. (2023) [18] tested the inclusion of GP in the diet of carp with an initial weight of 7 g over a duration of 56 days and observed a significant increase in growth parameters, such as WG, along with a decrease in FCR at 15% grape pomace inclusion. Additionally, in the same study, VSI and CF showed significant growth. Peña et al. [19] reported positive results on growth performances and FCR at an 18% inclusion level of GP in rainbow trout.

The biochemical analysis of carp meat revealed significant variations in moisture, protein, fat, and collagen content among dietary treatments. Notably, the highest GP inclusion (10%) led to an increased protein level in carp meat, indicating a potential enhancement in nutritional quality. It is well known that fish is an important source of protein for the human diet, with the nutritional value of fish meat being also given by its protein content [24]. Conversely, lower GP inclusion (5%) resulted in higher fat content, which may affect the sensory attributes and lipid profile of carp meat. These findings are in agreement with other reports on carp [18] and under-score the potential of GP supplementation to modulate the nutritional composition of carp meat, thereby influencing its quality and consumer acceptability.

Analyzing the results obtained via hematologic examination in this study, slight changes can be observed. In both groups, lymphopenia was associated with monocytosis. In the GP 5% group, the lymphocyte number was reduced almost by half, possibly because of the stress or intestinal microbiota as a result of the action of active molecules from administered diets on the gut microorganisms. Depending on their sensitivity or nutritional substrate, the multiplication of some bacteria species to others' detriment is favored, causing microbial population ratio changing, resulting in effects on blood cell populations. Other factors can be related with the environment parameters. In the GP 10% group, the platelet number was reduced. On the other hand, the RBC number and increased hemoglobin can be a sign for improving oxygen circulation into the tissues.

The increase in the number of enterobacteria creates, for shorter or longer periods of time, a change in intestinal homeostasis, with an effect on immune cells. Local immunity is served by cells (macrophages) located in the intestinal wall, which serve as sentinel cells and feature special phagocytic abilities (type 1 macrophages). Equally, some bacterial cells have the ability to survive inside macrophages and can multiply at this level [25,26]. Monocytes represent the circulating form of macrophages, with polarization occurring at the tissue level under the influence of differentiating factors. Enterobacteria, as Gram-negative cells, have in their wall structure the so-called lipopolysaccharides (LPSs), highly effective stimulators of Toll-like receptors (TLRs), on the surface of macrophages, activating them and causing the release of pro-inflammatory cytokines that attract circulating monocytes and, at the same time, intensify their differentiation from precursor cells, thus explaining monocytosis [27].

Under physiological processes in the body, the elevated intracellular levels of re-active oxygen species (ROS) related to oxidative stress can cause destructive effects on lipids, proteins, and DNA [23]. It is known that, in fish, like other organisms, the lack of balance between production of ROS and the antioxidant defense related to oxidative stress can cause DNA hydroxylation, protein denaturation, lipid peroxidation, apoptosis, and cell damage.

The hydroxyl radicals and hydrogen peroxides are among the most important free oxygen radicals within the ROS. The formation of the ROS is crucial for the maintenance of cell homeostasis, and living organisms achieve this by a system of antioxidant defense, allowing them to maintain a balance between oxidative stress and antioxidant protection [28,29].

To avoid the negative effects of naturally producing ROS, the living organisms developed an antioxidant defense system with two distinct classes: (a) the enzymatic antioxidant system, including different enzymes such as SOD, CAT and GPx, and (b) the non-enzyme antioxidants (glutathione, thioredoxin, vitamin C and vitamin E [23,30]). This system is very important, as malfunctioning can cause an imbalance in the system of homeostasis and oxidative stress [31,32].

The literature data indicate that the improvement of the antioxidant capacity can be stimulated by different techniques, one of the approaches referring to the administration of synthetic antioxidants or from different natural sources [28].

Grape pomace is one of the most important residues resulting from the wine-making process, being considered an abundant source of phenolic acids (gallic acid, protocatechuic acid (p-hydroxyphenylacetic acid, vanillic acid, homovanillic acid, homoprotocatechuic acid, gentisic acid, syringic acid, 4-O-methylgallic acid, 3-O-methylgallic acid, dihydro-3-coumaric acid, hydroferulic acid, hydrocaffeic acid, isoferulic acid), proanthocyanidins, anthocyanins (delphinidyl-3-O-glucosides, cyanidin-3-O-glucosides, petunidin-3-O-glucosides, peonidin-3-O-glucosides, malvidin-3-O-glucosides), resveratrol, and stilbenes [33], hence the multitude of beneficial effects on the animal body, including pronounced antioxidant, neuroprotective, and antitumor effects, as well as effects in regulating metabolic syndrome, regulating endothelial function, protecting blood vessels, and regulating bile acid metabolism [34,35]. In addition, grape pomace maintains lipid homeostasis, with an anti-obesity effect, and anti-insulin resistance activity, and it also features pronounced anti-inflammatory activity, manifested, in particular, in ischemic heart disease [36].

In recent years, humanity has made frequent use of ecological food resources, with aquaculture being the fastest-growing food production industry. Furthermore, it is known that fish nutrition management is essential to minimizing costs and maximizing growth in sustainable aquaculture practices. The literature data [18] indicate that, in fish, a diet rich in pomace grape improves the antioxidant status, growth performance, as well as the body's resistance to various diseases, the use of useful and economical foods being necessary to keep production costs as low as possible.

At the same time, the multitude of bioactive compounds from the grape pomace used in the food of *Ctenopharyngodon idella* specimens infected with *Pseudomonas aeruginosa* exerted an effect of mitigating liver lipid peroxidation and protein carbonylation, as well as stimulating the activity of antioxidant enzymes such as CAT and SOD [37].

Regarding the activity of SOD, as can be seen from Figure 1, the enzyme records wide variations depending on both the investigated tissue and the quantity of the administered grape pomace. Thus, when, in the control group, SOD reached activity levels of 4.052 ± 0.171 USOD/mg protein in the muscle, 4.723 ± 0.195 USOD/mg protein in the liver tissue, respectively, 2.496 ± 0.203 USOD/mg protein in the intestine samples, and the experimental variants featured food rations supplemented with grape pomace (5 and, respectively, 10%), a decrease in enzyme activity was observed, closely related to the dose of administered grape pomace. The minimum activity was recorded in the intestine, followed by muscle and liver, with the V2 variant (with 10% pomace in the daily feed), being marked by lower activities, in the case of all types of tissue, compared to the other groups.

The CAT activity, featuring an enzyme with a role in protecting cells from the toxic effects of hydrogen peroxide (which it mobilizes at high speed to convert to O_2 and H_2O without producing other free radicals [38]), registers fluctuations depending on the analyzed tissue and the amount of grape pomace administered. Thus, in the case of muscle and intestinal tissue, the enzyme shows a maximum level of activity in the variant in which

grape pomace was administered in a concentration of 10% (45.341 ± 2.631 UCAT/mg protein, respectively, 32.769 ± 2.808 UCAT/mg protein).

Another enzyme taken to be into account is GPx, an antioxidant intracellular enzyme present in various animal tissues, blood plasma, and erythrocytes that catalyzes the reduction in hydrogen peroxide in water and lipid hydroperoxides at the corresponding alcohols to limit its harmful effects [39]. The enzyme modulates the balance between necessary and harmful levels of ROS, using glutathione as a reducing agent. Figure 1 shows the fluctuation of the enzyme activity in the experimental variants of *Cyprinus carpio* L. in close dependence with the dose of grape pomace added to the daily diet, but also with the type of tissue analyzed. Thus, if, in the control group, the average activity of GPx in the liver tissue was 4.678 ± 0.183 UGPx/mg protein, in the case of the groups fed with a diet supplemented with grape pomace, the activity decreased in an inversely proportional relationship with the concentration of the supplement administered (4.596 ± 0.202 UGPx/mg protein in the V1 variant and 3.551 ± 0.258 UGPx/mg protein in the case of the group with 10% grape pomace). In contrast, at the level of muscle and intestinal tissue, a completely different situation was observed, with the highest average activity being recorded in the groups of carp fed with pomace at a 5% concentration (3.753 ± 0.55 UGPx/mg protein in muscle, respectively, 3.330 ± 0.214 UGPx/mg protein in intestine). At the same time, in the experimental variant V2, the activity of the enzyme was clearly lower compared to the control group both in the case of muscle tissue (0.993 ± 0.173 UGPx/mg protein compared to 1.536 ± 0.189 UGPx/mg protein in the control) and in the intestine (1.499 ± 0.181 UGPx/mg protein compared to 2.885 ± 0.181 UGPx/mg protein).

Another objective of our study was to determine the level of GSH—a tripeptide that directly or indirectly regulates the elimination of ROS and their reaction products [40]. Both grape pomace-based diets, especially the one with a content of 10% (V2), lowered the GSH level in the muscle, liver, and intestine compared to the fish fed with standard food (Figure 1). Thus, in the experimental variant V1, the average concentration of GSH registered an average threshold of 34.455 ± 3.819 $\mu\text{g GSH}/\mu\text{g protein}$ in the hepatic tissue, 30.674 ± 3.995 $\mu\text{g GSH}/\mu\text{g protein}$ at the muscle level, and 21.519 ± 3.074 $\mu\text{g GSH}/\mu\text{g protein}$ in the intestine.

At the same time, it is known that grape pomace has been used in aquaculture nutrition due to the presence of various active compounds (flavonoids, alkaloids, phenolics, pigments, steroids, terpenoids), enhancing appetite, combating microbes, and other stressful factors [41]. Moreover, the literature on the field [42] indicates that antioxidants are essential compounds in foods that are very efficient in preventing body-damaging reactions, such as the oxidation of lipids caused by oxidative stress, with MDA being a marker of oxidative stress and oxidative status of the animal and human organism [43,44].

From Figure 1, we can observe that the administration of grape pomace in the daily food ration of individuals of *Cyprinus carpio* L. had a different influence on the content of this biochemical indicator in the analyzed samples. Thus, in the case of the muscle tissue, we observe that, in the control group, the average concentration of MDA showed an average value of 4.645 ± 0.457 nmoles MDA/mg protein, while, in the experimental variant V1, there was a decrease in the content of this indicator of lipid peroxidation up to 2.651 ± 0.559 nmoles MDA/mg protein. A similar situation can also be observed in the case of the samples analyzed from the intestinal tissue; in the reference group, an average concentration of MDA equal to 3.724 ± 0.332 nmoles MDA/mg protein was recorded, and in the variant in which grape pomace was administered at a concentration of 5%, the average MDA level decreased by up to 50% of the value of the reference group (1.887 ± 0.244 nmoles MDA/mg protein).

The highest concentrations of MDA were recorded in the liver tissue, with the literature data indicating the significant influence of dietary supplementation with different agents on the concentration of MDA in various organs, the highest values being, as a rule, at the level of the liver [45]. Thus, in the control group, the average concentration of MDA was 7.365 ± 0.806 nmoles MDA/mg protein, while, in the experimental variant V1, there was a

slight decrease (6.591 ± 0.615 nmoles MDA/mg protein), additionally, in the V2 variant, this parameter reached only 66% of the value detected in the batch to which no grape pomace was added in the daily diet.

In explaining the results, we must take into account the influence of climate and environment due to their capacity to pollute the soils and reduce the availability of other ingredients in regard to obtaining high quality grape varieties and, implicitly, a high content of nutritive compounds in the grape pomace [46].

In addition, the experimental results obtained by us, in our case regarding carp specimens fed with a diet supplemented with grape pomace, correlate with the literature data, which indicate the importance of using grape pomace as an additive in piglet feed, the importance of which resides both in the development of innovative feeds and their potential beneficial effects on growth rate, productivity, and meat quality, with the antioxidant mechanisms being significantly stimulated compared to the reference group [47].

At the same time, it was demonstrated that the inclusion of this secondary solid product of the oenological industry in the cattle diet led to a significant positive increase in the ratio of polyunsaturated fatty acids-saturated fatty acids and an interesting improvement in the oxidative stability of meat, probably as an effect of the antioxidant activity achieved by the bioactive compounds in which grape pomace is rich [48].

Last, but not least, in a study that evaluated the effects of including in the diet of *Liza aurata* fry some products of the winemaking industry, including grape pomace—with a demonstrated high content of phenolic compounds—the antioxidant and immunostimulant capacity of this secondary product was remarked, as well as the positive effect that it exerted on the growth, immunity and metabolism of the golden gray mullet fry [49].

Fish microbiome is shaped by a multitude of host-associated factors actively participating in strengthening the digestive and immune systems [50]. Microbial diversity was reported in various specialized studies [51,52], showing that taxonomy variability fluctuates with diet [53]. Nevertheless, the results are difficult compare, as the tested fish species, diet, type of food, and microorganism groups are different. The major role of microorganisms in nutrient absorption and digestion is well known, influencing the health [42] and metabolic capacity [54] in all animal species. Evaluation of microbiological indicators provides general data on how these microorganisms can fluctuate under the action of a nutritional supplement. Grape pomace is an agro-industrial waste obtained from grapes with great potential for valorization in aquaculture, and our experiment results supports other studies [55], showing that the high amounts of fiber, as well as phenolic compounds, in grape pomace have some effects on the intestinal microbiota. Regarding the biological functionality of this product, studies performed on simulated digestion show that grape pomace has the necessary properties to maintain physical integrity until reaching the large intestine, where microorganisms use phenolic compounds to obtain metabolites that are easily absorbed in the intestine [55].

The results of our study confirm bacterial metabolism activation, inducing aerobic bacteria (NT-A) and Enterobacteriaceae (NT-E) increases. In opposition, a decrease in the number of anaerobic bacteria (NT-AN), and especially sulfite-reducing bacteria (N-SRC), was observed. The reduction in the number of pathogenic sulphite-reducing clostridia is an important result considering the implications of this microbiological indicator in regard to public health [56], an aspect also signaled by other authors [57]. The quantitative evaluation of the selected microbiological indicators provides general data on the effect of the tested diets on the intestinal microbiota, aspects that have also been highlighted in previous studies [58,59]. Comparing the experimental results with those obtained in the control group, it appears that GP provides important nutrients that favor the multiplication of commensal microflora in the digestive tract. The gut microbiota can alter its ratio of aerobic and anaerobic bacteria. As a result of diet administration, it has been observed that aerobic bacteria are favored, increasing their density, in opposition to populations of sulphite-reducing clostridia, whose density decreases. It is normal for this change in the local microbiome, which maintains a state of homeostasis, to generate some reactions from

the immune system, increasing the differentiation of some cell populations or decreasing the number of circulating cells.

5. Conclusions

Incorporating grape pomace into carp diets had varied effects on growth performance, physiological parameters, and biochemical composition. While most growth parameters remained unaffected, significant differences were observed in the hepatosomatic index, indicating a notable impact on liver size relative to body weight. The biochemical composition of carp meat was also altered, with differences in the moisture, protein, fat, collagen, and salt content between the control and experimental groups being noted. Furthermore, grape pomace supplementation showed potential in modulating oxidative stress and microbial parameters, suggesting its role in enhancing antioxidant defenses and influencing intestinal microbiota. These findings underscore the importance of grape pomace as a sustainable feed ingredient in aquaculture, offering insights into its potential benefits for fish health and nutrition. Further research is warranted to elucidate the underlying mechanisms and optimize the inclusion levels of grape pomace in fish diets for maximizing its nutritional and health-promoting effects.

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