Effect of Garlic Supplementation to Diet on Performance and Intestinal Morphology of Broiler Chickens under High Stocking Density

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Abstract
This study was designed to evaluate the impact of garlic powder (GAR) on performance and intestinal morphology when chicken were subjected to different stocking densities. A total 100 one-day old Cobb 500 male chicks were housed in cages as 10 birds/m² and 15 birds/m². The chicks received feed as (i) basal diet + 0 g/kg garlic powder and (ii) basal diet + 5 g/kg garlic powder supplementation from 1-42d. At the end of experiment, ten chicks per treatment randomly selected to collect duodenal samples. The results showed significant improvement in body weight and villi length when diet supplemented by GAR. High stocking density (HD) had negative impact on growth performance and villi length in basal diet, but chicken supplemented by GAR diet did not affect by HD in the both parameters. In conclusion, diet supplemented by GAR can improve performance and villi length when the chickens were subjected to HD condition.

Introduction
Over the past two decades, the poultry meat market has faced with high number of customers because of price and healthy meat compare to other meat products (Taha, 2003). Therefore, the market needs to present more products to customers than before in the meat market. In order to produce more products, producers attempts to increase the number of chicken per square meter. In spite of the some positive points of raring of chickens in high number per square meter, some negative points could affect the chicken performance such as ascites and mortality (Bessei, 2006). Stocking density is also undesirable in term of animal welfare and negative impacts of this factor can be directly related to the chicken performance (Feddes et al., 2002). Although,
the poultry market still prefer to have high numbers of chicken compare to optimum number per square meters. In poultry industry is common to supplement diet by antibiotic to eliminate negative impacts of stressful conditions (Joerger, 2003). Therefore, it necessarily needs to investigate other alternatives to reduce negative impacts of high stocking density (HD). HD has considered as one of the major factor that can directly or indirectly affect the chicken performance. It can increase competition among the chicken to access to feed and water and the most importantly causes heat stress (Imaeda, 2000). Moreover, it can negatively affect the performance during the rearing period (Bessei, 2006; Feddes et al., 2002). Garlic (Allium sativum), (GAR) is a species in the onion genus and has been used for many years as food flavour and medicine (Marilynn, 2001; Sallam et al., 2004). The benefits of garlic might be related to some active compounds such as thiosulfimates (Lawson and Wang, 2001) which has Allicin. Allicin has antibacterial, antifungal and antiviral activity in body (Salama et al., 2014). Moreover, the active compound in garlic has antioxidant activities and hypocholesterolemic effects (Lewis et al., 2003). Moreover, there are many other benefits have mentioned that garlic has in the body such as lower serum cholesterol (Choi et al., 2010; Rahimi et al., 2011), decrease egg yolk cholesterol level (Kocaoglu-Gucu et al., 2004), prevent Escherichia coli growth (Cavalitto and Bailey, 1944; Rahimi et al., 2011) and improve growth performance (Onibi et al., 2009; Tollba and Hassan, 2003). Moreover, it has positive impact on intestinal morphology which can be related to the chicken performance (Adimbmoradi et al., 2006). The present study was attempted to investigate the effect of garlic powder on growth performance and intestinal morphology of broiler chickens when were subjected to different stocking densities.

Materials and Methods

Birds, husbandry and housing

The project was undertaken under department of agriculture at University Putra Malaysia, on total of 100 one-day-old Cobb 500 male broiler chicks were obtained from the local hatchery. On d 1, the chickens were weighted and allocated to battery cages (0.7 m x 0.6m) as 10 birds/m² and 15birds/m² (in each treatment 20 birds as normal stocking density and 30 birds as high stocking density), (approximate temperature and humidity during the whole experiment were , 24°C to 35°C and 75 to 90%, respectively). Digital Thermohygrometer (Testo, Malaysia) was used to determine temperature and humidity of the environment. The cages walls were movable to change the space of cages when mortality occurred. Optimum number of chicken per square meter is related to climate condition and environment. The light was provided continuously during the experiment.

Experimental treatments

The project was designed as 2×2 factorial with or without GAR supplementation subjected to two different densities with 5 replicates for each density. The diets were as: (1) basal diet + 0 g/kg GAR powder, (2) basal diet + 5 g/kg GAR powder (0.5%) from 1-42 d. The reason of using 5 g/kg in this study was based on other studies results which showed this amount of GAR in diet can have positive effect on chicks performance (Canogullari et al., 2009; Gbenga et al., 2009) and less than this amount (0.25%) has no effect on chicken performance (Doley et al., 2009). GAR powder were prepared with raw fresh GAR bulb, dried in the sun for 10 days and grained to the smash form. In this study tried to use simple ways to dry garlics which is more functional in commercial projects. The diets prepared in mash form as starter from d 1 to d 21 and finisher from d 22 to d 42. GAR powder was added to the balanced starter and finisher diet. Feed and water were provided ad-libitum. The nutrition composition treatment is presented in Table 1.

Performance and feed consumption

On d 1 and d 42 chicks and feed weighted (during the six weeks) to measure the feed conversion ratio (FCR). The chicken weighted on arrival and at the end of the experiment to achieve total gain weight during the whole experiment. The mortality was recorded daily. Mortality date were analysed by chi-square test.

Intestinal morphology

The duodenal samples were fixed in 10% formalin and embedded horizontally in paraffin wax (Baddeley et al., 1986). For each treatment 20 slides were prepared by using 5 μm sections, stained by haematoxylin and eosin (H&E) to measure the villi length and crypt depth by a light microscope (Olympus) (Bancroft and Gamble, 2002).

Statistical analysis

All data were analysed by ANOVA using the GLM procedure of SAS (SAS Institute Inc., NC, USA). The data were analysed according to diet supplementation, stocking density, and interactions. In significant interactions, comparisons were made within each experimental variable. In significant effects, multiple means were modelled by Duncan’s multiple range test used for comparisons.
Table 1. Ingredients and nutrient matter composition diets used in this study.

<table>
<thead>
<tr>
<th>Diet</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Starter diet (1-3 weeks)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>61.20</td>
<td>64.50</td>
</tr>
<tr>
<td>Soybean meal (CP=33.9 %)</td>
<td>28.30</td>
<td>24.50</td>
</tr>
<tr>
<td>Fish meal(^1)</td>
<td>5.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Palm oil</td>
<td>2.00</td>
<td>3.50</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>Vitamin premix(^2)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Mineral premix(^3)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Nutrients Composition\(^3\)**

<table>
<thead>
<tr>
<th>Analyzed values of diets</th>
<th>Starter diet</th>
<th>Finisher diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME (kcal/kg)(^4)</td>
<td>3000</td>
<td>3000</td>
</tr>
<tr>
<td>CP(^5)</td>
<td>21.5</td>
<td>21.5</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.00</td>
<td>0.96</td>
</tr>
<tr>
<td>AVP(^6)</td>
<td>0.46</td>
<td>0.44</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.57</td>
<td>0.54</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.16</td>
<td>1.02</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.13</td>
<td>0.13</td>
</tr>
</tbody>
</table>

\(^1\)Provide sufficient protein to ensure the chicken performance will not affect by lacking of amino acids.
\(^2\)Supplied per kilogram: vitamin A: 4.500 IU; vitamin D3: 1000 IU; vitamin E, 50 mg; vitamin K, 1.5 mg; vitamin B12, 0.02 mg; vitamin B2, 3 mg; pantothenic acid, 5 mg; \(^3\)per kilogram of diet; zinc, 40 mg; iron, 80 mg; iodine, 80 mg; manganese, 60 mg; copper, 8 mg; selenium, 0.2; and cobalt, 0.4 mg.
\(^3\)Calculated based on NRC.
\(^4\)Metabolizable Energy
\(^5\)Crude Protein
\(^6\)Available Phosphorus

Results

Growth Performance

The present study results showed significant differences (P<0.05) between diet × density for weight gain (WG), feed intake (FI) and FCR at the end of the experiment (Table 2). HD had no significant effect (P>0.05) on WG when diet supplemented by GAR, but significantly (P<0.05) decreased for the basal diet. The number of mortality was not different between the diets, but the basal diet under HD showed higher rate of mortality.
Morphometric variables

The results indicated that chicken supplemented by GAR had positive impact on villi length (VL) compare to the basal diet in the both densities. The results of crypt depth (CD) did not show any significant differences between the diets, but the densities showed some differences (Table 3, Figure 1).

Table 3. Mean (±SEM) of villi length (VL) and crypt depth (CD) of chickens supplemented with or without garlic powder (GAR) reared two different densities.

<table>
<thead>
<tr>
<th>Density</th>
<th>Effect, P-value</th>
<th>VL (µm)</th>
<th>CD (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 birds/m²</td>
<td></td>
<td>BAS 1</td>
<td>GAR 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1005±67</td>
<td>1202±33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>15 birds/m²</td>
<td></td>
<td>132±11</td>
<td>147±12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0001</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* *±SEM within a row-subgroup with no common superscripts are different at P<0.05.

BAS: Basal diet.

Figure 1. Depth of crypt (CD) and length of villus (VL) are shown. GAR had positive impact on VL but not on CD (200× magnifications). (A) CD in basal diet; (B) VL in basal diet; (C) CD in chicken supplemented by GAR; (D) VL in chickens supplemented by GAR.

Şekil 1. Kript derinliği (CD) ve villus uzunluğu (VL) gösterilmiştir. GAR VL üzerine pozitif etkimiş ancak CD üzerine etkimiş değildir (CD (200× büyütmede). (A) Bazal diyetteki CD; (B) Bazal diyetteki VL; (C) Diyetlerine GAR ilavesi yapılan tavuklarda CD; (D) Diyetlerine GAR ilavesi yapılan tavuklarda VL.

Discussion

Garlic (GAR) has been used for several years as herbal medicine and flavour for food (Sallam et al., 2004) and consists of active compounds such as allicin that has positive biological properties in animal bodies (Chowdhury and Smith, 2002). Moreover, many positive effects about garlic have indicated in previous studies such as inhibit bacteria (E.coli) growth (Cavallito and Bailey, 1994) and improve gut and performance (Adibmoradi et al., 2006). According to our knowledge, this is a first work about chicken supplemented by garlic under high stocking density. high stocking density (HD) may adverse performance and feed efficiency (Dozier et al., 2005; Skrbic et al., 2009) and reduce the amount of products due to economic losses (Braun et al., 2010). High stocking density is considered as negative factor
which can cause heat stress and increase the rate of mortality (Bessei, 2006). The current results about weight gain (WG) are in agreement with (Adibmoradi et al., 2006; Khan et al., 2012; Pourali et al., 2010) that showed diet chicken supplemented by GAR has better performance than chicks without GAR supplementation. However, (Choi et al., 2010) showed that chicken supplemented by GAR powder did not show any differences with the basal diet. The reason of these differences in results may come back to used dosage of GAR (Canogullari et al., 2009; Doley et al., 2009; Gbenga et al., 2009). Moreover, longer villi length (VL) in chicken supplemented by GAR can confirm the data about the performance, because longer villus can increase the intestine surface and in follow increase absorption (Adibmoradi et al., 2006) (Table 3). The present data for the basal diet are in agreement and for GAR diet are in disagreement with a study that showed stressful conditions such as HD can impair intestinal morphology (Burkholder et al., 2008). This phenomenon can be justified by positive role of GAR on gut health which can decrease adverse effects of HD. In conclusion, under condition of HD in the present study, GAR supplementation showed better effect on growth performance and intestinal morphology of broiler chickens reared in battery cages.

Acknowledgement

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REFERENCES


