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NUTRITIONAL COMPOSITION AND QUALITY OF AFRICAN CATFISH (*Clarias gariepinus*) FED WITH *Melaleuca cajuputi* LEAVES EXTRACTS AS A NATURAL FEED ADDITIVE

KARIM, N.U.[1,2*], MOHD KHAIRUL SAHIMI, M.B.[2] and HASSAN, M.[2]

[1]*School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia*
[2]*Institute of Tropical Aquaculture (AQUATROP), Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia*
[*]*E-mail: ulfah@umt.edu.my*

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ABSTRACT

African catfish, *Clarias gariepinus* were treated as; controls (fed with commercial feed); T1, (fed with 5 mg kg^{-1} Melaleuca leaves extract formulated in commercial feed); and T2 (fed with 10 mg kg^{-1} Melaleuca leaves extract formulated in commercial feed) and fed twice daily for 21 days. The effects of the treatments on nutritional composition, microbiological and physicochemical quality of the catfish were analysed. The nutritional composition of the catfish fed with Melaleuca leaves extract formulated in commercial feed were not significantly modified ($p>0.05$). However, lipid and fibre content in T2 catfish showed a significantly ($p<0.05$) higher composition compared to the controls. The total bacteria load of T2 were successfully reduced ($p<0.05$) compared to T1 and the controls. Water holding capacity (WHC) of T2 catfish were recorded higher (77.93±6.95 %) but not significantly ($p>0.05$) different to the T1 and controls. pH value in all samples were recorded at range of 5.2-5.6. Meanwhile the colour value (L*, a* and b*) were not significantly ($p>0.05$) different in all samples. Feed additive of 10 mg kg^{-1} Melaleuca leaves extract are potential invention of aquafeed industry to be applied in aquaculture practice as it offers a good microbiological and physicochemical quality to cultured African catfish.

Key words: *Clarias gariepinus*, *Melaleuca cajuputi*, aquafeed, microbiological quality, physicochemical quality

INTRODUCTION

African catfish (*Clarias gariepinus*) is an omnivorous freshwater fish and high-value food fish. It is one of the most popular cultured fish species among the aqua culturist due to its fast growth rate, resistance to disease and tolerance to low oxygen levels (Wan Norhana *et al*., 2012). Therefore, the aquafeed industry grow rapidly to entail the successful in aquaculture growth and intensification. However, aquafeed industry is confronted with the rising costs. Meanwhile, aquaculture sector facing stressful problems in combating fish disease. The usage of antibiotics aroused for possible antibiotic residues and antibiotic resistance in fish. In addition, the use of chemical materials such as malachite green, formalin and sodium chloride in aquaculture systems may arise high level residue in fish (Mohamadi *et al*., 2013). Hence, a new aquafeed formulated with natural ingredients from sustainable and renewable resources is crucial (Workaagegn *et al*., 2013) as its offers safe, biodegradable, inexpensive and locally available. In addition, plant extracts play an important role in the stimulating the immune system of fish in challenged with bacterial, parasitic and fungal agents.

Melaleuca cajuputi commonly known as cajuput or *gelam* is medicinal herbal plants that has potential to be used as a natural additive in fish feed. Melaleuca leaves extracts is a non-toxic and auspicious as a strong disinfectant against bacteria (Nuyim, 1998). Jedlickova *et al.* (1993) documented that Melaleuca leaves extract effectively inhibit *Pseudomonas aeruginosa, Candida albicans, Streptococcus* spp., *Enterobacter* spp., *Klebsiella pnuemoniae* and *E. coli*. Interestingly, a recent studies by Shivappa *et al.* (2015) suggesting that Melaleuca extract might be an immunostimulant

* To whom correspondence should be addressed.

rather than an antimicrobial agent for ornamental fish such as goldfish (*Carassius auratus*) and false percula clownfish (*Amphiprion ocellaris*). Yoshpa (2002) also stated that Melaleuca extract positively enhance skin and tissue healing in injured fish or other aquatic animals. Othman (2013) added that Melaleuca leaves extract formulated in fish feed significantly promote the growth of African catfish (*Clarias gariepinus*).

It has been widely documented that the fish flesh quality is influenced by the type and nature of feed ingested, particularly the chemical composition of the diet (Srikar *et al*., 1989). High quality of food fish provide safe food, high nutritional composition, desirable flavour and textures, and able to inhibit growing micro-organisms that decaying the flesh. In conjunction with various benefits provide by the Melaleuca leaves extracts, a study on the flesh quality of African catfish, *Clarias gariepinus* fed with Melaleuca leaves extract as natural additives in commercial feed were conducted. This study revealed the nutritional composition, the total bacteria colony found in the flesh and the physicochemical quality of the fish flesh after feeding with the formulated Melaleuca leaves extract in commercial feed.

MATERIALS AND METHODS

Aquafeed preparation

Melaleuca cajuputi leaves were obtained from Marang, Terengganu and transported to the laboratory. The leaves were cleaned and dried in the oven (Ecocell EC111, Germany) at 30°C for 48 hours. The dried leaves were grinded to form coarse powder. Approximately (100±0.1 g) Melaleuca powder were mixed with 80% (v/v) methanol and shake in the incubator shaker at 200 rpm for 24 hours at 27°C. The mixture was filtered through Whatman filter paper before evaporated in rotary evaporator (IKA, Germany) at 30°C. The feed was prepared by mixing the crushed commercial pellet and the Melaleuca leaves extract at 5 mg kg^{-1} and 10 mg kg^{-1} for T1 and T2, respectively. The dry mixture was added to distilled water at ratio of 1.5: 1 (w/v) before moulded to pellet form using pelletizes machine. The pellet was dried in oven (Ecocell EC111, Germany) at 50°C for 24 hours before kept in airtight containers at -20°C upon fish feeding.

Sample preparation

Thirty African catfish of homogenous size were individually placed in 50 L tank which was maintained at 22-28°C, 5.0-7.0 mg l^{-1} oxygen level and 6.5-8.0 pH value. Upon experiments, all samples were acclimatized in the tanks and fed with the basal diet for a week. All samples were randomly divided into three treatment categories; controls, fed with commercial feed; T1, fed with 5 mg kg^{-1} Melaleuca leaves extract formulated in commercial feed; and T2, fed with 10 mg kg^{-1} Melaleuca leaves extract formulated in commercial feed. All samples were fed twice daily at proportion of 5% body weight for 21 days at well-maintained water quality.

Nutritional composition determination

The protein content of fish flesh was analysed using Kjeldahl method (Kjetlec TM 2100, Denmark) according to AOAC (1990). Lipid content of all samples were determined using Soxlhet method (AOAC, 1990). The moisture content was determined according to the method by AOAC (1994).

Microbiological analysis

The total bacterial counts were performed according to method described by Linton *et al.* (2003) and Karim *et al.* (2011). The incubation conditions used for total aerobe counts were 35°C for 48 hours. Plates with 30 to 300 colonies were selected and bacteria were counted using colony counter (Stuart Colony Counter, UK).

Water holding capacity (WHC) determination

Approximately 5±0.1 g of flesh sample was filled into 15 ml of weighed centrifuge tube. The tube was weighed (W_1). The samples were then centrifuged for 20 minutes at 1500 rcf g^{-1} at 10°C. The water remaining in the tubes were discarded and the tubes were weighed again (W_2). WHC of flesh was calculated as the weight of sample remaining after centrifugation. This process was done to 3 replicates and the average was expressed as % WHC in weight basis.

pH value measurement

The pH value in the fish muscle were measured by using pH electrode (ROSS flat pH electrode, Thermo Fisher Scientific).

Colour measurement

The colour of fillet were measured by using colorimeter (Konika Minolta CR-400, Japan). The measurement of L*, a* and b* value was taken at three different locations; close to the head, in the middle and at the tail of the fillet.

Statistical analysis

All measurements were performed in triplicate and the values were express as mean ± standard deviation. Data were analysed statistically using IBM SPSS Statistic software (Version 20). Significance difference was taken at $p<0.05$.

RESULTS AND DISCUSSION

Nutritional composition of African catfish

Protein content in fish flesh fed with commercial feed recorded an amount of 66.84±1.57 %. Meanwhile protein content in African catfish fed with 5 and 10 mg kg^{-1} Melalueca leaves extract formulated in commercial feed were 66.99±4.87 and 65.43±5.67 %, respectively. However, protein content in all samples show no significant ($p>0.05$) different among others (Table 1). African catfish fed with 10 mg kg^{-1} Melalueca leaves extract formulated in commercial feed showed a significantly ($p<0.05$) higher amount of lipid content compared to fish fed with commercial feed (Table 1). Meanwhile fish fed with 5 mg kg^{-1} Melalueca leaves extracted formulated in commercial feed showed no significant ($p>0.05$) different of lipid content from two other treatments (Table 1). In addition, fibre and moisture content showed no significant ($p>0.05$) different among all samples of the catfish (Table 1).

Flesh quality of fishes are influenced by the chemical composition of the diet (Srikar *et al*., 1979). A similar finding was documented by Omoniyi *et al*. (2013). They found that protein content in African catfish were 69.35±0.08 %. However, several researcher documented a lower amount of protein content found in African catfish. Ayeloja *et al.* (2013), Essa *et al.* (2011) and Ersoy & Ozeren (2009) documented protein content in African catfish were 16.38±0.00, 17.45±0.38, 16.20±0.66 % respectively. Lipid content found in current studies were higher compared from previous research by Omoniyi *et al.* (2013). They documented an amount of 16.03±0.08 % lipid content was found in cultured African catfish. In addition, Ayeloja *et al.* (2013), Essa *et al.* (2011) and Ersoy & Ozeren (2009) reported lipid content were below 5 % were found in African catfish. This obvious difference finding may be due to the myriad of oil constituent present in the Melaleuca leaves (Brophy & Doran, 2004) that may influenced the lipid content of the fish. Janskowska *et al.* (2007) also stated the feed offered to the cultured fishes may had direct impact on its body fat increment. Previous study by Oladipo & Bankole (2013) documented that, the fibre content in African catfish is 1.02 %, which is higher than the current study. Ayeloja *et al.* (2013), Essa *et al.* (2011) and Ersoy & Ozeren (2009) reported the moisture content were higher than in current findings which were at range of 75 to 78 %.

Microbiological quality of African catfish

The total bacteria count in control samples were recorded at amount of 4.15 ± 0.08 $\log_{10}$ CFU g^{-1}. The bacteria loads in African catfish fed with formulated 10 mg kg^{-1} Melaleuca leaves extracts with commercial feed showed a significant ($p<0.05$) reduced amount of 0.2 $\log_{10}$ CFU g^{-1} compared to the control samples. Meanwhile fishes fed with formulated 5 mg kg^{-1} Melaleuca leaves extracts with commercial feed showed no significant different ($p>0.05$) compared to control samples even though there is a slight reduction of bacteria loads (Table 2).

A similar finding were documented by Ekundayo *et al.* (2014) as they found bacteria loads in catfish were at the range of 3.57–5.54 $\log_{10}$ CFU g^{-1}. However, various researcher documented a higher amount of total bacteria found in raw African catfish. For example, Oladipo & Bankole (2013) documented bacteria count of raw African catfish were 6.16 $\log_{10}$ CFU g^{-1}. In addition, Ibrahim *et al.* (2014) also found the total bacteria loads in African catfish were 6.01 $\log_{10}$ CFU g^{-1}. Meanwhile,

Table 1. Nutritional composition of African catfish, *Clarias gariepinus* which fed with commercial diet (control) and diet treatment containing *Melaleuca cajuputi* leaves extracts

Sample treatments	Protein (%)	Lipid (%)	Fibre (%)	Moisture (%)
African catfish fed with commercial feed (Controls)	66.84±1.57^{a}	19.99±1.66^{a}	0.22±0.01^{a}	53.18±2.66^{a}
African catfish fed with formulated 5 mg kg^{-1} Melaleuca extracts with commercial feed (T1)	66.99±4.87^{a}	24.37±2.29^{ab}	0.25±0.02^{a}	58.15±0.94^{a}
African catfish fed with formulated 10 mg kg^{-1} Melaleuca extracts with commercial feed (T2)	65.43±5.67^{a}	27.55±2.14^{b}	0.29±0.01^{b}	57.73±2.52^{a}

The values are mean ± SD. Different letters in the same column, indicate statistically significant differences ($p < 0.05$) among the sample treatments.

Table 2. Total bacteria count on African catfish, *Clarias gariepinus* which fed with control and diet treatment containing *Melaleuca cajuputi* leaves extracts for 21 days

Sample treatments	Bacteria count (log CFU ml^{-1})
African catfish fed with commercial feed (Controls)	4.15±0.08[a]
African catfish fed with formulated 5 mg kg^{-1} Melaleuca extracts with commercial feed (T1)	4.04±0.08[ab]
African catfish fed with formulated 10 mg kg^{-1} Melaleuca extracts with commercial feed (T2)	3.97±0.08[b]

The values are mean ± SD. Different letters in the same column, indicate statistically significant differences ($p < 0.05$) among the sample treatments.

Emikpe *et al.* (2011) reported the total bacteria loads in *Clarias gariepinus* heavily contaminated and give amount at ranged of 12-13 $\log_{10}$ CFU g^{-1}.

The significant reducing amount of bacteria loads in treated samples may gives a new understanding that *Melaleuca cajuputi* leaves extract has properties to reduce the bacteria loads on a fish muscles. Carson *et al.* (2006) and Hammer *et al.* (2006) stated that *M. cajuputi* extract capable on deep penetrating power, eliminating subcutaneous infections and promoting fast healing. Doran (1999) identified that the chemical constituents of *M. cajuputi*; viz, 1, 8-cineole, (-)-linalool, (-)-terpinen-4-ol, (+/-)-α-terpinol, 3, 5-dimethyl-4, 6-di-O methylphloroacetophenone may potentially act as the antimicrobial agent. Thus, the use of *M. cajuputi* in fish diet is a benefit as it can reduce bacteria count in a fresh fish.

Water holding capacity and pH value of African catfish

Water holding capacity (WHC) in African catfish showed the highest amount in samples fed with formulated 10 mg kg^{-1} Melaleuca leaves extracts with commercial feed; 77.93±6.95 % followed by T1 and controls; 74.50±4.51 % and 72.49±3.64 % (Table 3). However, all samples showed no significant different to each other. A similar finding of WHC in African catfish was documented by Fadlalmola (2007) and Fiengul *et al.* (2001). WHC is importance to the quality characteristics of muscle foods and their acceptance by consumers. Parry (1993) stated that there is interrelation of WHC to pH value and texture of fish muscle. Fish muscle texture may defect due to the low pH value and WHC (Parry, 1993) where it increases in toughness and dryness (Tiffney & Mills, 1982). pH value of all samples range between 5.2 and 5.61 (Table 3). pH value recorded in current study were similar to Huss (1995) as it is stated that pH of post-mortem fish may vary considerably at pH 5.4 – 7.2.

Colour of African catfish

The value of lightness (L*) on control samples were recorded to give amount of 43.77±2.38. Meanwhile, the L* value in samples fed with formulated 5 and 10 mg kg^{-1} Melaleuca leaves extracts with commercial feed were recorded to give amount of 45.35±2.09 and 44.69±2.53, respectively (Table 4). However, all samples were not significantly ($p>.05$) different to each other. Similar trend was found in both a* and b* value in all samples (Table 4). Hardy & Lee (2010) stated that

Table 3. Physical quality test on WHC and pH of African catfish, *Clarias gariepinus* which fed with control and diet treatment containing *Melaleuca cajuputi* leaves extracts for 21 days

Sample treatments	Water holding capacity (%)	pH
African catfish fed with commercial feed (Controls)	72.90±3.64[a]	5.28±0.04[a]
African catfish fed with formulated 5 mg kg^{-1} Melaleuca extracts with commercial feed (T1)	74.50±4.51[a]	5.61±0.03[ab]
African catfish fed with formulated 10 mg kg^{-1} Melaleuca extracts with commercial feed (T2)	77.93±6.95[a]	5.48±0.03[b]

The values are mean ± SD. Different letters in the same column, indicate statistically significant differences ($p < 0.05$) among the sample treatments.

Table 4. The colour value (L*, lightness; b*, yellowness; and a*, redness) of flesh of African catfish, *Clarias gariepinus*

Sample treatments	L*	a*	b*
African catfish fed with commercial feed (Controls)	43.77±2.38[a]	1.84±0.67[a]	4.22±1.06[a]
African catfish fed with formulated 5 mg kg^{-1} Melaleuca extracts with commercial feed (T1)	45.35±2.09[a]	2.05±1.26[a]	4.27±0.87[a]
African catfish fed with formulated 10 mg kg^{-1} Melaleuca extracts with commercial feed (T2)	44.69±2.53[a]	2.47±1.18[a]	4.32±0.86[a]

The values are mean ± SD. Different letters in the same column, indicate statistically significant differences ($p < 0.05$) among the sample treatments.

the colour of fish fillet can be readily modified by the feed formulation. Fillet appearance differs due to affect reflectance properties of the muscle. The opacity of fish muscle is influence by the protein solubility and denaturation (Einen *et al*., 2002).

CONCLUSION

Natural feed additive of 10 mg kg^{-1} Melaleuca leaves extract is potential invention of aquafeed industry to be applied in aquaculture practice as it offers a good microbiological and physicochemical quality to cultured African catfish.

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