

## Trophic Effect of Bee Pollen on Small Intestine in Broiler Chickens

Jue Wang,<sup>1</sup> Shenghe Li,<sup>1</sup> Qifa Wang,<sup>1</sup> Baozhong Xin,<sup>2</sup> and Heng Wang<sup>2</sup>

<sup>1</sup>Anhui Science and Technology University, Fenyang, Anhui, People's Republic of China; and <sup>2</sup>Das Deutsch Center Clinic for Special Needs Children, Middlefield, Ohio

**ABSTRACT** In this study, the effects of bee pollen on the development of digestive organs were evaluated in broiler chickens. A total of 144 1-day-old AA broiler chickens were randomly and equally divided into two groups, assigned as the control group and the pollen group, respectively. The control group was fed with a basic diet, while the pollen group was fed with a basic diet supplemented with 1.5% bee pollen over a period of 6 weeks. At the end of each week, the digestive organs were obtained for comparison from 12 broilers randomly selected from each group. The results demonstrated that compared to the control group, the small intestine villi from the duodenum, jejunum, and ileum were longer and thicker in the pollen group. This difference was more significant during early development, especially through the first 2 weeks. Bee pollen increased the length of the villi by 37.1% and 29.4% in the duodenum, 28.1% and 33.7% in the jejunum, and 18.6% and 16.2% in the ileum in week 1 and 2, respectively. Furthermore, the small intestinal glands were developed at a higher density in the pollen group, and the depth of the glands was significantly increased by bee pollen in the first 2 weeks. These findings suggest that bee pollen could promote the early development of the digestive system and therefore is a potentially beneficial food supplement for certain conditions, such as short bowel syndrome.

**KEY WORDS:** • bee pollen • duodenum • ileum • jejunum • small intestine gland • small intestine villi

## INTRODUCTION

AS PART OF COMPLEMENTARY and alternative medicine, natural supplements have been increasingly used in the general population not only for health and well-being, but also for potential therapeutic effects on certain diseases or conditions.<sup>1</sup> However, many of these therapies are not recognized or supported by conventional health care providers. Besides safety concerns, one of the major reasons that such practices have difficulty gaining acceptance by conventional medicine is the lack of supporting data, such as experiments utilizing animal studies and double-blind controlled human studies.

One of those widely used natural supplements is bee pollen. It seems that bee pollen contains most of the essential nutritional elements to provide for growth and development in animals and humans.<sup>2,3</sup> Its efficacy in treating benign prostate hyperplasia and chronic nonbacterial prostatitis has been extensively studied.<sup>4–8</sup> It has also been revealed that pollen extract lowers blood lipid levels in both animals and humans, and thus may potentially slow the development of atherosclerosis.<sup>9,10</sup> In addition, consumption of pollen or its extract preparations has been proven to have

antitoxicant effects and to protect the liver and small intestine from damage caused by some poisoning agents.<sup>11–13</sup> Here we report, for the first time, a trophic effect of bee pollen on the small intestine in broilers. This trophic effect on the small intestine seems organ-specific, and its potential applications have been discussed in this report.

## MATERIALS AND METHODS

### Animals and diets

This study followed the general guidelines for the care and use of laboratory animals set by Anhui Science and Technology University, Fenyang, Anhui, People's Republic of China. A total of 144 commercial AA broilers were purchased at 1 day of age. These broilers were randomly divided into two groups: control group and bee pollen group. The control group was fed with a standard basic diet, and its components are shown in Table 1. Some minor component changes were made to suit the broilers' growth and development. The pollen group was fed with the standard basic diet supplemented with 1.5% bee pollen. The animals were housed under optimal conditions throughout the experimental period. Routine vaccines were given as part of animal care. Bee pollen was purchased in its natural state from Fenyang Farm Store (Anhui). The contents of main macronutrients (in percentage of weight) are as follows: water (18.07%), total carbohydrate (17.60%), protein (24.57%), fat (6.26%), fiber (3.12%), and others (30.38%).

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Address reprint requests to: Heng Wang, M.D., Ph.D., Das Deutsch Center Clinic for Special Needs Children, 15809 Madison Road, Middlefield, OH 44062, E-mail: wang@ddcclinic.org

TABLE 1. STANDARD BASIC DIET

Component	Content (%)	
	Week 0-3	Week 4-6
Maize	54	62
Soybean	24	20
Barley	10	10
Wheat bran	2.1	2.1
Fish meal	8	4
Bone powder	0.8	0.8
Stone powder	0.8	0.8
Minerals	0.3	0.3

The compositions of other nutrients and elements were determined and listed in Table 2.

#### Experimental design

The entire experiment lasted 6 weeks. At the end of each week from the first week, 12 broilers from each group were collected by random picking, and the animals were subjected to examination and comparison. A total of six time points

TABLE 2. CONTENTS OF AMINO ACIDS, VITAMINS, AND TRACE ELEMENTS IN BEE POLLEN

	Level
Amino acids (g/100 g)	
Arginine	0.0251
Serine	0.0672
Valine	0.0138
Methionine	0.0251
Isoleucine	0.0176
Leucine	0.0151
Tyrosine	0.0343
Phenylalanine	0.0084
Threonine	0.0087
Vitamin (mg/100 g)	
B1	9.07
B2	2.01
B5	4.08
B6	2.31
B7	0.07
C	70.52
A	0.21
E	7.47
D2	0.79
K	0.42
Trace elements (μg/g)	
K	5,600
Na	188
Ca	5,510
Mg	2,034
Zn	120
Cu	4.62
Fe	63
Cr	0.29
Pb	0.96

(weeks 1-6) were taken throughout the experiment period. For each examination, the animals were weighed, sacrificed, and dissected. The whole gut, liver, and pancreas were collected. Length of duodenum, jejunum, and ileum and weight of liver and pancreas were measured before further examination.

#### Histological study

After the measurements were taken as described above, a sample from each section of the gut was collected, fixed in Bouin's solution for 24 hours at room temperature, and embedded in paraffin. The paraffin blocks were then cut into 5-μm sections, stained with hematoxylin and eosin, and observed under an Olympus-CH30 optical microscope (Olympus, Melville, NY). Villus height and crypt depth were measured under the microscope. The representative fields were photographed.

#### Statistical analysis

All measurements were expressed as mean ± SD values. The data were assessed by analysis of variance followed by Fisher's Least Significant Difference test. Differences were considered significant at  $P < .05$ .

## RESULTS

#### Effect of bee pollen on animal growth

As shown in Table 3, body weight of the broilers increased gradually with time in both control and pollen groups, but the broilers in the bee pollen group grew significantly faster than those in the control group ( $P < .05$ ). After a 6-week growing period, the average body weight for the broilers fed with bee pollen-supplemented diet was 35.1% higher than that of the control group.

#### Effect of bee pollen on development of small intestine

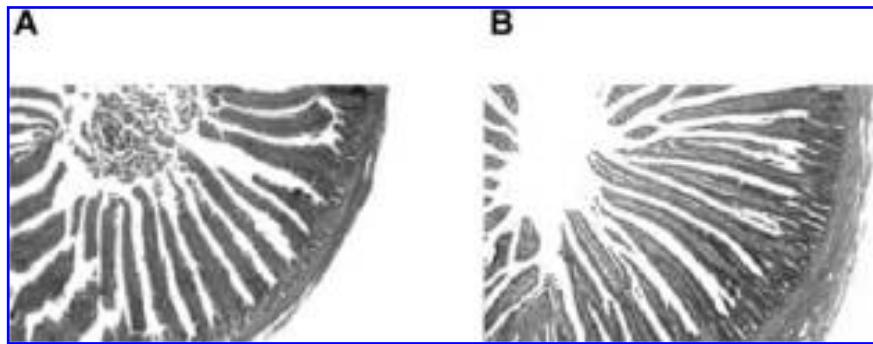
Dissection and examination of the digestive system of the broilers showed that the small intestine was the main target

TABLE 3. BODY WEIGHT OF BROILERS IN THE COURSE OF THE EXPERIMENT

Week	Control group (g)	Pollen group (g)
1	115.22 ± 5.78	133.57 ± 3.89*
2	281.33 ± 17.30	321.17 ± 13.29*
3	503.33 ± 16.72	537.17 ± 16.72*
4	578.33 ± 15.23	639.50 ± 14.15**
5	991.50 ± 18.95	1,132.17 ± 39.29**
6	1,173.33 ± 44.13	1,585.67 ± 68.27**

Data are mean ± SD values of 12 experimental broilers at each time point.

Significantly different from the control group: \* $P < .05$ , \*\* $P < .01$ .



**FIG. 1.** Histological representation of small intestinal villus structure, shown with hematoxylin and eosin staining of jejunum villi from 2-week-old broilers. (A) Control group, broilers fed on standard basic diet. (B) Pollen group, broilers fed on standard basic diet supplemented with 1.5% bee pollen. The intestinal villi were significantly longer and thicker in the pollen group than in the control group. Magnification  $\times 40$ .

affected by the bee pollen. Although there was no significant difference in the length of each section of small intestine between the pollen and control group (data not shown), significant changes were found at the tissue structure level. Small intestine villi were much longer and thicker in the pollen group than in the control group (Fig. 1). Bee pollen significantly increased the length of villus development in the duodenum during the first 3 weeks ( $P < .05$ ), especially at weeks 1 and 2 ( $P < .01$ ) (Fig. 2). The increase from week 1 to week 3 was 37.1%, 29.1%, and 22.0%, respectively. Starting in week 4, there was no significant difference between the two groups ( $P > .05$ ). In the jejunum, bee pollen also significantly increased villus development in the first 2 weeks ( $P < .05$ ), with the effect becoming significant by the end of the first week ( $P < .01$ ) (Fig. 2). The length of jejunum villi increased by 28.1% and 33.7%, respectively, during weeks 1 and 2. In the ileum, the effect of pollen on villus growth was less pronounced, with significant increases in length by 18.6% and 16.2%, respectively, at weeks 1 and 2 ( $P < .05$ ) (Fig. 2).

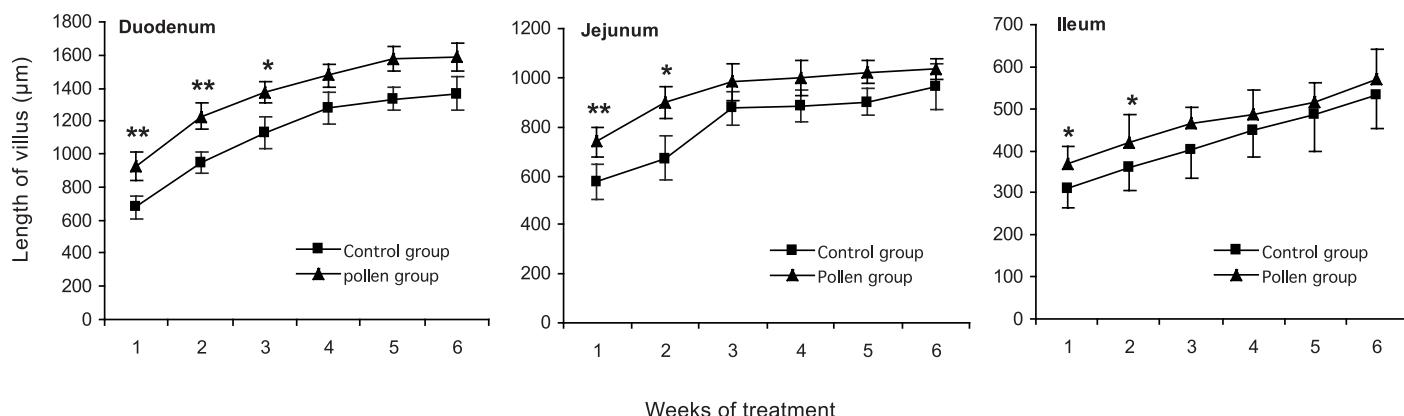
The pollen group's intestinal glands, as measured by the depth of small intestinal crypts, were much deeper and of higher density than those of the control group (Fig. 3). Microscopic observation showed that intestinal glands from all three parts of the small intestine were significantly affected

during early stages of development. In the duodenum, bee pollen significantly increased the depth of gland development by 67% in the first week ( $P < .01$ ) and by 30.3% in the second week ( $P < .05$ ) (Fig. 4). In the jejunum, the increase was 38.6% and 38.5%, respectively, in the first and second week ( $P < .01$ ) and 31.2% in week 3 ( $P < .05$ ) (Fig. 4). In the ileum, we only observed a significant increase in crypt depth by 42.9% in the first week ( $P < .05$ ) (Fig. 4). After 3 weeks, differences in intestinal gland measurements between the two groups did not achieve statistical significance ( $P > .05$ ).

## DISCUSSION

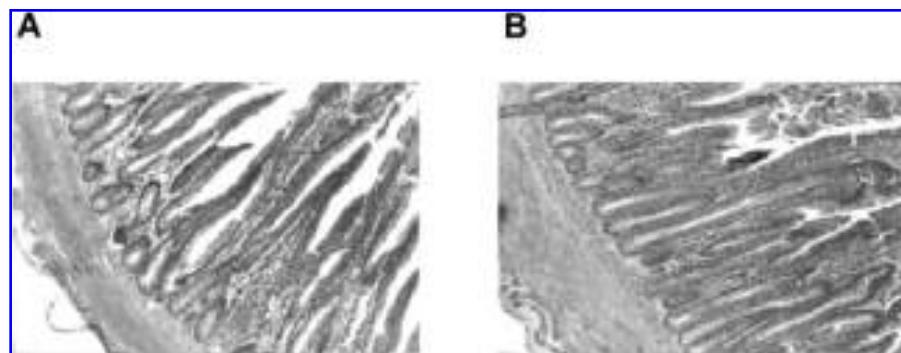
Bee pollen contains a wide spectrum of amino acids, vitamins, hormones, and minerals, as well as enzymes and coenzymes necessary for good digestion and cell growth. In this study, we report the effects of bee pollen *in vivo* on the development of the digestive system using broiler chickens as the animal model. Our data clearly demonstrate that bee pollen has significant trophic effects on the small intestine and consequently promotes growth in broilers.

The small intestine is the main site for digestion and absorption of nutrients. It consists of the duodenum, jejunum, and ileum. As a dietary supplement, bee pollen added into



**FIG. 2.** Effects of bee pollen on the length development of villus in small intestine: duodenum, jejunum, and ileum. Data are mean  $\pm$  SD values of 12 experimental broilers. Significantly different from the control group: \* $P < .05$ , \*\* $P < .01$ .

**FIG. 3.** Histological representation of small intestinal crypt structure, shown with hematoxylin and eosin staining of duodenum crypts from week 2 broilers. (A) Control group, broilers fed on standard basic diet. (B) Pollen group, broilers fed on standard basic diet supplemented with 1.5% bee pollen. The intestinal crypts were significantly deeper and of higher density in the pollen group than in the control group. Magnification  $\times 100$ .



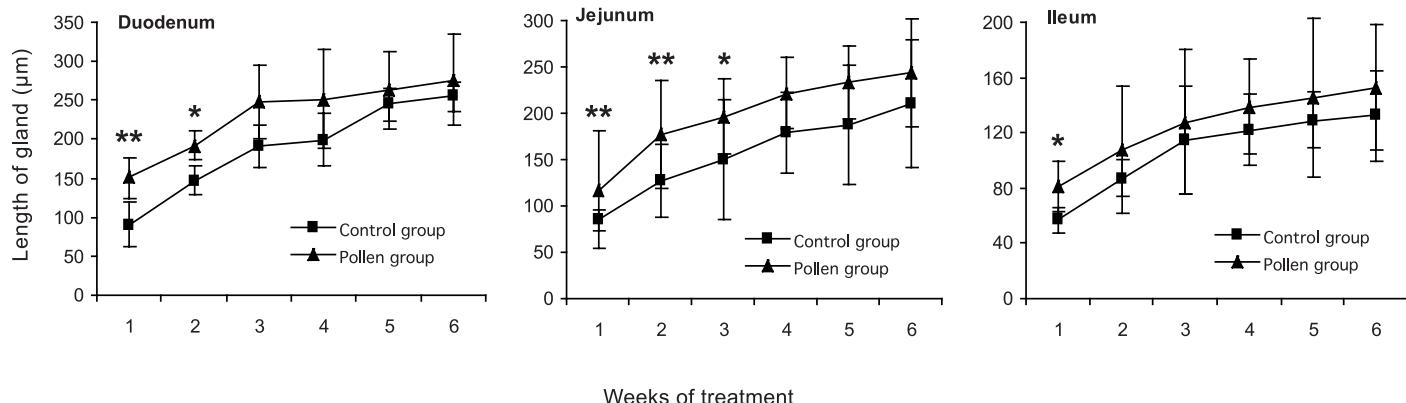
the regular diet efficiently promotes the development of all three parts of the small intestine during early developmental stages in broilers. Although the length of the small intestine was not increased in any section, the mucosa was more developed in the pollen group of broilers. The intestinal villi in the pollen group were longer and thicker than those of the control group. This enhanced intestinal villi development greatly increases the functional surface area of the mucosa for nutrient absorption in the small intestine. As a consequence, the absorption efficiency of the small intestine is accordingly increased. Furthermore, the glands of the small intestine play an important role in the digestion of nutrients. The increased function of these glands also significantly contributes to the absorption of the nutrients in small intestines. Thus, addition of bee pollen to the basic daily diet stimulates the digestive and absorptive functions of broilers and, in turn, promotes growth and development.

During the experiment, we noticed that two important digestive organs, the liver and pancreas, were larger in the pollen group compared to the control group. However, when adjusted relative to their body weights, there was no significant difference between the two groups (data not shown). Furthermore, histological observation of liver and pancreas sections did not show obvious microstructural differences between the two groups throughout the experiment period (data not shown).

Therefore, the trophic effect of bee pollen on the digestive system is most likely specific to the small intestine.

Our data also demonstrated that the trophic effect of bee pollen on the small intestine is an early event during the development of broilers. The most significant stimulation on the development of both the villi and glands of the small intestine took effect within 1–2 weeks after introduction of bee pollen, and body weight increase occurred at almost the same time. It would be interesting to know if the trophic effect persists if bee pollen is discontinued after 1–2 weeks. From a poultry production standpoint, it might not be cost-efficient to continuously add pollen to the diet to enhance production, but it would become attractive if the addition of pollen was needed for only 1 or 2 weeks and the growth promotion effect remained. Further investigation is needed to address this important issue.

The mechanism of the trophic effect of bee pollen on the small intestine remains unknown. Although the nutritional composition of bee pollen has been well studied,<sup>2</sup> the spectrum of biologically active substances is seldom studied. We speculate that certain biologically active substances might be responsible for such a trophic effect. It has been reported that exogenous insulin, growth hormone, and glutamine all have a gut-trophic effect, which has been applied clinically in treating short bowel syndrome.<sup>14,15</sup> Short bowel syndrome,



**FIG. 4.** Effects of bee pollen on the depth development of crypt in small intestine: duodenum, jejunum, and ileum. Data are mean  $\pm$  SD values of 12 experimental broilers. Significantly different from the control group: \* $P < .05$ , \*\* $P < .01$ .

characterized by the inability to maintain protein-energy, fluid, electrolyte, or micronutrient balance when on a conventionally accepted, normal diet, is a group of highly disabling pathological conditions resulting from surgical resection, congenital defects, or disease-associated loss of absorption.<sup>16</sup> Clinically, the treatment of this group of diseases remains challenging. Considering this first report of the trophic effect of bee pollen on the small intestine in an animal study, further work seems warranted to explore the potential use of bee pollen in the treatment of short bowel syndrome.

In conclusion, our study provides evidence that addition of 1.5% bee pollen to the diet stimulates the early development of the small intestine of experimental broilers and subsequently promotes their digestion and absorption functions, body growth, and development. Thus bee pollen might potentially work as a trophic agent to enhance small intestinal function, and may be of possible benefit for some pathological conditions such as short bowel syndrome.

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