



Short Communication

Dietary Formic Acid and Vitamin D₃ as Growth Effective Supplement for Grass Carp Fingerlings

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ABSTRACT

The present research was carried out to evaluate the effectiveness of dietary formic acid and vitamin D₃ on growth performance and muscle proximate analyses of grass carp (*Ctenopharyngodon idella*). Four experimental diets viz. FD1, FD2, FD3 and FD4 having formic acid (%) and vitamin D₃ (IU/Kg) 0, 0; 2, 0; 0, 5000 and 2, 5000, respectively were prepared. Fish were fed with experimental diets for 90 days. Growth performance of fish was observed on fortnightly basis throughout the trail. At the end of feeding trail, fish were dissected to obtain the samples of muscles for further analysis. Two-way analysis of variance under RCBD were applied on obtained data. Results demonstrated that growth performance (weight gain, specific growth rate and absolute weight gain) of *C. idella* fingerlings showed significant improvement by feeding formic acid and vitamin D₃. Result showed that *C. idella* fed with vitamin D₃ increased the crude protein, ash and decreased the fat contents in muscles while same result was observed by supplementation of formic acid. In conclusion, dietary formic acid and vitamin D₃ improves the growth performance and muscle proximate analysis for the *C. idella* fingerlings.

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Authors' Contribution

LS executed the research. MF helped in compiling the data. SZHS did statistical analysis. MA supervised the study. HN and SR helped in writing the article. YW and QL reviewed the manuscript.

Key words

Ctenopharyngodon idella, Growth, Muscles, Formic acid, Vitamin D₃.

Aquaculture is the world's fastest-growing field of agriculture (FAO, 2010). The main objective of aquaculture is to improve the health and growth performance of animals. Feed additives are the best choice (NRC, 1993) to enhance feed properties and growth performance. Antibiotics have long been used in aqua feeds, however, owing to side effects of antibiotics, European Union banned their use in animal production (FAO, 2002; Heuer *et al.*, 2009). Alternatively, antibiotics such as natural immune stimulants, pathogen inhibitors and growth promoters which include plant extracts, probiotics, prebiotics and acidifiers (Devasree *et al.*, 2014; Reda and Selim, 2015; Romano *et al.*, 2015; Selim and Reda, 2015) were used.

Fish meal is an important component of fish diet due to high protein and presence of essential nutrients (Zhou *et al.*, 2004). In the global market the price of fish meal is so high because of its rising demand and limited stock; therefore, it is necessary to search for alternative plant protein sources (Pereira and Oliva-Teles, 2003).

In aquaculture, the replacement of high quality fish meal with plant meal in animal feed is the most cost-effective approach because of reasonable price, high protein content, comparatively balanced amino acid profile and stable supply of plant meals (Storebakken *et al.*, 2000). Plant protein sources contain phytate which cannot be digested (Oliva-Teles *et al.*, 1998), and decreases the digestibility of protein (Spinelli *et al.*, 1983) and utilization of supplementary mineral (Gatlin and Phillips, 1989; Sugiura *et al.*, 2001). Phytate is hydrolysed with the addition of organic acid in the diet (Hossain *et al.*, 2007).

Organic acids (propionic, butyric, formic, citric, malic, sorbic, acetic and lactic acids) and their salts can

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be used as acidifier in the livestock feed industry and aquaculture, (Defoirdt *et al.*, 2009). The addition of sufficient quantity of organic acids and their salts in the diet can modulate disease resistance and feed quality and also improve the health and growth (Ng and Koh, 2011, 2016).

Acidifiers lower the pH of gastrointestinal tract, enhance the hydrolysis of phytate, kill harmful pathogens, reduce emptying rate of the gastrointestinal tract and improve pepsin activity, nutrient absorption, nitrogen retention and mineral absorption as well as transportation (Ravindran and Kornegay, 1993; Overland *et al.*, 2000; Lucksteadt, 2008).

Cholecalciferol is fat-soluble vitamin (vitamin D₃) and essential nutrient for the growth of aquatic organisms. It performs many physiological functions in organisms, due to its vital role in bone formation (Li, 1994). Fish cannot synthesize vitamin D₃ directly from sunlight like other vertebrates, hence obtains it from diet only (Darias *et al.*, 2011). So, vitamin D₃ added in fish diet for proper growth. If excess amount of vitamin D₃ is supplemented in the diet, it causes metabolic disorders and calcinosis (Hollis and Wagner, 2004; Horst *et al.*, 2003), while less amount of vitamin D₃ can cause tetany (Cho and Slinger, 1979; Barnett *et al.*, 1982; George *et al.*, 1981).

The present study was undertaken to analyze the effect of dietary formic acid and vitamin D₃ supplementation on proximate composition, intestinal digestive enzyme activities and bone mineralization in grass carp, *Ctenopharyngodon idella*.

Materials and methods

The study was carried out at Fisheries Research Farm, University of Agriculture, Faisalabad. Four earthen ponds, each having dimensions 19m×6m×1.5 m were selected and fixed with fine mesh to avoid any unwanted animals to enter the ponds. The ponds were filled with tube well water. *Ctenopharyngodon idella* (grass carp) (n=25) were stocked after measuring their weight and length in each experimental pond on 1st May 2016. Mixture of inorganic fertilizers that is urea and single superphosphate (1:1), was added in each pond on weekly basis for one month prior to fish stocking. The fish were fed with the fish feed 2% body weight of fishes twice a day. The water level of ponds was maintained throughout the study period. The sampling was done on fortnightly basis and feed ratio was adjusted accordingly. The fish were finally harvested and measured after 90 days feeding period of 1st August 2016.

For feed formulation four experimental diets (FD₁, FD₂, FD₃ and FD₄) were prepared that contained formic acid (%) and vitamin D₃ (IU/Kg) at following proportion

0, 0; 2, 0; 0, 5000 and 2, 5000, respectively. The duration of experiment was three months. For experimental diet formulations, cereal grinding machine (FFC-45, JIMO, China) was used for grinding and sieving of (0.05 mm) the diet ingredients. The minerals mixed fish oil and vitamins were added after grinding of ingredients. Appropriate amount of water was used for pellet formation. Experimental extruder (Model SYSLG30-IV) was used for the preparation of pellets (3.5 mm), which were dried upto 10% moisture and packed in plastic bags and stored at 20°C during the entire feeding experiment.

Growth performance was assessed on fortnightly basis from each pond in terms of weight gain by the fish. At the end of feeding trial eight fish were starved for a day and dipped in clove oil (3000 mg/L) for 40-60sec to anesthetise (Khajepour *et al.*, 2012). Fish was sacrificed by a sharp blow on head. For proximate analysis standard method of AOAC (1995) were followed to determined moisture at 105°C for 12 h in micro Kjeldahl apparatus used for the determination of crude protein (N x 6.25), Crude fat was estimated by petroleum ether extraction method (Soxtec system HT2 1045) and ash content were measured in electric furnace (Eyela-TMF 3100) by ignition at 650°C for 12 h to constant weight.

The data was analyzed by Two-way ANOVA under RCBD was applied on resulted data (Steel *et al.*, 1996). For the comparison of means Student-Newman-Keuls test was used, when significant differences occurred (Snedecor and Cochran, 1991). For analyzing statistical data CoStat computer package (Version 6.303, PMB320 Monterey, CA 93940, USA) was used.

Weight gain and specific growth rate (SGR) were calculated as:

$$\text{Weight gain\%} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}}$$

$$\text{SGR} = \frac{\ln \{ \text{Final weight(g)} - \text{Initial weight(g)} \}}{\text{Experimental periods in days}} \times 100$$

Result and discussion

Table I shows growth performance of fish. All growth parameters were significantly improved by feeding formic acid and vitamin D₃ supplemented diet. Muscle proximate analysis is given in Table II. Result showed that *C. idella* fed with vitamin D₃ increased the crude protein (18.30±0.01), ash (2.43±0.02) and decreased the fat (3.84±0.02) contents while same results were observed by supplementation of formic acid for crude protein (18.20±0.02) and ash (2.18±0.02) contents.

The growth rate increased with acidification, because acids increase the mineral availability and their utilization in body. The Organic acids reduce the gastric pH, which improves the activity of many metabolic enzymes leading

to improve growth rate (DeWet, 2005; Park *et al.*, 2009). Similarly, Hossain *et al.* (2007) reported the higher growth rate of *pagrus major* with the supplementation of citric acid which results in the higher mineral dissociation and forms chelated mineral complexes that are easily absorbed in body. Moreover, organic acid lowers the gastric emptying rate. Present research work showed that supplementation vitamin D₃ significantly increased growth rate which may owe to increase utilization of phosphorous. Because of phosphorous utilization Ling-Hong *et al.* (2015). Similar results were also reported by Ling-Hong *et al.* (2015) that supplementation of vitamin D increased the growth rate in Wuchang bream. A similar increase in weight gain % of rain bow trout was observed by feeding vitamin D (Coloso *et al.*, 2001).

Table I.- Growth performance of *Ctenopharyngodon idella*.

Diet	FD ₁	FD ₂	FD ₃	FD ₄
FA (%) + VD ₃ (IU/kg)	(0+0) Control	(2+0)	(0+500)	(500+2)
Initial weight (g)	19.48±1.05	19.13±0.63	19.74±0.15	19.50±0.75
Final WG (g)	181.52±8.18 ^d	203.38±5.31 ^b	195.36±2.89 ^c	220.4±4.56 ^a
Absolute WG (g)	162.04±7.14 ^d	184.26±4.68 ^b	175.62±2.74 ^c	200.89±3.81 ^a
WG (%)	832.04±8.03 ^d	963.57±7.23 ^b	889.63±6.88 ^c	1030.36±0.30 ^a
SGR	3.72±0.01 ^d	3.94±0.01 ^b	3.82±0.01 ^c	4.04±0.03 ^a

Values sharing similar letters in a column are non-significant at P<0.05. FA, formic acid; VD₃, Vitamin D₃; FD, FA+VD₃; WG, weight gain; SGR, specific growth rate.

Table II.- Muscle proximate analysis of *C. idella*.

Diet	FD ₁	FD ₂	FD ₃	FD ₄
FA (%) + VD ₃ (IU/kg)	(0+0) Control	(2+0)	(0+500)	(500+2)
Moisture (%)	75.3±0.04 ^a	74.8±0.03 ^b	74.5±0.04 ^b	75.1±0.04 ^a
Protein (%)	17.74±0.04 ^d	18.20±0.02 ^a	18.30±0.01 ^a	17.91±0.02 ^c
Crude fat (%)	3.63±0.05 ^d	4.13±0.03 ^a	3.84±0.02 ^c	3.90±0.04 ^b
Ash (%)	2.01±0.03 ^b	2.18±0.02 ^{ab}	2.43±0.02 ^a	1.81±0.03 ^c

Values sharing similar letters in a column are non-significant at P<0.05. For abbreviations, see Table I.

In present study crude protein, crude fat and ash content of muscle were affected by supplementation of vitamin D₃ and formic acid. A similar decrease in the

lipid content in the muscle of fish were observed by citric acid supplementation, however the yet action is unknown Khajepour *et al.* (2012). In present research work the effect of vitamin D₃ supplementation showed not significant effect on moisture content in muscle of *Ctenopharyngodon idella*. Similar results were observed by Ling-Hong *et al.* (2015) who said that addition of vitamin D₃ did not affect the moisture content in the muscle of fish while lipid contents, crude protein and ash contents were significantly affected. Moisture is not sensitive indices of dietary vitamin D₃. Supplementation of vitamin D in fish diet had non-significant effects on crude lipid in grass carp. This indicated that deficiency of vitamin D in fish body and also decreases the bioavailability of lipids (Jiang *et al.*, 2009).

CONCLUSION

In conclusion, dietary formic acid and vitamin D₃ improves the growth performance, muscle proximate analysis, bone mineralization and digestive enzyme activities in the *Ctenopharyngodon idella* fingerlings.

Statement of conflict of interest

The authors declare no conflict of interest.

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