Aquaculture International (2021) 29:261–273
https://doi.org/10.1007/s10499-020-00625-x

Growth, body composition, and hematology of yellowfin seabream (*Acanthopagrus latus*) given feeds supplemented with organic acid salts (sodium acetate and sodium propionate)

Mohammad Sangari[1] · Ebrahim Sotoudeh[1] · Dara Bagheri[1] · Salim Morammazi[2] · Mansour Torfi Mozanzadeh[3]

Received: 14 July 2020 / Accepted: 18 November 2020 / Published online: 26 November 2020

Abstract
Effects of adding organic acid salts (OAS) to plant protein (PP)–rich feeds were evaluated in a trial with juvenile yellowfin seabream (*Acanthopagrus latus*) (initial weight ca 6.5 g). Fish were given iso-nitrogenous (ca. 48% protein) and iso-caloric (ca. 19.6 kJ g^{-1}) feeds supplemented with sodium acetate (SA) and sodium propionate (SP): SP5 (5 g SP kg^{-1}), SP10 (10 g SP kg^{-1}), SA5 (5 g SA kg^{-1}), SA10 (10 g SA kg^{-1}), and SP+SA (5 g SP kg^{-1} + 5 g SA kg^{-1}). A PP-rich feed without OAS supplementation was the control. There were 3 replicates for each treatment and trial duration was 8 weeks, during which time the fish were kept in 60-L tanks (10 fish per tank) and fed to satiation twice each day. At the end of the trial, growth performance, feed utilization, whole-body proximate composition, and hemato-biochemical parameters were analyzed. Final weights in SP5 (14.61 g), SP10 (14.14 g), and SP+SA (14.29 g) groups were remarkably higher than the control (11.18 g). The highest and the least feed conversion ratio values were in the control (1.71) and SP5 (1.19) groups, respectively. Whole-body proximate composition did not change among groups. Blood hemoglobin contents in fish fed the OAS-supplemented diets were between 7.44 and 7.88 g dL^{-1} that was higher than the control (6.47 g dL^{-1}). Fish fed on the OAS-incorporated diets had greater amounts of plasma total protein (6.0–6.94 g dL^{-1}) compared to the control (5.06 g dL^{-1}). According to the findings of this study, administrating 5 g SP kg^{-1} of a PP-rich diet is recommended for improving growth and welfare of *A. latus* juveniles.

✉ Ebrahim Sotoudeh
E.sotoudeh@pgu.ac.ir

[1] Department of Fisheries, Faculty of Marine Science and Technology, Persian Gulf University, Bushehr 75169, Iran

[2] Department of Animal Science, Faculty of Agricultural and Natural Resources, Persian Gulf University, Bushehr 75169, Iran

[3] Agriculture Research, Education and Extension, South Iran Aquaculture Research Center, Iran Fisheries Science Research Institution (IFSRI), Ahwaz, Iran

Keywords Feed additives · Feed utilization · Health indices · Plasma metabolites · Sparidae

Introduction

Terrestrial plant ingredients make up a major part of aquafeeds and supported the sustainability of aquafeed industry (Gatlin et al. 2007; Hardy 2010). However, formulation of plant protein (PP)–rich diets especially for carnivorous fish species is challenging due to the existence of anti-nutrients (e.g., tannins, saponins, lectins, phytate, and protease inhibitors) and non-starch polysaccharides (e.g., cellulose, xylan and pectin), deficiencies or imbalances in their amino acid profiles, and low bioavailability of some minerals (Francis et al. 2001; Gatlin et al. 2007). A plethora of studies demonstrated that feeding various marine carnivorous fish species with PP-rich diets resulted in growth retardation, nutrients digestibility reduction, anemia, metabolic disorders, gut inflammation, and immunosuppression (Silva-Carrillo et al. 2012; Song et al. 2014; Sotoudeh et al. 2016; Yaghoubi et al. 2016; Zhang et al. 2016; Abbasi et al. 2020; Hernández et al. 2020). There is an increasing interest in using organic acids particularly short chain fatty acids (SCFA) for alleviating the negative impacts of PP-rich diets (Hoseinifar et al. 2017; Ng and Koh 2017; Tran et al. 2018). Organic acids particularly SCFA or their salts have been shown to alleviate some problems brought by inclusion of PP sources in diet such as mitigation of gut inflammatory response, oxidative damage, intestinal microecological imbalance, and healing gut tight junction disruption (Rimoldi et al. 2016; Liu et al. 2019). Organic acid salts (OAS) are chelated form of SCFA to minerals (Ng and Koh 2017). Short chain fatty acids such as acetic acid and propionic acid are generally passively absorbed by the intestinal epithelia and they are preferred energy sources for enterocytes through the citric or carboxylic acid cycles (Luckstadt 2008; Ng and Koh 2017; Tran et al. 2018). It has been reported that SCFA or their salts increase the bioavailability of dietary minerals by chelating them. Also, OAS can enhance the digestibility of dietary nutrients by acidifying diet and the gastrointestinal tract through delivering H^+ ions (Lim et al. 2015; Ng and Koh 2017). Furthermore, supplementing aquafeeds with OAS can promote the physical quality of diet such as expansion ratio, durability, and water stability of pellets (Morken et al. 2011). Besides, OAS can beneficially modulate the fish intestinal microbiome by reducing pathogenic bacteria and increasing lactic acid bacteria (LAB) colonization (Luckstadt 2008; Katya et al. 2018; Reyshahri et al. 2019). In addition, due to their antimicrobial characteristics, OAS are considered as ecofriendly and biodegrable alternatives for antibiotics in aquaculture (Hoseinifar et al. 2017; Ng and Koh 2017).

Yellowfin seabream, *Acanthopagrus latus*, is a valuable carnivorous species and considered as a promising candidate for extending marine cage culture in the tropical and subtropical regions. Furthermore, in recent years, this species was considered as good alternative to shrimp culture especially in the south-west of Iran where shrimp culture industry drastically suffered from white spot disease (Vahabnezhad et al. 2016; Esmaeili et al. 2019). A recent work by Abbasi et al. (2020) demonstrated that about 57% of FM and FO can be replaced with mixtures of PP and vegetal oils in diet for this species, indicating yellowfin seabream has great potential in utilizing PP feedstuffs. It has been speculated that inclusion of OAS in a PP-rich diet can enhance the tolerance of carnivorous fish species to high levels of PP in diet (Zhang et al. 2016; Lin and Cheng 2017; Chen et al. 2018). Thus, in the present study, it was aimed to

evaluate the influence of supplementing a PP-rich diet with individual or blend of OAS including sodium propionate and acetate on growth rate, hemato-biochemical parameters, and body composition of *A. latus* juveniles.

Materials and methods

Feed preparation

Six iso-nitrogenous (ca. 48% crude protein) and iso-caloric (ca. 19.6 kJ g^{-1}) PP-rich feeds were formulated (WUFFDA software, Ver 2.1, USA) (Table 1). About 75% of dietary protein was derived from blends of PP sources and crystalline amino acids. The PP-rich diet was supplemented with different levels of OAS including sodium propionate (SP) and sodium acetate (SA) to design experimental feeds as follows: SP5 (5 g SP kg^{-1} diet), SP10 (10 g SP kg^{-1} diet), SA5 (5 g SA kg^{-1} diet), SA10 (10 g SA kg^{-1} diet), and SP + SA (5 g SP kg^{-1} + 5 g SA kg^{-1} diet). Organic acid salts were added in the experimental diets at the expense of cellulose. The PP-rich diet without OAS supplementation was used as a control. Dry feedstuffs and OAS were blended for 20 min, then oils were added to the mixture and blended for more 10 min. Enough distilled water was added to form a dough and pellets (1 mm) were made with a meat grinder. Finally, pellets were dried (60° C for 12 h) then kept in a freezer (− 18° C).

Standard methods were conducted to examine proximate biochemical composition of feed stuffs, experimental diets, and fish whole body (AOAC 2005). Samples were dried at 105° C to a constant weight to determine their moisture content in an oven (D-63450; Heraeus, Hanau, Germany). Crude protein content was determined using an Auto Kjeldahl System (N × 6.25; Kjeltec Auto Analyzer; FOSS, Hillerød, Denmark) and estimated by multiplying nitrogen by 6.25. Crude lipid was measured following the chloroform-methanol method (Folch et al. 1957). Ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace (Muffle furnace, Isuzu, Tokyo, Japan) at 550° C for 8 h. Dietary crud fiber was measured according to the method of AOAC (2000), using an automatic fiber analyzer (Fibertec System M, Tecator, Sweden).

Husbandry trial

The present study was performed at Aquatic Research Laboratory of Agriculture and Natural Resources College of Persian Gulf University (29°26′ N, 51°23′ E, Borazjan, Bushehr, Iran). A total of 250 yellowfin seabream were purchased from the Mariculture Research Station of Iranian Fisheries Science Institute (30°32′ N, 49°20′ E, Sarbandar, Khuzestan, Iran) and stocked in a 2000-L circular fiberglass tank and acclimated to the husbandry system for 2 weeks. During a 2-week acclimation period, fish were fed with the control diet to apparent satiation, twice (at 10:00 and 16:00 h) daily. One hundred and eighty fish (6.5 ± 0.2 g, mean ± standard error) were individually weighed then distributed into 18 fiberglass tanks with volume of 60 L (10 fish $tank^{-1}$) and each treatment replicated in triplicate. Husbandry system was filled with sand-filtered and chlorine disinfected brackish water and about 30% of water was exchanged daily. The temperature, pH, ammonia-nitrogen, and salinity of water were 22.0 ± 0.5° C, 7.5 ± 0.5, 0.09 ppm, and 10.0 ± 0.2 ppt, respectively. The photoperiod was 12L:12D (light:darkness). Fish were hand-fed on the experimental feeds twice daily (10:00 and 16:00 h) to apparent satiation for 56 days. Every day in the morning, the bottom of the tanks was

Table 1 Ingredients and proximate composition of the experimental diets (g kg^{-1} dry diet)

Ingredients[a]	Experimental diets					
	Control	SP5	SP10	SA5	SA10	SP + SA
Fish meal[1]	170.0	170.0	170.0	170.0	170.0	170.0
Soybean meal[2]	402.3	402.3	402.3	402.3	402.3	402.3
Corn gluten[1]	167.5	167.5	167.5	167.5	167.5	167.5
Wheat gluten[1]	90.0	90.0	90.0	90.0	90.0	90.0
Wheat middlings[1]	20.0	20.0	20.0	20.0	20.0	20.0
Fish oil[1]	70.0	70.0	70.0	70.0	70.0	70.0
Soybean oil[2]	20.0	20.0	20.0	20.0	20.0	20.0
Soy lecithin[2]	10.0	10.0	10.0	10.0	10.0	10.0
Mineral mix[3]	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin mix[3]	5.0	10.0	10.0	10.0	10.0	10.0
Antioxidant	0.20	0.20	0.20	0.20	0.20	0.20
Methionine	5.0	5.0	5.0	5.0	5.0	5.0
Lysine	5.0	5.0	5.0	5.0	5.0	5.0
Dicalcium phosphate	5.0	5.0	5.0	5.0	5.0	5.0
Sodium acetate[4]	0.00	0.00	0.00	5.0	10.0	5.0
Sodium propionate[4]	0.00	5.0	10.0	0.00	0.00	5.0
Cellulose	10.0	5.0	0.00	5.0	0.00	0.00
Proximate analysis (g/kg)						
Protein	482.1	480.0	480.2	481.9	482.6	479.8
Lipid	123.8	124.5	125.1	122.6	121.6	123.2
Moisture	103.0	99.0	101.0	98.0	102.0	96.0
Ash	96.1	96.5	97.2	96.2	95.5	96.1
NFE[5]	232.0	230.0	226.5	231.3	228.3	232.9
Gross energy (kJ g^{-1})[6]	19.62	19.69	19.65	19.68	19.60	19.71

Diet abbreviations: SP5 (5 g kg^{-1} SP), SP10 (10 g kg^{-1} SP), SA5 (5 g kg^{-1} SA), SA10 (10 g kg^{-1} SA), and SP + SA (5 g kg^{-1} SP + 5 g kg^{-1} SA)

[a] Composition of ingredients as % dry weight basis [fish meal (73.5% crude protein, 10.7% crude lipid); soybean meal (41.0% crude protein, 4.2% crude lipid); corn gluten (71.4% crude protein, 4.1% crude lipid); wheat gluten (52.4% crude protein, 3% crude lipid)

[1] Beyza Feed Mill 21, Shiraz, Iran

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

[3] Vitamin and mineral premix U kg^{-1} of premix: vitamin A 5,000,000 IU, vitamin D3 500,000 IU, vitamin E 3000 mg, vitamin K3 1500 mg, vitamin, 6000 mg, vitamin B2 24,000 mg, vitamin B5 52,000 mg, vitamin B6 18,000 mg, vitamin B12 60,000 mg, folic acid 3000 mg, nicotinamide 180,000 mg, antioxidant 500 mg, copper 3000 mg, zinc 15,000 mg, manganese 20,000 mg, iron 10,000 mg, potassium iodate 300 mg, career up to1 kg, Damloran pharmaceutical company, Broujerd, Iran

[4] Sigma-Aldrich (99% purity, Germany)

[5] Nitrogen-free extract, calculated as 100 − (crude protein + crude lipid + crude ash + crude fiber)

[6] Calculated on gross energy values of 23.6 k g^{-1} proteins, 39.5 kJ g^{-1} fat, and 17.2 kJ g^{-1} carbohydrates (NRC 2011)

siphoned for cleaning up the feces. Thirty minutes after each feeding, the unfed pellets were siphoned from bottom of the tanks then dried in an oven (50° C, 24 h) and weighed.

Sampling

At the beginning of the feeding trial, 10 fish were collected from the initial population for the analysis of whole-body proximate composition. After 56 days of feeding trial, fish were unfed for a day. Biometry of fish regarding their weight (g) and total length (cm) were done

individually for all treatments. For evaluating complete blood counts, nine fish per treatment (3 fish of each tank) were anesthetized with an anesthetic (clove powder, 500 ppm) and bled from the caudal vein using heparinized syringes and the collected blood was transferred into heparinized vials. For examining plasma biochemical parameters, nine fish in treatment (3 fish of each tank) were anesthetized with the same anesthetic and bled with heparinized syringes and blood was transferred into heparinised vials, then centrifuged (1600*g*, 5 min) and plasma was extracted and kept in a freezer (− 80° C). Six fish per treatment (2 fish from each tank) were sacrificed with an overdose of the anesthetic (1000 ppm) and kept in a freezer (− 20° C) to determine the whole-body proximate composition.

Calculations

Standard formulae were used to determine growth performance, feed utilization, and somatic indices: SGR: specific growth rate (%) = ((ln BW_f – ln BW_i)/t) × 100, where t is the experimental period = 8 weeks; WG: weight gain (%) = ((BW_f – BW_i)/BW_i) × 100; feed intake = total feed intake per tank (g)/number of fish; FCR: feed conversion ratio = (feed intake (g)/weight gain (g)); PER: protein efficiency ratio = (protein intake (g)/weight gain (g)); K: Fulton's condition factor = (BW_f (g)/standard length (cm)^3) × 100; in which BW_i and BW_f are initial body weight and final body weight, respectively. For determining nutrients retention in the whole body, the following formulae were used: protein retention (PR) = [BW_f (g) × crude protein of final fish (%) − BW_i (g) × crude protein of initial fish (%)]/protein intake (g) and lipid retention (LR) = [BW_f (g) × crude lipid of final fish (%) − BW_i (g) × crude lipid of initial fish (%)]/lipid intake (g).

Hematology

Complete blood counts were examined as described by Blaxhall and Daisley (1973) and Dacie and Lewis (2001). The number of blood red cells (RBC) and white blood cells (WBC) were counted manually using a hemocytometer. Hematocrit (Hct) was analyzed using the standard microhematocrit method (centrifuging 13,000 rpm for 3 min) and reported as percentages. Hemoglobin (Hb) concentration was determined using a cyanomethemoglobin method. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the following formulae:

$$\begin{gathered}\mathrm{MCV\ (nm3)} = \mathrm{Hct\ (\%)/RBC\ (\times 106\ \mu L)} \times 10 \\ \mathrm{MCH\ (pg\ cell{-}1)} = \mathrm{Hb\ (g\ dL{-}1)/RBC\ (\times 106\ \mu L)} \times 10 \\ \mathrm{MCHC\ (g\ cell{-}1)} = \mathrm{Hb\ (g\ dL{-}1)/Hct\ (\%)}\end{gathered}$$

Plasma metabolites including total protein, albumin, glucose, and triglyceride were measured using an autoanalyzer (Technicon RA-1000, USA) by clinical kits (Pars Azmoon Kit, Iran).

Statistics

A SPSS ver. 20 (Chicago, Illinois, USA) software was used for data analyses. Normality of data tested with Kolmogorov-Smirnov test and homogeneity of variance was carried out with Levene test. One-way ANOVA at α = 0.05 used for finding significant differences among groups and Duncan's procedure was used for multiple comparisons.

Results

All fish survived the growth trial (Table 2). Growth of fish given SA5 did not differ significantly from that of the fish given the control feed, but the other treatments had higher growth than the control ($P < 0.05$). The growth performance of fish fed SA-supplemented diets did not significantly different, regardless of its inclusion levels. Fulton's condition factor was not affected by diets. Feed intake in fish fed SP-supplemented and SP + SA diets was higher than those in the control and SA10 groups ($P < 0.05$). In addition, there was no significant difference in feed intake values between SP10 and SA5 groups ($P > 0.05$). Feed conversion ratio was increased in the control compared to OAS-supplemented diets. Protein efficiency ratio in fish fed on SP-supplemented diets (5 and 10 g kg^{-1}) was greater than those fed the control and SA-supplemented diets; meanwhile, fish fed on the SP + SA-supplemented diet showed intermediate value. The whole-body biochemical composition of fish was not affected by supplementing diets with single or mixture of OAS ($P > 0.05$, Table 3), but protein retention increased in fish fed on the OAS-supplemented diets.

Regarding hematological parameters, inclusion of OAS in diet elevated blood Hb content in comparison with the control ($P < 0.05$, Table 4). However, other factors including RBC and WBC, Hct, and blood indices were not influenced by diets. The amounts of plasma total protein and albumin in fish fed on the OAS-incorporated diets were greater than the control ($P < 0.05$, Table 5); meanwhile, plasma glucose and triglyceride were not affected by experimental feeds.

Discussion

Organic acid salts are considered as growth promoter supplements for farmed aquatic species as they providing energy for gut epithelial cells, adjusting the gut microbiome, and improving intestinal health and nutrients utilization (Silva et al. 2013; Hoseinifar et al. 2017; Ng and Koh 2017). The results of the current research demonstrated that administrating SP (5 or 10 g kg^{-1} diet) in PP-rich diets pronouncedly enhanced growth in *A. latus* compared with the control may as consequence of increased feed intake and feed utilization. Likewise, supplementing diet with SP in Caspian white fish (*Rutilus frisii kutum*, Hoseinifar et al. 2016) remarkably increased growth rate in this species. However, in this research, the growth-promoting effects of SA in diet was negligible compared to SP because of lower feed intake in fish fed SA-supplemented diets. Similarly, several studies reported insignificant effect of incorporating acidifiers in diet on the growth performance of aquaculture species such as catfish (*Clarias gariepinus*; Owen et al. 2006), rainbow trout (*Oncorhynchus mykiss*; Pandey and Satoh 2007), hybrid tilapia (Ng et al. 2009; Zhou et al. 2009; Ebrahimi et al. 2017), and black tiger shrimp (*Penaeus monodon*; Ng et al. 2015). It has been suggested that an appropriate mixture of different OAS could enhance their positive synergistic impacts (Katya et al. 2018) that was noticed in our study in fish fed on the diet containing a combination of SP and SA compared to the fish fed the SA diets. In this context, it has been demonstrated that blends of different OA enhanced growth rate in various fish species such as: calcium propionate and formic acid in Mrigal carp (*Cirrhinus mrigala,* Kumar et al. 2017) and mixture of propionic acid, ammonium format, and formic acid in olive flounder (*Paralichthys olivaceus*, Katya et al. 2018). Likewise, Huan et al. (2018) reported that hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) fed a PP-base (without FM) diet supplemented with blends of Ca-propionate, Ca-formate, and SA had

Table 2 Growth performance and feed utilization in *A. latus* juveniles fed diets containing different organic acids salts for 8 weeks (mean values of 3 replicates ± SEM). A different superscript in the same row denotes statistically significant differences ($P < 0.05$)

Parameters	Dietary treatments						
	Control	SP5	SP10	SA5	SA10	SP + SA	*P* value
BW_f (g)	11.18 ± 0.9^{d}	14.61 ± 0.6^{a}	14.14 ± 0.2^{ab}	12.11 ± 0.8^{cd}	12.87 ± 0.9^{bc}	14.29 ± 0.9^{a}	0.001
WG (%)	72.1 ± 8.1^{d}	124.8 ± 5.1^{a}	117.5 ± 2.1^{ab}	86.3 ± 6.9^{cd}	97.9 ± 7.9^{bc}	119.8 ± 8.0^{a}	0.001
SGR (% BW day^{-1})	0.84 ± 0.07^{d}	1.26 ± 0.04^{a}	1.21 ± 0.02^{ab}	0.97 ± 0.05^{cd}	1.06 ± 0.06^{bc}	1.23 ± 0.05^{ab}	0.001
K	1.42 ± 0.05	1.58 ± 0.01	1.64 ± 0.06	1.47 ± 0.06	1.56 ± 0.10	1.63 ± 0.10	0.478
FCR	1.71 ± 0.09^{a}	1.19 ± 0.06^{c}	1.23 ± 0.04^{bc}	1.55 ± 0.10^{ab}	1.33 ± 0.08^{bc}	1.30 ± 0.11^{bc}	0.019
Feed intake (g $fish^{-1}$)	15.83 ± 1.2^{c}	19.31 ± 0.7^{a}	18.83 ± 0.6^{ab}	17.16 ± 0.6^{bc}	16.80 ± 0.5^{c}	20.03 ± 0.7^{a}	0.002
PER	1.22 ± 0.06^{c}	1.75 ± 0.09^{a}	1.69 ± 0.05^{a}	1.36 ± 0.11^{bc}	1.57 ± 0.09^{bc}	1.62 ± 0.13^{ab}	0.018

Diet abbreviations: SP5 (5 g SP kg^{-1} diet), SP10 (10 g SP kg^{-1} diet), SA5 (5 g SA kg^{-1} diet), SA10 (10 g SA kg^{-1} diet), and SP + SA (5 g SP kg^{-1} + 5 g SA kg^{-1} diet). BW_f: final body weight, *WG* weight gain, *SGR* specific growth rate, *K* Fulton's condition factor, *FCR* feed conversion ratio, *PER* protein efficiency ratio

Table 3 Whole-body proximate composition and nutrients retention of *A. latus* juveniles fed diets containing different organic acids salts for 8 weeks (mean values of 3 replicates ± SEM). A different superscript in the same row denotes statistically significant differences ($P < 0.05$)

Parameters	Initial	Dietary treatments						
		Control	SP5	SP10	SA5	SA10	SP + SA	*P* value
Moisture	74.11	74.36 ± 0.5	73.86 ± 0.5	75.40 ± 1.0	73.53 ± 0.9	74.73 ± 1.0	73.66 ± 1.4	0.222
Crude protein	14.81	13.80 ± 1.1	14.93 ± 0.5	15.20 ± 0.5	15.19 ± 0.3	15.15 ± 1.4	14.49 1.1	0.347
Crude lipid	4.85	5.46 ± 1.0	4.93 ± 0.4	4.37 ± 0.7	4.91 ± 0.4	5.05 ± 0.4	4.79 ± 0.9	0.623
Ash	2.52	2.86 ± 0.2	3.15 ± 0.3	3.17 ± 0.5	3.37 ± 0.6	3.19 ± 0.2	3.44 ± 0.2	0.523
Nutrient retention								
PR[a]		0.38 ± 0.02[b]	0.58 ± 0.02[a]	0.58 ± 0.05[a]	0.46 ± 0.04[ab]	0.54 ± 0.08[a]	0.57 ± 0.02[a]	0.044
LR[b]		0.59 ± 0.03	0.61 ± 0.02	0.58 ± 0.02	0.57 ± 0.01	0.60 ± 0.02	0.59 ± 0.04	0.863

Diet abbreviations: SP5 (5 g SP kg^{-1} diet), SP10 (10 g SP kg^{-1} diet), SA5 (5 g SA kg^{-1} diet), SA10 (10 g SA kg^{-1} diet), and SP + SA (5 g SP kg^{-1} + 5 g SA kg^{-1} diet). *PR* protein retention, *LR* lipid retention

Table 4 Hematological parameters of *A. latus* juveniles fed diets containing different organic acids salts for 8 weeks (mean values of 3 replicates ± SEM). A different superscript in the same row denotes statistically significant differences ($P < 0.05$)

Parameters	Dietary treatments						
	Control	SP5	SP10	SA5	SA10	SP + SA	*P* value
RBC ($\times 10^6$ μL^{-1})	2.22 ± 0.02	2.36 ± 0.07	2.35 ± 0.09	2.25 ± 0.12	2.25 ± 0.08	2.29 ± 0.05	0.207
WBC ($\times 10^3$ μL^{-1})	123.8 ± 5.0	122.1 ± 3.0	128.1 ± 8.0	122.4 ± 2.8	123.7 ± 2.1	121.9 ± 3.0	0.592
Hct (%)	28.4 ± 2.4	29.5 ± 2.8	29.6 ± 2.5	29.4 ± 2.0	29.5 ± 4.7	32.0 ± 4.0	0.833
Hb (g dL^{-1})	6.47 ± 0.5^{b}	7.51 ± 0.3^{a}	7.53 ± 0.4^{a}	7.44 ± 0.3^{a}	7.88 ± 0.4^{a}	7.79 ± 0.8^{a}	0.040
MCV (nm^3)	128.3 ± 11.3	124.5 ± 8.7	125.6 ± 6.8	130.6 ± 4.3	130.9 ± 15.9	139.7 ± 16.9	0.684
MCH (pg cell^{-1})	29.2 ± 2.2	31.8 ± 1.6	32. 0 ± 0.6	33.1 ± 2.8	35.1 ± 0.6	33.9 ± 3.2	0.069
MCHC (g dL^{-1})	23.0 ± 3.6	25.6 ± 2.1	20.7 ± 1.6	25.4 ± 2.1	27.0 ± 2.9	24.4 ± 0.7	0.460

Diet abbreviations: SP5 (5 g SP kg^{-1} diet), SP10 (10 g SP kg^{-1} diet), SA5 (5 g SA kg^{-1} diet), SA10 (10 g SA kg^{-1} diet), and SP + SA (5 g SP kg^{-1} + 5 g SA kg^{-1} diet). *RBC* red blood cell, *WBC* white blood cell, *Hct* hematocrit, *Hb* hemoglobin, *MCV* mean cell volume, *MCH* mean cell hemoglobin, *MCHC* mean cell hemoglobin concentration

similar growth rate compared to fish fed PP-rich diet (contained 80 g FM kg^{-1} diet). These discrepancies could be related to differences in the types and doses of acidifiers applied in different trials, fish species, and their health status as well as rearing conditions (Hoseinifar et al. 2017).

In the present study, the enhanced feed utilization observed in OAS-supplemented groups could be associated with the reduction of pH in the gut and feed that could increase the digesting capacity of gastrointestinal tract that may improve pepsin activity (Castillo et al. 2014; Ng and Koh 2017). In addition, reduction in pH could improve mineral solubilization in the gut that eventually enhance their availability and absorbance. In addition, supplementing a PP-rich diet with OAS can increase bioavailability of phosphorous by de-phosphorylation of phytic acid (Hoseinifar et al. 2017). In agreement with the results of the present study, it has been reported that sodium diformat (Reyshahri et al. 2019) or butyric acid (Aalamifar et al. 2020) improved growth performance and feed utilization in Asian seabass (*Lates calcarifer*). It has been postulated that the low molecular weight of OAS increase palatability of feed by

Table 5 Plasma biochemical parameters of *A. latus* juveniles fed diets containing different organic acids salts for eight weeks (mean values of 3 replicates ± SEM). A different superscript in the same row denotes statistically significant differences ($P < 0.05$)

Parameters	Dietary treatments						
	Control	SP5	SP10	SA5	SA10	SP + SA	*P* value
Total protein (g dL^{-1})	5.06 ± 0.2^{b}	6.0 ± 0.2^{a}	6.20 ± 0.2^{a}	6.03 ± 0.3^{a}	6.94 ± 0.3^{a}	6.22 ± 0.3^{a}	0.002
Albumin (g dL^{-1})	2.50 ± 0.1^{b}	3.28 ± 0.2^{a}	3.21 ± 0.3^{a}	3.16 ± 0.4^{a}	3.26 ± 0.2^{a}	3.33 ± 0.3^{a}	0.038
Glucose (mg dL^{-1})	53.06 ± 5.9	51.07 ± 3.8	46.71 ± 5.3	51.43 ± 3.4	54.81 ± 6.3	52.31 ± 4.6	0.526
Triglycerides (mg dL^{-1})	199.3 ± 11.6	199.5 ± 7.6	203.7 ± 7.4	206.7 ± 8.4	203.8 ± 8.0	205.5 ± 10.3	0.578

Diet abbreviations: SP5 (5 g SP kg^{-1} diet), SP10 (10 g SP kg^{-1} diet), SA5 (5 g SA kg^{-1} diet), SA10 (10 g SA kg^{-1} diet), and SP + SA (5 g SP kg^{-1} + 5 g SA kg^{-1} diet)

leaching from feed and inducing feeding behavior of aquatic species (Ng and Koh 2017; Tran et al. 2018). In addition, the results of the present study showed that PA-supplemented diets induced higher FI in *A. latus* compared to the control and SA-supplemented diets that resulted in higher growth performance. Previous studies also demonstrated that OAS could act as attractants in different fish species such as Nile tilapia (*Oreochromis niloticus*) (Xie et al. 2003) and Asian seabass (Reyshahri et al. 2019).

In the current study, whole-body biochemical composition of fish was not affected by supplementation of different OAS in diet. Similarly, it has been demonstrated that supplementing different OA in diets did not change whole body in various farmed fish species (Castillo et al. 2014; Rodriguez et al. 2017; Wassef et al. 2017; Katya et al. 2018). It seems that differences in experimental feed composition, feeding trial period, source, and concentration of OA as well as fish species might result in such discrepancies in the findings of the mentioned studies.

The findings of this study revealed that blood Hb content elevated in fish fed on the OAS-incorporated diets indicating greater oxygen-carrying capacity of blood. These results could be associated with increasing and liberating of minerals especially in PP due to the presence of phytate in these ingredients (Reda et al. 2016). In this regard, Benedito-Palos et al. (2016) reported that replacement of a large portion of FM and FO in diet with vegetal sources induced signs of anemia in gilthead seabream (*Sparus aurata*), but supplementing butyric acid (4 g kg^{-1} diet) in diet restored Hb level in fish. Positive influence of incorporating acidifiers in diets on hematological parameters such as increase in Hb, Htc, RBC, and WBC counts was also reported in other cultured fish species (Baruah et al. 2009; Kumar et al. 2017; Hassaan et al. 2018; Abdel-Mohsen et al. 2018).

In the current study, plasma total protein and albumin pronouncedly enhanced in *A. latus* fed OAS-administered diets. In this regard, inclusion of dietary acidifiers such as malic acid (Hassaan et al. 2018), blends of OA (formic acid and calcium propionate, Kumar et al. 2017), and sodium diformate (Wassef et al. 2017) increased serum total protein and albumin in Nile tilapia, mrigal carp, and European sea bass, respectively. The findings of our study showed that the concentrations of plasma cholesterol and triglycerides were not influenced by different levels or sources of OAS as also reported in red hybrid tilapia (Ebrahimi et al. 2017) and olive flounder (Katya et al. 2018). In contrast, it has been reported that dietary humic acid (Yilmaz et al. 2018) and sodium diformate (Krome et al. 2018; Reyshahri et al. 2019) reduced serum total triglyceride and cholesterol in different fish. These contradictory results might arise from differences in SCFA type, fish species, and life stage (Ng and Koh 2017).

Conclusion

Overall, as supplementing 5 or 10 g kg^{-1} of SP in PP-rich diets had the same influence on growth and physiological responses of *A. latus*, thus administrating 5 g SP kg^{-1} of a PP-rich diet is recommended for this species. However, the influence of including SA in PP-diet on growth performance and feed utilization of *A. latus* was negligible. It seems that growth-promoting effects of SP or blends of OAS were associated with feed and protein efficiency as well as health indices in *A. latus*.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

References

Aalamifar H, Soltanian S, Vazirzadeh A, Akhlaghi M, Morshedi V, Gholamhosseini A, Mozanzadeh MT (2020) Dietary butyric acid improved growth, digestive enzyme activities and humoral immune parameters in Barramundi (*Lates calcarifer*). Aquac Nutr 26:156–114. https://doi.org/10.1111/anu.12977

Abbasi A, Oujifard A, Mozanzadeh MT, Habibi H, Nafisi Bahabadi M (2020) Dietary simultaneous replacement of fish meal and fish oil with blends of plant proteins and vegetable oils in yellowfin seabream (*Acanthopagrus latus*) fry: Growth, digestive enzymes, antioxidant status and skin mucosal immunity. Aquac Nutr 26:1131–1142. https://doi.org/10.1111/anu.13070

Abdel-Mohsen HH, Wassef EA, El-Bermawy NM, Abdel-Meguid NE, Saleh NE, Barakat KM, Shaltout OE (2018) Advantageous effects of dietary butyrate on growth, immunity response, intestinal microbiota and histomorphology of European Seabass (*Dicentrarchus labrax*) fry. Egypt J Aqua Biol Fish 22:93–110. https://doi.org/10.21608/ejabf.2018.12055

AOAC (2005) Official methods of analysis of the association of official analytical chemists, 18th edn. Association of Official Analytical Chemist, Arlington

Baruah K, Pal AK, Sahu NP, Debnath D, Yengkokpam S, Norouzitallab P, Sorgeloos P (2009) Dietary crude protein, citric acid and microbial phytase interacts to influence the hemato-immunological parameters of Rohu, *Labeo rohita*, juveniles. J World Aquacult Soc 40:824–831. https://doi.org/10.1111/j.1749-7345.2009.00304.x

Benedito-Palos L, Ballester-Lozano GF, Simó P, Karalazos V, Ortiz A, Calduch-Giner J, Pérez-Sánchez J (2016) Lasting effects of butyrate and low FM/FO diets on growth performance, blood haematology/biochemistry and molecular growth-related markers in gilthead sea bream (*Sparus aurata*). Aquaculture 454:8–18. https://doi.org/10.1016/j.aquaculture.2015.12.008

Blaxhall PC, Daisley KW (1973) Routine hematological methods for use fish with blood. J Fish Biol 5:771–781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Castillo S, Rosales M, Pohlenz C, Gatlin DM (2014) Effects of organic acids on growth performance and digestive enzyme activities of juvenile red drum *Sciaenops ocellatus*. Aquaculture 433:6–12. https://doi.org/10.1016/j.aquaculture.2014.05.038

Chen Z, Zhao S, Liu Y, Yang P, Ai Q, Zhang W, Xu W, Zhang Y, Zhang Y, Mai K (2018) Dietary citric acid supplementation alleviates soybean meal-induced intestinal oxidative damage and micro-ecological imbalance in juvenile turbot, *Scophthalmus maximus* L. Aquac Res 49:3804–3816. https://doi.org/10.1111/are.13847

Dacie JV, Lewis SM (2001) Practical Hematology, 9th edn. Churchill Livingstone, London

Ebrahimi M, Daeman HN, Chong CM, Karami A, Kumar V, Hoseinifar SH, Romano N (2017) Comparing the effects of different dietary organic acids on the growth, intestinal short-chain fatty acids, and liver histopathology of red hybrid tilapia (*Oreochromis* sp.) and potential use of these as preservatives. Fish Physiol Biochem 43:1195–1207. https://doi.org/10.1007/s10695-017-0365-0

Esmaeili A, Sotoudeh E, Morshedi V, Bagheri D, Dorafshan S (2019) Effects of dietary supplementation of bovine lactoferrin on antioxidant status, immune response and disease resistance of yellowfin sea bream (*Acanthopagrus latus*) against *Vibrio harveyi*. Fish Shellfish Immunol 93:917–923. https://doi.org/10.1016/j.fsi.2019.08.045

Folch J, Lees M, Stanley G (1957) A simple method of the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Francis G, Makkar HP, Becker K (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199:197–227. https://doi.org/10.1016/S0044-8486(01)00526-9

Gatlin DM, Barrows FT, Brown P, Dabrowski K, Gaylord TG, Hardy RW, Herman E, Hu G, Krogdahl Å, Nelson R (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac Res 38:551–579. https://doi.org/10.1111/j.1365-2109.2007.01704.x

Hardy RW (2010) Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquac Res 41:770–776. https://doi.org/10.1111/j.1365-2109.2009.02349.x

Hassaan MS, Soltan MA, Jarmołowicz S, Abdo HS (2018) Combined effects of dietary malic acid and *Bacillus subtilis* on growth, gut microbiota and blood parameters of Nile tilapia (*Oreochromis nilotcus*). Aquac Nutr 24:83–93. https://doi.org/10.1111/anu.12536

Hernández C, Lizárraga-Velázquez CE, Contreras-Rojas D, Sánchez-Gutiérrez EY, Martínez-Montaño E, Ibarra-Castro L, Peña-Marín ES (2020) Fish meal replacement by corn gluten in feeds for juvenile spotted rose snapper (*Lutjanus guttatus*): Effect on growth performance, feed efficiency, hematological parameters, protease activity, body composition, and nutrient digestibility. Aquaculture 531:735896. https://doi.org/10.1016/j.aquaculture.2020.735896

Hoseinifar SH, Zoheiri F, Caipang CM (2016) Dietary sodium propionate improved performance, mucosal and humoral immune responses in Caspian white fish (*Rutilus frisii kutum*) fry. Fish Shellfish Immunol 55:523–528. https://doi.org/10.1016/j.fsi.2016.06.027

Hoseinifar SH, Sun YZ, Caipang CM (2017) Short chain fatty acids as feed supplements for sustainable aquaculture: an updated review. Aquac Res 48:1380–1391. https://doi.org/10.1111/are.13239

Huan D, Li X, Chowdhury MAK, Yang H, Liang G, Leng X (2018) Organic acid salts, protease and their combination in fish meal-free diets improved growth, nutrient retention and digestibility of tilapia (*Oreochromis niloticus* × *O. aureus*). Aquac Nutr 24:1813–1821. https://doi.org/10.1111/anu.12820

Katya K, Park G, Bharadwaj AS, Browdy CL, Vazquez-Anon M, Bai SC (2018) Organic acids blend as dietary antibiotic replacer in marine fish olive flounder, *Paralichthys olivaceus*. Aquac Res 49:2861–2868. https://doi.org/10.1111/are.13749

Krome C, Schuele S, Jauncey K, Focken U (2018) Influence of a sodium formate/formic acid mixture on growth of juvenile common carp (*Cyprinus carpio*) fed different fishmeal replacement levels of detoxified *Jatropha curcas* kernel meal in practical, mixed diets. J Appl Aquac 30:137–156. https://doi.org/10.1080/10454438.2017.1412845

Kumar P, Jain P, Sardar P, Sahu NP, Gupta S (2017) Dietary supplementation of acidifier: effect on growth performance and haemato-biochemical parameters in the diet of *Cirrhinus mrigala* juvenile. Aquac Int 25:2101–2116. https://doi.org/10.1007/s10499-017-0176-4

Lim C, Lückstädt C, Webster CD, Kesius P (2015) Organic acids and their salts. In: Lee CS, Lim C, Gatlin DM, Webster C (eds) Dietary Nutrients, Additives, and Fish Health. Willey-Blackwell, Hoboken, pp 305–320

Lin Y-H, Cheng M-Y (2017) Effects of dietary organic acid supplementation on the growth, nutrient digestibility and intestinal histology of the giant grouper *Epinephelus lanceolatus* fed a diet with soybean meal. Aquaculture 469:106–111. https://doi.org/10.1016/j.aquaculture.2016.11.032

Liu Y, Chen Z, Dai J, Yang P, Xu W, Ai Q, Zhang W, Zhang Y, Zhang Y, Mai K (2019) Sodium butyrate supplementation in high-soybean meal diets for turbot (*Scophthalmus maximus* L.): Effects on inflammatory status, mucosal barriers and microbiota in the intestine. Fish Shellfish Immunol 88:65–75. https://doi.org/10.1016/j.fsi.2019.02.064

Luckstadt C (2008) The use of acidifiers in fish nutrition. CAB Rev Per Agric Vet Sci Nutr Nat Resour 3:1–8. https://doi.org/10.1079/PAVSNNR20083044

Morken T, Kraugerud OF, Barrows FT, Sørensen M, Storebakken T, Øverland M (2011) Sodium diformate and extrusion temperature affect nutrient digestibility and physical quality of diets with fish meal and barley protein concentrate for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 317:138–145. https://doi.org/10.1016/j.aquaculture.2011.04.020

Ng WK, Koh CB (2017) The utilization and mode of action of organic acids in the feeds of cultured aquatic animals. Rev Aquac 9:342–368. https://doi.org/10.1111/raq.12141

Ng WK, Koh C, Sudesh K, Siti-Zahrah A (2009) Effects of dietary organic acids on growth, nutrient digestibility and gut microflora of red hybrid tilapia, *Oreochromis* sp., and subsequent survival during a challenge test with *Streptococcus agalactiae*. Aquac Res 40:1490–1500. https://doi.org/10.1111/j.1365-2109.2009.02249.x

Ng WK, Koh CB, Teoh CY, Romano N (2015) Farm-raised tiger shrimp, *Penaeus monodon*, fed commercial feeds with added organic acids showed enhanced nutrient utilization, immune response and resistance to *Vibrio harveyi* challenge. Aquaculture 449:69–77. https://doi.org/10.1016/j.aquaculture.2015.02.006

NRC (2011) Nutrient requirements of fish and shrimp. National Academies Press, Washington, DC

Owen MAG, Waines P, Bradley G, Davies S (2006) The effect of dietary supplementation of sodium butyrate on the growth and microplora of *Clarias gariepinus* (Burchell 1822). Abstract, XII International Symposium on Fish Nutrition and Feeding, Biarritz, France, 149

Pandey A, Satoh S (2007) Effects of organic acids on growth and phosphorus utilization in rainbow trout *Oncorhynchus mykiss*. Fish Sci 74:867–874. https://doi.org/10.1111/j.1444-2906.2008.01601.x

Reda RM, Mahmoud R, Selim KM, El-Araby IE (2016) Effects of dietary acidifiers on growth, hematology, immune response and disease resistance of Nile tilapia, *Oreochromis niloticus*. Fish Shellfish Immunol 50:255–262. https://doi.org/10.1016/j.fsi.2016.01.040

Reyshahri A, Mohammadiazarm H, Mohammadian T, Mozanzadeh MT (2019) Effects of sodium diformate on growth performance, gut microflora, digestive enzymes and innate immunological parameters of Asian sea bass (*Lates calcarifer*) juveniles. Aquac Nutr 25:1135–1144. https://doi.org/10.1111/anu.12929

Rimoldi S, Finzi G, Ceccotti C, Girardello R, Grimaldi A, Ascione C, Terova G (2016) Butyrate and taurine exert a mitigating effect on the inflamed distal intestine of European sea bass fed with a high percentage of soybean meal. Fish Aqua Sci 19:40. https://doi.org/10.1186/s41240-016-0041-9

Rodriguez MGM, Pohlenz C, Gatlin DM (2017) Supplementation of organic acids and algae extracts in the diet of red drum *Sciaenops ocellatus*: immunological impacts. Aquac Res 48:1778–1786. https://doi.org/10.1111/are.13015

Silva BCD, Vieira FDN, Mouriño JLP, Ferreira GS, Seiffert WQ (2013) Salts of organic acids selection by multiple characteristics for marine shrimp nutrition. Aquaculture 384–387:104–110. https://doi.org/10.1016/j.aquaculture.2012.12.017

Silva-Carrillo Y, Hernández C, Hardy RW, González-Rodríguez B, Castillo-Vargasmachuca S (2012) The effect of substituting fish meal with soybean meal on growth, feed efficiency, body composition and blood chemistry in juvenile spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869). Aquaculture 364:180–185. https://doi.org/10.1016/j.aquaculture.2012.08.007

Song Z, Li H, Wang J, Li P, Sun Y, Zhang L (2014) Effects of fishmeal replacement with soy protein hydrolysates on growth performance, blood biochemistry, gastrointestinal digestion and muscle composition of juvenile starry flounder (*Platichthys stellatus*). Aquaculture 426:96–104. https://doi.org/10.1016/j.aquaculture.2014.01.002

Sotoudeh E, Amiri Moghaddam J, Shahhosseini G, Aramli MS (2016) Effect of dietary gamma-irradiated and fermented soybean meal on the growth performance, body composition, and digestive enzymes activity of Caspian brown trout, *Salmo trutta caspius*, Juvenile. J World Aquacult Soc 47:830–842. https://doi.org/10.1111/jwas.12297

Tran NT, Li Z, Wang S, Zheng H, Aweya JJ, Wen X, Li S (2018) Progress and perspectives of short-chain fatty acids in aquaculture. Rev Aquac 12:283–298. https://doi.org/10.1111/raq.12317

Vahabnezhad A, Kaymaram F, Taghavi Motlagh SA, Valinassab T, Fatemi SMR (2016) The reproduction biology and feeding habits of yellowfin seabream, *Acanthopagrus latus* (Huttuyn, 1782), in the Northern Persian Gulf. Iran J Fish Sci 15:16–30

Wassef EA, Abdel-Momen SAG, Saleh NES, Al-Zayat AM, Ashry AM (2017) Is sodium diformate a beneficial feed supplement for European seabass (*Dicentrarchus labrax*)? Effect on growth performance and health status. Egypt J Aquat Res 43:229–234. https://doi.org/10.1016/j.ejar.2017.09.005

Xie BS, Zhang L, Wang D (2003) Effects of several organic acids on the feeding behavior of *Tilapia nilotica*. J Appl Ichthyol 19:255–257. https://doi.org/10.1046/j.1439-0426.2003.00451.x

Yaghoubi M, Mozanzadeh MT, Marammazi JG, Safari O, Gisbert E (2016) Dietary replacement of fish meal by soy products (soybean meal and isolated soy protein) in silvery-black porgy juveniles (*Sparidentex hasta*). Aquaculture 468:50–59. https://doi.org/10.1016/j.aquaculture.2016.06.002

Yilmaz S, Ergun S, Çelik ES, Yigit M (2018) Effects of dietary humic acid on growth performance, haemato-immunological and physiological responses and resistance of Rainbow trout, *Oncorhynchus mykiss* to *Yersinia ruckeri*. Aquac Res 49:3338–3349. https://doi.org/10.1111/are.13798

Zhang Y, Ji W, Wu Y, Han H, Qin J, Wang Y (2016) Replacement of dietary fish meal by soybean meal supplemented with crystalline methionine for Japanese seabass (*Lateolabrax japonicus*). Aquac Res 47:243–252. https://doi.org/10.1111/are.12486

Zhou Z, Liu Y, He S, Shi P, Gao X, Yao B, Ringo E (2009) Effects of dietary potassium diformate (KDF) on growth performance, feed conversion and intestinal bacterial community of hybrid tilapia *O. niloticus* x *O. aureus*. Aquaculture 291:89–94. https://doi.org/10.1016/j.aquaculture.2009.02.043