Effect of Activated Charcoal-Supplemented Diet on Growth Performance and Intestinal Morphology of Nile Tilapia

(Oreochromis niloticus)

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Abstract

The effect of tilapia diet supplemented with activated charcoal (AC) on growth performance and intestinal morphology of Nile Tilapia was examined. The fish were divided into four groups: a control group and three AC-supplementation groups (1%, 2% and 3% of AC in diet), and fed approximately 3% of body weight twice a day for 30 days. Growth performance and intestinal villus height were measured. Results revealed that the 2% AC-supplemented diet group was significantly ($P<0.05$) better in terms of percentage weight gain, specific growth rate (SGR) and feed conversion ratio (FCR), among the experimental groups. The foregut and midgut villus height of the 3% and 2% AC-supplemented diet groups was similar, but was significantly higher than the control group ($P<0.05$). Consequently, the supplementation of diet with 2% AC was found to be the most suitable for improving the growth performance and intestinal morphology in tilapia.

Keywords: activated charcoal, growth performance, intestinal villi, tilapia

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**Introduction**

Nile tilapia (*Oreochromis niloticus*) is the world’s most important warm water farmed species because of its large size, rapid growth and its value as a good source of protein for human consumption. Currently, the practice of tilapia culture has been expanding throughout Thailand, and it has become the biggest freshwater aquaculture fishery in the country with a volume of 1.39 million metric tons in 2009. In addition, the world aquaculture production of Nile tilapia has dramatically increased from 124,000 metric tons in 1997 to 3 million metric tons in 2010 (FAO, 2010).

Although tilapia is one of the important fisheries in Thailand with the potential for export, the quality and safety of the product need to be ensured in domestic and international markets (ACFS, 2004). Various problems such as infection, contamination with toxins, improper waste product disposal and improper management practices still remain, 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 stricter current standards for food safety (ACFS, 2004).

Activated charcoal (AC), a solid, porous, carbonaceous material prepared by carbonizing and activating organic substances, is recognized as an adsorbent of drugs and other toxic substances from the gastrointestinal tract and has been used in medicine since ancient times (Levy, 1982; JECFA, 2010). AC is the most powerful non-selective adsorbent that has a very large surface area available for adsorption or chemical reactions. AC effectively adsorbs pesticides, environmental hydrocarbons, pharmaceutical agents, mycotoxins, phytotoxins, feed additives, antibacterials and most bacterial toxins (Buck and Bratich, 1986). AC binds its positively charged molecular surfaces with negatively charged molecular surfaces of toxins (Edwards and McCredie, 1967). The AC raw materials, which include sawdust, peat, lignite, coal, cellulose residues, coconut shells, petroleum coke, etc., may be carbonized and activated at high temperature with or without the addition of inorganic salts in a stream of activating gases such as steam or carbon dioxide (JECFA, 2010). Alternatively, carbonaceous matter may be treated with a chemical activating agent such as phosphoric acid or zinc chloride and the mixture is carbonized at an elevated temperature, followed by removal of the chemical activating agent by washing in water (JECFA, 2010).

AC has been used in animal feed formulation as an additive because it absorbs ammonia and nitrogen, and activates the intestinal function through elimination of poisons and impurities from the gastrointestinal tract of land animals (Mekbungwan et al., 2004; Van et al., 2006). The use of AC at high dosages for mycotoxin binding has been shown to reduce aflatoxicosis in goats (Hatch et al., 1982). Calvano et al. (1995) reported reduced aflatoxin residues in milk of cows consuming different sources of activated carbon. AC may also be important in binding mycotoxins, zearalenone and deoxynivalenol (Döll et al., 2004; Bueno et al., 2005), and was shown in an *in vitro* gastrointestinal model to reduce the availability of deoxynivalenol and nivalenol (Avantaggiato et al., 2004). It has also been shown to successfully treat a variety of toxicity problems in ruminants (Bisson et al., 2001; Banner et al., 2000; Poage et al., 2000), including those from bacterial toxins (Buck and Bratich, 1986). AC has been found to be useful in removing *E. coli* O157:H7 cells and the toxin, both *in vitro* and *in vivo* (Pegues et al., 1979; Marks et al., 1986; Naka et al., 2001; Knutson et al., 2006). AC supplementation in diet could become a counter measure for preventing chemical contamination in farmed fish. Therefore, the optimum levels of AC needed in a diet should be examined by means of growth performance of the fish. Furthermore, the toxicological evaluation of AC needs to be done because of its non-selective absorbance. Moreover, Thu et al. (2010) reported that diet supplemented with 0.5% bamboo charcoal (BC) was found to be suitable to elicit maximum growth performance in Juvenile Japanese Flounder (*Paralichthys olivaceus*) and to decrease the amount of nitrogenous compound excreted.

Although AC supplementation in feed has been widely used in aquaculture, there have been few reports documenting its use in the case of Nile tilapia. This study was aimed at finding a suitable level of AC supplementation as an effective and safe additive to Nile tilapia feed, and understanding the influence of AC-supplemented diet on the growth performance and intestinal morphology in Nile tilapia.

**Materials and Methods**

**Experimental design:** Three hundred and sixty *Oreochromis niloticus* individuals, with an average body weight of 5.8 g, were randomly placed in four 300-L tanks (30 fish per tank), one for the control group and three for the activated charcoal supplementation groups (1%, 2% and 3%). All healthy tilapias were obtained from a certified tilapia farm for Good Agricultural Practices (GAP). The tank was filled with recycled water which was maintained at 25-28°C, with a dissolved oxygen (DO) content of 5.8-6.8 ppm and a pH of 6.5-7.0 throughout the experiment. The experiment was done in triplicate.

The fish in the control group was fed on commercial dry pellets, and the three experimental groups were fed on the commercial dry pellets mixed with 1%, 2%, and 3% activated charcoal (SKU V-1400, Charcoal House LLC, Crawford, NE, USA), respectively. All experimental ingredients were obtained from animal feedstuff companies. In order to produce isonitrogenous and isocaloric experimental diets, all feed ingredients were determined and formulated proximate compositions (moisture, protein, lipid and ash) according to AOAC (1990) (Table 1). Experimental diets were produced using a hammer grinder, mixer, and extruder (Paktonchai Pasusat, Nakhon Ratchasima, Thailand). The dry ingredients were ground using a grinder and mixed by a ribbon screw mixer (22 rpm). The floating experimental diets were produced by a single screw extruder (an extruding temperature of 120-160°C). All the fish were fed approximately 3% of their body
weight twice a day. They were weighed at the beginning (day 0) and the end (day 30) of the experiment for the growth performance.

**Growth parameters**: Weight gain (%), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated using the following equations (Yanbo and Zirong, 2006):

- **Weight gain (%)**
  \[ \text{Weight gain} = \frac{100 \times (\text{final mean body weight} - \text{initial mean body weight})}{\text{initial mean body weight}} \]

- **Specific growth rate**
  \[ \text{Specific growth rate} = \frac{\ln(\text{final body weight}) - \ln(\text{initial body weight})}{\text{number of days between weighing}} \times 100 \]

- **Feed conversion ratio**
  \[ \text{Feed conversion ratio} = \frac{\text{feed intake (g)}}{\text{weight gain (g)}} \times 100 \]

**Histopathology**: Liver, head kidney, spleen, trunk kidney, heart and gill from each fish (n=5) were collected, fixed in 10% neutral buffered formalin and processed according to standard histopathological techniques and tissue sections were stained with haematoxylin and eosin (H&E). The histopathological study revealed significant change in the other organs. Histopathological changes were evaluated as negligible, mild, moderate or severe based on frequency and severity of lesions.

**Measurement of villus height**: After 30 days of feeding, three portions of the intestines (n=5), namely, the foregut (from after the pyloric part of the stomach to before the spiral part of the intestines), midgut (the spiral part of intestines) and hindgut (from after the spiral part of the intestine to 2 cm. before anus) from the experimental and control fish were collected and fixed in 10% buffered formalin. Fixed tissues were processed according to standard histological techniques and tissue sections were stained with haematoxylin and eosin (H&E). For villus height measurement, the five highest villi were selected per section, and the villus length measured from the villus tip to the bottom for each villus. These measurements were averaged and expressed as mean villus height per section (Pirarat et al., 2011).

**Statistical analysis**: Data were expressed as a mean ± S.D. and were analyzed using ANOVA followed by Duncan’s multiple range test. All tests used a significance level of 0.05.

### Table 1 Ingredients and chemical composition (%) of four experimental diets

<table>
<thead>
<tr>
<th>Ingredient (g kg⁻¹)</th>
<th>0%</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>30.0</td>
<td>30.2</td>
<td>30.5</td>
<td>30.7</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>27.0</td>
<td>26.8</td>
<td>26.8</td>
<td>26.8</td>
</tr>
<tr>
<td>Rice bran</td>
<td>15.0</td>
<td>14.5</td>
<td>13.5</td>
<td>12.0</td>
</tr>
<tr>
<td>Corn meal</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
</tr>
<tr>
<td>Cassava chips</td>
<td>12.0</td>
<td>11.5</td>
<td>11.2</td>
<td>11.0</td>
</tr>
<tr>
<td>Premix¹</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td></td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Activated charcoal</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

**Proximate composition (g kg⁻¹ dry weight)**

<table>
<thead>
<tr>
<th></th>
<th>0%</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>92.5</td>
<td>91.0</td>
<td>93.5</td>
<td>94.0</td>
</tr>
<tr>
<td>Crude protein</td>
<td>32.5</td>
<td>32.2</td>
<td>32.4</td>
<td>32.8</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>6.4</td>
<td>6.4</td>
<td>6.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Crude ash</td>
<td>9.4</td>
<td>9.5</td>
<td>10.2</td>
<td>10.3</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>4.0</td>
<td>4.0</td>
<td>4.2</td>
<td>4.3</td>
</tr>
</tbody>
</table>

¹Vitamin and trace mineral mix provided the following (IU kg⁻¹ or g kg⁻¹ diet): biotin, 0.25 g; folic acid, 0.003 g; inositol, 0.25 mg; niacin, 0.0215 g; pantothenic acid, 0.03 g; vitamin A, 5,000 IU; vitamin B₁, 0.0025 g; vitamin B₂, 0.0012 g; vitamin B₆, 0.0075 g; vitamin B₁₂, 0.0005 mg; vitamin C, 1 g; vitamin D₃, 1,000 IU; vitamin E, 100 IU; vitamin K, 0.008 g; copper, 0.02 g; iron, 0.2 g; selenium, 0.3 mg; zinc, 0.32 g.

### Results

**Growth performance**: The growth performance, including weight gain (%), specific growth rate (SGR) and feed conversion ratio (FCR) of the tilapia are shown in Table 2. While there were no significant differences in growth among the AC-supplemented groups, all these groups showed significantly better growth than the control fish.

**Histopathology**: The histopathological study revealed mild fatty change in the liver of AC fish. However, the degree of fatty change was not related to the dose. No significant change was observed in the other organs.

**Measurement of villus height**: The intestinal villi characterized by mucosal folds that appeared swollen and bulging towards the lumen and contained numerous mucous cells were measured. The data on villus height measured from the foregut, midgut, and hindgut of the fish are shown in Table 3. The villus height from all three parts of the intestine in the 3% AC-supplemented group was the longest when compared with the other groups. In the foregut and midgut, the villus height of the control group was significantly lower than the AC-supplemented groups. No significant difference in villus height was observed in all parts of the intestine among.
the AC-supplemented groups. At the hindgut of the intestine, there was no statistic difference in villus height among the experimental groups.

**Discussion**

Supplementation of activated charcoal in feed has been known to enhance the growth performance and intestinal function of terrestrial and aquatic animals (Mekbungiwan et al., 2004; Van et al., 2006; Thu et al., 2010). However, activated charcoal supplemented in diet can adsorb not only various harmful chemicals but also beneficial nutrients. Previous studies in fish have shown that the highest weight gain was obtained with bamboo charcoal supplementation at 4% in tiger puffer fish (Moe et al., 2009) and 0.5% in juvenile Japanese Flounder (Paralichthys olivaceus) (Thu et al., 2010). Thus, the optimum level of AC supplementation in fish diet appears to be different among fish species and growth stages. Our study revealed that the supplementation of activated charcoal in feed (1-3%) can also increase the growth performance and improve the intestinal morphology in Nile tilapias. The maximum growth enhancement was noticed when the supplementation was done at the 2% level. However, no significant changes were seen in the liver, head kidney, spleen, trunk kidney, heart and gall. These results suggest that AC supplementation in feed is unlikely to cause any toxic effects in Nile tilapia. Therefore, we conclude, based on our results, that the optimum AC-supplementation level in feed for Nile tilapia is 2% (W/W).

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (0%)</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW, g</td>
<td>6.38±0.58</td>
<td>5.47±0.26</td>
<td>5.42±0.60</td>
<td>5.51±0.23</td>
</tr>
<tr>
<td>Final BW, g</td>
<td>12.90±1.63</td>
<td>15.87±1.15</td>
<td>17.08±2.55</td>
<td>14.26±0.50</td>
</tr>
<tr>
<td>Weight gain (%)</td>
<td>101.73±9.51</td>
<td>190.04±10.96*</td>
<td>211.53±22.60*</td>
<td>158.77±47.6*</td>
</tr>
<tr>
<td>SGR</td>
<td>2.81±0.39</td>
<td>3.55±0.13*</td>
<td>3.80±0.24*</td>
<td>3.17±0.06*</td>
</tr>
<tr>
<td>FCR</td>
<td>0.03±0.003</td>
<td>0.01±0.001*</td>
<td>0.01±0.001*</td>
<td>0.01±0.00*</td>
</tr>
</tbody>
</table>

**BW:** Body weight; **SGR:** Specific growth rate; **FCR:** Feed conversion ratio

Table 3 Villus height of tilapias fed activated charcoal supplemented diets for 30 days (Values are means ± SD, n=5)

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foregut</td>
<td>183.941</td>
<td>237.566</td>
<td>261.927</td>
<td>308.510</td>
</tr>
<tr>
<td>Midgut</td>
<td>158.547</td>
<td>220.671</td>
<td>265.811</td>
<td>284.584</td>
</tr>
<tr>
<td>Hindgut</td>
<td>110.137</td>
<td>146.309</td>
<td>142.629</td>
<td>148.964</td>
</tr>
</tbody>
</table>

*significantly higher than the control (P<0.05)

**significantly higher than the other groups (P<0.05)

The mechanism of action on growth enhancement by AC-supplemented diet is perhaps explained by the adsorption of ammonia and nitrogen. Similarly, the improvement in intestinal function is likely through the elimination of toxins and impurities from the gastrointestinal tract (Mekbungiwan et al., 2004; Van et al., 2006). AC can also be expected to have the potential to condition intestinal cell membranes, reduce surface tension by eliminating gases and toxins or noxious substances along the intestine, and consequently improve the utilization and absorption of nutrients across the cell membranes (Thu et al., 2010). Van et al. (2006) revealed that apparent dry matter and protein digestibility were significantly increased by dietary supplementation of AC in goats. Although the mechanism of AC in fish digestion is unknown, the results of our study indicate that dietary AC supplementation may have some positive effects on the functions of digestion and absorption of fish.

Although the difference in growth performance parameters between the 2% and 3% AC-supplemented diet groups was not statistically significant (P>0.05), the 3% AC-supplemented diet group showed lower weight gain, specific growth rate and higher feed conversion ratio than the 2% AC group. These results also suggest an interaction between the nutrients and the powerful non-selective adsorbent of AC in the feed, such that there is a temporary loss of the nutrients and the beneficial substances due to non-selective adsorption in the feed with the higher level of AC supplementation (Mikhalkovsky and Nikolaev, 2006). However, there have been no reports on orally administered AC associated with depletion of nutrients or other useful substances.

Furthermore, the tilapia fed on 1%, 2% and 3% AC-supplemented diet revealed longer villus height and greater weight gain than the control group. The AC-supplemented diet might elevate the
absorptive function of intestinal villi and epithelial cells in the foregut and midgut of the intestine. According to a previous study that used 3% CWVC (dietary charcoal powder including wood vinegar compound liquid) in piglet diet, it was speculated that the CWVC might activate the intestinal function both at villus and cellular levels, thus improving feeding efficiency (Mekbungwan et al., 2004).

Longer villi 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. Furthermore, shorter villi result in a reduction in the villus surface area and have been associated with decreased body weight (Zijlstra et al., 1997). Short villi are also associated with a reduction in the activity of digestive enzymes such as mucosal lactase, sucrose, alkaline phosphatase and disaccharidase, and also with a reduction in the total lactase phlorizin hydrolyase enzyme activity and mucosal protein concentration (Yamauchi, 2000).

Together, our data show that the supplementation of AC in feed can enhance the growth performance and intestinal function of Nile tilapia. The supplementation of the diet with 2% AC was found to be the most suitable for improving the growth performance and intestinal morphology in tilapia reared in fiber-tank condition. However, further studies to determine the mechanism by which AC-supplementation appears to be preventing the absorption of harmful chemicals in aqua feed will be undertaken at the 2% of supplementation level.

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References
Mikhalsky SV and Nikolev VG 2006. Activated carbons as medical adsorbents. Interface Science and Technology 7: 529-561
Mikhalsky SV and Nikolev VG 2006. Activated carbons as medical adsorbents. Interface Science and Technology 7: 529-561


บทคัดย่อ

ประสิทธิภาพของอาหารเสริมผงถ่านต่อการเจริญเติบโตและสัณฐานวิทยาของลำไส้ปลานิล

บทคัดย่อ

ประสิทธิภาพของอาหารเสริมผงถ่านต่อการเจริญเติบโตและสัณฐานวิทยาของลำไส้ปลานิล แบ่งการทดลองออกเป็น 4 กลุ่ม ได้แก่ กลุ่มควบคุมอาหารปกติและกลุ่มได้รับอาหารเสริมผงถ่าน 1% 2% และ 3% ต่อน้ำหนักอาหาร ตามลำดับ ทดลองให้อาหารประมาณ 3% ต่อน้ำหนักตัว 2 ครั้งในเวลา 30 วัน ทำการวัดการเจริญเติบโตและความสูงของวิลไลในลำไส้ การทดลองพบว่า ปลาในกลุ่มที่เสริมผงถ่านที่ระดับ 2% ในอาหารให้ผลการเจริญเติบโตที่สูงขึ้นอย่างมีนัยสำคัญทางสถิติ ทั้งเบอร์ลิ่มและน้ำหนักที่เพิ่มขึ้น ยิ่งตามการเจริญเติบโตและอัตราการเปลี่ยนน้ำหนักลำไส้ส่วนต้นและลำไส้ส่วนกลางของปลานิลในกลุ่มที่เสริมผงถ่านที่ระดับ 2% และ 3% แตกต่างกันขึ้นอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุม จากผลการทดลองสามารถสรุปได้ว่า การเสริมผงถ่านในอาหารที่ระดับความเข้มข้น 2% ให้ผลเหมาะสมที่สุดในการเพิ่มอัตราการเจริญเติบโต และความสูงของวิลไลในลำไส้ปลานิล

ค่าสำคัญ: ผงถ่าน การเจริญเติบโต วิลไลของลำไส้ปลานิล

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