Original Article

Dietary supplementation of condensed tannin decreased lipid peroxidation in muscle of nile tilapias (*Oreochromis niloticus*)

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Abstract

The effect of condensed tannin supplementation in diet on lipid peroxidation in the muscle was evaluated in Nile tilapias (*Oreochromis niloticus*). In total, 250 fish, aged 21 days old, were randomly allocated into 10 experimental tanks, 25 fish per tank. Five tanks were set as the control group and 5 tanks as the supplemented group. The control fish were fed a commercial diet, while the supplemented fish were fed the same control diet plus 4 g/kg of condensed tannin. The diets were offered at a rate of 8% of estimated fish body weight twice daily. The experimental period lasted for 120 days. At the end of the experiment, 3 fish from each tank were randomly sampled and euthanized by hypothermic method. Epaxial and hypaxial muscles were collected and divided into 2 subsamples to determine lipid peroxidation by thiobarbituric acid reactive substances (TBARS) assay. The first subsamples were measured for TBARS values immediately after euthanasia within 1 h. The second subsamples were kept at 4°C for 48 h before TBARS value determination (48 h). Average TBARS values measured at 1 h post euthanasia were lower in the supplemented fish [0.269±0.028 mg malondialdehyde (MDA)/kg of fresh meat] than in the control fish (0.480±0.025 mg MDA/kg). Similar results were also observed for TBARS values measured at 48 h. In conclusion, the dietary supplementation of condensed tannin significantly reduced lipid peroxidation in the muscle.

Keywords: condensed tannin, lipid peroxidation, Nile tilapia, thiobarbituric acid reaction assay

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Introduction

It is noted that fish cultivation under an extensive production system could induce stress to the fish, particularly when the cultivation management is inappropriate (Barton and Iwama, 1991). Fish under stress condition will have impaired health and immune function, and this situation may make the fish susceptible to opportunistic bacterial infections (Naylor and Burke, 2005). To control these bacteria, fish cultivators introduce antimicrobials into their cultivation system. This practice, however, might promote the problem of antibiotic-resistant bacteria, with which the environment might be contaminated (Aarestrup, 2000; Alderman and Hastings, 1998; Furushita et al., 2005; Miranda et al., 2003; Petersen et al., 2002; Schmidt et al., 2000; Tendencia and Pena, 2001; Wegener et al., 1999). Budiati et al. (2013) reported that the occurrence of plasmids of *Salmonella* spp. isolated from catfish, tilapia and water was a potential health hazard because the plasmids could transfer antibiotic resistance gene to other bacteria in aquaculture environment.

To reduce inevitable use of antimicrobials, alternative substances are selected as replacements. Phenolic compounds, natural extracts from plants, have been used to promote animal and human health. For example, robientic, myricetin and epigallocatechin could inhibit DNA synthesis of *Proteus vulgaris* (Mori et al., 1987). Catechin, a phenolic compound in green tea, inhibits growth of both gram positive and gram negative bacteria (Ikigai et al., 1993). In an *in vitro* study by Maisak et al. (2013), hydrolysable tannin extracted from sweet chestnut wood could inhibit fish bacterial pathogens, including those that are resistant to oxytetracycline. Some phenolic compounds have antiviral properties (Middleton and Chithan, 1993). Moreover, phenolic compounds have been noted for their antioxidant properties, which could stimulate the immune functions (Alonso et al., 2007; Facino et al., 1999; Hertog et al., 1993). As mentioned, mismanagement of the cultivation predisposes fish to stress, which then affects their physiological function. Fish under stress will increase the cellular reactive oxygen species, leading to alleviation of the cellular functions. In literature, stress could effectively increase lipid peroxidation in the muscles and livers (Hegazi et al., 2010).

Nowadays, tilapias are important exported fish of Thailand. Most products are exported as frozen fish. During freezing at -18°C, lipid peroxidation reaction in the muscles is ongoing which results in impaired quality of the fish meat (Sánchez-Alonso et al., 2007). Application of phenolic compounds in tilapia cultivation is hypothesized to be beneficial to both fish health during cultivation and fish meat quality after harvesting. Therefore, the objective of this study was to determine the effect of dietary supplementation of condensed tannin on lipid peroxidation in the muscles of Nile tilapias (*Oreochromis niloticus*).

Materials and Methods

Experimental fish and culture system: In total, 250 juvenile Nile tilapias aged approximately 21 days old were brought from a commercial nursery farm to the Aquatic Unit, Faculty of Veterinary Medicine, Kasetsart University. All fish were apparently healthy without any ectoparasites and any clinical signs of bacterial infection.

In the laboratory facilities, 10 experimental glass tanks (90x50x45 cm) containing 120 L of fresh water were prepared. Aeration of the tanks was maintained by aquarium air pump throughout the experiment. Every 3 days, 80% of the water in each tank was replaced with clean water by flushing technique. Ambient temperature ranged from 28-30°C.

Twenty-five fish were randomly assigned to each tank; 5 tanks were assigned as control and 5 tanks as supplemented group. The control fish received a commercial diet, while the supplemented fish were fed the same diet plus condensed tannin at 4 g/kg of the diet. The condensed tannin used in this study was a commercial product containing approximately 86% of plant-extracted polyphenols, of which 65% was condensed tannin. Chemical analyses of the control diet are presented in Table 1. During the period of 120 days, the fish in all tanks were allowed to acclimatize in the cultivating system for 15 days. During this period, they also adapted to the commercial diet. For the experimental period of 105 days, the fish were fed twice daily either control or condensed tannin supplemented diets at a rate of 8% of estimated body weight. During the entire experimental period, water quality was controlled as described in Table 2.

Table 1 Chemical composition of commercial diet (g/100g)

Item	Composition
Dry matter	91.66
Protein	42.8
Fat	8.18
Ash	12.34
Calcium	2.53
Phosphorus	1.49
Total energy (Calories/g)	4817.18

Table 2 Quality control of water during the experiment

Parameter	Value
Physical quality	
Temperature (°C)	27-30
pH[1]	7.5-8.5
Chemical quality	
Ammonia[2] (mg/L)	less than 0.1
Nitrite[3] (mg/L)	less than 0.1
Nitrate[4] (mg/L)	less than 0.1

[1]pH Test Kit, API® Aquarium Pharmaceuticals Inc., Pennsylvania, USA
[2]Ammonia Test Kit, API® Aquarium Pharmaceuticals Inc., Pennsylvania, USA
[3]Nitrite Test Kit, API® Aquarium Pharmaceuticals Inc., Pennsylvania, USA
[4]Nitrate Test Kit, API® Aquarium Pharmaceuticals Inc., Pennsylvania, USA

Muscle samples for lipid peroxidation determination: At the end of the experiment, 3 fish from each tank were randomly selected and were euthanized using hypothermic procedure. Immediately after euthanasia, epaxial and hypaxial muscles from all fish were collected and divided into 2 subsamples to determine lipid peroxidation by thiobarbituric acid reactive

substances (TBARS) assay using the procedure described by Witte et al. (1970). Briefly, approximately 1 g of fish meat (n = 15 per group) was homogenized with 25 mL of 7.5% trichloroacetic acid. The homogenates were filtered on filter paper (Whatman® Qualitative Filter Paper Grade 4, Sigma-Aldrich Pte Ltd., Singapore). The filtrates were then determined for lipid peroxidation using a modified TBARS method (Witte et al., 1970). Results were expressed as milligram malondialdehyde per kilogram of fresh meat, using a standard curve that covered concentration ranges of 0.5 to 10 μM of 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, Steinheim, Germany). Absorbance was measured at 532 nm by means of a spectrophotometer (UV-1601, Shimadzu Corporation, Kyoto, Japan). The first subsamples were measured for TBARS values immediately after euthanasia within 1 hour. The second subsamples were covered by polyvinyl chloride wrap and kept at 4°C for 48 h before TBARS value determination (48 hours).

Statistical analysis: Data were explored for normal distribution using Shapiro-Wilk test. Data between the two groups of fish at each analysis period were compared using Student's *t* test. Differences were considered to be significant if $P < 0.05$.

Results and Discussion

During the entire experimental period (120 days), the fish in all tanks remained healthy without any clinical abnormalities. The fish received the diets at the rate of 8% of the estimated body weight, and the amount of diets was adjusted accordingly every week. As observed in this study, the condensed tannin supplementation did not affect the intake of the fish, which was in agreement with the findings of Aiura and de Carvalho (2007). In a previous study, condensed tannin did not affect digestibility of tilapias because it did not bind to proteins or any nutrients (Buyukcapar et al., 2011). Results from the study of Buyukcapar et al. (2011) demonstrated that tilapias supplemented with condensed tannin up to 25 g/kg diet had similar growth performance to the control fish. In our study, the supplemented fish received condensed tannin only 4 g/kg diet; although the body weights were not measured, it might be implied that this supplemented dose had no effects on growth performance.

The lipid peroxidation activities measured in the muscles by TBARS assay at 1 h and 48 h are demonstrated in Figures 1 and 2. Immediately 1 h after euthanasia, the mean TBARS value (expressed as mg MDA/kg fresh meat) in the meat samples collected from the fish supplemented daily with 4 g condensed tannin/kg diet was significantly lower than the mean value from the control fish. Similar results were also observed for the samples measured at 48 h after euthanasia. However, the TBARS values increased from 1 h to 48 h in both groups, i.e. from 0.480 to 0.628 for the control fish and from 0.269 to 0.446 mg MDA/kg fresh meat for the supplemented fish. The TBARS assay has been used to determine lipid peroxidation activities of lipids. Therefore, increased TBARS values are positively related to increased lipid peroxidation activities (Łukaszewicz-Hussain et al., 2007).

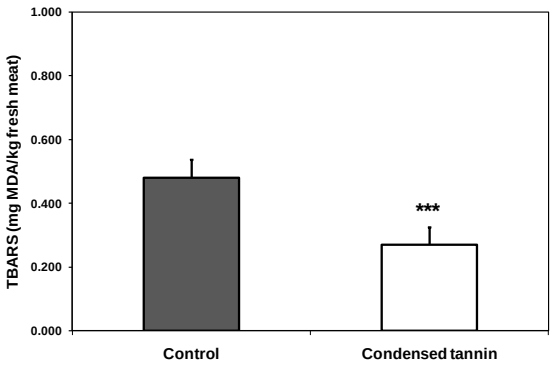

Figure 1 Comparison of thiobarbituric acid reactive substances (TBARS) values expressed as milligram malondialdehyde (MDA) per kilogram of fresh meat between control fish (n = 15) and supplemented fish (n = 15) at 1 h after euthanasia. The asterisks indicate that means between the two groups differ at $P < 0.001$.

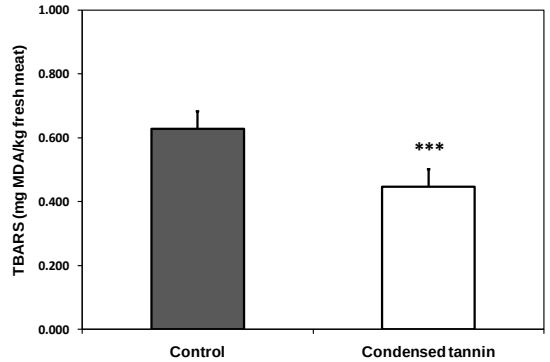

Figure 2 Comparison of thiobarbituric acid reactive substances (TBARS) values expressed as milligram malondialdehyde (MDA) per kilogram of fresh meat between control fish (n = 15) and supplemented fish (n = 15) at 48 h after euthanasia. The asterisks indicate that means between the two groups differ at $P < 0.001$.

Condensed tannin is classified as a bioflavonoid. Absorption of bioflavonoids starts during chewing the plants; however factors affecting absorption include molecular weights, complexity of the molecular structure, and solubility. Costa et al. (2015) studied phytochemical screening of *Phthirusa pyrifolia* leaf extract and observed high phenol, flavonoid and condensed tannin constituents in the aqueous extract of leaves. Most of these phytochemical extracts demonstrated excellent scavenging properties of reactive oxygen species (Costa et al., 2015). Condensed tannin has antioxidant activities, which could reduce the adverse effects from oxidative stress. As indicated in this study, the fish supplemented with condensed tannin in the diet had lower lipid peroxidation activities in the muscles than the control fish. The antioxidant activities of tannin depend on alignment of the molecules in its structure, total numbers of hydroxyl groups, and hydrogen ion replacement ability. Possible mechanisms of condensed tannin as antioxidants are 1) reduction in reactive oxygen species by impairing enzyme system or by binding to trace minerals essential to form free radicals, 2) removal of reactive oxygen species, and 3) prevention or control of antioxidant activities (Halliwell and Gutteridge, 1998; Mishra et al., 2013). In agreement with the study by Sánchez-Alonso et al. (2007), grape by-products, which are rich in bioflavonoids, could prevent lipid peroxidation in minced fish. In that study, the grape by-products were prepared as grape antioxidant dietary fiber before being dispersed into the minced fish muscle. After storage for 30 days at -20°C, minced fish meat containing 2% and 4% grape antioxidant dietary fiber had higher rate of oxidation inhibition than the control meat. It is, therefore, suggested that when fish are fed condensed tannin-supplemented diet, the antioxidant activities in the muscles increase, finally leading to the maintenance of meat quality during storage. However, further research on the application of condensed tannin in field practices will guarantee its beneficial effects.

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บทคัดย่อ

การเสริมคอนเดนส์แทนนินในอาหารช่วยลดลิปิดเปอร์ออกซิเดชันในกล้ามเนื้อของปลานิล (*Oreochromis niloticus*)

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ประเมินผลของการเสริมคอนเดนส์แทนนินในอาหารต่อลิปิดเปอร์ออกซิเดชันในกล้ามเนื้อของปลานิล ใช้ปลานิลอายุ 21 วัน จำนวน 250 ตัว สุ่มแบ่งออกเป็นกลุ่มละ 25 ตัว เลี้ยงในตู้ปลาทดลองจำนวน 10 ตู้ โดยเป็นกลุ่มควบคุมจำนวน 5 ตู้และกลุ่มทดลองจำนวน 5 ตู้ กลุ่มควบคุมให้อาหารสำเร็จรูป และกลุ่มทดลองให้อาหารสำเร็จรูปที่เสริมด้วยคอนเดนส์แทนนินในอัตรา 4 กรัม/กิโลกรัม ให้อาหารในอัตราร้อยละ 8 ของน้ำหนักตัว วันละ 2 ครั้ง ระยะทดลองนาน 120 วัน เมื่อสิ้นสุดการทดลอง สุ่มเลือกปลา 3 ตัวจากแต่ละตู้ปลาทดลอง และเมตตาฆาตด้วยความเย็น เก็บตัวอย่างกล้ามเนื้อสันหลัง โดยแบ่งออกเป็นสองส่วน เพื่อวิเคราะห์ลิปิดเปอร์ออกซิเดชันโดยวิธีหา thiobarbituric acid reactive substances หรือ TBARS โดยตัวอย่างส่วนแรกวิเคราะห์ค่า TBARS ทันทีภายใน 1 ชั่วโมงหลังเมตตาฆาต ส่วนตัวอย่างส่วนที่สองเก็บไว้ที่อุณหภูมิ 4°C เป็นเวลา 48 ชั่วโมงก่อนนำไปวิเคราะห์ค่า TBARS การศึกษาพบว่าค่าเฉลี่ย TBARS ของกล้ามเนื้อปลานิลที่เสริมคอนเดนส์แทนนินเมื่อวัดที่ 1 ชั่วโมงหลังเมตตาฆาต (0.269±0.028 มิลลิกรัม มาลอนไดอัลดีไฮด์/กิโลกรัมเนื้อปลาสด) มีค่าต่ำกว่าค่าเฉลี่ย TBARS ของปลานิลในกลุ่มควบคุม (0.480±0.025 มิลลิกรัม มาลอนไดอัลดีไฮด์/กิโลกรัมเนื้อปลาสด) ผลค่าเฉลี่ย TBARS เกิดขึ้นเหมือนกันเมื่อวัดที่ 48 ชั่วโมงหลังเมตตาฆาต กล่าวโดยสรุป การเสริมคอนเดนส์แทนนินในอาหารสามารถลดลิปิดเปอร์ออกซิเดชันในกล้ามเนื้อได้อย่างมีนัยสำคัญทางสถิติ

คำสำคัญ: คอนเดนส์แทนนิน ลิปิดเปอร์ออกซิเดชัน ปลานิล thiobarbituric acid reaction assay

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