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Effects of Thyme and Thyme Oil on Growth of White Shrimp, *Litopenaeus vannamei*

Kekik ve Kekik Yağının Beyaz Karides Büyümesi Üzerine Etkileri, *Litopenaeus vannamei*

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Abstract: In this study, it was aimed to evaluate the effectiveness of different levels of thyme leaf and thyme oil as feed additives in white shrimp (*Litopenaeus vannamei*) culture. In the experiment, five groups were formed as 1% and 2% thyme leaves (ThyL1% and ThyL2%), 0.5% and 1% thyme oil (ThyO0.5% and ThyO1%), and control group. Shrimps (6.67±1.52 g) were stocked at a stocking rate of 15 shrimp/250 L (100% water change day^{-1}) in a three-replications. At the end of the trial, the highest final body weight (14.90±2.77) and weight gain (8.22±0.72) were determined in the ThyO1% group and were statistically different compared to the control group (Final weight: 13.54±3.37, weight gain: 6.87±0.54) ($p<0.05$). While the best feed conversion ratio between the groups was similarly determined in the ThyO1% (2.59±0.92) group, there was no difference between the groups in terms of survival rates. Histological examination of hepatopancreatic tissues showed no difference in tissue morphology between the groups. Dry matter, crude protein, and crude ash contents did not differ significantly between the groups. The highest lipid content was found in the ThyO1% group (1.64) compared to the other groups ($p<0.05$). Saturated and unsaturated fatty acids composition values differed significantly for all groups. ($p<0.05$). As a result, it can be stated that 1% thyme oil added feed can be used as a growth promoter in white shrimp culture.

Keywords

- Aromatic plants
- fatty acids
- histology
- nutrition
- shrimp

Özet: The Bu çalışmada, beyaz karides (*Litopenaeus vannamei*) yetiştiriciliğinde yem katkı maddesi olarak kekik yaprağı ve kekik yağının farklı besin takviyesi düzeylerinin etkinliğinin değerlendirilmesi amaçlanmıştır. Denemede, %1 ve %2 kekik yaprağı (ThyL1% ve ThyL2%), %0,5 ve %1 kekik yağı (ThyO0.5% ve ThyO1%) ve kontrol grubu olmak üzere beş grup oluşturulmuştur. Karidesler (6,67±1,52 g), üç tekerrürlü deneme düzeninde stoklama oranı 15 karides/250 L (günlük %100 su değişimi) olacak şekilde stoklanmıştır. Deneme sonunda, en yüksek final vücut ağırlığı (14,90±2,77) ve ağırlık artışı (8,22±0,72) ThyO1% grubunda belirlenmiş ve kontrol grubu (Final ağırlık: 13,54±3.37, ağırlık artışı: 6.87±0.54) ile karşılaştırıldığında istatistiksel olarak farklı bulunmuştur ($p<0.05$). Gruplar arasında en iyi yemden değerlendirme oranı benzer şekilde ThyO1% (2,59±0,92) grubunda belirlenirken, yaşama oranları bakımından gruplar arasında fark bulunmamıştır. Hepatopankreas dokularının histolojik incelemesi,

Anahtar kelimeler

- Aromatik bitkiler
- yağ asitleri
- histoloji
- beslenme
- karides

gruplar arasında doku morfolojisi açısından fark olmadığını göstermiştir. Kuru madde, ham protein ve ham kül içerikleri gruplar arasında önemli ölçüde farklılık göstermemiştir. En yüksek lipid içeriği ThyO1% grubunda (1,64) saptanmış ve diğer gruplarla karşılaştırıldığında istatistiki fark oluşturmuştur ($p<0.05$). Doymuş ve doymamış yağ asitleri kompozisyon değerleri tüm gruplar için anlamlı farklılık göstermiştir. ($p<0.05$). Sonuç olarak, %1 kekik yağı katkılı yemin beyaz karideslerde büyüme destekleyicisi olarak kullanılabileceği ifade edilebilir.

1. INTRODUCTION

Artificial chemicals such as hormones and antibiotics have been used on growth promoters, disinfection, and other purposes in aquaculture (Jayaprakas et al., 1996; Citarasu, 2010). However, because of the residual and resistance effects, most of the hormones and antibiotics have been banned, limited, and not recommended for fish, mollusk, and crustacean culture. Therefore, new approaches have emerged which may be more effective in aquaculture applications. Natural food additives such as medicinal plants can be considered as an alternative tool to reduce or prevent the problems caused by infectious diseases in aquaculture (Zargar et al., 2019). Medicinal/aromatic plant applications in aquaculture practice can reduce the side effects of applying the synthetic compounds and the cost and also make it eco-friendly. Lamiaceae (Labiatae) plant family, as its most popular members with oregano, thyme and sage have attracted important attention (Burt, 2004). Thyme (*Thymus vulgaris*, Linnaeus) is rich in essential oils, widely used, and distributed in the world (Davis 1965). It is well known that thyme and its essential oil are consumed as food for humans. In addition to being consumed as food, the plant has been reported to promote various activities like appetite stimulation, growth promotion, immune stimulation, and have aphrodisiac and antimicrobial properties (Kroismayr et al., 2008; Windisch et al., 2008; Lee et al., 2009; Zheng et al., 2009; Tiihonen et al., 2010; Villeda, 2013). The essential oil of the thyme plant is the most known and used essential oil because of its antibacterial activity. Active substances such as carvacrol and thymol in this oil are effective on *Escherichia coli* and many other pathogenic microorganisms (Dorman and Deans, 2000). The use of thyme oil as a feed additive for poultry can provide many benefits such as weight gain, better feed conversion ratio, inhibition of intestinal pathogenic microorganisms, and enhancement of digestive enzymes activity (Sugiharto, 2016). However, there is limited research on aquaculture (Júnior et al., 2018; Valladão et al., 2019).

According to Food and Agricultural Organisation (FAO), global shrimp farming production currently reached 4.58 million tonnes, which could go up to 11-18 million tons in 2030 (FAO, 2016). The white shrimp (*Litopenaeus vannamei*, Boone, 1931) is one of the most widely cultured shrimps; represents over 50% all over the world. The shrimp culture industry could be assumable as a sector already entering its mature stage, but still needs development such as in feed, feeding strategy, and culture methods for sustainability. Therefore, the general objective of this study was to evaluate the efficacy of dietary supplemental different levels of thyme leaves and thyme oil as a feed additive in white shrimp.

2. MATERIAL and METHODS

2.1. Experimental animals, design and diet preparation

The experiment was carried out at the Research and Application Unit of the Faculty of Marine Sciences and Technology, Iskenderun Technical University, Iskenderun, Hatay, Turkey.

In the experiment, thyme leaf and thyme oil were added to the experimental diet in 2 different concentrations. Trial groups were constituted as 1% and 2% of thyme leaf (ThyL 1%, ThyL 2%), 0.5% and 1% of thyme oil (ThyO 0.5%, ThyO 1%) supplemented groups, and a control group were created to compare the effects of these plants.

Juvenile white shrimps (initial average weight of 6.67±1.52 g) were stocked with three replicates per treatment tank (cylindrical R: 120 cm, h: 40 cm, water volume: 200 L) and the stocking rate 15 shrimps/tank (100% water exchange day^{-1}). The thyme (*Tyhmus vulgaris*) used in the experiment was freshly supplied (Talya Natural Products Ltd. Antalya, Turkey) and dried in the shade (28±1°C air temperature). The leaves were ground into a flour/powder in the feed machine (Lavion Grinder, Akse Ltd. Turkey). The thyme oil was obtained from the commercial firm (Talya Natural Products Ltd. Antalya, Turkey). Thyme leaf and thyme oil contents are shown in Table 1.

Table 1. The proximate contents of thyme oil.

Thyme leaf	
Raw materials	**Average percentage (%)**
Dry matter	8.82
Crude protein	5.01
Ether extract	3.14
Crude fibre	18.02
Ash	6.84
Thyme oil	
Compound	**Average percentage (%)**
Carvacrol	74.09
Linalool	5.46
Para Cymen	4.78
Gamma Terpinen	3.17
Beta Bisabolene	2.92
Thymol	2.87
Trans Caryophyllene	2.41
(+) Barneol	1.39
Alpha Pinen	1.00
Alpha Terpinen	14,24
Beta Myrcene	0.91

*Talya ® Herbal (Certificate of analysis 2017).

Experimental shrimp feed containing 37% of crude protein was used as a basal diet (Table 2). The thyme which was made into flour was mixed with 1% and 2% of the powdered feed, and then the feed was moistened (300 ml drinking water per 1 kg dry mixture) and made into dough. Feeds that were pelleted 2 mm in diameters from the kitchen-type pellet machine (Arcelik Ltd., Turkey) were dried in a fume cupboard (28±1°C). For the 0.5% and 1% of thyme oil supplementation, commercial thyme oil was sprayed and mixed with dried powdered feed and pelletized in the method given before. The same protocol (without additives) was used for the control group diet. Shrimp were fed with the experimental diet twice a day (4% feeding rate).

2.2. Water quality and growth performance

Throughout the experimental period, salinity, temperature, dissolved oxygen, and pH were measured daily using a multi-parameter instrument (YSI® 556, YSI Inc., Yellow Springs, OH, USA) at 10:00 hours in all tanks. Salinity, temperature, dissolved oxygen, and pH values varied between 19.4-21.2 ppt, 24.3-25.1°C, 6.92-7.23 mg L$^{-1,}$ and 7.8-8.3 respectively.

The following formulas were used in the calculation of growth parameters and survival rate.

Weight gain = (final weight − initial weight)

Specific growth rate (SGR, % day^{-1}) = (Ln (final weight) – Ln (initial weight)/duration) × 100

Survival (%) = 100 × (final number of shrimp / initial number of shrimp)

Feed conversion ratio (FCR) = live weight gain (g) / dry feed intake (g)

Table 2. Ingredients of the experimental feed and proximate analysis of diet (% dry matter).

	Control	ThyL 1%	ThyL 2%	ThyO 0.5%	ThyO 1%
					Ingredients %
Fish meal	29	29	29	29	29
Soybean	12	12	12	12	12
Corn gluten	13	13	13	13	13
Wheat flour	36.3	35.3	34.3	35.8	35.3
Fish oil	3	3	3	3	3
Soy lecithin	2	2	2	2	2
Thyme leaf	0	1	2	0	0
Thyme oil	0	0	0	0.5	1
Vitamin's premix	0.8	0.8	0.8	0.8	0.8
Mineral's premix	0.2	0.2	0.2	0.2	0.2
Vitamin C	0.1	0.1	0.1	0.1	0.1
Cholesterol	0.6	0.6	0.6	0.6	0.6
Binder (guar gam)	3	3	3	3	3
Proximate analysis (%)					
Crude protein	36.93±0.31	36.98±0.21	37.03±0.20	36.95±0.28	36.90±0.25
Crude lipid	8.07±0.26	8.10±0.24	8.13±0.22	8.57±0.25	9.07±0.18
Crude ash	15.73±0.35	15.34±0.30	15.82±0.29	15.98±0.31	15.61±0.33

Vit amounts/453 g Vitamin Premix (Vit A: 325,000 USP Units, Vit D_3: 65 USP Units, Vit E: 32 USP Units, Vit K 793.65 mg, Vit B_{12}: 10.08 mg, Riboflavin: 3.250 mg, p-Panthothenic acid: 15.600, Niacin: 19.500 mg, Cholin: 2.600 mg, Thiamine: 2.600 mg, Pridoxine Folic acid: 780 mg, Ascorbic acid: 87.100 mg Biotin: 40 mg, BHT: 2 mg, Inositol: 13. Minerals; Manganese 60 ppm, Zinc 50 ppm, Iron 40 ppm, Copper 4 ppm, Cobalt 0.5 ppm, Iodine 40 ppm, Selenium 0.4 ppm (Formulated and Packaged By Florida Aqua Farms Inc. Dade City, Florida).

2.3. Proximate and fatty acid analysis

At the end of the study shrimp proximate analysis; dry matter, crude ash, and protein were determined according to AOAC (1990). Lipid and fatty acid contents were freshly analyzed by Bligh and Dyer (1959) method. Samples placed in GC tubes were read in a GC device (Agilent Technologies 7820A GC System, USA) to determine the fatty acid contents of each sample represented the groups. Live weight measurements were made with a digital scale of 0.01 sensitivity to determine growth performance.

2.4. Total bacteria count and hepatopancreas histology

Two shrimps from each replicate were randomly sampled and anesthetized on ice for total bacteria counts and histological analysis. The decapitated shrimp samples (abdomen ~15 g) and the whole hepatopancreas and anterior part of intestine samples (~3 g) of shrimps were removed in aseptic condition. Samples were mixed and homogenized in sterile saline (NaCl 0.85%: 27 mL for ~3 g sample, 135 mL for ~15 g sample) as representative of each group (serially diluted from 10^{-1} to 10^{-7} in sterile saline). Dilutions (0.1 mL) were spread on three replicates plate count agar (Difco™). Plates were incubated for 48 h at 36±1°C for the total aerobic bacteria counts (APC) (Okpala et al., 2014). The bacteria count results (means of triplicates) were given as conventional colony forming units (CFU g^{-1}) and also logarithm of CFU (Log CFU g^{-1}). The hepatopancreas samples were fixed in 4% buffered formaldehyde solution (1 cm^3/20 ml). After dehydration with ethanol, the routine paraffine protocol was conducted. Blocked tissues were cut in 4-5 μm by a microtome (Thermo Shandon, Germany). Sections were stained with haematoxylin & eosin (H&E) and analyzed with a trinocular microscope (Leica CME, Germany) (Takashima and Hibiya, 1995; Roberts and Smail, 2004).

2.5. Statistical analysis

Statistical analysis was performed using SPSS package program (IBM, Statistics 17.0 for Windows). Analysis of variance (ANOVA) was used on shrimp performance parameters data to look at differences of thyme leaf and thyme oil groups. The 5% significance level was used for all tests.

3. RESULTS

3.1. Growth and Feed Utilization

The growth performance parameters of the groups are presented in Table 3. At the end of the experiment, the highest final body weight (14.90±2.77 g) and weight gain (8.22±0.72 g) were determined in the ThyO 1% group and statistically different when compared with the control group (FBW: 13.54±3.37 g, WG: 6.87±0.54%) ($p < 0.05$). The best feed conversion ratio among the groups was similarly detected in the ThyO 1% group (2.59±0.92), while the lowest survival rate was also determined in the ThyO 1% group as 86.45±3.85% and the best survival rate was determined in the control group as 90.11±7.70% ($p > 0.05$).

Table 3. Growth and feed utilization in white shrimp-fed test diets for 84 days.

	Control	ThyL 1%	ThyL 2%	ThyO 0.5%	ThyO 1%
IBW g	6.67±1.72	6.66±1.41	6.67±1.57	6.66±1.31	6.68±1.61
FBW g	13.54±3.37^{a}	13.44±2.42^{a}	14.28±2.84^{ab}	14.02±2.21^{ab}	14.90±2.77^{b}
WG g	6.87±0.54^{a}	6.78±0.44^{a}	7.61±1.16^{ab}	7.36±0.96^{ab}	8.22±0,72^{b}
WG (%)	102.97±8.3^{a}	102.01±7.2^{a}	113.73±16.9^{ab}	110.48±14.9^{ab}	123.05±10.8^{b}
SGR % day^{-1}	0.84±0.50^{a}	0.84±.0.45^{a}	0.90±0.92^{ab}	0.89±0.81^{ab}	0.95±0.56^{b}
FCR	3.28±0.42	3.36±0.25	2.68±0.56	2.71±0.83	2.59±0.92
SR %	90.11±7.70	88.89±7.70	86.67±6.67	88.89±7.70	86.45±3.85

Values are means of triplicate groups ± s.d. within a row, means with different letters are significantly different ($p<0.05$). The absence of letters indicates no significant difference between treatments. IBW: initial body weight (g), FBW: final body weight (g), WG: percent weight gain (%), SGR: specific growth rate (% day^{-1}), FCR: feed conversion ratio, SR: survival rate (%).

3.2. Proximate compositions and fatty acids

The chemical composition of the basal diet and shrimps' carcasses are displayed in Table 4. Dry matter, crude protein, and crude ash contents were not significantly different among the groups. The highest lipid content was observed in the ThyO %1 group (1.64) and was different when compared to the other groups. According to the results of the analysis of fatty acids, the control group (except C16:1, n-7 Palmitoleate) were determined in higher values in terms of saturated fatty acid (C16:0 Palmitate n-hexadecanoate, C18:0 Stearate n-Octadecanoate) ($p<0.05$). Similar results were also observed for unsaturated fatty acid values in this study. The fatty acid profile is shown in Table 5.

3.3. Microbiological and histological results

Total aerobic bacterial count (APC) was determined for edible parts (except the head, posterior intestine including the abdominal region), and hepatopancreas-anterior intestinal tract of tested shrimp. As a result of the evaluation, it was found that there was no significant difference between the groups. APC values of the consumable portion; control: 5.04±0.05 log CFU g^{-1} (1.10±0.13 x10^{6} log CFU g^{-1}), ThyL 1%: 5.03±0.05 log CFU g^{-1} (1.08±0.13 x10^{6} log CFU g^{-1}), ThyL 2%: 5.03±0.05 log CFU g^{-1} (1.08±0.13 x10^{6} log CFU g^{-1}), ThyO 0.5%: 5.03±0.04 log CFU g^{-1} (1.07±0.10 x10^{6} log CFU g^{-1}) and ThyO 1%: 5.01±0.03 log CFU g^{-1} (1.03±0.08 x10^{6} log CFU g^{-1}). The ThyO 1% supplemented group's shrimp edible part APC value was found relatively low compared to the control group.

Table 4. Proximate composition (%) of the whole shrimp (triplicate composite samples of three shrimps fed with different supplemented experimental diets).

	Control	ThyL %1	ThyL %2	ThyO %0.5	ThyO %1
Dry matter	23.69	23.92	23.91	23.93	23.89
Crude protein	20.92	20.30	20.70	20.28	21.10
Lipid	1.52^{b}	1.46^{a}	1.45^{a}	1.44^{a}	1.64^{c}
Crude ash	1.61	1.69	1.69	1.70	1.66

Values are within a row means with different letters are significantly different ($p<0.05$).
The absence of letters indicates no significant difference between treatments.

Table 5. Effect of thyme leaf and thyme oil on the fatty acid composition of shrimp.

	Fatty acids	Control	ThyL 1%	ThyL 2%	ThyO 0.5%	ThyO 1%
Saturated f.a.	C16:0	23.54±0.48^{c}	17.07±0.06^{a}	17.94±0.37^{ab}	17.40±0.46^{ab}	18.79±0.91^{b}
	C16:1, n-7	1.19±0.04	1.12±0.02	1.18±0.00	1.35±0.32	1.27±0.17
	C18:0	19.98±2.49^{b}	14.00±0.02^{a}	15.35±0.71^{a}	14.17±0.44^{a}	15.20±0.48^{a}
Unsaturated f.a.	C18:1, n-9c	18.55±0.06^{c}	14.19±0.43^{a}	15.33±0.38^{ab}	17.89±0.89^{c}	15.90±0.32^{b}
	C18:1, n-9t	5.66±0.19^{b}	4.24±0.03^{a}	4.14±0.16^{a}	4.76±0.75^{ab}	4.68±0.07^{a}
	C18:2, n-6c	5.18±0.03^{b}	4.05±0.12^{ab}	4.07±0.35^{ab}	4.81±1.04^{ab}	3.78±0.14^{a}
	C20:0	2.33±0.35^{b}	1.60±0.03^{a}	1.97±0.07^{ab}	1.93±0.29^{ab}	1.80±0.06^{ab}
	C20:2, n-6	1.94±0.26^{b}	1.38±0.02^{a}	1.51±0.09^{a}	1.58±0.21^{ab}	1.38±0.01^{a}
	C20:4, n-6	3.53±0.44^{b}	2.58±0.09^{ab}	2.49±0.11^{a}	3.01±0.68^{ab}	2.50±0.03^{a}
	C20:5, n-3	7.43±0.21^{b}	7.25±0.36^{b}	6.53±0.00^{a}	6.46±0.46^{a}	6.28±0.01^{a}
	C22:6, n-3	9.08±0.63	9.97±0.06	9.51±0.56	9.80±2.24	9.30±0.58

Values are means of triplicate groups±s.d. within a row means with different letters are significantly different ($p<0.05$).
The absence of letters indicates no significant difference between treatments. **Saturated f.a.**: C16:0 Palmitate n-hexadecanoate, C16:1, n-7 Palmitoleate, C18:0 Stearate n-Octadecanoate, **Unsaturated f.a.**: C18:1, n-9 c, Oleic acid cis-9-octadecenoic acid (ω9), C18:1 n9 t Olaidic acid trans-9-octadecenoic acid, C18:2, n-6 c Linoleate (ω6), C20:0 Arachidic acid, C20:2, n-6 Eicosadienoic acid, C20:4, n-6 Arachidonate (ω6), C20:5, n-3 EPA (Eicosapentaenoic acid) (ω3), C22:6, n-3 DHA (Docosahexaenoic acid) (ω3).

APC values of hepatopancreas-anterior intestinal tract samples were determined as 7.45±0.02 log CFU g^{-1} (2.80±0.10 x10^{7} log CFU g^{-1}) in the control group. ThyL 1%, ThyL 2%, ThyO 0.5% and ThyO 1% for APC values respectively; 7.47±0.02 log CFU g^{-1} (2.93±0.15 x10^{7} log CFU g^{-1}), 7.46 ± 0.04 log CFU g^{-1} (2.87±0.23 x10^{7} log CFU g^{-1}), 7.47±0.05 log CFU g^{-1} (2.97±0.32 x10^{7} log CFU g^{-1}) and 7.47±0.01 log CFU g^{-1} (2.97±0.06 x10^{7} log CFU g^{-1}). These values were found to be slightly increased in all treatment groups compared to the control group.

At the end of the experiment, histological examination showed that there was no difference with regards to tissue morphology between the shrimp fed with thyme and thyme oil supplemented feed and the hepatopancreas tissues of the control group shrimp (Figure 1).

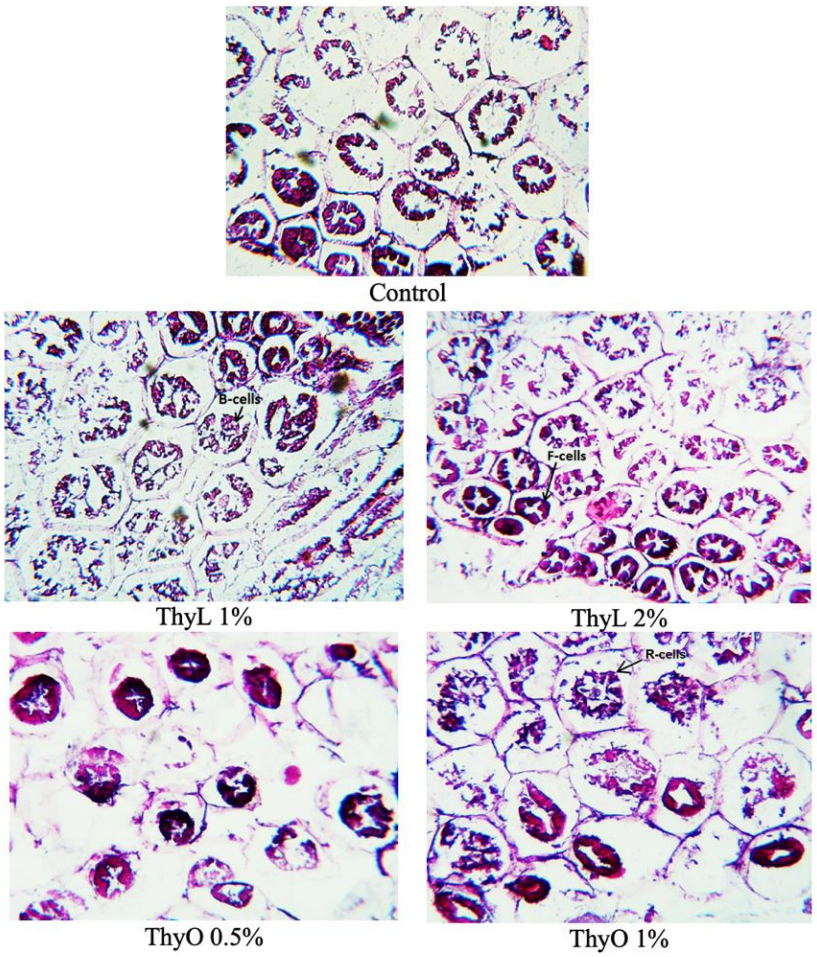

Figure 1. Histological sections hepatopancreatic tissues of *Litopenaeus vannamei*, fed on diets containing control, %1 thyme leaf wheat (ThyL 1%), %2 thyme leaf wheat (ThyL 2%), %0.5 thyme oil (ThyO 0.5%) and %1 thyme oil (ThyO 1%). The epithelial cells of lumen and tubule cells show normal morphology (large vacuole: B-cells; small vacuole: R-cells and basophilic non-vacuolated: F-cells), H&E x10.

4. DISCUSSION

Thyme essential oils are herbal products with important antimicrobial activity. Although this product has applications in terrestrial animals thanks to its positive effects on both the immune system and growth performance, there is limited information in aquaculture (Zargar et al., 2019). In the current study, the highest final body weight and weight gain were gained in the ThyO1% group compared to the control. It can be argued that the thyme oil improves the nutrient utilization of shrimp with better growth. Similarly, Zargar et al. (2019) stated that rainbow trout fed with the *Thymus vulgaris* essential oils at 0.5 ml/kg feed showed a better weight gain and specific growth rate. Sönmez et al. (2015) also recorded that the weight gain percentage of rainbow trout fed the diets containing thyme oils was significantly higher than that of the control group. There is only one study examining the effects of essential oils on growth in shrimp. Kim et al. (2011) investigated the effects of essential oils on the growth parameters of white shrimp in recirculating aquaculture systems. The researchers examined four experimental diets (commercial diet: control, phytoncyte oil, oregano oil, and fermented garlic liquid) with 0.62 g initial weight of shrimp (12 tanks 75 shrimp 500 L^{-1} stocks) for 16 weeks (27.8±1°C, pH: 7.6±0.3, salinity: 15.5±0.3 ppt and oxygen: 6.1±0.3 g L^{-1}). They stated that the final weights of shrimp were between 21.9-23.6 g and there was no difference between the groups in terms of FCR and SGR, also the highest survival rate was 55.11% in the oregano oil group. Therefore,

it can be assumed that the content and amount of some essential oils used in the diet may not positively affect the growth performance.

In general, the results of previous studies have referred to the effects of medicinal aromatic plants on growth and healthy development in aquatic organisms. It is understood from the research that mainly the antimicrobial properties of thyme oil have been taken into account (Kroismayr et al., 2008; Windisch et al., 2008; Lee et al., 2009; Zheng et al., 2009; Tiihonen et al., 2010; Villeda, 2013). According to the growth results in the present study, shrimps fed with 1% thyme oil supplementation showed better growth compared with the control group. Because of its antimicrobial properties, thyme oil could make a limitation the development of pathogenic organisms in the digestive tract, and it could be suggested that the growth performance may also increase in this way.

Fatty acid analysis results of the control group (compared to the feed additives groups) in general were statistically better regarding the saturated and unsaturated fatty acid values. Although these results stated that the thyme leaf and thyme oil improved live weight gain, fatty acid composition slightly decreased statistically except for the C16:1, n-7 Palmitoleate C22:6, n-3 DHA (Docosahexaenoic acid). The omega-3 fatty acids C20:5, n-3 EPA (Eicosapentaenoic acid) were significantly higher in the control group and the ThyL 1% group than the other experimental groups ($p<0.05$). As a result, it could be argued that there was no significant difference between the groups in terms of essential fatty acids, in other words, the thyme leaf and thyme oil additive was not effective in enriching the fatty acid composition.

Zeng et al. (2005) reported the total bacterial count for *Pandalus borealis* shrimp as 2.4×10^5 CFU g^{-1} (~ 5.38 log CFU g^{-1}), and Cadun et al. (2008), 5.76 log CFU g^{-1} for *Parapenaeus longirostris*. Additionally, Farajzadeh et al. (2016), reported that the total bacteria count of *Litopenaeus vannamei* as 2.5 log CFU g^{-1} (which was washed after the decapitation). The APC values obtained from this study (5.01-5.05 log CFU g^{-1}) were found similar to the international ($1x10^6$ CFU g^{-1}) and Japan ($1x10^5$ CFU g^{-1}) standards for the consumable shrimp (as ~ 5-6 log CFU g-1).

About APC values of the digestive tract content, Zhang et al. (2011), reported the APC amount for *Penaeus japonicus* as 7.28±0.09 log CFU $shrimp^{-1}$. Rengpipat et al. (1998) reported 10^7-10^8 CFU g^{-1} (7-8 log CFU g^{-1}) for *Penaeus monodon*. According to the references, intestinal total bacterial counts for *Litopenaeus vannamei*; Rengpipat et al. (2000), reported as 1.1×10^7 to 1.7×10^8 CFU g^{-1} (~ 7-8 log CFU g^{-1}), Ziaei-Nejad et al. (2006), 1.0 ± 0.1 $x10^6$ CFU g^{-1} (6 log CFU g^{-1}) and Li et al (2009), $1x10^{10}$ CFU g^{-1} (10 log CFU g^{-1}). In the current study, the total bacterial count of the samples from the hepatopancreas-anterior intestine region for *Litopenaeus vannamei* (7.45-7.47 log CFU g-1) was similar to the values reported in the references.

In conclusion, according to all data obtained, it can be stated that the feeding of white shrimp with thyme leaf and thyme oil added with 1% levels does not negatively affect the general health of the shrimps.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Authors declare that they have equally contributed to the article.

ETHICAL STATEMENTS

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

DATA AVAILABILITY STATEMENT

Authors declare that the data is not available.

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