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Effects of dietary short chain fatty acids on growth, antioxidant capacity and digestive enzymes activity in Sobaity seabream (*Sparidentex hasta*) Juveniles

Torfi Mozanzadeh M.[1]; Mehrjooyan S.[1]; Mohammadian T.[2]

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Abstract

An eight-week study was conducted to investigate the effects of dietary butyric acid (BA) and propionic acid (PA) supplementation levels on growth and gut microflora in juvenile Sobaity seabream (*Sparidentex hasta*) (28.5 g). Seven experimental diets were prepared by adding different levels of BA and PA to a basal diet including (1) control (without organic acid supplementation); (2) BAU0.5; (3) BAU1.0; (4) PRA0.5; (5) PRA1.0; (6) a mixture of 0.25% BUA and 0.25% PRA; and (7) a mixture of 0.5% BUA and 0.5% PRA. A total of 315 fish, with an initial body weight of 28.5±0.5 g, were distributed among twenty-one 300-L polyethylene tanks. Fish were fed the experimental diets twice daily to apparent satiation, ensuring that no feed remained in the tanks. Water temperature and salinity were 31.3 °C and 46 g/L, respectively. Fish fed diets containing 0.5% or 1% PA, or the 0.5% BA+PA blend, showed higher growth rates than the other groups. Feed efficiency ratio also improved in fish receiving 0.5% PA and 1% BA. Based on the findings, 0.5% PA or a 0.5% blend of PA and BA is recommended to improve growth and intestinal health in juvenile Sobaity seabream. The PRA0.5 treatment produced the most pronounced improvements in hepatic GSH and CAT activities, while BUA0.5 resulted in the highest SOD activity. Dietary PRA at 0.5% and especially the combined supplementation of BUA and PRA at 0.5%, increased alkaline phosphatase, trypsin, chymotrypsin, amylase, lipase, acid and alkaline proteases in sobaity seabream (p<0.05). Based on the results of the present study supplementing diet with PRA at 0.5% or a combination of 0.25% BUA and 0.25% PRA could be beneficial for growth, antioxidant capacity and digestive function of sobaity seabream.

Keywords: Antioxidant defense, Digestive enzymes activity, Growth performance, Organic acids; Sobaity seabream

1-Aquaculture Research Center -South of Iran, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Ahvaz, Iran
2-Department of Livestock, Poultry and Aquatic Animal Health, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
*Corresponding author's Emial: Mansour.torfi@gmail.com

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Introduction

Disease outbreaks on aquaculture farms are difficult to avoid under culture conditions such as high stocking density, inappropriate management, marked fluctuations in the physicochemical properties of water, and unsuitable diets (Ringo and Song 2016). Specific treatments have not yet been established for a number of diseases, and the outcomes of drug treatments under experimental conditions often differ from those achieved under natural and practical conditions. Some treatments cannot be readily applied during the growing season or winter, or in certain production facilities, particularly large-scale marine cage farms with very high biomass, where treatment costs may also be substantial. Furthermore, the persistence of chemicals and pharmaceuticals in aquatic animals may have adverse effects on consumers and the environment. Therefore, disease prevention is considerably more important than treatment (Wang *et al.,* 2017). Because of these challenges, immunostimulants and other feed additives, including organic acids, probiotics and prebiotics, are increasingly used in aquaculture to prevent infectious and non-infectious diseases as alternatives to medication (Wang *et al.,* 2017; Dawood *et al.,* 2018). Various organic acids (formic, propionic, acetic, butyric, citric, lactic, malic and sorbic acids) and their salts occur in plant and animal tissues and have been used as acidifiers in livestock and poultry nutrition, and more recently in aquaculture (Mohammadi *et al.,* 2020). Organic acids and their sodium, potassium and calcium salts can stimulate growth and improve animal health (Defoirdt *et al.,* 2009). They may also increase disease resistance and feed efficiency when incorporated into diets (Asgharzadeh and Taati, 2020). Among feed additives and immunostimulants, commercial use of organic acids in fish and other animal diets has received particular attention as a means of controlling disease and improving growth performance. Studies in aquatic animals have shown that growth performance, nutrient digestibility and intestinal health can be improved through the use of certain organic acids and their salts (Tran *et al.,* 2020). The effects of organic acids and their salts on fish growth performance and health depend on the fish species and on the type and inclusion level of the acid or salt used (Ng and Koh, 2017; Tran *et al.,* 2020). In animal nutrition, organic acids exert their effects through the feed, gastrointestinal tract and animal metabolism (Hoseinifar *et al.,* 2017). These compounds may also reduce intestinal pH and bind minerals, thereby increasing mineral absorption and promoting the conversion of pepsinogen to pepsin. For instance, dietary supplementation with butyric (BUA) and propionic (PRA) acids has been reported to positively influence digestive enzyme activities and antioxidant capacity in fish. These short-chain fatty acids (SCFA) may enhance intestinal digestive functions by stimulating the activities of proteolytic enzymes, such as trypsin and chymotrypsin, as well as

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lipase and α-amylase, thereby improving nutrient digestion and utilization (Alamifar *et al.,* 2020; Yousefi *et al.,* 2024). Butyric acid, in particular, can serve as an energy source for intestinal epithelial cells, contributing to intestinal mucosal development, epithelial integrity, and brush-border function (Abdel-Latif *et al.,* 2020). In addition to their effects on digestion, both organic acids may contribute to maintaining cellular redox homeostasis by enhancing antioxidant defense mechanisms, including superoxide dismutase (SOD) and catalase (CAT) activities, and reducing lipid peroxidation (Alamifar *et al.,* 2020; Yousefi *et al.,* 2024; Abdel-Latif *et al.,* 2020). These physiological responses may ultimately improve intestinal functionality, nutrient utilization, and the overall health status of fish.

Sobaity seabream (*Sparidentex hasta*), a member of the family Sparidae, is an important native species of the Persian Gulf and Oman Sea. Given its biological characteristics and ability to tolerate captivity, it is considered a suitable candidate for developing marine cage aquaculture in the Persian Gulf and the Sea of Oman. Sobaity seabream efficiently utilizes pelleted diets and, depending on the culture system and diet quality, particularly protein content and source—can achieve a feed conversion ratio of approximately 1.4–1.8. The species reaches a body weight of about 700 g from 50 g over a 14–18-month rearing period. Its macronutrient requirements have been investigated in several studies (Mozanzadeh *et al.,* 2017a,b). However, no study had previously examined the use of dietary supplements such as organic acids in this species. Therefore, the present study investigated the effects of dietary BAU and PRA on growth parameters and gut microflora in juvenile Sobaity seabream.

Materials and methods

Experimental diets

Butyric and propionic acids (Merck, Germany) were used at two inclusion levels, 0.5% and 1.0% of the diet (Table 1). Seven experimental diets were prepared: (1) control, with no organic acids; (2) 0.5% BAU; (3) 1.0% BAU; (4) 0.5% PRA; (5) 1.0% PRA; (6) 0.25% BAU+0.25% PRA (total organic acid inclusion of 0.5%); and (7) 0.5% BAU+0.5% PRA (total inclusion of 1.0%). The diet was formulated based on the nutritional requirements of Sobaity seabream as reported by Mozanzadeh *et al.* (2017a,b) The dry ingredients were thoroughly mixed for 20 min, followed by the addition of oils and further mixing for 10 min. Subsequently, the organic acids were dissolved in distilled water, incorporated into the mixture, and homogenized for an additional 10 min to obtain a uniform, soft dough. The resulting dough was cold-pelleted through a meat grinder fitted with a 3-mm die. The pellets were then dried at room temperature under continuous fan ventilation and stored at −20°C until use (Alamifar *et al.,* 2020).

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Table 1: Ingredients and nutrient composition (g/kg) of the basal diet.

Ingredients (g/kg diet)	Control	BUA0.5	BUA1.0	PRA0.5	PRA1.0	BUA+PRA0.5	BUA+PRA1.0
Fish meal[1]	500	500	500	500	500	500	500
Wheat gluten meal	200	200	200	200	200	200	200
Soybean meal[3]	100	100	100	100	100	100	100
Beef gelatin	20	20	20	20	20	20	20
Wheat middling	80	75	70	75	70	75	70
Butyric acid[4]	-	5	10	-	-	2.5	5
Propionic acid[4]	-	-	-	5	10	2.5	5
Fish oil	55	55	55	55	55	55	55
Soy lecithin[2]	20	20	20	20	20	20	20
Vitamin and mineral remix[5]	20	20	20	20	20	20	20
Vitamin C[6]	5	5	5	5	5	5	5
Total	1000	1000	1000	1000	1000	1000	1000
Nutrient composition							
Dry matter	90.5	91.0	91.3	90.8	90.4	91.1	91.2
Crude protein	48.3	48.0	47.8	48.5	48.1	47.8	47.9
Crude lipid	16.3	16.6	16.1	16.1	16.8	16.7	15.8
Ash	9.1	9.0	8.7	9.2	9.6	8.9	8.8
Carbohydrates[7]	16.8	17.4	18.7	17.0	15.9	17.7	18.7

*Composition of ingredients as % Dry-weight basis [fish meal (60.5% crude protein, 18.0% crude lipid); corn gluten (71.4% crude protein, 4.1% crude lipid); wheat gluten (53.3% crude protein, 2.8% crude lipid); soybean meal (41% crude protein, 4.2% crude lipid); gelatin (85% crude protein, crude lipid, 4.2); wheat middling (12% crude protein, 3.0% crude lipid)]. Diet abbreviations: BAU0.5 (butyric acid 0.5%); BUA1.0 (butyric acid 1.0%); PRA0.5 (propionic acid 0.5%); PRA1.0 (propionic acid 1.0%); BUA+PRA0.5 (butyric acid 0.25% + propionic acid 0.25%); BUA+PRA1.0 (butyric acid 0.5% + propionic acid 0.5%).

[1] Parskilka Mazandaran, Iran (Clupeonella sp.).

[2] Product of Kesht Va Sanat Shomal Vegetable Oil Factories Complex (Neca, Iran).

[3] Behpak indastrial company, Behshahr, Mazandaran, Iran.

[4] Merck (Germany)

[5] Included are as follows: (as mg / kg of premix) vitamin A, 50000 (IU/kg); vitamin D3, 10,000 (IU/kg); vitamin E, 30; vitamin B1, 20; vitamin B2, 10; vitamin B6, 3; vitamin K3, 15; nicotinamide, 150; calcium pantothenate, 40; copper (Cu^{++}), 30; iron (Fe^{++}), 100; zinc (Zn^{++}), 150; manganese (Mn^{++}).

[6]Rooyan Darou, Semnan, Iran.

[7]Calculated as 100 − (moisture (%) +protein (%) + lipid (%) + ash (%))

Fish and experimental conditions

The experiment was conducted at the Bandar Imam Marine Fish Research Station, South Iran Aquaculture Research Institute. Before the experiment, juvenile fish were acclimated to laboratory conditions for two weeks. A total of 315 juvenile Sobaity seabream (15 fish per tank), with an approximate initial body weight of

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28.5±0.5 g, were randomly distributed among twenty-one 300-L tanks filled with filtered and disinfected seawater. Fish were fed the experimental diets twice daily, at 08:00 and 14:00, to apparent satiation for eight weeks, ensuring that no pellets remained on the tank bottom. Water temperature, salinity and dissolved oxygen were 31.3±0.5°C, 46±0.3 g/L and 6.2±0.2 mg/L, respectively. Each dietary treatment was tested in triplicate. The photoperiod was maintained at 12 h light:12 h dark.

Growth performance and survival

Feeding was stopped one day before sampling. Fish length and body weight were measured individually at the beginning and end of the experiment. At the end of the trial, fish were anaesthetized with 2-phenoxyethanol (100 mg/L), after which individual length and weight were recorded. For gut and liver sampling, six fish from each tank were anesthetized and eviscerated then gut and liver were dissected on ice and finally transferred to -80°C for further analyses.

Antioxidant capacity parameters

For liver antioxidant parameters analyses, liver samples were thawed, weighed, and homogenized for 60 s in ice-cold potassium phosphate buffer (0.1 M, pH 7.4) at a tissue-to-buffer ratio of 1:9 (w/v), using a homogenizer operating at 10,000 × g. The homogenates were subsequently centrifuged at 9,000 × g for 30 min at 4°C. The supernatants were collected, aliquoted, and stored at −80°C until analysis. Liver CAT (Aebi, 1974) and SOD (McCord and Fridovich, 1969) activities, as well as glutathione (GSH) levels (Beutler *et al.,* 1963), were determined using standard analytical methods.

Digestive enzymes activity assessment

Gut samples were homogenized for 60 s in ice-cold (0–4°C) mannitol buffer (50 mM mannitol and 2 mM Tris-HCl, pH 7.0) at a tissue-to-buffer ratio of 1:30 (w/v) (Castro-Ruiz *et al.,* 2019). The homogenates were initially centrifuged at 9,000 × g for 10 min at 4°C. The resulting supernatants were collected and subjected to a second centrifugation at 34,000 × g for 30 min at 4°C. The final pellets were resuspended in 1 mL of buffer containing 0.1 M KCl, 5 mM Tris-Hepes, and 1 mM DTT (pH 7.5) for the assessment of intestinal brush-border alkaline phosphatase (ALP) activity (Gisbert *et al.,* 2019). Soluble protein concentration (Bradford, 1976) and the activities of trypsin (Bergmeyer, 1974), chymotrypsin (CHYM, Hummel, 1959), acid protease (AP, Folin and Ciocalteu, 1929), alkaline protease (AKP, Bessey *et al*., 1946), α-amylase (AMYL, Bernfeld, 1955), and lipase (LIP, Tietz and Fiereck, 1966) were determined following established analytical procedures.

Statistical analysis

Data were analysed using SPSS version 21. Data normality and homogeneity of variances were assessed using the Kolmogorov–Smirnov and Levene tests, respectively. Data were analysed by

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two-way analysis of variance (ANOVA) to define the independent effects and the possible interaction between dietary BAU and PRA. A one-way ANOVA, and differences among means were evaluated using Duncan's multiple range test. The significance level was set at 0.05. Excel software was used to prepare the graphs.

Results

Growth performance and feed utilization

The effects of dietary BUA, PRA, and their combination on growth performance and feed utilization of Sobaity seabream after eight weeks are presented in Table 2. Fish fed the PRA0.5 and BUA+PRA0.5 diets exhibited the highest final body weight (FBW) (78.7±2.4 and 77.4±0.7 g, respectively) and weight gain percentage (WGP) (166.3±7.9 and 164.5±7.2%, respectively), with significantly higher values than those of the control, BUA0.5, and BUA+PRA1.0 groups (p<0.05). Similarly, the highest total length (TL) values were recorded in the PRA0.5 and BUA+PRA0.5 groups. Fulton's condition factor (K) was not significantly affected by dietary treatments (p>0.05). Survival rates ranged from 92.5 to 100%, with the highest values observed in the BUA+PRA0.5 and BUA+PRA1.0 groups (100%). The lowest FI was recorded in the BUA1.0 and PRA0.5 groups (55.3±1.1 and 51.8±2.9 g, respectively), whereas the highest feed efficiency ratio (FER) was observed in fish fed PRA0.5 (94.9±2.1%), followed by BUA1.0 (83.1±3.1%).

Table 2: Growth performance and feed utilization in Sobaity seabream (*Sparidentex hasta*) fed the experimental diets for 8 weeks (n = 3).

	IBW	FBW	WGP	DWG	TL	K	SUR	FI	FER
Control	29.8 ± 0.1	71.8 ± 2.2^{b}	141.0 ± 7.8^{b}	0.75 ± 0.0^{b}	16.7 ± 0.2^{b}	1.63 ± 0.0	97.5 ± 1.4^{ab}	59.2 ± 0.4^{b}	70.9 ± 4.2^{b}
BUA0.5	29.6 ± 0.2	72.7 ± 1.4^{b}	145.4 ± 3.7^{b}	0.77 ± 0.0^{b}	17.0 ± 0.3^{b}	1.64 ± 0.0	95.0 ± 2.9^{ab}	60.7 ± 0.4^{b}	71.0 ± 2.5^{b}
BUA1.0	29.5 ± 0.1	75.4 ± 1.5^{ab}	155.4 ± 5.4^{ab}	0.82 ± 0.0^{ab}	17.1 ± 0.2^{b}	1.58 ± 0.0	95.0 ± 2.9^{ab}	55.3 ± 1.1^{c}	83.1 ± 3.1^{ab}
PRA0.5	29.5 ± 0.1	78.7 ± 2.4^{a}	166.3 ± 7.9^{a}	0.88 ± 0.0^{b}	17.7 ± 0.4^{a}	1.62 ± 0.0	97.5 ± 1.4^{ab}	51.8 ± 2.9^{c}	94.9 ± 2.1^{a}
PRA1.0	29.6 ± 0.2	76.8 ± 1.1^{ab}	159.5 ± 2.4^{ab}	0.84 ± 0.0^{ab}	17.1 ± 0.2^{b}	1.68 ± 0.0	92.5 ± 1.4^{b}	71.6 ± 2.8^{a}	66.2 ±3.5^{c}
BUA+PRA0.5	29.2 ± 0.1	77.4 ± 0.7^{a}	164.5 ± 7.2^{a}	0.86 ± 0.0^{a}	17.6 ± 0.3^{a}	1.55 ± 0.0	100.0 ± 0.0^{a}	71.9 ± 3.0^{a}	67.3 ± 5.1^{c}
BUA+PRA1.0	29.6 ± 0.1	72.8 ± 1.2^{b}	145.7 ± 5.2^{b}	0.77 ± 0.0^{b}	17.1 ± 0.3^{b}	1.56 ± 0.0	100.0 ± 0.0^{a}	58.6 ± 3.0^{b}	73.9 ± 3.2^{b}
	Two-Way ANOVA								
BUA	0.240	0.826	0.906	0.862	0.796	0.063	**0.001**	**0.001**	**0.001**
PRA	0.065	**0.047**	**0.020**	**0.038**	**0.038**	0.335	0.149	**0.001**	**0.001**
Interaction	0.301	0.158	0.097	0.130	0.558	0.566	**0.001**	**0.001**	**0.004**

Values are presented as the means ± SE (n = 3 tanks). Different superscripts in each row show significant differences among groups (P < 0.05). Diet abbreviations: BAU0.5 (butyric acid 0.5%); BUA1.0 (butyric acid 1.0%); PRA0.5 (propionic acid 0.5%); PRA1.0 (propionic acid 1.0%); BUA+PRA0.5 (butyric acid 0.25% + propionic acid 0.25%); BUA+PRA1.0 (butyric acid 0.5% + propionic acid 0.5%).
IBW: initial body weight; FBW: final body weight; WGP: weight gain percentage = [FBW (g) - IBW (g) / IBW (g)] × 100; DWG: daily weight gain = [FBW (g) - IBW (g) / 56)]; TL: total length (cm); K: Fulton's condition factor = FBW (g) / L ^{3} × 100; SUR: survival = final number of fish / initial number of fish × 100; FI: feed intake = total feed intake (g) / number of fish; FER: feed conversion ratio = WG (g) /FI (g) × 100

In contrast, the PRA1.0 and BUA+PRA0.5 groups exhibited the highest FI (71.6±2.8 and 71.9±3.0 g, respectively) and comparatively lower

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FER values (66.2±3.5 and 67.3±5.1%, respectively). Two-way ANOVA revealed that BUA supplementation significantly affected survival, FI, and FER (p<0.05), whereas PRA supplementation significantly influenced FBW, WGP, DWG, TL, FI, and FER. Significant interactions between BUA and PRA were detected for survival, FI, and FER (p<0.05).

Liver antioxidant responses

The effects of dietary organic acid supplementation on liver antioxidant responses are presented in Table 3. GSH content was highest in the PRA0.5 group (35.8±1.8 μmol/ g tissue), which was significantly greater than that of the control group (14.9±0.9 μmol/ g tissue; p<0.05). Similarly, CAT activity reached its highest value in fish fed PRA0.5 (121.5±15.4 U/mg protein), which was significantly higher than that of the control and BUA+PRA1.0 groups (p<0.05). SOD activity was significantly higher in the BUA0.5 group (44.0±1.4 U/mg protein) than in the control and BUA+PRA1.0 groups (31.6±3.2 and 29.5±1.7 U/mg protein, respectively; p<0.05). According to the two-way ANOVA, both BUA and PRA supplementation significantly affected GSH and CAT values (p<0.05). PRA also exerted a significant effect on SOD activity (p<0.05), whereas BUA had no significant main effect on this parameter (p>0.05). A significant interaction between BUA and PRA was detected for GSH and CAT values (p<0.05), but not for SOD activity (p>0.05).

Table 3: Liver antioxidant responses in Sobaity seabream (*Sparidentex hasta*) fed the experimental diets for 8 weeks (n = 3).

	IBW	FBW	WGP
Control	14.9 ± 0.9^{b}	63.0 ± 13.5^{b}	31.6 ± 3.2^{b}
BUA0.5	24.5 ± 2.3^{ab}	94.4 ± 17.5^{ab}	44.0 ± 1.4^{a}
BUA1.0	30.1 ± 2.8^{ab}	87.8 ± 15.8^{ab}	41.6 ± 1.3^{ab}
PRA0.5	35.8 ± 1.8^{a}	121.5 ± 15.4^{a}	38.1 ± 2.8^{ab}
PRA1	27.9 ± 3.2^{ab}	86.8 ± 12.7^{ab}	40.9 ± 0.6^{ab}
BUA+PRA0.5	28.0 ± 1.4^{ab}	90.6 ± 14.8^{ab}	38.7 ± 2.3^{ab}
BUA+PRA1.0	27.3 ± 1.8^{ab}	54.4 ± 15.5^{b}	29.5 ± 1.7^{b}
Two-Way ANOVA			
BUA	**0.011**	**0.001**	0.735
PRA	**0.001**	**0.011**	**0.001**
Interaction	**0.001**	**0.025**	0.097

Values are presented as the means ± SE (n = 3 tanks). Different superscripts in each row show significant differences among groups (p<0.05). Abbreviations: GSH: glutathione; CT: catalase; SOD: superoxide dismutase. Diet abbreviations: BAU0.5 (butyric acid 0.5%); BUA1.0 (butyric acid 1.0%); PRA0.5 (propionic acid 0.5%); PRA1.0 (propionic acid 1.0%); BUA+PRA0.5 (butyric acid 0.25% + propionic acid 0.25%); BUA+PRA1.0 (butyric acid 0.5% + propionic acid 0.5%).

Digestive enzyme activities

The effects of dietary organic acid supplementation on digestive enzyme activities are presented in Table 4. ALP activity was significantly affected by dietary treatments, with the highest value recorded in the BUA0.5 group (79.2±13.1 U/mg protein), followed by

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PRA1.0 (54.1±4.5 U/mg protein) and BUA+PRA1.0 (45.5±5.6 U/mg protein). For trypsin activity, the highest numerical value was observed in the BUA+PRA0.5 group (29.3±7.2 U/mg protein), which was significantly higher than that of the control group (8.2±2.2 U/mg protein; $p<0.05$). Chymotrypsin activity was also significantly enhanced in the PRA0.5 and BUA+PRA0.5 groups (17.8±3.6 and 18.6±p1.9 mU/mg protein, respectively) compared with the control and BUA0.5 groups ($p<0.05$). Acid protease activity was significantly higher in the BUA1.0, PRA1.0, and BUA+PRA0.5 groups (2.6±0.3, 2.7±0.2, and 2.8±0.3 U/mg protein, respectively) than in the control and BUA0.5 groups (1.7±0.1 U/mg protein; $p<0.05$). Similarly, AKP activity was highest in the BUA0.5 group (18.1±1.1 U/mg protein), followed by the PRA1.0 group (16.2±1.1 U/mg protein). The highest AMYL activities were recorded in the PRA0.5 and BUA+PRA0.5 groups (495.1±74.0 and 428.0±108.0 mU/mg protein, respectively), which were significantly higher than those in the control, BUA0.5, and BUA+PRA1.0 groups ($p<0.05$).

Table 4: Digestive enzymes activity in Sobaity seabream (*Sparidentex hasta*) fed the experimental diets for 8 weeks (n = 3).

	ALP (U/mg protein)	TRYP (U/mg protein)	CHY (mU/mg protein)	AP (U/mg protein)	AKP (U/mg protein)	AMYL (mU/mg protein)	LIP (U/mg protein)
Control	18.8 ± 2.1^{f}	8.2 ± 2.2^{b}	9.9 ± 1.1^{b}	1.7 ± 0.1^{b}	9.2 ± 1.4^{c}	62.6 ± 5.4^{c}	6.5 ± 0.6^{b}
BUA0.5	79.2 ± 13.1^{a}	17.3 ± 3.3^{ab}	10.0 ± 0.8^{b}	1.7 ± 0.1^{b}	18.1 ± 1.1^{a}	104.6 ± 9.3^{b}	7.6 ± 1.3^{b}
BUA1.0	37.1 ± 9.2^{d}	16.7 ± 5.9^{ab}	13.1 ± 1.5^{ab}	2.6 ± 0.3^{a}	11.5 ± 1.1^{b}	119.4 ± 12.8^{b}	10.2 ± 1.1^{ab}
PRA0.5	31.7 ± 3.0^{e}	14.4 ± 1.9^{ab}	17.8 ± 3.6^{a}	2.0 ± 0.1^{ab}	11.5 ± 0.7^{b}	495.1 ± 74.0^{a}	8.7 ± 1.0^{ab}
PRA1.0	54.1 ± 4.5^{b}	16.3 ± 2.2^{ab}	10.7 ± 1.2^{b}	2.7 ± 0.2^{a}	16.2 ± 1.1^{ab}	262.2 ± 34.9^{ab}	8.8 ± 1.8^{ab}
BUA+PRA0.5	29.2 ± 5.0^{e}	29.3 ± 7.2^{a}	18.6 ± 1.9^{a}	2.8 ± 0.3^{a}	14.4 ± 1.2^{ab}	428.0 ± 108^{a}	12.5 ± 1.5^{a}
BUA+PRA1.0	45.5 ± 5.6^{c}	13.0 ± 1.9^{ab}	14.2 ± 1.4^{ab}	2.0 ± 0.4^{ab}	14.4 ± 1.3^{ab}	57.0 ± 5.0^{c}	10.0 ± 1.2^{ab}
Two-Way ANOVA							
BUA	**0.001**	**0.001**	**0.001**	0.190	**0.001**	0.072	**0.039**
PRA	**0.002**	0.368	**0.006**	**0.014**	**0.001**	**0.001**	**0.029**
Interaction	**0.001**	**0.002**	0.080	0.666	**0.001**	0.282	0.261

Values are presented as the means±SE (n=3 tanks). Different superscripts in each row show significant differences among groups ($P<0.05$). Abbreviations: ALP: alkaline phosphatase; TRYP: trypsin; CHY: chymotrypsin; AP: acid protease; AKP: alkaline protease; AMYL: amylase; LIP: lipase. Diet abbreviations: BAU0.5 (butyric acid 0.5%); BUA1.0 (butyric acid 1.0%); PRA0.5 (propionic acid 0.5%); PRA1.0 (propionic acid 1.0%); BUA+PRA0.5 (butyric acid 0.25% + propionic acid 0.25%); BUA+PRA1.0 (butyric acid 0.5% + propionic acid 0.5%).

The PRA1.0 group exhibited an intermediate AMYL activity (262.2±34.9 mU/mg protein). Lipase activity was highest in the BUA+PRA0.5 group (12.5±1.5 U/mg protein), which was significantly higher than that of the control and BUA0.5 groups (6.5±0.6 and 7.6±1.3 U/mg protein, respectively; $p<0.05$). Two-way ANOVA indicated that BUA significantly affected ALP, TRYP, CHY, AKP, and LIP activities, whereas PRA significantly influenced ALP, CHY, AP, AKP, AMYL, and LIP

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activities. Significant BUA×PRA interactions were detected for ALP, TRYP, and AKP activities (p<0.05), but not for CHY, AP, AMYL, or LIP activities.

Discussion

Effects of dietary organic acids on growth performance and feed utilization

In the present study, fish fed diets containing 0.5% or 1.0% PRA, or a 0.5% blend of BAU and PRA, showed higher growth rates than the other groups. Studies have shown that adding organic acids to diets can increase the bioavailability of nutrients, including amino acids and minerals such as phosphorus, zinc and calcium, by improving digestibility, modifying intestinal bacterial flora and chelating minerals (Ng *et al.,* 2009). Furthermore, lowering intestinal pH through the use of organic acids increases mineral solubility in the intestine and improves their bioavailability (Hoseinifar *et al.,* 2017). Short-chain fatty acids are absorbed by passive diffusion through the intestinal epithelium and participate in energy production through the Krebs cycle (Lückstadt, 2008). Intestinal epithelial cells can use these compounds for tissue repair and to support nutrient digestion and absorption (Ng and Koh, 2017). This may increase the size of intestinal brush-border structures, expand the absorptive surface area and consequently improve feed utilization, as reported for red seabream (*Pagrus major*, Sarker *et al.,* 2007). An initial explanation for improvements in fish growth efficiency and FER is the effect of these compounds on pH in the stomach and intestine (Fabay *et al.,* 2022). The improved growth performance observed in fish fed PRA0.5 suggests that PRA may have a particularly beneficial role in nutrient utilization and metabolic regulation in Sobaity seabream. Fish receiving 0.5% PRA exhibited the highest FBW, WGP, and FER, together with the lowest FI among the experimental groups. This combination is particularly noteworthy because it indicates that improved growth was achieved without a corresponding increase in feed consumption. Therefore, the growth-promoting effect of PRA0.5 may be associated more closely with improved feed utilization than with increased appetite. Propionate and butyrate can also participate in host metabolic pathways and serve as substrates for cellular energy production. These physiological functions may contribute to improved nutrient utilization and growth performance in aquatic animals (Hoseinifar *et al.,* 2017; Tran *et al.,* 2020). Similarly, Estensoro *et al*. (2016) demonstrated that dietary sodium butyrate could help restore intestinal condition in gilthead seabream (*Sparus aurata*) fed diets containing high levels of plant-derived ingredients, supporting the potential role of butyrate in maintaining intestinal functionality and nutrient utilization. Interestingly, the combination of BUA and PRA at 0.5% exhibited one of the highest FI values and a comparatively low FER. This observation suggests that the simultaneous inclusion of both SCFA

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may have influenced feeding behavior or nutrient utilization differently from individual supplementation. Furthermore, the reduced growth response at the higher combined inclusion level (BUA+PRA1.0) indicates that the biological effects of these acids may depend on their dietary concentration and relative proportions.

Effects of dietary organic acids on hepatic antioxidant status

Oxidative balance is an important component of physiological health in fish because reactive oxygen species (ROS), when produced in excess, can damage cellular membranes, proteins, and nucleic acids. The antioxidant defense system includes enzymatic components such as SOD and CAT, as well as non-enzymatic antioxidants, including GSH (Birnie-Gauvin *et al.,* 2017). In the present study, the most pronounced responses in GSH and CAT were observed in fish fed PRA0.5, whereas the highest SOD activity was recorded in the BUA0.5 group. These findings suggest that BUA and PRA may influence different components of the liver antioxidant defense system. The enhanced GSH and CAT activities in fish receiving PRA0.5 may indicate an improved capacity to maintain intracellular redox balance. Butyrate, in particular, has attracted attention because of its potential to modulate oxidative stress responses and intestinal health in aquatic animals (Tran *et al.,* 2020). In this sense, Estensoro *et al*. (2016) reported beneficial effects of sodium butyrate on intestinal condition in gilthead seabream. These findings emphasize that the antioxidant effects of organic acids may depend on their chemical form, dietary concentration, and fish species. In addition, BAU, particularly in its sodium salt form (sodium butyrate), has demonstrated beneficial effects on antioxidant defense mechanisms in Asian seabass (*Lates calcarifer*). Alamifar *et al.* (2020) reported that dietary BAU supplementation increased liver CAT activity, while SOD activity was significantly elevated in fish receiving 0.5 and 1.0% dietary BAU. An interesting observation in the present study was the relatively low GSH and CAT activities in fish fed the higher combined inclusion level (BUA+PRA1.0). In fact, CAT activity in this group was numerically lower than that of the control group. This response suggests that the simultaneous inclusion of higher levels of BUA and PRA did not provide additional antioxidant benefits. One possible explanation is that the biological response to organic acids follows a concentration-dependent pattern, whereby an appropriate inclusion level supports physiological regulation, whereas excessive supplementation may alter metabolic homeostasis. The significant BUA × PRA interaction for GSH and CAT further indicates that the effects of these SCFA on antioxidant responses were dependent on their combined dietary inclusion levels. Taken together, the results suggest that moderate dietary SCFA supplementation, particularly 0.5% PRA, may support hepatic

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antioxidant defense in juvenile Sobaity seabream.

Effects of dietary organic acids on digestive enzyme activities

Digestive enzyme activities are important indicators of the digestive and absorptive capacity of fish and provide information about the potential effects of dietary additives on nutrient utilization. Several studies reported the positive effects of SCFA on digestive enzymes in numerous marine fish species. For instance, BAU (0.25-1.0%) enhanced LIP and AKP activities in Asian seabass (Alamifar *et al.,* 2020), sodium butyrate (0.1-0.3%) enhanced LIP, TRYP and AKP in orange-spotted grouper (*Epinephelus coioides*, Ke *et al.,* 2022) or sodium butyrate (0.05-0.4%) increased protease, lipase and amylase in thinlip grey mullet (*Liza ramada*, El-Sharkawy *et al.,* 2023). Furthermore, organic acid salts can mitigate inflammatory responses associated with plant-protein-rich diets in yellowfin seabream (*Acanthopagrus latus*), suggesting their potential to improve intestinal health and facilitate the utilization of alternative protein sources (Sotoudeh *et al.,* 2020).One of the most notable findings was the substantial increase in amylase activity in fish fed PRA0.5 and BUA+PRA0.5. Amylase activity in these groups was approximately 7.9- and 6.8-fold higher than in the control group, respectively. This finding suggests that moderate organic acid supplementation may be associated with enhanced carbohydrate digestive capacity. SCFA can influence gastrointestinal physicochemical conditions and microbial activity, potentially modifying the digestive environment and nutrient availability (Hoseinifar *et al.,* 2017). Moreover, SCFA can interact with host metabolic pathways and contribute to intestinal energy metabolism (Tran *et al.,* 2020). The increased amylase activity in the PRA0.5 group is particularly interesting because this treatment also resulted in the highest growth performance and feed efficiency. These simultaneous responses suggest a possible association between improved digestive functionality and enhanced growth. In this study, the highest trypsin activity was recorded in the BUA+PRA0.5 group, whereas CHYM activity was significantly elevated in the PRA0.5 and BUA+PRA0.5 groups compared with the control. These findings indicate that moderate supplementation, particularly when the two acids were combined, may influence protein digestion. Since dietary protein is a major determinant of growth in carnivorous marine fish, improvements in proteolytic activity could potentially contribute to better utilization of dietary protein. ALP is associated with intestinal epithelial function, nutrient transport, and phosphate metabolism, and its activity can provide information about intestinal physiological status (Lallès, 2020). The elevated ALP and AKP activities observed following BUA0.5 supplementation may therefore reflect changes in digestive and intestinal metabolic processes. Similarly, LIP activity was highest in fish receiving the

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combined BUA+PRA0.5 treatment, suggesting a possible stimulatory effect of the SCFA combination on lipid digestion. This response may be relevant to the utilization of dietary lipids as an important energy source. Nevertheless, the absence of a significant interaction between BUA and PRA for lipase activity indicates that the observed differences should not be interpreted as evidence of a statistically confirmed synergistic effect. An important finding was that higher combined supplementation (BUA+PRA1.0) did not consistently enhance digestive enzyme activities. For example, AMYL activity in this group was comparable to the control, despite the relatively high activities recorded in the BUA+PRA0.5 group. This observation further supports the hypothesis that the physiological effects of SCFA are dependent on inclusion level and that increasing dietary concentrations does not necessarily improve digestive performance. Overall, the digestive enzyme results indicate that moderate organic acid supplementation can modulate different components of the digestive system, with PRA0.5 and BUA+PRA0.5 showing particularly favorable responses in carbohydrate and protein-digesting enzymes, respectively.

Conclusion

Overall, the present findings demonstrate that dietary BUA and PRA can differentially influence growth performance, feed utilization, hepatic antioxidant responses, and digestive enzyme activities in juvenile Sobaity seabream. Among the tested treatments, 0.5% PRA produced the most consistent overall benefits, particularly in terms of growth performance, feed efficiency, live GSH and CAT activities, and several digestive enzyme activities. Although combined supplementation at the lower inclusion level improved some growth and digestive responses, it did not provide a consistent advantage over individual PRA supplementation. These findings suggest that the selection of an appropriate organic acid and inclusion level is more important than simply increasing the total concentration or combining different acids.

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