

Article

Slight Increases in Salinity Improve Muscle Quality of Grass Carp (*Ctenopharyngodon idellus*)

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Abstract: Fish muscle quality is an important parameter in the aquaculture industry. In this study, we analyzed and compared the muscle quality of grass carp (*Ctenopharyngodon idellus*) cultured at salinities of 0‰, 3‰, and 6‰ (GC0, GC3, GC6). There was no significant difference in crude protein and crude fat content of muscle between GC0 and GC3. Crude fat was significantly lower in GC6 compared to the other groups. GC3 and GC6 had higher hydroxyproline content, which suggested that these groups had higher collagen content. GC3 and GC6 had higher contents of free amino acids and umami amino acids than GC0, but there was no significant difference in sweet or sour amino-acid content among groups. GC3 and GC6 had better texture properties, including hardness, gumminess, chewiness, resilience, and springiness, than GC0. GC3 had the highest water-holding capacity among the groups. As the salinity increased, the diameter of muscle fibers decreased and the sarcolemma showed a thickening trend. These results suggest that a slight increase in salinity (i.e., 3‰) can effectively improve the muscle quality of grass carp.

Keywords: muscle quality; grass carp; composition; salinity; texture property



Citation: Zhang, X.; Shen, Z.; Qi, T.; Xi, R.; Liang, X.; Li, L.; Tang, R.; Li, D. Slight Increases in Salinity Improve Muscle Quality of Grass Carp (*Ctenopharyngodon idellus*). *Fishes* **2021**, *6*, 7. <https://doi.org/10.3390/fishes6010007>

Academic Editor: Maria Angeles Esteban

Received: 22 January 2021
Accepted: 21 February 2021
Published: 24 February 2021

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1. Introduction

The grass carp (*Ctenopharyngodon idellus*) is an economically important freshwater fish in China, and its production is the highest among aquaculture fish species worldwide [1,2]. As the production of this species continues to grow, improving muscle quality has become an important goal for further development of the aquaculture industry [3–5]. Fish growth and muscle quality are affected by external and internal factors, including culture environment, nutrition, and genetics [1,5–7]. To date, most studies of grass carp muscle quality have focused on nutrition [1,3,5,8], and little is known about the effects of environmental conditions on grass carp muscle quality.

In our previous studies, we found that the muscle quality of grass carp could be improved by a better culture environment, such as one that includes the presence of bio-floating beds in the culture ponds [4]. However, little is known about the influence of water salinity on grass carp muscle quality. In recent years, climate change has accelerated sea-level rise and has increased the frequency of extreme events such as coastal flooding, cyclones, and storm surges, which indirectly affect the salinity in fresh water [9,10]. Water salinity is an important factor in the aquaculture environment. Kilambi [11] reported that grass carp can withstand a range of salinities, as he found no difference in the growth rate of grass carp cultured at 3–9‰. However, the details and mechanisms responsible for the effect of salinity on muscle quality are not clear.

Fish quality is defined by a combination of characteristics such as wholesomeness, freshness, and integrity. Freshness, which can be reflected by appearance, taste, and texture

of muscle, is crucial to the consumer [4,12]. The chemical composition and water-holding capacity (WHC) of muscle have significant impacts on fish quality [13–15]. The amino-acid composition is important for nutrition and flavor, as the total amino-acid (TAA) composition affects the nutritional value of the food, and the free amino acids (FAAs) affect its flavor [16]. Additionally, texture is one of the most important quality indicators of fish-muscle products for producers, processors, and consumers [1,17]. Therefore, these indicators are generally used for comprehensive evaluations of muscle quality of fish.

The purpose of this study was to investigate the muscle quality of grass carp cultured in different salinities. We measured chemical composition, amino-acid composition, WHC, and texture properties of grass carp muscle, and we conducted histological analysis to observe muscle structure. This study provides a theoretical basis for changes of muscle quality of freshwater fishes cultured in different salinities.

2. Results

2.1. Chemical Composition

GC0, GC3, and GC6 had a weight loss of 5.5%, 6.3%, and 8.4% compared to initial fish weights, respectively, and there was no significant difference among GC0–6. Figure 1 shows the chemical composition of muscle of grass carp cultured at different salinities. There was no significant difference found in the chemical composition of muscle between GC0 and GC3. No significant difference in water content was detected among the different salinity groups. Fat content was significantly lower in GC6 compared with GC0 and GC3.

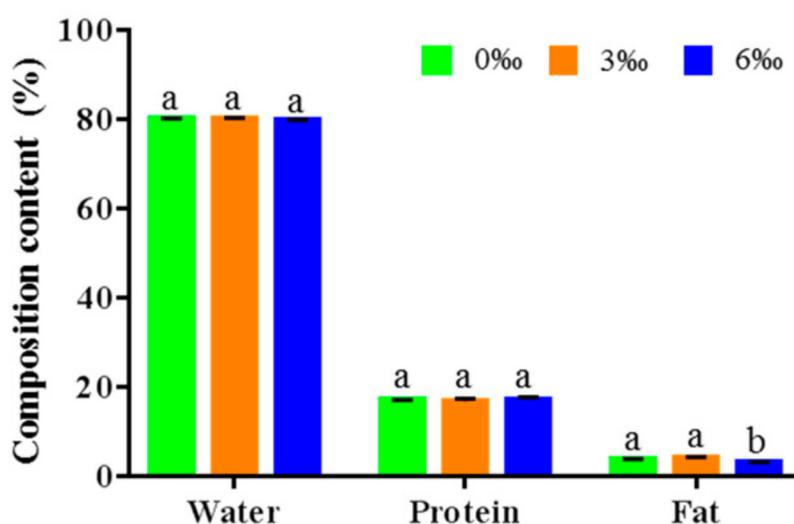


Figure 1. Composition of muscle in grass carp cultured at different salinities (0‰, 3‰, and 6‰). For the same parameter, different lowercase letters (a, b) indicate significant differences among different salinities ($p < 0.05$).

2.2. Amino-Acid Analysis

Tables 1 and 2 show the amino-acid composition and FAA composition, respectively, of muscle from the three groups. Of the TAAs in this study (Table 1), glutamic acid dominated, accounting for 15.6–17.4% to the total. The essential amino acids (EAAs) histidine, threonine, valine, methionine, phenylalanine, isoleucine, leucine, and lysine all were present in the muscle of grass carp. For all groups, EAAs accounted for 40–42% of the TAAs. The ratio of EAA/NEAA (nonessential amino acids) was 70–78%, which indicated good protein quality according to the FAO/WHO reference. The only significant differences in amino-acid content among groups were for aspartic acid, glutamic acid, tyrosine, proline, and hydroxyproline. There was no significant difference in the composition of TAAs, EAAs, and NEAAs among the three groups. The dominant FAA in this study (Table 2) was glycine, which contributed 36.2–43.1% to the total FAAs in the three groups. Significant differences

in quantities of most amino acids were found among the three groups; the exceptions were glutamic acid, histidine, glycine, alanine, and cysteine. The contents of total free amino acids (TFAA), umami amino acids (UMAA), and bitter amino acids (BAA) in grass carp muscle increased with increasing salinity. GC6 had significantly higher TFAA and BAA than GC0 and GC3. Additionally, GC3 and GC6 had significantly higher UMAA than GC0. There was no significant difference in sweet or sour amino-acid compositions among GC0, GC3, and GC6.

Table 1. Amino-acid composition of muscle in grass carp cultured at different salinities (mg/g dry weight basis).

Amino Acid	0‰	3‰	6‰
Asp	89.90 ± 0.77 ^a	82.36 ± 0.02 ^b	85.66 ± 2.52 ^{ab}
Glu	145.14 ± 0.83 ^a	134.28 ± 0.15 ^b	137.97 ± 3.94 ^{ab}
Ser	31.45 ± 0.71 ^a	32.74 ± 0.03 ^a	32.35 ± 0.29 ^a
His	16.16 ± 0.46 ^a	17.47 ± 0.15 ^a	16.56 ± 0.18 ^a
Gly	43.37 ± 1.61 ^a	45.82 ± 0.44 ^a	46.89 ± 1.43 ^a
Thr	36.12 ± 0.32 ^a	36.40 ± 0.00 ^a	35.72 ± 0.33 ^a
Arg	51.13 ± 0.28 ^a	51.16 ± 0.12 ^a	50.84 ± 0.16 ^a
Ala	48.38 ± 0.38 ^a	48.78 ± 0.13 ^a	48.78 ± 0.33 ^a
Tyr	23.50 ± 0.42 ^a	26.98 ± 0.20 ^b	24.15 ± 0.98 ^a
Cys-s	2.43 ± 0.55 ^a	2.90 ± 0.14 ^a	2.37 ± 0.53 ^a
Val	44.44 ± 1.00 ^a	43.20 ± 0.08 ^a	42.92 ± 0.23 ^a
Met	21.75 ± 0.68 ^a	24.76 ± 0.06 ^a	22.62 ± 0.96 ^a
Phe	35.62 ± 0.73 ^a	34.57 ± 0.10 ^a	34.44 ± 0.13 ^a
Ile	44.09 ± 1.78 ^a	40.33 ± 0.01 ^a	40.18 ± 0.18 ^a
Leu	70.83 ± 1.94 ^a	67.56 ± 0.14 ^a	66.56 ± 0.39 ^a
Lys	86.92 ± 2.83 ^a	88.27 ± 0.27 ^a	84.21 ± 1.82 ^a
Pro	22.66 ± 3.06 ^a	37.32 ± 1.50 ^b	35.02 ± 3.50 ^b
Hyp	26.87 ± 0.55 ^a	33.05 ± 0.95 ^b	32.96 ± 2.22 ^b
TAA	840.76 ± 4.45 ^a	847.97 ± 1.17 ^a	840.20 ± 4.67 ^a
EAA	355.93 ± 8.12 ^a	352.56 ± 0.65 ^a	343.21 ± 3.17 ^a
NEAA	458.90 ± 5.39 ^a	432.47 ± 1.80 ^a	437.51 ± 3.99 ^a
EAA/TAA	0.42 ± 0.01 ^a	0.42 ± 0.00 ^a	0.41 ± 0.00 ^a
EAA/NEAA	0.78 ± 0.02 ^a	0.76 ± 0.00 ^a	0.73 ± 0.01 ^a

Data are mean ± SE. Different lowercase letters (a, b) in a row indicate significant differences among different salinities ($p < 0.05$). TAA = total amino acids; EAA = essential amino acids (His, Thr, Val, Met, Phe, Ile, Leu, Lys); NEAA = non-essential amino acids (Asp, Glu, Ser, Gly, Arg, Ala, Pro, Hyp).

Table 2. Free-amino-acid composition of muscle of grass carp cultured at different salinities (mg/g dry weight basis).

Amino Acid	0‰	3‰	6‰
Asp	1.04 ± 0.19 ^a	4.49 ± 0.80 ^b	3.69 ± 0.34 ^b
Glu	3.86 ± 0.51 ^a	6.29 ± 1.22 ^a	5.70 ± 0.42 ^a
Ser	5.32 ± 0.33 ^a	3.01 ± 0.01 ^b	3.99 ± 0.06 ^c
His	24.01 ± 4.14 ^a	23.83 ± 5.64 ^a	22.74 ± 0.44 ^a
Gly	62.97 ± 8.42 ^a	76.52 ± 8.48 ^a	78.61 ± 5.55 ^a
Thr	13.90 ± 0.14 ^a	17.01 ± 0.14 ^a	23.63 ± 1.28 ^b
Arg	1.45 ± 0.01 ^a	3.01 ± 0.25 ^b	6.63 ± 0.10 ^c
Ala	10.99 ± 0.16 ^a	13.88 ± 1.21 ^a	11.48 ± 0.46 ^a
Tyr	1.35 ± 0.06 ^a	2.25 ± 0.12 ^b	2.67 ± 0.01 ^c
Cys-s	0.40 ± 0.06 ^a	1.13 ± 0.23 ^a	0.65 ± 0.11 ^a
Val	4.41 ± 0.34 ^a	7.03 ± 1.03 ^b	9.01 ± 0.03 ^b
Met	1.66 ± 0.05 ^a	2.35 ± 0.11 ^b	2.77 ± 0.04 ^c

Table 2. Cont.

Amino Acid	0‰	3‰	6‰
Phe	1.53 ± 0.00 ^a	2.30 ± 0.23 ^b	2.94 ± 0.06 ^c
Ile	1.68 ± 0.05 ^a	1.94 ± 0.15 ^a	2.76 ± 0.05 ^b
Leu	2.90 ± 0.05 ^a	1.68 ± 0.28 ^b	7.72 ± 0.24 ^c
Lys	4.43 ± 0.13 ^a	5.48 ± 0.32 ^a	20.43 ± 0.73 ^b
Pro	4.28 ± 0.28 ^a	7.16 ± 0.65 ^a	12.01 ± 0.98 ^b
TFAA	146.21 ± 12.75 ^a	179.70 ± 12.37 ^{ab}	217.44 ± 15.62 ^b
UMAA	4.90 ± 0.32 ^a	11.12 ± 1.73 ^b	9.38 ± 1.32 ^b
SWAA	101.90 ± 9.47 ^a	123.06 ± 10.17 ^a	150.16 ± 14.11 ^a
BIAA	38.98 ± 3.57 ^a	44.40 ± 3.69 ^a	57.25 ± 0.39 ^b
SOAA	28.91 ± 3.82 ^a	34.95 ± 3.91 ^a	32.13 ± 2.08 ^b

Data are mean ± SE. Different lowercase letters (a, b, c) in a row indicate significant differences among different salinities ($p < 0.05$). TFAA = total free amino acids; UMAA = umami amino acids (Asp, Glu); SWAA = sweet amino acids (Ser, Gly, Thr, Ala, Lys, Pro); BIAA = bitter amino acids (His, Arg, Tyr, Val, Met, Phe, Ile, Leu); SOAA = sour amino acids (Asp, Glu, His).

2.3. WHC

Environmental salinity strongly influenced the muscle WHC (Figure 2). DL did not differ significantly among GC0, GC3, and GC6. There was no significant difference in FLR between GC0 and GC3, but FLR increased significantly in GC6. Additionally, the FLR increased as thawing progressed from 1 h to 2 h. As the environmental salinity increased, CL first decreased and then increased. CL was the lowest in GC3. Therefore, GC3 had the highest WHC among the groups.

2.4. Analysis of Muscle-Texture Profiles

Grass carp in the different salinity groups exhibited significant differences in muscle texture (Figure 3). Muscle-texture properties of hardness, gumminess, and chewiness increased first and then decreased with the increase of environmental salinity. The highest value of these indicators was found in GC3, followed by GC6, and the value in GC0 was significantly lower compared to the other groups. Muscle resilience of GC3 and GC6 was significantly higher than that of GC0. The springiness value of GC6 was significantly higher than that of GC3, and the lowest value was found in GC0. GC3 and GC0 showed no significant difference in muscle cohesiveness, and the value in GC6 was lower. These results indicate that GC3 and GC6 had better muscle-texture properties than GC0.

2.5. Histological Analysis

Muscle histological sections revealed that as salinity increased, the sarcolemma thickened (Figure 4). Muscle tissue from GC3 and GC6 fish appeared to be tighter than that in GC0 fish. In addition, the diameter of white muscle fibers showed a downward trend with increasing salinity; GC6 had significantly a smaller diameter than those of GC3 and GC0. There was no significant difference in fiber diameter between GC3 and GC0. The density of the number of fibers per tissue sectional area was $19 \pm 3/\text{mm}^2$, $20 \pm 3/\text{mm}^2$, and $22 \pm 4/\text{mm}^2$ (GC0, GC3, and GC6, respectively), and there was no significant difference among GC0–6.

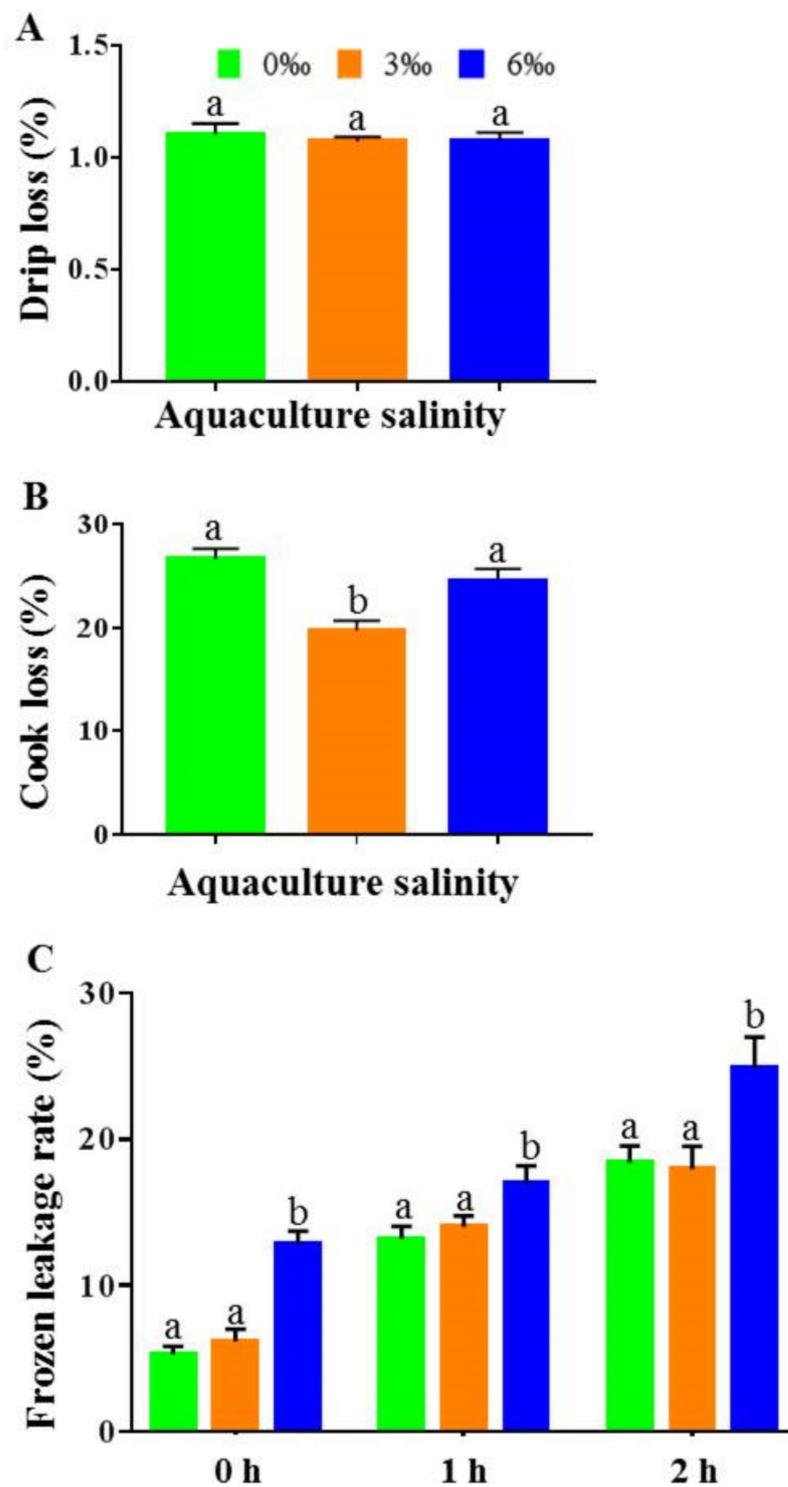


Figure 2. Water-holding capacity of muscle of grass carp cultured at different salinities (0‰, 3‰, and 6‰). (A), Drip loss; (B), Cook loss; (C), Frozen leakage rate. Different lowercase letters indicate significant differences among different salinities ($p < 0.05$).

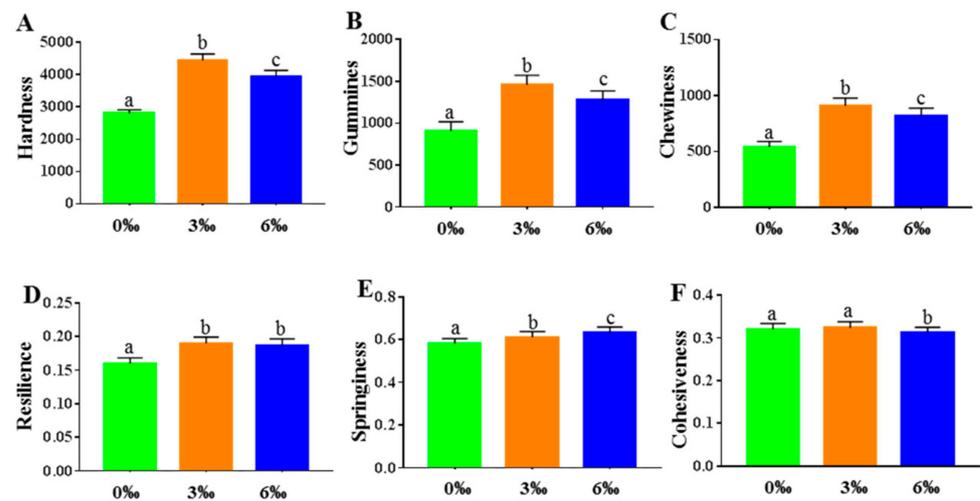


Figure 3. Results of texture-profile analysis of muscle of grass carp cultured at different salinities (0‰, 3‰, and 6‰). (A), Hardness; (B), Gumminess; (C), Chewiness; (D), Resilience; (E), Springiness; (F), Cohesiveness. Different lowercase letters indicate significant differences among different salinities ($p < 0.05$).

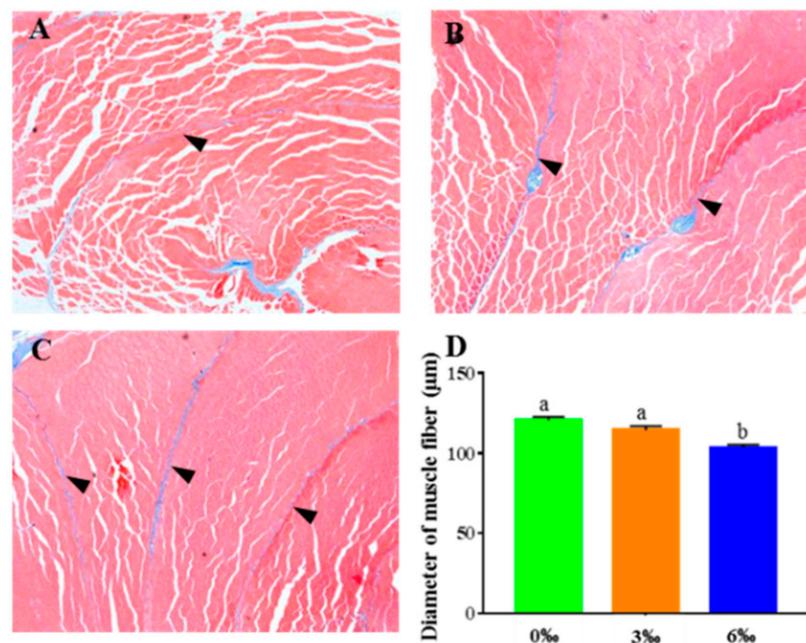


Figure 4. Tissue sections of muscle of grass carp cultured at different salinities (40× magnification). (A–C) correspond to aquaculture salinities of 0‰, 3‰, and 6‰, respectively. Muscle fiber is stained red, and collagen in intramuscular connective tissue is stained blue. The arrows indicate sarcolemma. (D) indicates statistics for muscle-fiber diameters. Different lowercase letters indicate significant differences among different salinities ($p < 0.05$).

3. Discussion

In this study, muscle quality of grass carp cultured at different salinities was analyzed. Grass carp had a slight weight loss due to the deprivation of food during the experiment. A period of food deprivation is usually used for improving the quality and flavor of the fish [18–20]. In the study of Murray cod, fish had an organoleptic quality amelioration after 2 and 4 weeks of food deprivation, and had a weight loss of 4.1% and 9.1%, respectively [18]. For grass carp, the fillets can be improved effectively by depuration and food deprivation after more than 20 days culture with a slight weight loss, and the food-deprivation time

should not be more than 50 days to avoid the excessive loss in protein and lipid contents of muscle [20]. Although there was no significant difference in weight loss among GC0–6, the weight loss showed an upward trend with the salinity increase, thus aquaculture salinity for grass carp was suggested to be lower than 6‰. Fish protein is an important determinant of the nutritional value of muscle [15,16]. In this study, no significant difference was found in protein and TAA content of muscle among the three groups. Thus, a slight increase of aquaculture salinity did not affect the protein nutritional value of grass carp muscle. In contrast, fat content was significantly decreased in muscle of GC6. Crude-fat content primarily depends on the feed, fish species, and rearing conditions [4,13]. We posit that the lower fat content in GC6 was due in part to some stress response of grass carp cultured in this higher salinity, as fats are an essential energy source for fish, and fat metabolism may be altered under stress conditions [21,22].

Amino-acid composition is generally important for nutrition and flavor. The composition of TAAs affects the nutritional value of the food, whereas the FAAs affect its flavor [16]. Among the FAAs, UMAAs, including aspartic and glutamic acid, are perceived to confer the savory umami taste, which affects the taste and quality of muscle tissue, as well as consumer preference [16]. As the salinity increased in this study, the content of UMAAs increased significantly. This finding illustrates that increased aquaculture salinity can improve the flavor of grass carp muscle. Although the three groups had similar nutritional value, GC3 and GC6 had better flavor than GC0. These results suggest that the aquaculture salinity mainly affected the content of FAAs in grass carp muscle, thereby affecting muscle flavor rather than muscle nutritional value.

WHC is one of the most important indicators of meat quality, especially in the process of muscle processing [23,24]. The majority of water in muscle is held within the myofibrils, between the myofibrils, between the myofibrils and the sarcolemma, between muscle cells, and between muscle bundles [25]. Water loss causes the loss of soluble protein to a certain extent, and high muscle WHC can reduce protein breakdown [26]. In this study, GC3 had the highest WHC among the groups, which suggests that a slight increase of aquaculture salinity could improve the WHC of grass carp muscle. In a study of European sea bass [27], the aquaculture environment was reported to affect the density of muscle fibers. In this study, although salinity of 3–6‰ did not suggest a significant difference in density of muscle fibers, fiber diameter of GC6 showed a significant decrease compared with GC0–3, which may be related to its lower WHC.

Texture is an important indicator of muscle quality, which is crucial for producers and processors and for customer satisfaction and acceptance of fish products [1,15]. Previous studies demonstrated that muscle from wild-caught fish had higher hardness and springiness values compared to cultured fish, and higher values are more attractive to the consumer [14,15,28]. Additionally, a better texture makes fish easier to process into high-quality products [17]. Grass carp with high textural property values have been reported to be preferred by consumers, resulting in a higher market price [3,29]. Therefore, we propose that grass carp cultured at 3 and 6‰ would have better market value because of their better textural properties compared to fish cultured at 0‰, normal fresh water. Muscle texture is affected by many factors, such as moisture, fat, and collagen content, and they affect the perception of texture features differently [14]. Water content and distribution have a profound effect on muscle-texture properties, such as hardness and juiciness, and higher WHC may be associated with harder muscle [4,30]. In our study, muscle samples from the GC3 group had the highest hardness, gumminess, and chewiness values, as well the highest WHC among the groups, which is consistent with the results of previous studies. On the other hand, Andersen et al. [20] reported that a higher fat content in fillets resulted in less resistance against compression, which indicated a softer texture of fish muscle. Thus, the higher springiness of muscle in GC6 may be due in part to the lower fat content. Our results suggest that a slight increase of aquaculture salinity could improve muscle-texture properties.

Collagen content and properties play an important role in the texture and integrity of fish muscle, as collagen is the most important constituent of fish intramuscular connective tissue [14,31]. Hydroxyproline content can be used to estimate collagen content because they are positively correlated [8,32]. In this study, the collagen content of muscle tissue from GC3 and GC6 was significantly higher than that from GC0, based on hydroxyproline content. Assessment of the quantity of collagen is a simple and practical method for assessing the quality of meat products [32,33]. Previous studies reported that higher collagen content is the main factor responsible for the texture properties of crisp grass carp that are popular with consumers [29]. Therefore, we hypothesized that the better muscle-texture properties of GC3 and GC6 compared with GC0 were positively correlated with their higher collagen content. This is also consistent with previous studies of sea bream (*Sparus aurata*) that showed a positive correlation between collagen and fish muscle firmness [4,34]. On the other hand, it has been found that during the process of collagen fibril formation in vitro, increased salt concentration can accelerate collagen cross-link formation and make collagen fibrils thicker [35]. Nishimura [36] reported that changes in collagen cross-links can increase the mechanical properties of intramuscular connective tissue, thereby contributing to the toughening of meat. Based on our results, we speculate that the increases of culture salinity may have affected these aspects of collagen, thus affecting muscle texture. Assessment of the quantity of connective tissue is a simple and practical method for assessing the quality of meat products [32,33]. The sarcolemma is the most important intramuscular connective tissue. Previous studies of crisp grass carp reported that the thickening of intramuscular connective tissue enhanced the texture properties of the muscle [1,29]. Therefore, the thickening and increase of the sarcolemma in GC3 and GC6 likely explain the better muscle-texture properties found in these groups compared to GC0. Thickening of the sarcolemma may be related to increased collagen content [14,31]. This trend was evident in the results of our study as well.

4. Materials and Methods

4.1. Experimental Protocol and Sampling

This experimental protocol was approved by the Institutional Animal Care and Use Committee of Huazhong Agricultural University (HZAUF-2020-001). A total of 120 fish with similar size (body weight, 80.01 ± 10.49 g) were procured from the Chonghu Fish Farm and transported to the aquaculture center of Huazhong Agricultural University. After 7 days of acclimatization, grass carp were randomly divided into 9 tanks at a density of 3.5–4 kg/m³ per tank. The brackish water culture experiment consisted of 3 groups with salt concentrations of 0‰, 3‰, and 6‰ (GC0, GC3, and GC6), each with 3 replicate tanks. To create the culture concentrations, salinity began at 1.5‰ and was increased daily by 1.5‰ until all tanks reached their target concentration. Grass carp were cultured for 30 days and all fish were deprived of food during the experimental period. There was no abnormality observed in fishes during the experimental period. At the end of the experiment, fish from each group were randomly selected and anesthetized with MS-222. Dorsal white muscle tissue of each fish was sampled and used for biochemical analysis.

4.2. Chemical Composition Measurements

The chemical composition of muscle was measured according to methods provided by the National Food Safety Standard of the People's Republic of China and our previous study [4]. Water content was measured by the GB 5009.3-2010 method, crude protein by the Kjeldahl method (GB 5009.5-2010), and lipid content by the Soxhlet method (GB/T 5009.6-2003).

4.3. Amino Acid Composition Measurements

For analysis of hydrolyzed amino acids, muscle samples were hydrolyzed in 6 N HCl at 110 °C for 24 h. The hydrolysates were evaporated, and the remaining materials were dissolved in citric acid buffer solution. The samples then were analyzed using high-

performance liquid chromatography with an ods hypersil column (250 × 4.6 mm, 5 µm) (HPLC, AG1100, Palo Alto, CA, USA). Pre-column derivatization with *o*-phthalaldehyde and 9-fluorenylmethyl chloroformate was used to identify the FAAs. Liquid nitrogen was added to muscle samples and the mixture was quickly ground and ultrasonically shaken in 5% trichloroacetic acid for 20 min. After standing for 1 h, the mixture was centrifuged at 10,000 × *g* for 10 min. The supernatant was filtered through a 0.22-µm membrane filter and subjected to HPLC.

4.4. WHC Measurements

WHC of the samples was measured by drip loss (DL), frozen leakage rate (FLR), and cook loss (CL). To eliminate the effects of different parts of the muscle on the WHC, we used 5 ± 0.5 g of muscle taken from the same location of each fish for the analyses. To calculate DL, we weighed the muscle, placed it in a hanging plastic bag for 24 h at 4 °C, and then weighed it again. To obtain the FLR, we weighed the muscle, froze it for 24 h at −20 °C, tightly sealed it in a plastic bag at 4 °C, and then weighed it at 0, 1, and 2 h. CL was calculated by weighing the muscle, cooking it for 15 min, and then weighing it again. DL, FLR, and CL were calculated as the percentage of original weight lost.

4.5. Texture Measurements

The texture of the muscle samples was evaluated using a TA-Xt Plus Micro TPA device (Stable Micro Systems, Godalming, UK) equipped with a flat-bottomed cylindrical probe P/36R and a load cell of 250 N. The assay was performed following the method described by Ma et al. [8]. The texture-profile analysis (TPA) and shear-force tests were carried out at room temperature; five fish from each group, with three parallel samples from each fish, were used. Texture curves were generated, and the maximum force was determined as an average of the three measurements.

4.6. Histological Analysis

Samples were fixed with 10% neutral formalin buffer, routinely processed, sectioned at 5 µm after paraffin embedding, and then stained with Masson's trichrome staining. Observation and analysis were performed with a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan). A total of 200–300 muscle fibers per fish were observed, and the muscle-fiber diameter and the density (200× magnification) was measured using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA).

4.7. Statistical Analysis

Statistical analyses were performed using SPSS Base 25.0 statistical software (IBM, Armonk, NY, USA). Values are expressed as mean ± standard error (SE). The significant differences were evaluated by one-way analysis of variance followed by Duncan's test. Normality and variance uniformity were verified. $p < 0.05$ was considered to be statistically significant for all analyses.

5. Conclusions

In this study, we assessed and compared the muscle quality of grass carp cultured at different salinities. Our results suggest that a slight increase in water salinity when culturing grass carp can help increase the FAA and UAA content of muscle, and at the same time improve the WHC and texture properties of the muscle. Increasing the culture salinity increased the collagen content in muscle, decreased the diameter of muscle fibers, and thickened the sarcolemma. Increasing the culture salinity from 0 to 6‰ did not have a negative impact on muscle nutrient composition. Considering all indicators assessed in this study, grass carp cultured at 3‰ had the best muscle quality among the salinities tested. These results indicate that grass carp muscle quality can be effectively improved by a slight increase in water salinity. These findings provide a theoretical basis for improving

the muscle quality of cultured freshwater fishes and for the development of fish farming technology in inland saline–alkali areas and coastal brackish-water areas.

Author Contributions: Conceptualization, X.Z. and D.L.; data curation, Z.S., T.Q., R.X., and X.L.; funding acquisition, X.Z. and D.L.; investigation, X.Z., Z.S., T.Q., R.X., and X.L.; methodology, X.Z.; supervision, D.L.; writing—original draft, X.Z.; writing—review and editing, L.L. and R.T. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the National Key Research and Development Program of China (2019YFD0900303), the National Natural Science Foundation of China (31902390), and the Earmarked Fund for China Agriculture Research System (CARS-45).

Institutional Review Board Statement: In the present study, all procedures were performed in accordance with the “Guidelines for Experimental Animals” of the Ministry of Science and Technology (Beijing, China), and were approved by the Institutional Animal Care and Use Committees of Huazhong Agricultural University (HZAUI-2020-001).

Conflicts of Interest: The authors declare no conflict of interest. This work has not been published previously.

References

- Chen, L.; Liu, J.; Kaneko, G.; Xie, J.; Wang, G.; Yu, D.; Li, Z.; Ma, L.; Qi, D.; Tian, J.; et al. Quantitative phosphoproteomic analysis of soft and firm grass carp muscle. *Food Chem.* **2020**, *303*, 125367. [[CrossRef](#)]
- Xie, C.; Li, J.; Li, D.; Shen, Y.; Gao, Y.; Zhang, Z. Grass Carp: The Fish that Feeds Half of China. *Aquac. China* **2018**, 93–115. [[CrossRef](#)]
- Yu, E.; Fu, B.; Wang, G.; Li, Z.; Ye, D.; Jiang, Y.; Ji, H.; Wang, X.; Yu, D.; Ehsan, H.; et al. Proteomic and metabolomic basis for improved textural quality in crisp grass carp (*Ctenopharyngodon idellus* C.et V) fed with a natural dietary pro-oxidant. *Food Chem.* **2020**, *325*, 126906. [[CrossRef](#)]
- Zhang, X.; Wang, J.; Tang, R.; He, X.; Li, L.; Takagi, Y.; Li, D. Improvement of Muscle Quality of Grass Carp (*Ctenopharyngodon idellus*) With a Bio-Floating Bed in Culture Ponds. *Front. Physiol.* **2019**, *10*, 683. [[CrossRef](#)]
- Zhao, H.; Xia, J.; Zhang, X.; He, X.; Li, L.; Tang, R.; Chi, W.; Li, D. Diet Affects Muscle Quality and Growth Traits of Grass Carp (*Ctenopharyngodon idellus*): A Comparison between Grass and Artificial Feed. *Front. Physiol.* **2018**, *9*, 283. [[CrossRef](#)]
- De Paula, T.G.; De Almeida, F.L.A.; Carani, F.R.; Vechetti-Júnior, I.J.; Padovani, C.R.; Salomão, R.A.S.; Mareco, E.A.; Dos Santos, V.B.; Dal-Pai-Silva, M. Rearing temperature induces changes in muscle growth and gene expression in juvenile pacu (*Piaractus mesopotamicus*). *Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol.* **2014**, *169*, 31–37. [[CrossRef](#)]
- Johnston, I.A. Genetic and Environmental Determinants of Muscle Growth Patterns. *Fish Physiology* **2001**, *18*, 141–186. [[CrossRef](#)]
- Ma, X.-Z.; Feng, L.; Wu, P.; Liu, Y.; Kuang, S.-Y.; Tang, L.; Zhou, X.-Q.; Jiang, W.-D. Enhancement of flavor and healthcare substances, mouthfeel parameters and collagen synthesis in the muscle of on-growing grass carp (*Ctenopharyngodon idella*) fed with graded levels of glutamine. *Aquaculture* **2020**, *528*, 735486. [[CrossRef](#)]
- Chand, B.; Trivedi, R.; Dubey, S.; Rout, S.; Beg, M.; Das, U. Effect of salinity on survival and growth of giant freshwater prawn *Macrobrachium rosenbergii* (de Man). *Aquac. Rep.* **2015**, *2*, 26–33. [[CrossRef](#)]
- Dubey, S.K.; Trivedi, R.K.; Chand, B.K.; Mandal, B.; Rout, S.K. Farmers’ perceptions of climate change, impacts on freshwater aquaculture and adaptation strategies in climatic change hotspots: A case of the Indian Sundarban delta. *Environ. Dev.* **2017**, *21*, 38–51. [[CrossRef](#)]
- Kilambi, R.V. Food consumption, growth and survival of grass carp *Ctenopharyngodon idella* Val at four salinities. *J. Fish Biol.* **1980**, *17*, 613–618. [[CrossRef](#)]
- Martin, R. Contaminants in relation to the quality of seafoods. *Food Technol.* **1998**, *42*, 104.
- Alasalvar, C.; Taylor, K.; Zubcov, E.; Shahidi, F.; Alexis, M. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): Total lipid content, fatty acid and trace mineral composition. *Food Chem.* **2002**, *79*, 145–150. [[CrossRef](#)]
- Cheng, J.-H.; Sun, D.-W.; Han, Z.; Zeng, X.-A. Texture and Structure Measurements and Analyses for Evaluation of Fish and Fillet Freshness Quality: A Review. *Compr. Rev. Food Sci. Food Saf.* **2013**, *13*, 52–61. [[CrossRef](#)] [[PubMed](#)]
- Fuentes, A.; Fernández-Segovia, I.; Serra, J.A.; Barat, J.M. Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality. *Food Chem.* **2010**, *119*, 1514–1518. [[CrossRef](#)]
- Liyana-Pathirana, C.; Shahidi, F.; Whittick, A.; Hooper, R. Effect of season and artificial diet on amino acids and nucleic acids in gonads of green sea urchin *Strongylocentrotus droebachiensis*. *Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol.* **2002**, *133*, 389–398. [[CrossRef](#)]
- Hyldig, G.; Nielsen, D. A Review of Sensory and Instrumental Methods Used to Evaluate the Texture of Fish Muscle. *J. Texture Stud.* **2001**, *32*, 219–242. [[CrossRef](#)]
- Palmeri, G.; Turchini, G.M.; Caprino, F.; Keast, R.; Moretti, V.M.; De Silva, S.S. Biometric, nutritional and sensory changes in intensively farmed Murray cod (*Maccullochella peelii peelii*, Mitchell) following different purging times. *Food Chem.* **2008**, *107*, 1605–1615. [[CrossRef](#)]

19. Du, H.; Lv, H.; Xu, Z.; Zhao, S.; Huang, T.; Manyande, A.; Xiong, S. The mechanism for improving the flesh quality of grass carp (*Ctenopharyngodon idella*) following the micro-flowing water treatment using a UPLC-QTOF/MS based metabolomics method. *Food Chem.* **2020**, *327*, 126777. [[CrossRef](#)] [[PubMed](#)]
20. Lv, H.; Hu, W.; Xiong, S.; You, J.; Fan, Q. Depuration and starvation improves flesh quality of grass carp (*Ctenopharyngodon idella*). *Aquac. Res.* **2018**, *49*, 3196–3206. [[CrossRef](#)]
21. Menezes, C.; Ruiz-Jarabo, I.; Martos-Sitcha, J.A.; Toni, C.; Salbego, J.; Becker, A.; Loro, V.L.; Martínez-Rodríguez, G.; Mancera, J.M.; Baldissotto, B. The influence of stocking density and food deprivation in silver catfish (*Rhamdia quelen*): A metabolic and endocrine approach. *Aquaculture* **2015**, *435*, 257–264. [[CrossRef](#)]
22. Montero, D.; Izquierdo, M.; Tort, L.; Robaina, L.; Vergara, J. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. *Fish Physiol. Biochem.* **1999**, *20*, 53–60. [[CrossRef](#)]
23. Andersen, U.B.; Thomassen, M.S.; Rørå, A.M.B. Texture Properties of Farmed Rainbow Trout (*Oncorhynchus mykiss*): Effects of Diet, Muscle Fat Content and Time of Storage on Ice. *J. Sci. Food Agric.* **1997**, *74*, 347–353. [[CrossRef](#)]
24. Otto, G.; Roehe, R.; Looft, H.; Thoelking, L.; Henning, M.; Plastow, G.; Kalm, E. Drip loss of case-ready meat and of premium cuts and their associations with earlier measured sample drip loss, meat quality and carcass traits in pigs. *Meat Sci.* **2006**, *72*, 680–687. [[CrossRef](#)] [[PubMed](#)]
25. Huff-Lonergan, E.; Lonergan, S.M. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Sci.* **2005**, *71*, 194–204. [[CrossRef](#)]
26. Bee, G.; Anderson, A.L.; Lonergan, S.M.; Huff-Lonergan, E. Rate and extent of pH decline affect proteolysis of cytoskeletal proteins and water-holding capacity in pork. *Meat Sci.* **2007**, *76*, 359–365. [[CrossRef](#)] [[PubMed](#)]
27. Nathanailides, C.; Lopez-Albors, O.; Abellán, E.; Vazquez, J.M.; Tyler, D.D.; Rowleron, A.; Stickland, N.C. Muscle cellularity in relation to somatic growth in the European sea bass *Dicentrarchus labrax* (L.). *Aquac. Res.* **1996**, *27*, 885–889. [[CrossRef](#)]
28. Periago, M.; Ayala, M.; López-Albors, O.; Abdel, I.; Martínez, C.; García-Alcázar, A.; Ros, G.; Gil, F. Muscle cellularity and flesh quality of wild and farmed sea bass, *Dicentrarchus labrax* L. *Aquaculture* **2005**, *249*, 175–188. [[CrossRef](#)]
29. Lin, W.L.; Zeng, Q.X.; Zhu, Z.W. Different changes in mastication between crisp grass carp (*Ctenopharyngodon idellus* C. et V) and grass carp (*Ctenopharyngodon idellus*) after heating: The relationship between texture and ultrastructure in muscle tissue. *Food Res. Int.* **2009**, *42*, 271–278. [[CrossRef](#)]
30. Pearce, K.L.; Rosenvold, K.; Andersen, H.J.; Hopkins, D.L. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes—A review. *Meat Sci.* **2011**, *89*, 111–124. [[CrossRef](#)]
31. Aussanasuwannakul, A.; Kenney, P.B.; Brannan, R.G.; Slider, S.D.; Salem, M.; Yao, J. Relating Instrumental Texture, Determined by Variable-Blade and Allo-Kramer Shear Attachments, to Sensory Analysis of Rainbow Trout, *Oncorhynchus mykiss*, Fillets. *J. Food Sci.* **2010**, *75*, S365–S374. [[CrossRef](#)]
32. Messia, M.C.; Di Falco, T.; Panfili, G.; Marconi, E. Rapid determination of collagen in meat-based foods by microwave hydrolysis of proteins and HPAEC–PAD analysis of 4-hydroxyproline. *Meat Sci.* **2008**, *80*, 401–409. [[CrossRef](#)] [[PubMed](#)]
33. Zarkadas, C.G.; Yu, Z.; Zarkadas, G.C.; Minero-Amador, A. Assessment of the Protein Quality of Beefstock Bone Isolates for Use as an Ingredient in Meat and Poultry Products. *J. Agric. Food Chem.* **1995**, *43*, 77–83. [[CrossRef](#)]
34. Suárez, M.; Abad, M.; Ruiz-Cara, T.; Estrada, J.; García-Gallego, M. Changes in muscle collagen content during post mortem storage of farmed sea bream (*Sparus aurata*): Influence on textural properties. *Aquac. Int.* **2005**, *13*, 315–325. [[CrossRef](#)]
35. Zhang, X.; Ookawa, M.; Tan, Y.; Ura, K.; Adachi, S.; Takagi, Y. Biochemical characterisation and assessment of fibril-forming ability of collagens extracted from Bester sturgeon *Huso huso* × *Acipenser ruthenus*. *Food Chem.* **2014**, *160*, 305–312. [[CrossRef](#)] [[PubMed](#)]
36. Nishimura, T. The role of intramuscular connective tissue in meat texture. *Anim. Sci. J.* **2010**, *81*, 21–27. [[CrossRef](#)] [[PubMed](#)]