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Araştırma Makalesi
Research Article

The Effects of the Carvacrol and Thymol Addition to *Pangasius hypophthalmus* Feeds in Different Amount on the Growth Performance

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Abstract

In the present study we investigated the effects of phytoadditive carvacrol and thymol on growth performance and feed utilization of *Pangasius hypophthalmus*. In trial, 840 *Pangasius hypophthalmus* which had average weight of 1.33 ± 0.05 gram was used. Fish were divided into seven groups before being fed for 90 days with 0, 1 (C1), 3 (C3), 5 (C5), 1(T1), 3(T3), 5(T5) g/kg of carvacrol and tymol. All treatment groups were planned three replicate and randomly plots method and fish were distributed to groups.It was determined that 3 g/kg timol addition to feed provided the best result in terms of growth performance.

Keywords*:Pangasius hypophthalmus, carvacrol, thymol, growth performance, feed utilization.*

Öz

Karvakrol ve Timolün *Pangasius hpophthalmus* Balıklarının Büyüme Performansı ve Yem Dönüşüm Oranı Üzerine Etkisi

Bu çalışmada,bitkisel kaynaklı karvakrol ve timol ektraktlarının *Pangasius hypophthalmus* balığında büyüme performansı ve yem tüketim üzerine etkileri araştırılmıştır. Çalışmada ortalama ağırlık 1.33 ± 0.05 golan 840 adet *Pangasius hpophthalmus* balığı kullanılmıştır. Çalışmada 0, 1 (C1), 3 (C3), 5 (C5), 1 (T1), 3 (T3), 5 (T5) g / kg yem oranında karvakrol ve timol ile 90 gün balıklar beslenmiştir. Tüm deneme grupları 3 tekerrürlü olarak planlanmış ve tesadüfî parselleme metoduna göre balıklar gruplara dağıtılmıştır. Büyüme performansı bakımından en iyi sonucu yeme 3 g/kg timol ilavesinin sağladığı belirlenmiştir.

***Anahtar Kelimeler*:** *Pangasius hypophthalmus, karvakrol, timol, büyüme performansı, yem dönüşümü.*

Introduction

In recent years, the aquaculture output of the striped catfish (*Pangasius hypophthalmus*), generally known as pangasius, has grown rapidly with production output in 2008 exceeding over 1 million. Vietnam is by far the largest pangasius producer, but Thailand, Cambodia, Lao People's Democratic Republic, Myanmar, Bangladesh and China also culture pangasius on a commercial scale (FAO, 2014).

Feed additives are supplements that are used to increase the utilization of the feed, increase the quality and quantity of the animal

products, protect the health of animals and to cut down on the costs of the product the animal provides. In addition, new approaches which bring the use of alternative feed additives into the forefront began to be adopted because of the problems arising from the extensive use of antibiotics in recent years. New alternative additives that are used in practice are: enzymes, organic acids, probiotics, oligosaccharides (prebiotics) and plant extracts (Kahraman, 2009).

Numerous researches have been reported on the supplementation of some herbal / medicinal plants or their extracts like cold-pressed oil, essential oil or effective compounds such as thymol and carvacrol, to large and small animal diets that improved productive and reproductive performance, immune functions, antioxidant status, carcass traits and quality, and lowered morbidity and mortality rates (Soković *et al.*, 2008; Khattak *et al.,* 2014; Aboelwafa and Yousef 2015; Dhama *et al.*, 2015).

In general, thymol [5-methyl-2-(1-methylethyl) phenol], a main component of thyme essential oil, and its isomer, carvacrol [2-methyl-5-(1-methylethyl) phenol], a main component of oregano essential oil, are principally responsible for these activities (Yanishlieva *et al.,* 1999).

Some studies have reported that oral administration of combination of carvacrol and thymol in *Ictalurus punctatus* (Zheng *et al.,* 2009) and *Oncorhynchus mykiss* (Ahmadifar *et al.,* 2011) improved growth performance, disease resistance and/or immunity. Ahmadifar *et al.* (2011) suggested that administration of 2.0 and 3.0 g/kg thymol-carvacrol in rainbow trout juveniles diet can improved some growth parameters (final weight, food conversion ratio, specific growth rate and daily growth rate) of fish. Also, Zheng *et al.* (2009) were reported that the essential oil of thymol has a good effect on growth performance of channel catfish (*Ictalurus panctatus*). In another study, it was seen that the carvacrol (12 g/kg) had a positive effect on the growth performance of trouts (Giannenas et al., 2012).

Therefore, the aim of the present study was to determine the effects of dietary phyto additi ve carvacrol and thymol (1. 3.or 5 g/kg) on growth performance in panga fish.

Materials and Methods

Fish and Experimental Protocol; Juvenile healty panga fish, which had not been vaccinated and which were not exposed to any diseases obtained from a private company were used in the study. 200 lt. aquariums were used in the test. In the trial, 840 *Pangasius hypophthalmus* which has average weight of ±SD = 1.33±0.05 g was used. Fish were fed by hand 2 times a day, in the ratio of %2 of their body weight during 90 days trial. Water temperature, dissolved oxygen and pH were determined as 28˚C, 7.56 $mg \cdot L^{-1}$, 7.04 respectively. The duration of the test was 90 days and the effects of the volatile oils on the growth, feed utilization and survival rate of the plant species were determined by carry out biometric measurements on the fish once every 30 days. The weighing of the fish were made with a ±0.001 g precise digital scale in the test.

Carvacrol (W224502, Sigma-Aldrich) and Thymol (16254, Sigma-Aldrich) were added to a commercial trout extrude feed (Kılıç Feed Company, Turkey, pellet size: 500-800 micron, Table 1), at a dose of 0 (Control), 1 (C1), 3 (C3), 5 (C5), 1(T1), 3(T3), 5(T5) g/kg by mixer. Briefly, after heating (40˚C) for 3 h the diet was top-dressed with fish oil containing the carvacrol and thymol by slowly mixing in a mixer. No herbal oil supplementation was made to control feed (Sönmez *et al.*, 2015).

Table 1. Commercial trout extruder feed ingredients (pellet size: 500-800 micron)

Parameters	Values
Proximate analyses	
Crude Protein (%)	50
Crude Lipid (%)	7
Crude Cellulose (%)	2
Crude Ash (%)	12
Energy (kcal/kg)	
Amino acids (%)	
Lysine %	2.5
Methionine + Cystine %	2
Vitamins (per kg feed)	
K (mg)	10
Macro elements (%)	
Calcium %	2
Total Phosphore %	5

Legend: Ingredients: Fish meal, fish oil, soybean and by products, wheat and by products, yeast and by products, amino acids, vitamins and minerals.

Growth Performance and Proximate Analyses; Growth performance of *P. hypophthalmus* with different diets was considered by calculating weight gain (WG), specific growth rate (SGR) and feed conversion rate (FCR).

WG (g)= final weight (FW) (g)-initial weight (IW) (g)

SGR = (%/d)[(ln final weight (g)-ln initial weight (g))/ days] x 100

FCR = feed intake (g) / weight gain (g)

Proximate analyses of the diets were performed using standard methods (AOAC, 1998). Moisture was detected after drying at 105˚C until a constant weight was achieved. Crude protein was analyzed by the Kjeldahl method, and crude ash by incineration at 525˚C in a muffle furnace for 12 h. Crude fat was analyzed by methanol/chloroform extraction (Folch *et al.*, 1957)

Statiscal Analysis; The variation analyses were carried out with Duncan comparison tests, and the differences between groups were carried out via the use of SPSS 17 statistics program in order to evaluate the relationships between the data of growth parameters and proximate composition obtained from the test group.

Results

Growth data are presented in Table 2 and 3.

Legend: Values are mean ±SE (n=6). Within a row, means with different letters are significantly different ($P<0.05$).

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While it was determined that the value of weight gain (WG) was higher in groups fed with timol and carvacrol added feed than the control group, there were not any differences with utilization of the feed (FCR).

Table 2. Growth performance and feed utilization in pangasius that were fed diets containing different levels of carvacrol (0 (Control), 1, 3, or 5 g/kg of feed; for 90 day)

	Control	C1	C3	C5
IW (g)	1.35 ± 0.009^{a}	1.25 ± 0.03^{a}	1.33 ± 0.09^{a}	1.35 ± 0.04^{a}
FW (g)	8.10 ± 0.44^{cb}	8.84 ± 0.31^{ab}	9.01 ± 0.28^{ab}	9.07 ± 0.54^{ab}
WG (g)	6.75 ± 0.33^{c}	7.58 ± 0.28^{abc}	7.67 ± 0.18^{ab}	7.71 ± 0.50^{ab}
FCR	0.77 ± 0.06^{a}	0.76 ± 0.03^{a}	0.76 ± 0.01^{a}	0.75 ± 0.04^{a}
SGR (%/d)	1.69 ± 0.05^{b}	1.75 ± 0.01^{ab}	1.71 ± 0.04^{b}	1.70 ± 0.02^{b}

The whole-body proximate compositions of fish presented in Table 4 and Table 5.

Legend: Values are mean ±SE (n=6). Within a row, means with different letters are significantly different ($P<0.05$).

There was not any significant differences were detected related to crude protein, crude lipid, crude ash and crude moisture between the experimental group and the control group. HSI, VSI and SSIvalues of the fish fed with timol and

Table 3. Growth performance and feed utilization in pangasius that were fed diets containing different levels of thymol (0 (Control), 1, 3, or 5 g/kg of feed; for 90 day)

	Control	**T1**	**T3**	**T5**
IW (g)	1.35±0.009^{a}	1.36±0.09^{a}	1.27±0.05^{a}	1.33±0.04^{a}
FW (g)	8.10±0.44^{cb}	9.07±0.67^{ab}	9.71±0.80^{a}	7.84±0.55^{c}
WG (g)	6.75±0.33^{c}	7.69±0.64^{ab}	8.43±0.84^{a}	7.39±0.10^{cb}
FCR	0.77±0.06^{a}	0.76±0.06^{a}	0.69±0.07^{a}	0.77±0.02^{a}
SGR (%/d)	1.69±0.05^{b}	1.69±0.07^{b}	1.82±0.10^{a}	1.68±0.01^{b}

Table 4. Whole-body proximate composition (%) of pangasius fed diets with different levels of carvacrol and thymol for 60 day

Composition (%)	Control	C1	C3	C5	T1	T3	T5
Crude Protein	13.31±0.45^{a}	13.57±0.27^{a}	13.39±0.05^{a}	13.61±1.78^{a}	14.19±1.41^{a}	13.26±0.17^{a}	12.73±0.32^{a}
Crude Lipid	3.38±0.19^{a}	3.55±0.22^{a}	3.30±0.50^{a}	3.90±0.51^{a}	3.37±0.37^{a}	3.87±0.11^{a}	3.52±0.28^{a}
Crude Ash	3.74±0.41^{a}	4.04±0.41^{a}	4.12±0.06^{a}	4.04±0.04^{a}	3.85±0.08^{a}	3.64±0.50^{a}	3.74±0.48^{a}
Moisture	76.25±0.94^{a}	74.77±1.19^{a}	74.57±0.58^{a}	73.88±3.29^{a}	74.33±2.65^{a}	73.94±0.27^{a}	75.28±0.72^{a}

Table 5. Whole-body proximate composition (%) of pangasius fed diets with different levels of carvacrol and thymol for 90 day

Composition (%)	Control	C1	C3	C5	T1	T3	T5
Crude Protein	13.41±0.21^{a}	13.45±0.27^{a}	13.85±0.49^{a}	13.49±0.28^{a}	14.34±0.54^{a}	13.61±0.25^{a}	13.39±0.32^{a}
Crude Lipid	4.27±1.00^{a}	4.35±1.14^{a}	4.78±1.10^{a}	4.70±1.12^{a}	3.78±1.03^{a}	4.18±0.97^{a}	4.73±1.31^{a}
Crude Ash	4.05±0.21^{a}	4.11±0.05^{a}	4.19±0.25^{a}	4.32±0.06^{a}	4.20±0.16^{a}	4.05±0.24^{a}	4.14±0.19^{a}
Moisture	79.80±0.23^{a}	79.28±0.69^{a}	79.01±0.46^{a}	79.13±0.61^{a}	79.31±0.34^{a}	79.18±1.77^{a}	79.61±0.16^{a}

Table 7. Viscerosomatic index, Hepatosomatic index and Spleen somatic index of pangasius fed different diets containing carvacrol and thymol extracts for 90 day

Diets	Control	C1	C3	C5	T1	T3	T5
VSI	4.85±0.13^{a}	4.36±0.29^{a}	4.51±0.42^{a}	4.71±0.05^{a}	4.58±0.18^{a}	4.76±0.13^{a}	4.53±0.29^{a}
HSI	0.48±0.02^{a}	0.48±0.03^{a}	0.49±0.00^{a}	0.46±0.023^{a}	0.47±0.042^{a}	0.45±0.054^{a}	0.47±0.018^{a}
DSI	0.055±0.00^{a}	0.058±0.00^{a}	0.057±0.00^{a}	0.057±0.00^{a}	0.057±0.00^{a}	0.055±0.00^{a}	0.060±0.00^{a}

carvacrol for 60 days and 90 days were compared to the control group and no significant difference was found (Table 6,Table 7; P<0.05).

Legend: Values are mean±SE (n=6). Within a row, means with different letters are significantly different (P <0.05).

Discussion

Previous studies reported that the carvacrol or thymol carvacrol powder have been reported to promote various health functions such as growth (Zheng *et al.*, 2009; Ahmadifar *et al.*, 2011; Yilmaz *et al.*, 2015). Giannenas e*t al.* (2012) studied on the effect of the carvacrol and timol components added into the feed on the growth in 113 g rainbow trout. For this purpose, 3 different diets were prepared (control, 6.0 g/kg carvacrol, 12.0 g/kg timol) and the fish were fed with these feeds for 8 weeks. While it was determined that the value of weight gain (WG) was higher in the group of fed with timol added feed than the control group, feed conversion rate (FCR) values were determined to be lower.

On the other hand it was determined, that there was no increase in growth parameters in the groups fed with carvacrol added feed.

Volpatti et al. (2012) determined that feed prepared with pure carvacrol added to fish oil in seabass had no effect on the growth performance of the fish after feeding them for 9 weeks at the rates of 0.025% and 0.05%.

Zheng *et al.* (2009) reported that carvacrol and thymol (0.05%) significantly improved weight gain and FCR in rainbow trout. Ahmedifar et al. (2011), reported that inclusion of dietary thymol-carvacrol significantly increased the final weight, specific growth rate (SGR) and weight gain (P<0.05) of great sturgeon compared to the control. In addition, while the weight gain value increased in rainbow trouts fed with 1, 3, 5 g/kg carvacrol, specific growth rate (SGR) and feed conversion rate (FCR) did not change (Yilmaz *et al.,* 2015). These results indicate that the action modes of different levels of carvacrol and thymol in small fish and big fish are different and this may result from physiological differences in different stages of the fish life cycle.

The panga fish were fed with carvacrol and timol added feed for 90 days for the first time were studied with this study. It was determined that 3 g/kg timol provided the best result in terms of growth performance. It can be said that volatile oils can be used as an alternative feed additive which has a prebiotic-like effect in aquaculture as a result of the positive effect of their antiicrobial and anti-oxidant characteristics on growth management when used in certain amounts.

When the growth-promoting effects of the volatile oils in fish, the probability that they may cause a strong inhibition in the pathogenic bacteria population in fish intestines, the possibility that the qualitative and quantitative histophysiologic characteristics of the cells in gastrointestinal system may positively affect the growth performance in fish are taken into consideration, it is clear that conducting more advanced bacterial microflora studies in this respect will be useful.

In addition, it is recommended to support detailed studies to determine the optimum practicing durations of prophylactic practices related to timol and carvacrol.

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