EFFECT OF SUPPLEMENTING BROILER DIET WITH ANISE AND THYME ESSENTIAL OILS ON HISTOLOGICAL CHANGES OF SMALL INTESTINE

By
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ABSTRACT: An experiment was conducted to study the effect of essential oils supplementation to broilers diet on small intestine histological changes. One hundred and twenty eight day-old (Cobb) broiler chicks were allocated randomly to four dietary treatments from 1-38 days of age, with two replicate pens (16 bird/pen). The experimental diets were as follows; control (T1), 300 mg/kg diet anise essential oil/kg diet (T2), 300 mg thyme essential oil/kg diet (T3) and 200 mg/kg diet anise and 200 mg/kg diet thyme essential oil mixture/kg diet (EOM)(T4). Three birds were isolated randomly from each treatment at 42 days and anesthetized by using chloroform inhalation in closed chambers and then the necropsy were applied to remove the small intestine to study the histological changes. The results indicated that feed conversion ratio were significantly (p>0.05) better in all supplemented groups. Duodenal wall and mucosal layer thickness were significantly (p>0.05) higher in (T4) while dietary supplementation of essential oil had no effect on jejunum wall and mucosal layer thickness. Supplementing 300 mg/kg diet anise oil (T2) resulted in significantly (p<0.05) increase in ileum wall and mucosal layer thickness. It could be concluded that supplementation of anise, thyme essential oils and their mixture in broiler diets improved the overall function and efficiency of small intestine. The improvement could be related to histological changes of small intestine.

INTRODUCTION

A wide range of spices, herbs, and their extract are known from medicine to exert beneficial action within the digestive tract (Chrubasik et al., 2005). Furthermore, stimulation of digestive secretion, bile and mucus, as well as enhanced enzyme activity are documented to be a core mode of nutritional action (Platel and Srinivasan, 2004). Similarly, essential oils used as feed additives for broiler were shown to enhance activities of trypsin and amylase (Lee et al., 2003). Glucose absorption from the intestine was accelerated in rats fed anise oil (Kreydiyyehet. al., 2003). Phytogenic feed additives were also reported to stimulate intestinal mucous secretin in broilers, an effect which was assumed to impair adhesion of pathogens and thus contribute to stabilize the microbial eubiosis in the animals gut (Jamrozet et al., 2006). These observations support the hypothesis that phytogenic feed additives favorably affect gut functions, but studies on poultry is not comprehended.

The antimicrobial mode of action is considered to arise mainly from the potential of the hydrophobic essential oils to intrude into the bacterial cell membrane, disintegrate membrane structure, and cause ion leakage. The beneficial effects of thyme essential oil on live performance of broiler have been shown in experiments either alone or in combination with other essential
oils (Denli et al., 2004; Halle et al., 2004 and AL-Mashhadani et al., 2011). There is limited data of the beneficial effect of essential oils from thyme on microbial load as well as of specific pathogen (Salmonella) on broiler carcasses (Aksit et al., 2006). Recent studies on poultry and swine indicated stabilizing effects of phytogenic feed additive on the ecosystem of gastrointestinal micro biota. Kroismayr et al., (2006) compared blend of essential oils from oregano, anise and citrus peel with an antibiotic growth promoter and reported a decrease in microbial activity in the terminal ileum, cecum and colon for both feed additives. Comparable observation for herbal essential oils were found also in other studies with pigs and broilers (Manzanilla et al., 2004; Mitsch et al., 2004; Namkung et al., 2004; Jamroz et al., 2005 and Castillo et al., 2006). However, limited researches has been performed on plant extracts including anise essential oils alone or in combination with thyme essential oils. Combining strategies may sometimes prove more beneficial than individual supplementation of feed additives. In broiler chicken (Zhang et al., 2005) speculated that, essential oil-organic acid blend proved to be promising alternative to antibiotic growth promoters.

The Mickel's diverticulum is often used as a landmark to separate the jejunum and ileum (Cervantes, 2006; Dorman and Deans, 2000).

Intestinal wall histological structure consists of seven layers: mucous membrane, tunica propria, muscularis mucosa, sub mucosa, circular and longitudinal layers of muscularis extern, and, serosa. The mucosa of small intestine forms villi which project into the lumen and greatly increase the overall absorption surface area of the organ. The epithelium of the villi is small columnar with numerous goblet cells. Intestinal absorptive cells have extensive microvilli on its apical surface. Goblet cells are scattered between the absorptive cells and produce the mucous.

Intestinal glands (Crypts of lieberkuhn) extend from the base of the villi into the underlying lamina properia. Undifferentiated epithelial cells that located in the glands divide and migrate up to renew the glandular and surface epithelium every 24-48 hr. Acidophilic granular cells (paneth cells) are present in the epithelium at the base of the gland, these cells produce peptidase and lysozyme and may be phagocytic . Enteroendocrine cells are also present in the epithelium of the intestinal gland. Tunica submucosa is very thin in chickens with absence of bronner gland, tunica muscularis is characterized by having two layers of smooth muscle, the inner circular are surrounded by an outer longitudinal folds .Mysentric plexus are often present between muscle layers. Atypical tunica serosa lies outside the tunica muscularis as the outermost layer of the organ (Ann, 2004; Elizabeth and Fredric, 2001).

In comparative of small intestinal villi between white layer hens and broiler chicken, the broiler have largervilli and more matured ultrastructure in the epithelial cells than those in white layer (Yamauchi, 2002a,b,2001; Yamauchi and Tarachai, 2000 ). The villi from both types of chickens form zig-zag arrangement which is thought to slow ingesta flow (Yamauchi and Isshiki, 1991; Altken, 1960).

The aim of the present trial was to study the effect of anise and thyme essential oils and their mixture on the wall thickness of duodenum, jejunum and ileum.

MATERIALS AND METHODS

This study was conducted at the Poultry Farms Animal Resources Department, University of Baghdad, College of Agriculture, from October 22 to November 30, 2010, to study the histological effect of inclusion of anise and thyme essential oils and their mixture (EOM) on broiler intestinal wall thickness. One hundred and twenty eight day-old
(Cobb) broiler chicks were allocated randomly utilizing a complete randomize Design (CRD) to four dietary treatments from 1-38 days of age, with two replicate pens (16 birds/pen). The experimental diets were as follows control (T1), 300 mg/kg diet anise essential oil/kg diet (T2), 300 mg/kg thyme essential oil/kg diet (T3) and 200 mg/kg diet anise and 200 mg/kg diet thyme essential oil mixture (EOM) (T4). The experimental diets were formulated to be isocaloric and isonitrogenic according to NRC (1994). Essential oils was dissolved in vegetable oil and then gently mixed with the standard diets. The diets were prepared freshly each week from 0-28 days (starter) and from 29-38 days of age were prepared twice a week. The experimental diets contained essential oils either derived from anise (Pipinellaanisum L.), Thyme (Thymus vulgaris) and the EOM contained two different essential oils derived from anise and thyme. Feed and water were provided ad libitum.

The birds were kept in floor pens (1.2 x 1.2m) broiler house containing wood shaving as litter material. Live body weight, weight gain, feed intake and feed conversion ratio (g. feed/g. gain) were measured at 14, 28 and 38 days of age.

Histological criteria, at the end of the experiment, 3 experimental birds were isolated randomly from each replicate at 42 day and anesthetized by using chloroform inhalation in closed chammers and then the necropsy were applied to removed the small intestine. The samples were immediately fixed by formalin (10%) for 24 hours. A standard dehydration and rehydration changes were follows using xylol as clearing agent and paraffin wax for embedding. Tissue blocks were trimmed and sectioned by the rotary microtome at 5µm. Tissue sections were stained by Hematoxylin and Eosin stains (Luna, 1968). An ocular micrometer was used to measure the thickness of the intestinal wall layers (Bancroft and Cook, 1984; Crossmon, 1937).

Data were subjected to analysis of variance (SAS, 2001) and significant treatment means were separated by Duncan’s multiple range tests(1955).

RESULTS AND DISCUSSION:

The effect of anise, thyme and their mixture (EOM) essential oils on feed conversion (g. feed/g. gain) is presented in Table 1. There were no significant differences (P>0.05) in feed conversion during the periods from 0 to 14, 14 to 28 and 28 to 38 days of age. While the overall (0-38 days) feed conversion was significantly (P<0.05) better in oils supplemented groups (T2, T3 and T4) as compared to the control (T1). Table 2 compares duodenum wall thickness among all treatments. Chicks in T4 had significantly (P<0.05) higher thickness in duodenum wall than other treatments. While there were no significant differences in jejunum wall thickness among treatments as shown in table 3. Data in table 4 show that the T2 had significantly (P<0.05) higher ileum wall thickness compared with T1, T3 and T4.

The effect of anise, thyme essential oils and their mixture (EOM) supplementation mucosal layer thickness in duodenum, jejunum and ileum were shown in tables (5, 6 and 7), respectively. Chicks in T4 had significantly (P<0.05) higher thickness of duodenal mucosal layer thickness than the other treatment groups (table 5). Table(6) shows that dietary inclusion of essential oils had no effect on jejunum mucosal layer thickness. Ileum mucosal layer thickness was significantly (P<0.05) increased in T2 as compared with T1, T3 and T4(table 7).
Table (1): Effect of anise, thyme essential oils and their mixture on feed conversion (g. feed/g. gain) of broiler chicken.

<table>
<thead>
<tr>
<th>Periods</th>
<th>Control</th>
<th>Essential oils mg/kg diet</th>
<th>Level of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>0-14</td>
<td>1.62±0.02</td>
<td>1.59±0.00</td>
<td>1.53±0.04</td>
</tr>
<tr>
<td>14-28</td>
<td>1.60±0.00</td>
<td>1.57±0.02</td>
<td>1.40±0.13</td>
</tr>
<tr>
<td>28-38</td>
<td>1.85±0.09</td>
<td>1.59±0.09</td>
<td>1.41±0.19</td>
</tr>
<tr>
<td>0-38</td>
<td>1.69±0.02 a</td>
<td>1.58±0.02 b</td>
<td>1.45±0.09 b</td>
</tr>
</tbody>
</table>

a,b, : Means in the same raw with different superscript are significantly different.
*(P<0.05).
Mean±Std. Error
T1: control(0%); T2:(300 mg/kg diet anise oil); T3:(300 mg/kg diet thyme oil) and T4:(200 mg/kg diet anise oil + 200 mg/kg diet thyme oil)
N.S.: not significant

Table (2): The comparison of duodenal wall layers thickness (µm) of broiler chicks at 42 day-old.

<table>
<thead>
<tr>
<th>Wall layers</th>
<th>Control T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>Level of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa</td>
<td>117.33±1.42 b</td>
<td>149.63±2.37 b</td>
<td>174.65±10.30 b</td>
<td>260.05±47.25 a</td>
<td>*</td>
</tr>
<tr>
<td>Sub mucosa</td>
<td>0.95±0.00</td>
<td>1.90±0.00</td>
<td>1.42±0.47</td>
<td>1.90±0.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>Muscularis</td>
<td>14.25±0.95 bc</td>
<td>11.87±0.47 c</td>
<td>24.22±0.47 a</td>
<td>19.95±2.85 ab</td>
<td>**</td>
</tr>
<tr>
<td>Serosa</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total wall thickness</td>
<td>134.43±0.47 b</td>
<td>165.30±2.85 b</td>
<td>202.20±10.30 ab</td>
<td>283.80±44.40 a</td>
<td>**</td>
</tr>
</tbody>
</table>

a,b,c, : Means in the same raw with different superscript are significantly different.
*(P<0.05).
Mean±Std. Error
T1: control(0%); T2:(300 mg/kg diet anise oil); T3:(300 mg/kg diet thyme oil) and T4:(200 mg/kg diet anise oil + 200 mg/kg diet thyme oil)
N.S.: not significant
Diet, anise, thyme, small intestine, microscopic study

Table (3): The comparison of jejunum wall layers thickness (µm) of broiler chicks at 42 day-old.

<table>
<thead>
<tr>
<th>Wall layers</th>
<th>Control T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>Level of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa</td>
<td>107.35±2.85</td>
<td>96.43±4.27</td>
<td>108.78±15.67</td>
<td>121.60±0.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>Sub mucosa</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>1.42±0.47</td>
<td>N.S.</td>
</tr>
<tr>
<td>Muscularis</td>
<td>8.55±0.00  c</td>
<td>9.02±0.47 c</td>
<td>19.47±0.47 a</td>
<td>15.67±1.42 b</td>
<td>**</td>
</tr>
<tr>
<td>Serosa</td>
<td>1.900.00</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total wall thickness</td>
<td>119.70±2.85</td>
<td>109.25±4.75</td>
<td>132.05±15.20</td>
<td>140.60±1.90</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

a,b,c, : Means in the same row with different superscript are significantly different.

*(P<0.05).

Mean±Std. Error
T1: control(0%); T2:(300 mg/kg diet anise oil); T3:(300 mg/kg diet thyme oil) and T4:(200 mg/kg diet anise oil + 200 mg/kg diet thyme oil)
N.S.: not significant

Table (4): The comparison of ileum wall layers thickness (µm) in of broiler chicks at 42 day-old.

<table>
<thead>
<tr>
<th>Wall layers</th>
<th>Control T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>Level of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa</td>
<td>44.65±0.95 c</td>
<td>57.95±0.00 a</td>
<td>50.35±1.90 b</td>
<td>43.70±1.90 c</td>
<td>**</td>
</tr>
<tr>
<td>Sub mucosa</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>Muscularis</td>
<td>11.87±0.47</td>
<td>10.45±2.85</td>
<td>11.40±0.95</td>
<td>14.72±4.27</td>
<td>N.S.</td>
</tr>
<tr>
<td>Serosa</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>1.90±0.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total wall thickness</td>
<td>60.32±1.42 b</td>
<td>72.20±2.85 a</td>
<td>65.55±2.85 b</td>
<td>62.22±2.37 b</td>
<td>*</td>
</tr>
</tbody>
</table>

a,b,c, : Means in the same row with different superscript are significantly different.

*(P<0.05).

Mean±Std. Error
T1: control(0%); T2:(300 mg/kg diet anise oil); T3:(300 mg/kg diet thyme oil) and T4:(200 mg/kg diet anise oil + 200 mg/kg diet thyme oil)
N.S.: not significant
Table (5): The effect of Anise, Thyme essential oils and their mixture (EOM) on duodenum mucosal layers thickness (µm) of broiler chicks at 42 day-old.

<table>
<thead>
<tr>
<th>Mucosal layer parts</th>
<th>Control T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>Level of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villi length</td>
<td>104.98±1.42 b</td>
<td>136.80±0.95 ab</td>
<td>152.95±8.55 ab</td>
<td>215.65±40.85 a</td>
<td>*</td>
</tr>
<tr>
<td>Crypts of lieberkuhn</td>
<td>11.40±0.00 c</td>
<td>11.87±1.42 c</td>
<td>20.90±1.90 b</td>
<td>38.95±1.90 a</td>
<td>**</td>
</tr>
<tr>
<td>Muscularis mucosa</td>
<td>0.95±0.00</td>
<td>0.95±0.00</td>
<td>0.95±0.00</td>
<td>0.95±0.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total mucosa thickness</td>
<td>117.33±1.42 b</td>
<td>151.13±0.87 b</td>
<td>174.80±10.45 ab</td>
<td>255.55±42.75 a</td>
<td>**</td>
</tr>
</tbody>
</table>

a,b,c,: Means in the same raw with different superscript are significantly different.
*(P<0.05).
Mean±Std. Error
T1: control(0%); T2:(300 mg/kg diet anise oil); T3:(300 mg/kg diet thyme oil) and T4:(200 mg/kg diet anise oil + 200 mg/kg diet thyme oil)
N.S.: not significant

Table (6): The effect of Anise, Thyme essential oils and their mixture (EOM) on jejunum mucosal layers thickness (µm) of broiler chicks at 42 day-old.

<table>
<thead>
<tr>
<th>Mucosal layer parts</th>
<th>Control T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>Level of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villi length</td>
<td>97.37±4.27 a</td>
<td>70.77±5.22 b</td>
<td>88.82±9.97 ab</td>
<td>101.17±1.42 a</td>
<td>*</td>
</tr>
<tr>
<td>Crypts of lieberkuhn</td>
<td>9.02±1.42 b</td>
<td>24.70±0.95 a</td>
<td>19.00±5.70 ab</td>
<td>19.47±1.42 ab</td>
<td>*</td>
</tr>
<tr>
<td>Muscularis mucosa</td>
<td>0.95±0.00</td>
<td>0.95±0.00</td>
<td>0.95±0.00</td>
<td>0.95±0.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total mucosa thickness</td>
<td>107.35±2.85</td>
<td>96.43±4.27</td>
<td>108.78±15.67</td>
<td>121.60±0.00</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

a,b,: Means in the same raw with different superscript are significantly different.
*(P<0.05).
Mean±Std. Error
T1: control(0%); T2:(300 mg/kg diet anise oil); T3:(300 mg/kg diet thyme oil) and T4:(200 mg/kg diet anise oil + 200 mg/kg diet thyme oil)
N.S.: not significant
Table (7): The effect of Anise, Thyme essential oils and their mixture (EOM) on ileum mucosal layers thickness (µm) of broiler chicks at 42 day-old.

<table>
<thead>
<tr>
<th>Mucosal layer parts</th>
<th>Control T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>Level of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villi length</td>
<td>38.95±0.00 a</td>
<td>44.65±0.95 a</td>
<td>39.42±1.42 a</td>
<td>32.30±2.85 b</td>
<td>**</td>
</tr>
<tr>
<td>Crypts of lieberkuhn</td>
<td>4.75±0.95 b</td>
<td>12.35±0.95 a</td>
<td>9.97±0.47 a</td>
<td>10.45±0.95 a</td>
<td>**</td>
</tr>
<tr>
<td>Muscularis mucosa</td>
<td>0.95±0.00</td>
<td>0.95±0.00</td>
<td>0.95±0.00</td>
<td>0.95±0.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total mucosa thickness</td>
<td>44.65±0.95 c</td>
<td>57.95±0.00 a</td>
<td>50.351.90 b</td>
<td>43.70±1.90 c</td>
<td>**</td>
</tr>
</tbody>
</table>

a,b,c,: Means in the same raw with different superscript are significantly different. 
*(P<0.05).
Mean±Std. Error
T1: control(0%); T2:(300 mg/kg diet anise oil); T3:(300 mg/kg diet thyme oil) and T4:(200 mg/kg diet anise oil + 200 mg/kg diet thyme oil)
N.S.: not significant

The positive improvement in average gain and feed conversion ratio in the treated groups may be related to anise and thyme oil active compound such as anathol and eugenol in anise oil which increase body weight by destroying of the pathogen microorganism in the digestive system, increasing production of digestive enzyme, improving utilization of digestion of digestive products and enhancing liver function (Langhout, 2000; Williams and Losa, 2001). In the present study the improvement in body weight, average gain and feed conversion in birds fed dietary anise, thyme essential oils and EOM (P<0.05) as compared to others fed the control group (T1) could be due to those positive effects of anise, thyme and EOM on digestive system. As shown by Hernandez et al., (2004) who reported that a supplementation of essential oil extract from oregano, cinnamon and pepper improved apparent whole tract and ileum digestibility of nutrients in broilers. Additionally, Ertaset al., (2005) reported that the addition of essential oils mix (Oregano, clove and anise) to the diet improved body weight gain, feed intake and feed conversion ratio in broilers. In addition, the improvement of broiler performance in this study may be due to the active ingredient in anise (anathole) and thyme (Listerine) which have antifungal, antimicrobial action.

Obtained results are in agreement with (Mass, 1974; Miller, 1975; Langhout et al., 1999; Yasar and Forbes, 1999) who showed that the poultry innards are affected by diet. Yamauchi and Zhou (1988) believes that the feeding habits rather than individual body weight difference account for gross anatomical difference in the intestine. Furthermore, these report suggest that, the nutritional value of diet may produce microscopic alteration in the intestinal mucosa although the general histological feature of the intestine are well known.

The results show the increase in the intestinal wall thickness indicating that the intestine is highly activate in digestion and absorption function and may lead to
improve of feed conversion ratio (Al-Tememy et al., 2011). Also the study proved that the duodenum was the mainly part of small intestine in digestive and absorptive function because the duodenum had more wall thickness compared with other parts of small intestine and it fallowed by jejunum then ileum in this respect. These results were agree with (Yamauchi et al., 1995, 1990) who suggest that the duodenum has the highest villi length followed by jejunum then ileum, the results were suggest that the vigorous absorptive part would be mainly the duodenum and then extend to the jejunum and ileum.

Differentiation of the intestinal wall thickness resulted mainly from difference in thickness of mucosal layer because presence of villi and intestinal glands (Crypts of lieberkuhn). Increasing of villi length increased the absorptive area. While the increasing in crypts depth lead to more activity in degeneration of absorptive epithelial cells which covered the villi, and more active in releasing the digestive enzymes. The villus morphological feature correspond with increase feed intake and rapid growth rate of broiler suggesting possibility of intestinal villus histological alterations related with intestinal function (Yamauchi and Isshiki, 1991; Ziswiler and Farner, 1972). The study agree with (William and Linda, 2000) who suggested that the villus were larger in duodenum but gradually shorten and thicken caudally. The ileal villi are shorter (Yamuchi and Isshiki, 1991; Yamauchi et al., 1993) and lower (Yamuchiet al., 1995, 1996) than those of the duodenum and this indicate that the absorptive function of ileal villi were less active than that of intestinal proximal parts, this may be due to fact that nutrient have already been absorbed by the time intestinal contents reach the intestinal proximal parts (Yamauchi, 2002).

The present study shows that the sub- mucosal layer in the wall of small intestine were lacking activity in birds due to absence of Brunner glands compared with mammals. That agree with (William and Linda, 2000) who suggest that the wall of intestine of the chicken was similar to that of the mammals but the absence of duodenal glands and the thin sub mucosa in the chicken are noticeable. The results of this study indicated that the inclusion of different levels of anise and thyme in broiler diet, resulted in increase of duodenum wall thickness in T3 and T4 groups, farther more, jejunum wall was thicker in T4 and T3 groups, while ileum wall thickness was higher in T2, T3 than the control and T4.

**REFERENCES**


Diet, anise, thyme, small intestine, microscopic study


Langhout D.J, J.B. Schutte, P. Vanleerwen, J. Wiebenga and S. Tamminga, 1999. Effect of dietary high and low methylated citrus pectin on the activity of the...

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**Yamauchi K. and Isshiki ,1991.** Scanning electron microscopic observation on the intestinal villi in growing White leghorn and Broiler chickens from 1 to 30 days of age. Br.Poult.Sci. 32:67-78.


