Abstract: This study examined the effect of whole bee venom (BV) as a potential stimulant of the piglet immune system, on growth performance, blood parameters, plasma protein and immune globulin content of serum. Piglets (n=97) received combinations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/kg of parenterally administered BV on 4 occasions between birth and day 30. In the apipuncture group (n=31), piglets were acupunctured with the worker honeybee. Two acupoints, GV-1 (Jiao-chao) and GV-20 (Bai-hui), were selected for apipuncture. All piglets (n=128) in the treatment groups were treated 4 times throughout the study period of 60 days. The control piglets received no treatments. Blood was taken via jugular venipuncture on day 30 after birth. Body weight and survivability were measured, and changes in haematological values were analysed. Both the BV injection group and apipuncture group demonstrated 26.6 and 21.8%, and 7.9 and 6.7% increase compared with controls in body weight and survivability, respectively. The number of leukocytes, erythrocytes, lymphocytes and monocytes were not influenced by treatments. However, a potential clinical benefit of high dose therapy was seen in increased populations of leukocytes, lymphocytes and monocytes compared with either the apipuncture or control groups. Other blood parameters such as total protein and albumin were not affected by treatment. However, IgG levels were generally higher in treated groups than in controls. These findings indicate that BV might be useful to stimulate immuno-competence in pig production, possibly via the primary bioactive components of melittin, phospholipase A2 and apamin. The administration of BV, either via injection or acupuncture, did not make any differences to growth performance of young pigs. These results would be useful for further purification and characterization of immune boosting agents from BV.

Author Address:
pchenoweth@csu.edu.au  spak@csu.edu.au

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Effects of Bee Venom Treatment on Growth Performance of Young Pigs

Sang Mi Han, Ph.D., Kwang Gill Lee, Ph.D., Joo Hong Yeo, Ph.D., Sung Jin Hwang, Ph.D., Chul Ho Jang, M.D., Ph.D., Peter J. Chenoweth, Ph.D., and Sok Cheon Pak, Ph.D.

1Laboratory of Applied Material Science, Department of Agricultural Biology, National Institute of Agricultural Science and Technology, RDA, Suwon, 441-100, South Korea
2Department of Biology, College of Natural Sciences, Chonnam National University, Gwangju, South Korea
3Department of Otolaryngology, Chonnam National University Medical School, Gwangju, South Korea
4School of Agricultural and Veterinary Sciences, Charles Sturt University, Wagga Wagga, Australia
5School of Biomedical Sciences, Charles Sturt University, Bathurst, Australia

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Correspondence

Dr. Sok Cheon Pak
School of Biomedical Sciences
Charles Sturt University
Panorama Avenue
Bathurst NSW 2795
Australia
Tel: 61-2-6338-4952, Fax: 61-2-6338-4993, Email: spak@csu.edu.au
Abstract: This study examined the effect of whole been venom (BV) as a potential stimulant of the piglet immune system, on growth performance, blood parameters, plasma protein and immune globulin content of serum. Piglets (n=97) received combinations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/kg of parenterally administered BV on 4 occasions between birth and day 30. In the apipuncture group (n=31), piglets were acupunctured with the worker honeybee. Two acupoints, GV-1 (Jiao-chao) and GV-20 (Bai-hui), were selected for apipuncture. All piglets (n=128) in the treatment groups were treated 4 times throughout the study period of 60 days. The control piglets received no treatments. Blood was taken via jugular venipuncture on day 30 after birth. Body weight and survivability were measured, and changes in haematological values were analysed. Both the BV injection group and apipuncture group demonstrated 26.6 and 21.8%, and 7.9 and 6.7% increase compared with controls in body weight and survivability, respectively. The number of leukocytes, erythrocytes, lymphocytes and monocytes were not influenced by treatments. However, a potential clinical benefit of high dose therapy was seen in increased populations of leukocytes, lymphocytes and monocytes compared with either the apipuncture or control groups. Other blood parameters such as total protein and albumin were not affected by treatment. However, IgG levels were generally higher in treated groups than in controls. These findings indicate that BV might be useful to stimulate immuno-competence in pig production, possibly via the primary bioactive components of melittin, phospholipase A₂ and apamin. The administration of BV, either via injection or acupuncture, did not make any differences to growth performance of young pigs. These results would be useful for further purification and characterization of immune boosting agents from BV.

Keywords: Acupuncture, Apipuncture, Melittin, Phospholipase A₂, Immune system
**Introduction**

From a clinical perspective, the search for alternatives to heavy usage of antibiotics which could result in unexpected resistant strains of pathogens in animals is an important task. Therefore, emphasis has always been laid on high standards of animal husbandry management to reduce the potential negative impact of pathogens on performance. One of the strategies is to increase resistance to pathogenic causes of multifactorial health problems which can occur in livestock production systems by strengthening the animal’s immune function (Maass et al., 2005). In this context, attention has been paid to activating the paraspecific immune system (Maass et al., 2005). One of the stimulants of the paraimmune system is the whole bee venom (BV).

The use of BV as therapy has long been part of folklore. Bee venom contains a number of bioactive substances such as melittin, phospholipase A₂, apamin and adolapin (Lariviere and Melzack, 1996). Melittin as a major component of BV induces membrane permeabilization by reorganizing lipid assemblies which includes vesicularization of multibilayers, fusion of small lipid vesicles, fragmentation into discs and micelles (Raghuraman and Chattopadhyay, 2007). These peptides demonstrated a significant antinociceptive and anti-inflammatory effect (Kwon et al., 2001), antibacterial action (Perumal Samy et al., 2007) and immunity boosting effect (Tarzi et al., 2006). Additionally, microarray analysis has shown BV to have pharmacologic actions in the treatment of arthritis (Yin et al., 2005) and a photoprotective action by reducing protein levels of matrix metalloproteinases which are main contributors to photoaging processes (Han et al., 2007).

In oriental medicine, acupuncture is one of the most commonly used therapies to treat a number of human (Kundu and Berman, 2007) and animal (Lin and Rogers, 1980) ailments. Generally it is believed that acupuncture stimulates natural defence
systems via involvement of peripheral and central nervous systems including the
autonomic nervous system and neuroendocrine system (Lin and Rogers, 1980).
Acupoint stimulation is thought to send signals via specific peripheral sensory nerves
and ascending spinal tracts to specific integration control centres in the brain,
especially in the hypothalamus. This region contains control centres which regulate
the immune response (Lin and Rogers, 1980). In this regard activation of specific
hypothalamic regions would release neurotransmitters and neuro-humoral factors
which in turn would activate the local terminals of the descending neural controls.
These neuro-humoral factors appear to stimulate antibody and tissue immune
response. Apipuncture defined as bee venom acupuncture can be used for the relief of
pain in rats (Kwon et al., 2001; Kim et al., 2003), for controlling bacterial diarrhea in
preweaning piglets (Choi et al., 2003a) and for therapy in sows with hypogalactia
(Choi and Kang, 2001) or oligogalactia (Choi et al., 2003b).

Since the immune system at the cellular level is regulated by the stimulation of
mitosis of progenitor cells (Drochner et al., 2004), the different blood parameters
were used as indicators of BV efficiency in the present study. Therefore, the paper
presents trials using BV, as a potential stimulant of immune function, to improve
production parameters in pigs and provide an alternative to antibiotic growth
promoters.
Materials and Methods

Honeybee and Venom Collection

Colonies of natural honey bees (*Apis mellifera* L.) used for apipuncture in this study were maintained at the National Institute of Agricultural Science and Technology (NIAST), Suwon, Korea. Bee venom was collected by a bee venom collecting device (Chunglin, Korea) in a sterile manner under strict laboratory conditions. In brief, the bee venom 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 bee venom was later scraped off. The collected venom was diluted in cold sterile water and then centrifuged at 10,000g for 5 minutes at 4 °C to discard residues from the supernatant. Bee venom was lyophilized by freeze dryer and refrigerated at 4 °C for later use. All the bioactive components of bee venom used in the experiment were confirmed with size exclusion gel chromatography (AKTAexplorer, Pharmacia, USA) by dissolving in 0.1 M ammonium formate adjusted to pH 4.5 and comparing with standard proteins using a Sephadex TM200 column (Amersham Biosciences, USA). Mellitin, apamin and phospholipase A2 purified from bee venom were obtained commercially (Sigma Aldrich, St Louis, MO, USA).

Animals

To produce piglets for the present study, a total of fifteen healthy sows (German Landrace × Pietrain) were selected. As shown in Table 1, ninety seven piglets from nine sows were used for BV injection, 31 piglets from three sows for apipuncture and 34 piglets from three sows for the control. For objective and non-prejudiced data, piglets from the same litter could not be used for different treatments. The BV injection group was further divided into three groups to compare different
combinations of BV dosage (Table 1). Those piglets in the control received no treatments. The sows received a total mixed ration specially formulated to meet the nutrient requirements at each stage of pregnancy and lactation. Castration of male piglets was performed three days after birth according to routine industry practice. At 14 day of age, piglets were weaned, weighed and ear tagged. At 30 day of age they were moved into individual cages. They were housed with an ambient temperature ranging between 28 (at weaning) to 22 °C and a relative humidity of 55-60%. Piglets were fed ad libitum and diets contained no antibiotic growth promoters. Piglets were vaccinated against microplasma, cholera and enzootic pneumonia based on standard timetable.

Treatments

In the BV injection and apipuncture groups (Table 1), piglets were treated 4 times as follows: time of birth (day 1), castration (day 3), weaning (day 14) and move to cages (day 30). Three different combinations of BV dosage were employed (Table 1). The subcutaneous BV injection point was at the indentation between the base of tail and the anus. Both acupoints of GV-1 (Jiao-chao) and GV-20 (Bai-hui) were chosen for apipuncture since these two acupoints are known to be involved in immune function. Two live honeybees were utilized for each piglet and for each acupoint, totalling four bee stings per animal. All of the methods used in the present study were approved by the Animal Care and Use Committee at NIAST.

Parameters

The body weight was measured on day 1, day 14, day 30 and day 60. Survivability was calculated by counting the living piglets per sow at each growth
phase. Blood was taken via jugular venipuncture at day 30 using K3EDTA vacuum tubes (Vacutainer, BD Diagnostics, USA). The hematological blood counts as well as analysis of clinical-chemical parameters (total protein, albumin and IgG) were determined with an automated blood counting machine (Hamat 8, SEAC, Italy) and automated biochemical machine (ADVIA1650, Byer, USA). Data were subjected to Duncan’s t-test using the SAS statistical package. In the tables, results are given as means ± standard deviation. Throughout, \( p < 0.05 \) was considered to be statistically significant.
**Results**

Three major bioactive components of BV, namely melittin, phospholipase A$_2$ and apamin were confirmed as being present in the bee venom used in this study by comparing with a commercial product (Fig. 1).

Weight gain of the piglets was 26.6 and 21.8% higher in the BV injection and apipuncture groups, respectively than in the control group (p < 0.05, Fig. 2). No differences in weight gain were found between the BV injection and apipuncture groups. Noticeable trends were groups with an incremental dose increase of BV injection with an advanced age, all of which demonstrated a significant weight gain compared with the control (p < 0.05). The survivability was somewhat influenced by BV injection (96.8% - 100%) and apipuncture (100%) compared with 93.8% of control at the time of weaning (Table 2). On day 60 of birth, survivability was 93.4% - 96.8%, 93.2% and 86.7% in BV injection group, apipuncture group and control group, respectively. The significant difference (p < 0.05) was only between BV injection group and the control.

Number of leukocytes, erythrocytes, lymphocytes and monocytes were not affected by treatment (Table 3). However, the high dose combination group of BV injection (group C) has demonstrated significant differences (p < 0.05) in numbers of leukocytes, lymphocytes and monocytes compared with either the apipuncture or control groups. Other blood parameters, i.e. total protein and albumin, were within physiological ranges and not affected by the treatment, although IgG levels were generally higher in treated groups than the control (Table 4). The value difference was significant (p < 0.05) only between high dose combination BV injection group (group C) and the control.
**Discussion**

No effect on piglet growth performance was observed from BV treatment. Previous studies have reported on the ability of BV to promote antinociception, antiinflammation, antimicroorganisms and immunity. Kwon and his colleagues have reported that long-term treatment with BV at a dose of 1 mg/kg/day resulted in a significant antinociceptive and antiinflammatory effect on the Freund’s complete adjuvant-induced arthritis in animal model (Kwon et al., 2001). Bee venom including several venoms of different snake species has shown antibacterial effects against both gram-positive and gram-negative bacteria (Perumal Samy et al., 2007). The effect of BV on the immune system is probably caused by phospholipase A$_2$ contained in the venom (Tarzi et al., 2006).

In the present study, we have shown that BV injection at incremental doses of 1.0, 1.5, 2.0 and 2.5 mg did boost the growth performance of piglets. The dose was based on the therapeutic regulation of BV and the fact that bees of about 15 days old after metamorphosis are known to have about 0.1 mg of venom in their poison sacs (Choi et al., 2003$^a$). Both BV injection group and api puncture group demonstrated significant increase compared to the control in body weight and survivability, respectively. Api puncture treatment into selected acupoints produced a similar growth performance to BV injection treatment. We hypothesized that BV injection and api puncture into acupoints might behave as a chemical stimulant that activates the acupoints and subsequently engages the endogenous immune modulating system. Because whole BV used in the present study contains stimulating compounds such as melittin and phospholipase A$_2$, it is possible that these components activate the acupoints directly with multiple biological effects. Furthermore, we assumed that the immune boosting effect of BV was from the melittin because it is major bioactive
component of whole BV (Lariviere and Melzack, 1996) as confirmed with a chromatography in the present study. It seems likely that the BV is potentiating an immune response to the normal environmental, social and nutritional challenges the newly weaned young pigs encounter. The increased stress with days after birth seemed counterbalanced with incremental doses of BV.

The enhanced IgG level of BV treated piglets, alongside the increased lymphocytes and monocytes, indicated that these animals had mounted an immune response. The similar haematological profiles in low dose BV treated piglets to those of the control suggest that BV required high dose to elicit any physiological effects. Antibodies take approximately one week after exposure to antigen before they can be detected in the serum as a primary immune response, and subsequently remain detectable for up to two weeks (Ilsley et al., 2005). Thus it is conceivable that antibody production in response to series of BV challenge resulted in higher concentration at the day 30 sampling time. The results of the presented experiment showed that the immune stimulating effect of BV can be achieved in the form of injection or acupuncture. This makes BV treatment interesting for practical application in livestock production.

It can be concluded that BV treatment as performance additive might have a stimulating effect on the immune system, especially in situations with increased stress for the immune system. The results would be useful for further purification and characterization of immune boosting agents from BV.
References


Figure legends

Figure 1. Gel filtration of 100 mg freeze-dried whole BV on Sepadex TM200 10/300. Elution with 0.1 M ammonium formate buffer, pH 4.5. Main components were compared with standard proteins by the optical density at 280 nm. A, Sigma whole BV; B, whole BV used for the current study; PA2, phospholipase A2; M, melittin; A, apamin.

Figure 2. Changes of body weight in piglets of BV injection, apipuncture and control group (*p < 0.05: significantly different from the control, **p < 0.01: significantly different from the control)
Figure 1