Al-Muthanna J. For Agric Sci Print ISSN: 2226-4086 Online ISSN:2572-5149
Vol. 10 , Issue. 02. 2023

https://muthjas.mu.edu.iq/ http://doi.org/10.52113/mjas04/10.2/19

Adding of different levels of propionic fatty acid in the diets of common carp *Cyprinus carpio* L. and its effect on productive, physiological and immunological characteristics

Abbas shanshool Alhamadany[1] Mariamm. J. mohamed[2]
University of AL-Muthanna / College of Agriculture / Department of Animal Production
Iraq / AL-Muthanna Governorate
Abbas.shanshol@mu.edu.iq
drmary300@mu.edu.iq

Received on 23/08/2023 Accepted on 25/8/2023 Published on 15/12/2023

Abstract

This study was conducted to find out the effect of propionic fatty acid on some growth indicators and blood immune traits in common carp fish *Cyprinus carpio* L. Four concentrations of propionic fatty acid in form of four treatments as, 0.00% represents first treatment as a control, 0.05% represents second treatment, 0.15% represents third treatment and 0.20% fourth treatment. These percentages were added to a standard diet with a protein content of 29.14%. A total 72 fish with an average weight of (25±0.08) g were distributed in 12 cages for each treatment with 3 replicate per treatment. The fish were fed 3% of their body weight during the experimental duration. The experiment continued for 84 day. The results of significant analysis showed that B 0.15% treatment was significantly ($p \leq 0.05$) exceeded on other treatments in all studied growth parameters such as FW, WG, DGR, RGR, SGR, MGR, FI, FCR, FCE, and PER, followed by first treatment. As for blood parameters also the B 0.15% treatment was exceeded on other treatments in RBC, Hb, PCV, MCV, MCH, and MCHC followed by B 0.05% treatment. As for the immunological parameters (WBC and IGM) the B 0.15% treatment also exceeded on other treatments followed by B 0.05% treatment. Hence concluded the adding of propionic fatty acid to the diet of common carp fish had a positive effect on all the studied parameters.

Keywords: Acidifier; propionic, Growth , Hematology, Immunity, common carp fish.

Introduction

One of the most important indicators of the success of fish farming is the presence of a regular and balanced diet, which is the basis for the possibility of increasing the density of fish farming per unit area. Intensive

fish farming led to the presence of many problems faced by intensive fish farming, including the spread of diseases, which means the inefficiency of the immune system of fish in the case of rearing at high densities, or that the immune system is unable to confront these diseases [1] [2]. When fish eat diets the concentration of hydrochloric acid decreases in the stomach, and this increases the pH levels. This increase has a detrimental effect on the activation of pepsin and pancreatic enzymes, which reduces the capacity of the alimentary canal and affects the growth performance of fish.Acidifiers, such as fatty acids and their salts, provide a potential alternative to antibiotics and to improve fish growth and health, as fatty acids are involved in many pathways of energy metabolism [3]. Also, the excessive and ill-conceived use of antibiotics led to an increase in resistance to pathogens as a result of genetic mutations, or finding new methods and mechanisms for resistance to used antibiotics, in addition to the accumulation of these antibiotics in the cells of the body [3]; [4]. For these reasons, the use of antibiotics as a growth stimulator was prohibited in different fish farming systems [5]. Also, the continuous use of antibiotics leads to the destruction of beneficial bacterial communities in fish, which leads to inhibition of the effectiveness of the immune system of fish [6]. Therefore, it has become necessary to have alternatives to enhance the immune system of fish and thus promote the growth of beneficial bacteria. Recently, focus has been placed on the use of many additives, including fatty acids (acidifiers) [7]. Fatty acids are also approved for use in fish farming by the European Union (EU) as they have been shown to be the most promising natural growth promoters in terms of controlling pathogens and promoting fish growth [8].

Material and methods

Environmental measurements

The following physicochemical parameters of water were measured during the duration of the experiment such as Water temperature, Dissolved oxygen, pH, Salinity, and total dissolved substances. Periodic measurements were made to assess the quality of the water and to indicate its suitability for fish rearing.

Fish

Fingerlings of common carp (*Cyprinus Carpio* L.) were brought from one of the local hatcheries, Babel Governorate, Al-Mahaweel district, with 200 fingerlings, with an average weight of (25 ± 0.08) gm, by means of a transport vehicle designated for transporting fish. The fish were sterilized with 3% saline solution for five minutes until signs of stress appeared on them. The fish were left for 24 hours to rest, then they were distributed among the experimental cages, with six fish per cage, after excluding the undesirable fish. After that, it was acclimatized for 14 days, as the fish were starved for three days, after which food was given to them at a rate of 1% of the biomass weight in each tank (two meals per day).

Preparing the ration

The components of the ration were brought from the local markets, mixed, and then pressed using a thermal press. Tables (1) show the components of the ration. Then, propionic acid was added at four levels: P 0.00%, P 0.05%, P 0.15%, and P 0.20%, with three replications for each treatment.

Statistical analysis

The experiment was designed according to the CRD design and the

results were analyzed using the SPSS data analysis software (21).

Studied parameters

Weight gain = Final Weight- Initial Weight

Daily growth rate = Final Weight- Initial Weight/ ΔT according to [9].

Relative Growth Rate = Final Weight*Initial Weight/ Initial Weight according to [10].

Specific growth Rate = Ln Final Weight- Ln Initial Weight / ΔT according to [11].

Metabolic growth Rate = Weight gain (g) /[{(Initial Weight/1000)$^{0.8}$ + Final Weight/1000)$^{0.8}$ }/2]/study duration (g/kg 0.8/d) according to [12].

Feed Conversion Ratio = weight of food intake / weight gain of fish according to [13].

Feed conversion efficiency = weight gain of fish / weight of food intake *100 according to [13].

Protein Efficiency Ratio = Weight gain / protein intake according to [14].

Table (1) Ingredients of experimental diet

No	Ingredients	percentage in the diet (%)
1	Soy bean meal	40
2	AL-Wafi protein concentration	20
3	Wheat bran	15
4	Corn	15
5	Barley	5
6	Wheat flour	3
7	Premix	1
8	Oil	1

Chemical composition of diet

Component	Result (%)
Moisture	5.03
Dry matter	94.97
Crude protein	29.14
Crude fat	1.74
Crude fiber	4.48
Nitrogen free extract (NEF)	51.1
Ash	8.51
Gross energy (Kcal/g) *	396.726
Digestible energy (Kcal/g)**	297.5445
Metabolizable energy (Kcal/g)***	323.7654
Protein : Calorie ratio ****	979.34

* Gross energy (Kcal/g) it was calculated according to [15] by using factors 5.65, 9.45 and 4.22 Kcal/g of protein, lipid and carbohydrate, respectively.

** Digestible energy (Kcal/g) it was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to [16] .

*** Metabolizable energy (Kcal/g) it was calculated using a value of 4.5 Kcal/g proteins, 8.51 Kcal/g fat and 3.48 Kcal/g carbohydrates according to [17].

**** Protein : Calorie ratio it was calculated by below equation

P:E = crude protein x 10000/digestible energy, according to [18].

Results:

1- **Environmental factors:** The environmental factors of the water in the breeding basin were measured, so the temperature ranged between 16.5 C at the beginning of the experiment and 30 C at the end of the experiment. The amount of oxygen ranged between (7.8-7.2), and the pH value ranged between (8.1-7). Salinity

ranged between (4.981 - 6.73). The amount of total suspended matter ranged (3015 - 4105), as shown in Table (2).

Table (2) Some environmental factors of water in the breeding pond

duration	Environmental factors				
	Temperature C^{o}	O_2 *Mg/l*	pH	salinity *Mg/l*	TDS
3-3 to 16-3-2022	16.50	7.8	8.1	4.981	3015
16-3 to 30-3-2022	18.00	7.5	7.8	5.380	3022
30-3 to 12-4-2022	22.70	7.5	7.5	5.370	3334
12-4 to 25-4-2022	24.30	7.4	7.3	5.501	3411
25-4 to 8-5-2022	25.00	7.5	7.2	5.622	3590
8- 5 to 21-5-2022	28.20	7.3	7.0	5.850	3701
21- 5 to 3-6-2022	30.00	7.2	7.0	6.730	4105

2- Growth parameters:

Table (3) shows that there are significant differences between the treatment in all studied parameters. P 0.15% was superior to the rest of the treatments in FW, which recorded (157.68 ± 0.433) gm, in WG recorded 125.31 ± 0.346) gm, in DGR recorded (1.49 ± 0.01) gm/day, and RGR recorded (387.35 ± 6.71)%. SGR recorded (1.88 ± 0.017)%/day MGR recorded (10.20 ± 0.06) µg/kg/day. The control treatment recorded the highest mean in FCR (2.37 ± 0.02), while in the FCE it was superior P0.20% with mean (43.840 ± 0.127)%, while in the PER the highest mean was record to P 0.20% (1.506 ± 0.003)%. Followed it significantly in most parameters P 0.05%, then P 0.20% and the control treatment recorded the lowest means in those parameters.

Table (3) Some of the studied growth parameters (mean ± standard error) for common carp fish fed on diets containing different levels of propionic acid during the experimental period.

Treat. & concent.	IW (gm)	FW (gm)	WG (gm)	DGR (gm/day)	RGR (%)	SGR (%/day)	FI (g/Fish)	MGR (g/Kg/day)	FCR	FCE (%)	PER (%)
P 0.00%	31.92 ±0.207	136.06 ±1.683 c	104.14 ±1.673 c	1.24 ±0.020 c	326.22 ±5.684 c	1.72 ±0.016 c	246.87 ±1.275 c	9.30 ±0.082 c	2.3700 ±0.02 a	42.18 ±0.463 b	1.4467 ±0.017 b
P 0.05%	32.33 ±0.192	151.47 ±0.534 b	119.14 ±0.648 b	1.41 ±0.008 b	368.57 ±3.856 b	1.83 ±0.008 b	283.25 ±1.305 a	9.94 ±0.04 b	2.3767 ±0.008 a	42.06 ±0.183 b	1.4433 ±0.008 b
P 0.15%	32.37 ±0.504	157.68 ±0.433 a	125.31 ±0.346 a	1.49 ±0.005 a	387.35 ±6.713 a	1.88 ±0.017 a	286.66 ±1.406 a	10.20 ±0.05 a	2.2867 ±0.014 b	43.7167 ±0.297 a	1.4933 ±0.006 a
P 0.20%	32.61 ±0.306	148.75 ±0.609 b	116.13 ±0.337 b	1.38 ±0.003 b	356.09 ±2.601 b	1.80 ±0.008 b	264.89 ±1.386 b	9.78 ±0.02 b	2.2800 ±0.005 b	43.8400 ±0.127 a	1.5067 ±0.003 a

3- Blood parameters:

The results of the statistical analysis in Table (4) indicate that there are significant differences ($p \leq 0.05$) between the concentration treatments used, the P 0.15% recorded the highest values among the three concentration treatments. P 0.15% was recorded in RBC (1.54 ± 0.01)10^6 cells/ μm^3, in Hb Table (5) shows that the two treatments, P 0.15% and P 0.05%, were significantly superior ($p \leq 0.05$) with values (9.58 ± 0.39),(8.92 ± 0.01) (g/dl), respectively, as there were no significant differences between them, but they differed significantly from the treatments, P 0.20%, and control. In PCV, the treatment achieved P 0.15% mean (23.80 ± 0.10)%, in MCV it achieved (213.85 ± 1.45) μm^3, in MCH recorded (92.15 ± 0.75%), and in MCHC the four treatments did not

record any significant difference between them. The treatment, P 0.15%, was followed by the treatment P 0.05% and P 0.20% in all the above criteria, while the control treatment recorded the lowest mean in the above criteria.

Table (4) Some studied blood parameters (mean ± standard error) for common carp fish fed on diets containing different percentages of propionic acid during the duration of the experiment.

Studied parameters	RBC 10^6/mm^3 ()	Hb g/dl) (	PCV (%)	MCV (µm^3)	MCH (pg)	MCHC (%)	WBC 10^3/mm^3 ()	TSP	IGM (gm/L)
P 0.00%	1.00 ±0.01 d	7.09 ±0.06 b	20.50 ±0.40 c	161.55 ±4.85 d	80.70 ±0.40 c	33.72 ±2.41 a	202.21 ±0.82 c	2.59 ±0.04 b	0.00227 ±0.00005 b
P 0.05%	1.27 ±0.05 b	8.92 ±0.01 a	22.15 ±0.25 b	196.70 ±1.40 b	87.30 ±1.60 b	33.84 ±1.07 a	211.39 ±2.33 b	2.86 ±0.00 a	0.00418 ±0.00007 a
P 0.15%	1.54 ±0.01 a	9.58 ±0.39 a	23.80 ±0.10 a	213.85 ±1.45 a	92.15 ±0.75 a	39.06 ±0.54 a	217.90 ±0.55 a	2.92 ±0.00 a	0.00444 ±0.00011 a
P 0.20%	1.13 ±0.00 c	7.72 ±0.18 b	20.80 ±0.10 c	178.30 ±0.60 c	85.80 ±0.60 b	36.35 ±0.44 a	203.33 ±0.18 c	2.59 ±0.01 b	0.00234 ±0.00010 b

4- Immunological parameters:

Table (4) shows that there are significant differences ($p \leq 0.05$) between the treatments. P0.15% excelled in all the studied immunological parameters, in WBC P 0.15% recorded the highest mean (217.90 ± 0.55) 10^3 cells/µm^3, followed by P 0.05%, in TSP P0.15% recorded (2.92 ± 0.00) g/100ml, followed by, without significant differences, P 0.05%, which recorded (2.86 ± 0.00) g/100ml. As for the immunoglobulin IGM treatment P 0.15%, recorded (0.00444 ± 0.00011) g/L which did not differ significantly from P 0.05%. The control treatment recorded the lowest means in all immunological traits (Table 4).

Discussion

1- Environmental measurements

The obtained results showed that the amount of oxygen was suitable for raising common carp fish, as its value ranged between 7.8 mg/l and 7.2 mg/l. This amount is very suitable for the growth of common carp, as Alabaster [19] indicated that the appropriate ranges for growth and other vital activities of carp fish from dissolved oxygen in water amounted to 6-8.4 mg / L. The results in Table (2) showed that the temperature was suitable for breeding common carp fish, as Froese and Pauly [20] confirmed that common carp fish can live in warm waters and in a wide range of temperatures ranging from (3-35) °C. The pH value was within the optimum limits for common carp breeding. FAO [21] showed that the appropriate pH for common carp breeding ranged between (6.5-9.5).

2- Growth parameters:

It seems that the acid concentration used in this study played an important and major role in the

studied growth parameters, as it is clear from Table (4) that the treatments, P 0.15% and P 0.05%, recorded the highest values in all parameters , final weight, weight gain, daily growth rate and Relative growth rate is a measure of the qualitative growth rate. It is also noted that all the above-studied criteria decreased in relation to the last treatment of propionic acid, this is evidence that increasing the concentration beyond certain limits can have a negative effect. The increase in growth parameters relative to the two initial concentrations of acid is consistent with several studies that confirm that the addition of acidifiers to fish feed can positively affect most, if not all, of the growth parameters from those studies, study El-Naby et al. [22] those who confirmed that the use of acidifiers led to an increase in all the studied growth parameters, the researchers instructed the reason that acidifiers could have the ability to increase the surface area of the alimentary canal by increasing the height and width of the intestinal villi, which leads to improved absorption of nutrients later in the intestine of fish. This was also confirmed by the study of Zhou et al. [23], in which they used three types of butyrate salts and were fed in one proportion to grass carp fish. They concluded that the different types of salts used had no effect, but they affected all the studied growth parameters. These results are also consistent with the findings of Wenshu et al. [24] when they conducted a study of the effect of sodium butyrate encapsulated in microcapsules on growth, intestinal mucosal formation, immune response and adherent bacteria in common carp (*Cyprinus carpio* L), where they found that sodium butyrate had an effect on All studied growth parameters, as these salts improved weight gain, specific growth coefficient, and feed conversion coefficient. The superiority of the treatment, P 0.15, is due to the presence of a good concentration suitable for the growth of Gram-positive bacteria, in addition to providing the appropriate pH for the action of digestive enzymes, because the presence of organic acids leads to a diversity of the activity of those acids inside the intestine as anti-activities for many types of bacteria, especially the pathological ones, and this It gives a wider space for beneficial bacteria to expand in the intestine due to reducing crowding out between different types of bacteria. The optimal pH value (optimal pH) which is (the pH value that provides the highest enzyme activity) and pH stability (the pH range that provides suitable enzyme stability) can greatly affect the enzyme activity due to an increase in the amount of acid in the diet fed to the fish. . Therefore, the activity of many enzymes diminishes when the pH drops or rises from optimal levels [25]. This is what happened here in this study, where it was observed that when the acid concentration was increased to high levels, the growth parameters began to decline. Acidifiers work in two important directions, the first is that they provide the ability to lower the pH of the gastrointestinal tract, which provides good ranges for the action of many enzymes, and the second is that they participate in many metabolic pathways to generate energy, which increases the metabolic processes within the body [3]. In both directions in which acidifiers work, acidifiers helped in this experiment to lower the pH value and thus increase digestion, which ultimately leads to an increase in metabolism, and this may explain the increase in the rate of metabolic growth of fish at certain levels of the concentrations used. In a study conducted by Nordrum et al.

[26], which dealt with the study of the effect of graduated levels of triglycerides on growth, digestive processes and nutrient utilization in Atlantic salmon, they found a decrease in the digestion of carbohydrates and an increase in the digestion of fats. The presence of digestive processes, which led to a high rate of metabolic growth. This supports the results of this study, where it was clear that the rate of metabolic growth increased for the treatment of P 0.15%. As for the low rate of metabolic growth in treatment P 0.20%, it may come from the effect of high concentrations of acidifiers on the growth of beneficial bacteria that live within medium acidity ranges, such as lactic bacteria, as confirmed by Busti et al. [27] that acidifiers change the properties of prebiotics in the gut microbiota. Or this decrease in the level of metabolic growth rate may be due to the palatability, as table (3) shows the decrease in the amount of feed intake due to the high concentration of acids in the diet, which may reduce the fish's palatability to the diets, as the addition of these materials in higher quantities or concentrations reduces the palatability of the food and thus leads to This leads to less food intake by fish due to the strong odor and flavor becoming undesirable to the fish [28][29].

It is also noted in Table (4) a significant decrease in the criterion of the food conversion rate by increasing the concentration, as the treatment exceeded P 0.15%, followed by the treatment P 0.05%, then P 0.20%. The reason may also be due to palatability or the reason may be due to the fact that a decrease in the pH value to low levels can affect the feed conversion rate as the optimal pH (as discussed previously) and the stability of the pH affect the activity of enzymes in fish [25]. These results are consistent with the findings of Wenshu et al. [24].

The presence of propionic acid may have improved, albeit slightly, the protein efficiency ratio. These results are consistent with the study of Yi et al. [30] in mirror carp (Cyprinus carpio) and confirmed by Zhang et al. [31] in young eel (Anguilla rostrata) and confirmed by Huang et al.[32] in the Japanese sea bass Lateolabrax japonicas..

3- blood parameters

The treatment, P 0.15%, was superior to the rest of the other concentration treatments in most of the studied characteristics, especially red blood cells, hemoglobin and hematocrit. This treatment was followed by a treatment of P 0.05%, then a treatment of P 0.20%. Certainly, those standards increased, but when the concentration increased beyond those limits, those standards decreased.

The most important functions of the blood are the supply of oxygen and nutrients to tissues, immune functions, coagulation, and hormone transport functions [33]. There is also a close correlation between the number of red blood cells and the amount of oxygen consumed and transported through hemoglobin, given the various important roles of blood as measuring blood parameters provides a more reliable picture of the metabolism of fish and the short and long-term effects of "suboptimal" farming conditions, water quality, and condition Nutrition [34]. It was found that there is a strong correlation between blood parameters and the rate of metabolic growth. Where the higher the metabolism, the greater the amount of oxygen that the body needs to cover the high rate of metabolism and thus increase the number of red blood cells accordingly. This is confirmed by the high rate of metabolic growth in Table (3),

accompanied by an increase in the number of red blood cells (Table 4). The close association between blood parameters, especially red blood cells and hemoglobin, with fish metabolism stems from the fact that increased metabolism requires sufficient amounts of oxygen for the purpose of metabolizing these nutrients in the body [35]. The higher the metabolism, the greater the amount of oxygen needed by the fish for the purpose of representing nutrients in the cells, which prompts the body to promote red blood cells and increase their levels in order to keep up with the body's need for oxygen [36] Which forces red blood cells to increase the amount of hemoglobin in the bodies of those cells because it is responsible for transporting oxygen and thus an increase in the volume of red blood cells in addition to the increase in the size of those cells because they contain a larger amount of hemoglobin. This explains the increase in the size of red blood cells in the above results. Also, hemoglobin is responsible for aerobic metabolism, which works to deliver oxygen to tissues, where tissues use these gases as a final receiver for electrons derived from oxidative catabolic reactions and metabolism, as the higher the metabolism, the greater the need for [37]. This phenomenon can be explained by the fact that the hematopoietic activity in animals, including fish, is the result of synergy between various essential nutritional factors, including vitamin B12, vitamin B6, vitamin K, vitamin D, and the presence of some acids, including folic, propionic, butyric, and some other acids, as well as some minerals such as iron. zinc, copper...etc [38]. From this, the role of acids in increasing blood activity becomes clear in two directions. The first is the ability of acids to increase the digestion of nutrients, which includes all digestible food in the diet and the factors that aid in their digestion. The second direction is the increase in growth as a natural result of increased digestion. In both directions, fish need large amounts of Greater than blood, in the study of Hongyan et al., [39] in the search for the relationship between body mass and blood parameters in young yellowfin tuna fish Thunnus albacares, they proved that the greater the body mass, the greater the amount of blood in those fish as an indicator of a high metabolism.

As the results show in Table (4), the two treatments of the second and first concentrations were superior in the standard of the number of white blood cells, followed by the treatment of the third concentration. direct effect of functional additions or as a result of improved health of fish) [36].

Immunoglobulin (IGM) is also considered the first barrier in fish resistance to diseases, and its function is to identify foreign bodies such as bacteria and viruses and weaken them [40]. Zarei et al. [41] also confirmed in his study the effect of dietary butyric acid glycerides on the performance of Acanthopagrus fingerlings. latus, the content of total immunoglobulins and lysozyme activity in the skin mucus increased with the increase of dietary butyric acid glyceride, as it was observed in our study and when dealing with fish that the fish had a high content of the amount of mucus in the skin, which is one of the most important first defense means in the [42].

Total serum proteins play an important role in the humoral immunity of fish and the innate immune response [43]. The amount of serum proteins increased with increasing acid concentration until reaching the highest concentration and then began to decrease. The results of this study confirmed the results

obtained by Rasha et al. [44], where they indicated that there was a significant increase in total erythrocytes, hemoglobin content, platelet count, hematocrit, average hemoglobin in the body, and total number of white blood cells in most treatments, and the average volume of lymphocytes and lymphocytes increased. neutrophils; Elham et al. [45] showed that acidifiers have a high effect on fish immunity.

Conclusions:

The results showed that propionic acid can be safely used in common carp diets and that this acid has a positive effect on growth parameters, blood parameters, and immunological parameters. The optimum acid addition percentage was 0.15% to the used fish diets.

Recommendations: The need to include this acid in the diet of common carp because of its positive effect on all the studied criteria, especially the immunological ones, because of its role in improving the health of fish, and this is what many breeders suffer from because of the large number of deaths as a result of breeding at high densities in our country, Iraq.

References

1. Liem, D.T. (2004). *E. coli* resistant to most antibiotics in Vietnam. *Asian Pork*, 8: 22-24.
2. Dhellal, Muhammed Halbos, (2018). Effect of additional foods and Feeding Ratio on Some Growth Indicators of fish Ctenopharyngodon idella. *Muthanna Agriculture*.6 (2). P 44-50.
3. Luckstadt, C. (2008).The use of acidifiers in fish nutrition. In: *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources.* 3, No. 044, pp. 1-8. Doi:10.1079/PAVSNNR20083044
4. Menconi, A., Kuttappan, V.A., Hernandez-Velasco, X., Urbano, T., Matté, F., Layton, S., G. Kallapura, G., Latorre, J., Morales, B.E., Prado, O., Vicente, J.L., Barton, J., Filho, R.L.A., Lovato, M., Hargis, B.M. and Tellez, G. (2014). Evaluation of a commercially available organic acid product on body weight loss, carcass yield, and meat quality during pre-slaughter feed withdrawal in broiler chickens: a poultry welfare and economic perspective. *Poultry Science*, 93: 448-455. Doi: 10.3382/ps.2013-03444
5. Hunter, P.A.; Dawson, S.; French, G.L.; Goossens, H.; Hawkey, P.M.; Kuijper, E.J., Nathwani, D., Taylor, D.J., Teale, C.J., Warren, R.E. and Wilcox, M.H. (2010). Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. *Journal of Antimicrobial Chemotherapy*, 65: 13-17. Doi.org/10.1093/jac/dkp433.
6. Sapkota, A., Sapkota, A.R., Kucharski, M., Burke, J., McKenzie, S., Walker, P. and Lawrence, R. (2008). Aquaculture practices and potential human health risks: current knowledge and futurepriorities. *Environment International*, 34: 1215-1226. Doi.org/10.1016/j.envint.2008.04.009.
7. Sardar, P., Shamna, N., Sahu, N.P. (2020). Acidifiers in aquafeed as an alternate growth promoter: A short review. *Anim. Nutr. Feed.Technol*. 20: 353–366.

Doi:10.5958/0974-181X.2020.00032.3
8. Browdy, L., Ei, O., Minh, P. and Bharadwaj, S. (2011). Organic acid supplementation in aqua feeds: effects on gut microflora, health and performance. Abstract CD-ROM. *World Aquaculture Society*, June 6-10, 2011, Natal, Brazil, (Abstr.). Doi:10.5958/0974-181X.2020.00032.3
9. Schmalhousen, L. (1926). Studien uber washtum and differenzierung III.Die embryonale wachsturm skurve des hiichens.Wilhem roux.Arch entwic klungsmech.Org Cited by Hoor W.S.،Randall،D.J.and Breett،J.R.(eds).Fish physiology. Vol.VIII, 322-387.
10. Velmurugan, S. and Rajagopal, S. (2009). Beneficial uses of probiotics in mass scale production of marine ornamental fish. *African Journal of Microbiology Research*, 3(4):185-190. Doi.org/10.5897/AJMR.9000124
11. Brown, M.E. (1957). Experimental studies on growth .In:Fish physiology, M.E. Brown (ed.) New York , N.Y. Academic press Vol . I ,p 361-400.
12. -Dabrowski K, Murai T, Becker K (1986). Physiological and nutritional aspects of intensive feeding of carp. In: Aquaculture of cyprinids (ed. by R. Billard & J. Marcel), *INRA, Paris*, pp 55–70.
13. Uten, F. (1978). Standard methods and terminology in finfish nutritions from: proc. World symp on finfish nutrition and fish feed technology. *Hamburg*، 20-23 June 1978. Vol. II Berlin.
14. Gerking, S.D. (1971). Influence of feeding and body weight on protein metabolism of bluegill sunfish. *Physiol. Zool* ، 44 : 9-19.
15. NRC. (1993). Nutrient requirements of fish. Washington (DC): National Academy Press.
16. Hepher, B. ; Liao, I.C. ; Cheng, S.H. and Hsieh, C.S.(1983). Food utilization by red tilapia - effects of diet composition, feeding level and temperature on utilization efficiencies for maintenance and growth. *Aquaculture*. 32(3–4):255–75. Doi.org/10.1016/0044-8486(83)90223-5.
17. Jauncey, K, and Ross, B. (1982). A guide to tilapia feeds and feeding Ins. Aquaculture, Univ. Sterling, *FK94 La, Scotland, U. K*.111.
18. Hepher, B. ; Liao, I.C. ; Cheng, S.H. and Hsieh, C.S.(1983). Food utilization by red tilapia - effects of diet composition, feeding level and temperature on utilization efficiencies for maintenance and growth. *Aquaculture*. 32(3–4):255–75. Doi.org/10.1016/0044-8486(83)90223-5.
19. Alabaster, J.S. (ed.), (1982). Report of the EIFAC Workshop on fish-farm effluents. Silkeborg, Denmark, 26–28 May 1981. EIFAC Tech. Pap., (41): p166.
20. Froese, R. and Pauly, D. (eds.).(2011). FishBase. World

Wide Web electronic publication, version (02/2011) (available at : www.fishbase.org/summary/speciessummary.php.

21. FAO, (1981). Report of the symposium on new developments in the utilization of the heated effluents in the circulation system for intensive aquaculture stavanger ,Rome. Italy.,29-30.
22. El‐Naby ASA, Khattaby AE, Samir F, Awad SM and Abdel ‐Tawwab M. (2019). Stimulatory effect of dietary butyrate on growth, immune response, and resistance of Nile tilapia، *Oreochromis niloticus* against *Aeromonas hydrophila* infection. *Anim Feed Sci. echnol: Jul*, 2019 (Epub ahead of print.(10.1016/j).114212. Doi.org/10.1016/j.anifeedsci.2019.114212.
23. Zhou Ji Shu, Pan Guo, Hai Bo Yu, Hong Ji, Zhou Wen Lai & Yi An.(2019).Chen Growth performance, lipid metabolism, and health status of grass carp (*Ctenopharyngodon idella*) fed three different forms of sodium butyrate .*Fish Physiology and Biochemistry* volume 45, pages287–298. Doi: 10.1007/s10695-018-0561-6.
24. Wenshu L., Yanou Y.,Jianli Z. ,Delbert M. G. ,Einar R., Zhigang Z. (2014). Effects of dietary microencapsulated sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (*Cyprinus carpio*) pre-fed with or without oxidised oil. *British Journal of Nutrition*. V. 112. I. 1. Published online by Cambridge University. Doi:10.1017/S0007114514000610.
25. Marquez, L., Robles, R., Morales, G.A. and Moyano, F.J. (2012). Gut pH as a limiting factor for digestive proteolysis in cultured juveniles of the gilthead sea bream (*Sparus aurata*). *Fish Physiol Biochem*. 38:859–869. Doi: 10.1007/s10695-011-9573-1
26. Nordrum, S. ; Olli, J. J. ; Røsjo, C. ; Holm, H. and Krogdahl, A.(2003). Effects of graded levels of medium chain triglycerides and cysteine on growth, digestive processes and nutrient utilization in sea water reared Atlantic salmon (*Salmo salar*, L.) under ad libitum feeding regime. *Aquac. Nutr*. 9, 263–274. Doi:10.1046/j.1365-2095.2003.00252.x
27. Busti, S.; Rossi, B.; Volpe, E.; Ciulli, S.; Piva, A.; D'Amico, F. and Parma, L. (2020). Effects of dietary organic acids and nature identical compounds on growth, immune parameters and gut microbiota of European sea bass. *Sci Rep*. 10:1–14. Doi: 10.1038/s41598-020-78441-9
28. Xie, S., Zhang, L. and Wang, D. (2003). Effects of several organic acids on the feeding behavior of *Tilapia nilotica*. *Journal of Applied Ichthyology*, 19: 255-257. Doi.org/10.1046/j.1439-0426.2003.00451.x
29. FAO, (2013). CODEX ALIMENTARIUS

COMMISSION, PROCEDURAL MANUAL, 21st- edition.
30. Yi, Du; Long, Cheng; Jianhua, Zhao; Clement, R. and de Cruz (2023). Effects of Clostridium butyricum and sodium butyrate on growth performance, immunity, and gut microbiota of mirror carp *Cyprinus carpio* fed with soybean meal based diet. *Aquaculture Reports*, V. 29, Iss , Pp 101501. [abst]. Doi.org/10.1016/j.aqrep.2023.101501.
31. Zhang, Mingliang. Yue Wang, Shaowei Zhai. (2021). Effects of dietary compound acidifiers supplementation on growth performance and intestinal health of juvenile American eels (*Anguilla rostrata*) cultured in cement tanks. *The Israeli Journal of Aquaculture IJA*.73.1520998, 12 p. Doi:10.3390/fishes7040203
32. Huang, Z.F.; Ye, Y.L.; Xu, A.L.; Li, Z.B. and Wang, Z. (2022). Dietary supplementation with an acidifier blend (citric, lactic, and phosphoric acids) influences growth, digestive enzymes, and blood chemistry of juvenile Japanese sea-bass (*Lateolabrax japonicus*). *Aquac. Int*., 30, 19–32.
33. Ciesla, B., (2007). Hematology in Practice; FA Davis Company: Philadelphia, PA, USA; p. 230. ISBN-13: 978-0-8036-6824-9.
34. Rebl, A.; Seibel, H. and Baßmann,(2021). B. Blood Will Tell: What Hematological Analyses Can Reveal About Fish Welfare. *Front. Vet. Sci*. 8, 194. Doi: 10.1016/j.fsi.2016.01.040.
35. Wilco, C. E. P.; Jeroen, F.; Iris L. E.; van de Pol, M. A. Urbina, R.W.; Wilson, D. J.; McKenzie, F. P. L.(2022). Body mass and cell size shape the tolerance of fishes to low oxygen in a temperature-dependent manner. Doi.org/10.1111/gcb.16319.
36. Moha Esmaeili. (2021). Blood Performance: A New Formula for Fish Growth and Health. Review. Hobart Private Bag 49, 15-21. Doi.org/10.3390/biology10121236
37. Wells, R.M. (2009). Blood-gas transport and hemoglobin function: Adaptations for functional and environmental hypoxia. *Fish Physiology*. Volume 27. Elsevier; Amsterdam, The Netherlands: pp. 255–299. Doi.org/10.1016/S1546-5098(08)00006-X.
38. Choi JW. and Kim SK. (2005). Relationships of Lead, Copper, Zinc, and Cadmium Levels versus Hematopoiesis and Iron Parameters in Healthy Adolescents. *Ann Clin Lab Sci* 35: 428-432.
39. Hongyan, LIU; Zhengyi, FU; Gang, YU and Zhenhua, MA. (2023). Study on relationship between body mass and blood indexes of juvenile *Thunnus albacares*[J]. *South China Fisheries Science*, 2023, 19(1): 173-178. Doi: 10.12131/20220077.
40. Giri, S. S. ; Sen, S. S. and Sukumaran, V.(2012).Effects of dietary supplementation of potential probiotic *Pseudomonas aeruginosa* VSG2 on the innate immunity and disease resistance of tropical freshwater fish, *Labeo rohita*. Fish Shellfish Immunol., 32, 1135–1140. Doi: 10.1016/j.fsi.2012.03.019

41. Zarei, S.; Badzohreh, G.; Davoodi, R.; Nafisi Bahabadi, M. and Salehi, F. (2021). Effects of dietary butyric acid glycerides on growth performance, haemato-immunological and antioxidant status of yellowfin seabream (*Acanthopagrus latus*) fingerlings. *Aquac*. *Res*. 52, 5840–5848.Doi.org/10.1111/are.15458
42. Ahmed, Hussain and Shashwati, Ghosh (2023). Fish Epidermal Mucus as a Source of Diverse Therapeutical Compounds. 29(3): 36. Doi: 10.1007/s10989-023-10505-6
43. Jha, A.K.; Pal, A.; Sahu, N.; Kumar, S. and Mukherjee, S.(2007). Haemato-immunological responses to dietary yeast RNA, ω-3 fatty acid and β-carotene in *Catla catla* juveniles. *Fish Shellfish Immunol*. 23, 917–927. DOI: 10.1016/j.fsi.2007.01.011
.
44. Rasha, M.; Reda , R. M.; Khaled, M.; Selim, IE. and El-Araby. (2016). Effects of dietary acidifiers on growth, hematology, immune response and disease resistance of Nile tilapia, *Oreochromis niloticus*. *Fish & Shellfish Immunology* Volume 50, P. 255-262. Doi.org/10.1016/j.fsi.2016.01.040
45. Elham, A.W.; Norhan, E.S. and Khouloud, B.(2018). Advantageous effects of dietary butyrate on growth, immunity response, intestinal microbiota and histomorphology of European Seabass (*Dicentrarchus labrax*) fry. *Egyptian Journal of Aquatic Biology and Fisheries*. I. 1110 6131 :22(4):93-110. Doi.org/10.1016/j.aqrep.2023.10150.