Effects of honeybee venom supplementation in drinking water on growth performance of broiler chickens

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ABSTRACT The effects of water supplementation of bee venom (BV) on performance, antioxidant activity, and liver function in Arbor Acres broiler chickens were investigated. Hence, 3 experimental treatment groups (control, 0.5 mg/L of BV, and 1 mg/L of BV) were allocated to 3 replicates of 5,000 one-day-old chicks each. The control group was kept on tap water, whereas the other 2 groups were supplied water supplemented with 0.5 and 1 mg of BV, respectively, per liter of drinking water. Broilers were provided ad libitum access to feed for the experimental period of 1 to 28 d of age. Supplementing drinking water with BV significantly increased BW gain at 28 d of age ($P < 0.05$). The average daily weight gain from d 1 to 28 was increased for birds supplemented with BV compared with control birds. The increase in BW gain was more pronounced with supplementation of 1 mg/L of BV compared with 0.5 mg/L of BV. An improved feed intake was noted in groups supplemented with BV as compared with control chicks. Liver function enzymes, aspartate aminotransferase, and alanine aminotransferase activities including total cholesterol, total protein, albumin, and globulin were not changed by BV supplementation. Tap water supplementation of BV did not alter the number of leukocytes, erythrocytes, heterophils, and lymphocytes. However, the antioxidative activities estimated as a superoxide dismutase-like activity of broiler chicks supplemented with BV was significantly increased ($P < 0.05$) in comparison with those without BV supplementation. These data indicate a possibility of better broiler performance through BV supplementation under conditions of severe stressful challenges the newly born chicks encounter.

Key words: bee venom, drinking water, broiler, performance, antioxidant activity

2010 Poultry Science 89:2396–2400
doi:10.3382/ps.2010-00915

INTRODUCTION

Antimicrobial agents have been used in agriculture including poultry for animal health. Controversy surrounds when large amounts of antimicrobial agents are given to food-producing livestock in subtherapeutic doses to stimulate the conversion of feed into body mass in the absence of diseases (Costa et al., 2010). This has led to the prevalence of resistance in animal bacteria and to be a risk factor for the emergence of antimicrobial resistance in human pathogens. Suspension of the use of some antimicrobial agents as growth promoters in animal feeds was followed by some regulatory authorities (Angulo et al., 2004).

Antimicrobial use in the poultry industry is mainly for the improvement of meat and egg production. Inevitably, it causes drug-resistant bacteria, drug residues in the body of the birds, and imbalance of normal microflora (Awad et al., 2009). As a consequence, emphasis has always been laid on the development of alternatives to reduce the potential negative effect of antimicrobial agents on performance. One such candidate is whole bee venom (**BV**). Bee venom comprises such peptides as melittin (the major active ingredient), apamin, adolapin, and mast-cell-degranulating peptide 401 (Lee

Received May 26, 2010.
Accepted July 17, 2010.
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et al., 2009). Several studies have shown BV to exert both an antiinflammatory effect, a property shared with nonsteroidal antiinflammatory drugs (Jang et al., 2003), and an antibacterial effect involving no side effects in animal models (Han et al., 2006). Furthermore apitherapy using live honeybee stings had therapeutic value for pigs with respiratory diseases such as atrophic rhinitis, pleuropneumonia, and Glasser's disease (Choi et al., 2003).

The effects of BV on BW gain of broilers in association to different blood parameters are not known. This is the first paper to present trials using BV as an alternative to antimicrobial growth promoter in the broiler production industry, which is highly sophisticated, concentrated, and with limited variation in breeds, nutrition, and production methods (Costa et al., 2010). This makes broiler flocks suitable for studying the effect of BV on growth performance. Therefore, the current study presents trials using BV, as a potential antimicrobial agent, to improve production parameters in broiler chickens and to provide an alternative to antimicrobial growth promoters.

MATERIALS AND METHODS

Honeybee Venom Collection and Analysis

Colonies of natural honey bees (*Apis mellifera* L.) used in this study were maintained at the National Institute of Agricultural Science and Technology, Suwon, Korea. Bee venom was collected by a BV collecting device (Chung-Jin Biotech Ltd., Ansan, Korea) in a sterile manner under strict laboratory conditions. In brief, the BV collector was placed on the hive, and the bees were given enough electric shock to cause them to sting a glass plate from which dried BV was later scraped off. The collected venom was diluted in cold sterile water and then centrifuged at 10,000 × *g* for 5 min at 4°C to discard residues from the supernatant. Bee venom was lyophilized by a freeze dryer and refrigerated at 4°C for later use. The methodology of BV analysis has been described previously (Han et al., 2006). Briefly, 100 mg of frozen and dried whole BV was dissolved in 0.1 *M* ammonium formate (pH 4.5). Particles were removed by centrifugation and filtration (0.2-μm membrane filter, Millipore, Billerica, MA) before sample application to a Sephadex TM200 column (AKTA Explorer, Pharmacia, Piscataway, NJ) equilibrated in 0.1 *M* ammonium formate (pH 4.5). Honeybee venom standard proteins were purchased commercially (Sigma-Aldrich, St Louis, MO). All fractions collected were examined for total protein, hyaluronidase, phospholipase A_2, melittin, and apamin. Protein concentration was determined by the Bradford method (BioRad, Hercules, CA). The purity of proteins and peptides was assessed by SDS-PAGE on 4 to 20% gradient tricine gels (Novex Tricine Gels, Invitrogen, Carlsbad, CA). Proteins and peptides were stained with Coomassie Blue R-250. Proteins from BV were separated by size exclusion gel chromatography (Pharmacia, Figure 1A). The verification of the main components of BV was confirmed by comparison with venom protein standards from Sigma (Figure 1B). Identification of each peak was performed by SDS-PAGE (Figure 1C). The comparison between peaks of BV standards and whole BV showed that all major venom proteins and peptides were found to be similar in terms of compositions, although with a slight difference in proportions.

Birds and Experimental Treatment

From commercial broiler chick hatcheries, located in Kyunggi Province, Republic of Korea, a total of 15,000 one-day-old broiler chicks (Arbor Acres) were used for this study from March through October 2009. The broilers were housed in chambers that contained identical-sized pens (20 × 80 m) fitted with wire mesh and side wall cloth curtains with a wood shavings floor. Environmental temperature was set at 33°C for the first week and 30°C for the second week, which was further decreased to 26°C until the end of the experiment. Relative humidity was set at 50% throughout the study. During the first week, the light regimen was 22L:2D, which was reduced to 20 h of light afterward. Due to facility locations and long study period, assignment of treatments was not completely randomized and environmental variables were kept constant for all chambers.

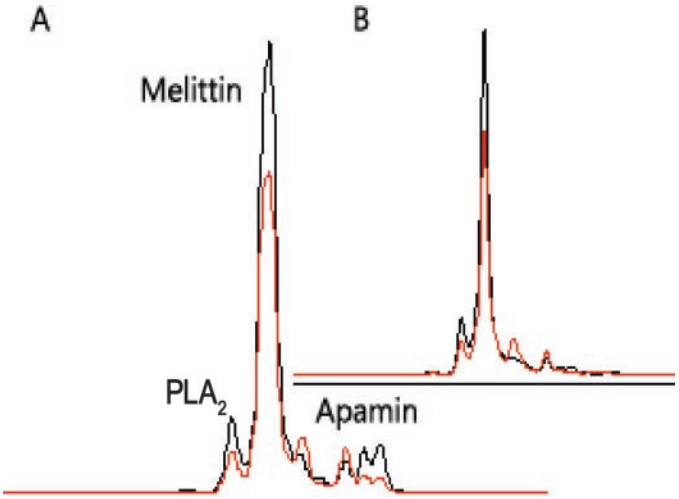
The birds were randomly divided into 3 groups (5,000 birds/group) and kept to 28 d of age. Each group had 3 replicates. Birds were fed ad libitum and diets contained no antimicrobial growth promoters. All experimental procedures used in the present study were approved by the Animal Care and Use Committee at the National Institute of Agricultural Science and Technology and conform to the US National Institutes of Health guidelines for the care and use of laboratory animals. The basal diet with ingredient composition is shown in Table 1. Water was supplemented with 0.5 and 1 mg of BV/L of drinking water for those chickens in the treatment groups (Table 2). The 2 time point methodology of BV supplementation was based on our preliminary study. Control birds received tap water throughout the experiment. Water not treated with chlorine was premixed with BV in the water tank and was delivered through a water line to a nozzle.

Growth Performance

Data on feed intake and BW gain were recorded on d 1 and 28, whereas mortality was recorded daily throughout the experimental period. Feed intake was corrected for mortality.

Determination of Blood Parameters

Blood sample was collected by brachial venipuncture at d 28 from 24 chicks of each group into an anticoag-



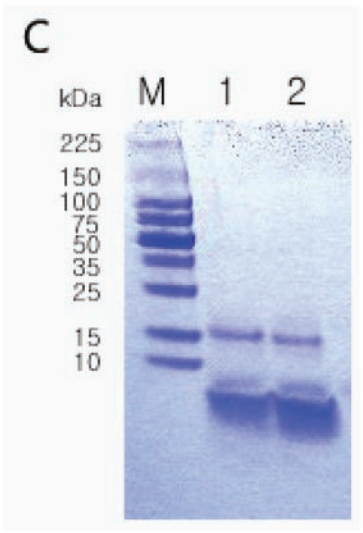


Figure 1. Gel filtration of 100 mg of frozen-dried whole bee venom on Sephadex TM200 10/300 (AKTA Explorer, Pharmacia, Piscataway, NJ). Elution with 0.1 *M* ammonium formate buffer (pH 4.5). Determination of main components was compared with standard proteins by optical density at 280 nm. A) Whole bee venom for current study. B) Sigma whole honeybee venom. C) Sodium dodecyl sulfate-PAGE on a 4 to 20% gradient gel that was stained with Coomassie Blue R-250; lane M = protein marker; lane 1 = Sigma whole honeybee venom; lane 2 = whole bee venom for current study. PLA_2 = phospholipase A_2. Color version available in the online PDF.

ulant-free vacuum tube (Vacutainer, BD Diagnostics, Sparks, MD) for the determination of clinical-chemical parameters as well as analysis of hematological blood counts. They were determined with an automated biochemical machine (Advia 1650, Bayer, Tarrytown, NY) and automated blood counting machine (Hamat 8, SEAC, Florence, Italy), respectively. Total cholesterol, aspartate aminotransferase, and alanine aminotransferase were measured enzymatically using a kit (Asan Pharmaceutical, Seoul, Korea; Omodeo et al., 1984).

Antioxidative Activity

The antioxidative activity was estimated as superoxide dismutase (**SOD**)-like activity. The analysis of SOD-like activity was based on the methodology of Marklund and Marklund (1974) with further modification. Blood was taken via brachial venipuncture at d 28 from 24 chicks of each group into anticoagulant K3 EDTA vacuum tubes (Vacutainer, BD Diagnostics). The reaction mixture contained 50 μL of Tris-HCl buffer and 7.2 m*M* pyrogallol, with or without sample. The mixed solution was incubated for 45 min at 25°C, which was followed by reading the absorbance at 405 nm against the blank. The SOD-like activity was calculated using the following equation: SOD like activity (%) = (1 − A/B) × 100, where A was the absorbance of sample and B the absorbance of the control.

Statistics

Data were subjected to Duncan's *t*-test using the SAS statistical package. In the tables, results are expressed as means ± SE. Throughout, $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The initial BW of chicks did not differ among treatments (Table 3). However, water treatment with BV resulted in significantly higher BW gain than in the control group ($P < 0.05$). The average daily weight gain from d 1 to 28 was increased (data not shown) for birds supplemented with BV compared with control birds. The increase in BW gain was more pronounced with supplementation of 1 ppm of BV compared with 0.5 ppm of BV, although it was not significant. But both BV groups were significantly higher than the control group at 28 d of age ($P < 0.05$). Feed intake was numerically higher, although not significantly higher, for birds supplemented with BV than control birds. A

Table 1. Ingredient composition of the basal diet

Item	Amount
Ingredient (%)	
Corn	49.05
Wheat middlings	15.00
Fish meal	14.22
Soybean meal	12.00
Poultry fat	5.42
Corn gluten meal	3.00
Limestone	0.62
Salt	0.40
Vitamin premix	0.24
Mineral premix	0.05
Composition estimated from basal diet	
ME (kcal/kg)	3,200
CP (g/kg)	230
Methionine + cysteine (g/kg)	0.87
Choline (g/kg)	1.22
Folic acid (mg/kg)	0.70

Table 2. Experimental scheme

	Period			
Treatment	1 to 7 d	8 to 14 d	15 to 21 d	22 to 28 d
Control	NT[1]	NT	NT	NT
0.5 mg/L of BV	0.1 mg/L of BV	NT	0.4 mg/L of BV	NT
1.0 mg/L of BV	0.2 mg/L of BV	NT	0.8 mg/L of BV	NT

[1]NT = treatment without antimicrobial agents and bee venom (BV).

positive correlation between BW gain and improved feed intake noted in our study was observed in other supplementation studies in drinking water (Celik et al., 2003; Ahmad et al., 2008). The mortality percentage was lower ($P < 0.05$) for the BV-supplemented group than the control group. These results indicate that BV supplementation promoted growth while showing a nonsignificant effect on mortality. This is in agreement with Han et al. (2009), who indicated a net increase in BW gain and survivability in piglets with BV injection. Pippia et al. (1989) reported that melittin promoted tumor growth in rat fibroblasts, whereas this alkaline polypeptide consisting of 26 amino acids was shown to have a fast and potent action of killing a variety of tumor cells (Ling et al., 2005). Further, melittin did not inhibit the growth and cloning efficiency of normal cells at a concentration that prevents the proliferation of tumor cells (Zhu et al., 1991). This difference in responsiveness suggests that different growth signaling pathways are triggered in histologically distinct tumor cell lines and normal cells (Son et al., 2007). In this regard, we hypothesized that melittin with the involvement of phospholipase A_2 might behave as a chemical promoter at the level of cellular membrane in this study because it induces membrane permeabilization by reorganizing phospholipid assemblies (Raghuraman and Chattopadhyay, 2007).

Furthermore, melittin does not injure the immune system (Lu et al., 1999), which must be another contributing factor for growth performance in a highly concentrated production environment in the broiler industry. It seems likely that the BV is potentiating an immune response to the normal environmental, social, and nutritional challenges the newly born chicks encounter. The increased stress with days after birth seemed counterbalanced with BV supplementation. Interestingly, heterophil:lymphocyte ratio did not differ between treatments. If BV reduced stress, a decrease in the ratio when the BV groups were compared with the control would be expected. However, it was not the case for our study. It might be related with other blood cell parameters because number of leukocytes and erythrocytes was not affected by BV supplementation (data not shown). At least it illustrates that BV supplementation did not cause stress to broilers. According to Post et al. (2003), the heterophil:lymphocyte ratio has proved to be a valuable tool in stress-related research in chickens.

No significant effects of BV supplementation were noticed on total cholesterol, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, and globulin. At the same time, no major organ damages were seen with BV supplementation via drinking water. Although globulin level was significantly elevated with high-dose BV treatment in piglets (Han et al., 2009), this was not the case for the present study, which suggests that BV requires a high dose to elicit any physiological effects. It could also explain why BV did not lower lipids in the current study. However, BV did decrease total cholesterol and triglyceride content in mice with atherosclerotic lesions when a physiologically optimal level of BV was administered (Lee et al., 2010). Moreover, the antioxidative activities of broiler chicks supplemented with BV significantly increased ($P < 0.05$) in comparison with those without BV supplementation (Table 4). Although our study did not investigate malondialdehyde level as an indicator of lipid peroxidation, the increased SOD-like activities indicate that BV plays a role in alleviating the adverse effect of stress-related oxidative activity. When lipid peroxidation was reversed with supplementation of ascorbic acid, levels of SOD and malondialdehyde were increased

Table 3. Effect of bee venom (BV) supplementation via drinking water on BW (g), daily BW gain (g), feed intake (g), and mortality rate (%) of broiler chicks[1]

	Treatment		
Item	Control (n = 90)	0.5 mg/L of BV (n = 90)	1.0 mg/L of BV (n = 90)
Initial BW at d 1	38 ± 0.3	38 ± 0.3	38 ± 0.6
Final BW at d 28	1,265 ± 22.7[b]	1,396 ± 4.9[a]	1,415 ± 5.4[a]
Feed intake	64.4 ± 1.6	66.5 ± 1.2	67.2 ± 1.6
Mortality	8.3[b]	4.7[a]	4.5[a]

[a,b]Within the same row, values with different superscripts are significantly different ($P < 0.05$).

[1]Data are expressed as means ± SE.

Table 4. Effect of bee venom (BV) supplementation via drinking water on superoxide dismutase (SOD)-like activity of broiler chicks[1]

Item	Treatment		
	Control (n = 24)	0.5 mg/L of BV (n = 24)	1.0 mg/L of BV (n = 24)
SOD-like activity	21.4 ± 1.1[b]	27.3 ± 0.5[a]	28.2 ± 1.3[a]

[a,b]Within the same row, values with different superscripts are significantly different ($P < 0.05$).

[1]Data are expressed as means ± SE.

and decreased, respectively, in broilers (Erdogan et al., 2005). Bee venom might influence the immune response through superoxide production.

In conclusion, BV supplementation via drinking water showed significant effects on overall performance of broilers during the early stage of life. This makes BV treatment interesting as an alternative to antimicrobial growth promoter. Furthermore, this method may be an alternative option to avoid frequent administration of antimicrobials.

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