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Garlic extract product enhancing growth performance, digestive and immune system in Nile tilapia (*Oreochromis niloticus*)

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Introduction

Nile tilapia (*Oreochromis niloticus*) is the world's most important freshwater fish species because of its large size, rapid growth; and its value as a good source of protein for human consumption. Although tilapia is very important fisheries business in Thailand with huge export, therefore the quality and safety of the product need to be ensured in domestic and international markets [1].

Farming the tilapia culture was often meets most important problems including infection and epidemic diseases. The farmers usually use drugs and chemicals to prevent this serious problem, and this affects a residual drug and spreading out of drug-resistant bacteria in pond and culturing environment, especially in the case of intensive tilapia farming, adversely affecting the potential revenue generated by this resource. Contamination with harmful chemicals through aquaculture feed is an especially important issue for safe aquaculture production, due to the rules and regulations of the current standards for food safety [1].

Many herbs are considered for alternative antimicrobial agents for prevention and treatment the diseases. One of the common medicinal plants is Garlic (*Allium sativam* L.), possess the wide ranges of antimicrobial properties. It has been proved for therapeutic effects to many viruses, bacteria, parasites, fungi and protozoans. For this study, our garlic extract product (GEP) was developed that containing high allicin, S-allylcysteine, and S-allylmercaptocysteine, the major bioactive compounds in garlic which can instead of antibiotics.

Materials and methods

Experimental fish
Nile tilapia with an average initial weight of 10 g were used. They were divided into three groups of 30 fish received each treatment as in the Experiment 1. The control group was provided with the same diet without GEP. The experimental 2 and 3 were adding the GEP 0.5 and GEP 1.0 using a commercial pellet feed for Nile tilapia. The growths were measured including weight and length for every week and continued for 8 weeks.

Non-specific immune assays
Haematocrit was determination method adopted from Blaxhall and Daisley [2]. Counting of white blood cells (WBC) and red blood cells (RBC) were determination procedure was used from Anderson and Siwicki [3] and: serum lysozyme activity was determined as followed from Obach et al. [4]. The results are given as units (U) ml^{-1} where one unit is the amount of sample causing a decrease in absorbance of 0.001 min^{-1}. Superoxide anion production ratio (SOD ratio) was determined as followed Munoz et al. [5].

Determinations of digestive enzymes
The enzymes extraction was performed according to Rungruangsak-Torrissen [6]. Protein concentrations in the crude enzyme extracts were determined according to Lowry et al. [7], using bovine serum albumin (BSA) as standard. Amylase specific activity was determined using the method from Areekijseree [8] based on Bernfeld [9]. Lipase specific activity was analyzed according to Winkler and Stuckmann [10]. Trypsin and chymotrypsin specific activity were determined according to Rungruangsak-Torrissen [6]. The digestive efficiency was expressed as the activity ratio of Trypsin to Chymotrypsin (T/C ratio), as described by Rungruangsak-Torrissen [11,12]. After 8 weeks of feeding, three parts of the intestines foregut, midgut and hindgut, were collected and fixed in 10% buffered formalin. Fixed tissues were processed according to Pirarat et al. [13]. For villus height measurement was estimated by the Motic Images Plus 3.0 ML program.

Results

Non-specific immune responses in Nile tilapia
Nile tilapia was showed significant increases ($P < 0.05$) in the RBC and WBC, serum lysozyme activity and

SOD ratio after feeding with GEP 0.5, whereas control group had no effect on these immune parameters (Table 1).

Table 1. Non-specific immune responses in Nile tilapia on these immune parameters

Immune parameters	Control	GEP 0.5	GEP 1.0
Haematocrit (%)	58.15±5.40[a]	54.76±6.79[a]	52.20±12.67[a]
RBC (×10^8 cell/ml)	13.89±1.50[b]	22.47±4.15[a]	15.58±3.26[b]
WBC (×10^6cell/ml)	149.78±2.46[a]	155.67±1.87[a]	114.11±6.16[b]
Lysozyme activity (units/ml serum)	6.93±0.61[b]	15.50±2.17[a]	7.99±0.71[b]
SOD ratio	1.76±0.01[b]	2.05±0.03[a]	1.95±0.02[ab]

Alphabet represents statistic value at p< 0.05.

Digestive system and feed efficiency in Nile tilapia

The specific activities of enzymes amylase, lipase and trypsin, in GEP 0.5 group significantly increased (P < 0.05) when compared with the other groups (Table 2). Contrastingly, the specific activities of chymotrypsin was significantly lowest (P < 0.05) in GEP 0.5 group. For villus height of GEP 5.0 group in the foregut and midgut also showed the significant longest (P < 0.05).

Table 2. Specific activity of digestive enzymes in Nile tilapia

Digestive enzymes	Control	GEP 0.5	GEP 1.0
Amylase	596.20±15.15[c]	1342.33±1.26[a]	1210.10±12.72[b]
Lipase	730.96±1.54[c]	2152.91±9.28[a]	1723.88±6.49[b]
Trypsin	3189.19±19.62[c]	4984.74±16.40[a]	4798.44±10.49[b]
Chymotrypsin	14485.57±13.62[a]	8601.97±18.53[c]	13549.04±10.90[b]

Alphabet represents statistic value at p< 0.05.

The digestive efficiency T/C ratio was significantly increased (P < 0.05) in the GEP 0.5 group related to the growth performances, i.e. weight gain (WG), feed efficiency (FE) and specific growth rate (SGR), compared with the other groups after feeding with GEP 0.5 for 8 weeks (Table 3).

Table 3. Feed efficiency of Nile tilapia on these: growth parameters; T/C ratio, FE ratio, SGR (%) and WG (%)

Growth parameters	Control	GEP 0.5	GEP 1.0
T/C ratio	0.32±0.03[c]	0.78±0.07[a]	0.47±0.03[b]
FE ratio	0.66±0.13[c]	1.53±.08[a]	1.39±0.15[b]
SGR (%)	0.23±0.003[b]	0.52±0.03[a]	0.47±0.05[ab]
WG (%)	129.88±0.16[c]	186.23±0.19[a]	155.22±0.14[ab]

Alphabet represents statistic value at p< 0.05.

Discussion

At 8 weeks after feeding, the results were revealed that all immune responses unless haematocrit, correspond with [14] which reported that the effects garlic can promote growth rate, decrease mortality rate and increase the SOD in Nile Tilapia.

The villus height result in an increased surface area that is capable of greater absorption of available nutrients, and greater villus height and numerous cell mitoses in the intestine are indicators that the function of the intestinal villi is enhanced [15].

Correlation of the production levels of specific activity on digestive enzymes were increases when compared with the other groups. Whereas that, the chymotrypsin specific activities were significantly decreased (P < 0.05) and specific activities correspond with [11] reported that the Chymotrypsin specific activity, on the other hand, increased when there was a reduction in growth rate. Whereas fish with higher growth had lower chymotrypsin specific activity resulting in lower T/C ratio value which is related to T/C ratio value in the GEP 0.5 group was significant higher than control group. This study results were corresponding with [16] reported that feed supplementary garlic extract can be help to stimulate the digestive enzymes, and be used to enhance feeding and growth rates in sand goby. Respectively, allicin in garlic has been identified as the major active pharmaceutical molecule found in crashed garlic, however it has a very short haft-life as it reacts with many of the surrounding proteins. Consequently, development of GEP was that containing high allicin, S-allylcysteine, and S-allylmercaptocysteine, the major biocompounds in garlic. Moreover, allicin can help to stimulate intestinal flora living in the local intestine, as well as help to improve the digestive system and also helps to maximize the energy utilization [17].

Conclusions

This experimental study suggested that GEP 0.5 added in feed could be significantly induce immune responses, villus height, digestive enzyme activities and growth performance in juvenile Nile tilapia. Therefore, this product might be benefited to apply for special feed additive for Nile tilapia aquaculture.

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References

1. Pirarat N (2015) Thai J Vet Med 45: 113–119
2. Blaxhall PC, Daisley KW (1973) J Fish Biol 5: 771–781
3. Anderson DP, Siwicki AK (1995) Proceeding of the Second Symposium on Disease in Asian Aquaculture, 2nd ed. (Arthur MJR, Subasinghe RP eds) Fish Health Section, Manila, pp. 185–202
4. Obach A et al. (1993) DAO 15: 175–185
5. Munoz M et al. (2000) Aquaculture 191: 89–107
6. Rungruangsak-Torrissen K (2007) J Food Biochem 31: 509–540
7. Lowry HO et al. (1951) J Biol Chem 193: 265–275
8. Areekijseree M et al. (2004) Aquaculture 234: 575–587
9. Bernfeld P (1951) Adv Enzyme 12: 379
10. Winkler UK, Stuckmann M (1979) J Bacteriol 138:663–670
11. Rungruangsak-Torrissen K et al. (2006) FISH 32: 7–23
12. Rungruangsak-Torrissen K (2012) Trypsin: Structure, Biosynthesis and Functions 2nd ed. (Weaver K, Kelley C eds), Nova Science Publishing, New York, pp. 1–59
13. Pirarat N et al. (2011) Res Vet Sci 91: e92–e97
14. Metwally M (2009) WJFMS 1: 56–64
15. Zijlstra RT et al. (1997) J Nutr 127: 118–127
16. Journal of Fisheries Technology Research. Available online: https://goo.gl/BcD8AY (accessed on 10 July 2017)
17. Khalil R H et al. (2001) J Egypt Med Vet 2: 381–392