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Effect of Bee Pollen on The Immunity and Tibia Characteristics in Broilers

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ABSTRACT

This study was carried out to evaluate the effect of bee pollen (BP) levels on the IgG and IgM titers, weight of lymphoid organs, and on the tibia morphometric measures and mineralization in broilers at 21 and 42 days of age. Four hundred birds were used in an entirely randomized design with four treatments (0, 0.5, 1 and 1.5% of BP feed inclusion) and five replicates. At 21 and 42 days of rearing, blood samples were collected for IgG and IgM analysis, as well as lymphoid organs (bursa, thymus and spleen) and the tibiae. There was no effect ($p>0.05$) of the BP inclusion on IgG titers, bursa and spleen weights, tibia morphometric measures and mineralization at 21 and 42 days, IgM titer at 42 days or thymus weight at 21 days. However, IgM titers at 21 days and the thymus weight at 42 days linearly increased with BP dietary inclusion. It was concluded that up to 1.5% BP can be included in broiler feeds until 21 days of age to enhance bird immunity.

INTRODUCTION

Bee pollen is an agglomerate of flower pollen collected by bees mixed with nectar and secretions from the hypofaryngean glands (Carpes *et al*., 2008). Bee pollen sold in Brazil must present the following physical-chemical characteristics: maximum moisture 30%, maximum ashes 4%, minimum ether extract 1.8%, minimum crude protein 8%, total sugar from 14.5 to 55%, minimum crude fiber 2%, and pH from 4 to 6 (Brasil, 2001).

Bee pollen is rich in oligoelements, including Fe, Cu, Zn, Cr and Mn; it contains active substances such as catalase, peroxidase, superoxide dismutase and antioxidants. Its use has been associated with increased humoral immune cells and phagocytes number and activity, increased red blood cells number, accelerated antibody production and delayed disappearing antibody (Zuo & Xu, 2003).

In experiments with broilers, Wang *et al*. (2005a) included 1.5% bee pollen in diets for broilers from one to 42 days of age and reported that there was an increase of the absolute and relative weights of the spleen, bursa and thymus. However, Zhang *et al*. (2005) studied the oral administration for 30 days of bee pollen to rats, at doses of 165, 330 and 660 mg/kg body weight, and did not notice any differences in spleen weight, but the authors reported higher antibody titers, higher macrophage and natural-killer cells activity.

There is evidence that the bee pollen improves bone mineralization due its high vitamin D content, which increases calcium absorption. According to Hamamoto *et al*. (2006) and Yamaguchi et al. (2007b), bee pollen has stimulatory effects on bone formation and inhibitory effects on bone resorption. When evaluating the effect of the dietary inclusion of bee pollen extracts on femoral components, Yamaguchi *et al.* (2006)

verified that there was an increase in calcium and DNA content, due the cellular proliferation, and of alkaline phosphatase in rats' femur with the administration of 5 and 10 mg/100 g body weight.

This study was carried out to evaluate the effects of different bee pollen dietary inclusion levels on IgG and IgM titers, lymphoid organs weight, and tibial morphometric measures and mineralization in broilers at 21 and 42 days of age.

MATERIAL AND METHODS

Four hundred one-day-old male (M) and female (F) chicks were used, with mean initial weight 52.44 ± 1.76 g. Birds were housed in a masonry shed divided into 21.85 m² experimental pens at a density of 10.8 birds/m².

A completely randomized experimental design was applied, with four treatments of five replicates of 12 birds (M and F) per experimental unit. Treatments consisted of the inclusion of increasing ground bee pollen (BP) levels in the diet. The inclusion levels assessed were 0, 5, 10 and 15 g/kg.

Birds were fed a starter diet from one to 21 days of age and a grower diet from 22 to 42 days of age (Table 1). The experimental feeds were isonutritive and were formulated to meet broilers' requirements as recommended by Rostagno *et al*. (2005), except for crude protein (CP), apparent metabolizable energy (AME), calcium (Ca) and phosphorus (P) levels, which corresponded to 97% of the recommendations. Water and feed were supplied *ad libitum* throughout the experimental period.

The BP used contained 3.83% moisture, 22.97% crude protein, 3953 kcal/kg gross energy, 0.39% calcium, 0.99% phosphorus, 3.14% ashes, 1.71% fat, and its pH was 4.68.

On days 21 and 42 of the rearing period, two birds per pen were feed fasted for 12 hours and then weighed. Blood samples were collected by jugular vein puncture for IgG and IgM analysis using the turbidimetry and nephelometry methods, respectively. Birds were then sacrificed by cervical dislocation and their tibiae, bursa, thymus and spleen were collected. Lymphoid organs were weighed and their relative weights were determined as a function of live weight.

Table 1 – Composition and cost of the experimental diets containing increasing levels of dehydrated bee pollen (BP)

	Starter				Growes			
	BP level (g/kg)				BP level (g/kg)			
Ingredients (g/kg)	0	5	10	15	0	5	10	15
Ground corn	561.0	561.0	561.0	561.0	600.0	600.0	600.0	600.0
Soybean meal	350.6	350.6	350.6	350.6	313.0	313.0	313.0	313.0
Soybean oil	31.0	31.0	31.0	31.0	36.5	36.5	36.5	36.5
Dicalcium phosphate	17.5	17.5	17.5	17.5	16.8	16.8	16.8	16.8
Limestone	7.8	7.8	7.8	7.8	8.0	8.0	8.0	8.0
L-Lysine 99%	-	-	-	-	1.0	1.0	1.0	1.0
Salt	5.0	5.0	5.0	5.0	4.8	4.8	4.8	4.8
Mineral/vitamin supplement[1,2]	4.01	4.01	4.01	4.01	4.02	4.02	4.02	4.02
Dehydrated bee pollen	0.00	5.0	10.0	15.0	0.0	5.0	10.0	15.0
Inert material[3]	23.1	18.1	13.1	8.1	15.9	10.9	5.9	0.9
Total	1000	1000	1000	1000	1000	1000	1000	1000
	Calculated composition[4]							
Crude protein (g/kg)	205.2	205.2	205.2	205.2	191.4	191.4	191.4	191.4
Metabolizable energy (kcal/kg)	2960	2960	2960	2960	3055	3055	3055	3055
Calcium (g/kg)	8.3	8.3	8.3	8.3	8.1	8.1	8.1	8.1
Available phosphorus (g/kg)	4.3	4.3	4.3	4.3	4.1	4.1	4.1	4.1
Total lysine (g/kg)	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Methionine (g/kg)	3.2	3.2	3.2	3.2	3.0	3.0	3.0	3.0
Methionine+cystine (g/kg)	6.5	6.5	6.5	6.5	6.1	6.1	6.1	6.1
Cost (R$/kg)	0.69	1.00	1.31	1.63	0.82	1.13	1.44	1.76

[1]Content per kg product: vit A 1,500,000 IU, vit D_3 375,000 IU, vit E 5,000 IU, vit K_3 375 mg, vit B_1 500 mg, vit. B_2 1625 mg, vit B_6 750 mg, vit B_{12} 3750 mcg, niacin 10000 mg, biotin 20 mg, folic acid 250 mg, calcium pantothenate 2500 mg, choline 65000 mg, methionine 450 g, Cu 2000 mg, Fe 8000 mg, I 250 mg, Mn 20000 mg, Se 75 mg, Zn 16500 mg, BHT 60 mg, avilamycin 2500 mg, nicarbazin 25000 mg. [2]Content per kg product: vit A 1125000 IU, vit D_3 300000 IU, vit E 3750 IU, vit K_3 250 mg, vit B_1 450 mg, vit B_2 1125 mg, vit B_6 500 mg, vit B_{12} 2500 mcg, niacin 3750 mg, folic acid 125 mg, calcium pantothenate 2500 mg, choline 65000 mg, methionine 440 g, Cu 2000 mg, Fe 8000 mg, I 250 mg, Mn 20000 mg, Se 75 mg, Zn 16500 mg, BHT 60 mg, avilamycin 1875 mg, salinomycin 16500 mg.

[3]kaolin [4]according to Rostagno *et al.* (2005).

Tibiae were cleaned of adherent soft tissues and the right tibiae were weighed and their length and diameter were weighed using a manual caliper. The weight/length index was also calculated by dividing bone weight, in mg, by its length, in mm. The left tibiae were evaluated for ashes, calcium and phosphorus content.

In order to determine ash content, tibiae were weighed before and after the drying in a forced-ventilation oven at 55ºC for 48 hours. Subsequently, tibiae were defatted, ground, and analyzed using the methodology of Silva & Queiroz (2002).

The results were submitted to analyses of variance using the system of statistical and genetic analyses (SAEG, 2007) at 5% probability level and the significant results were submitted to the polynomial regression model to estimate the best BP level.

RESULTS AND DISCUSSION

There was no effect (p>0.05) of BP inclusion in the broilers diets on IgG titers at 21 and 42 days of age and on IgM titers at 42 days of age (Table 2); however, IgM titers at 21 days of age linearly increased (p<0.05) with the BP inclusion levels (Figure 1).

Table 2 – IgG and IgM titers of broilers fed diets containing different bee pollen levels

	Bee pollen level (%)				CV[1]
Parameters	0.0	0.5	1.0	1.5	(%)
21 days of age					
IgG (mg/dL)	16.80	4.30	10.46	7.00	11.90
IgM (mg/dL)2	1.68	7.67	14.80	18.33	5.85
42 days of age					
IgG (mg/dL)	10.02	15.02	8.70	6.95	8.19
IgM (mg/dL)	4.20	2.07	2.42	4.83	8.65

[1]coefficient of variation, obtained with log-transformed means (log X).
[2]Linear effect ($\hat{Y}$ = 1.93 + 11.52x, r2 = 0.30)

Chicks were vaccinated against infectious bursal disease at 7 and 21 days of age and against Newcastle disease at 14 days of age, via drinking water, which may explain the results above. IgM is the first antibody to appear in response to an infection or vaccination, reaching its highest concentration in 4 to 5 days, approximately, and then it diminishes after 10 or 12 days. As the immune response progresses, the cells that produce IgM, start producing IgG or IgA. On day 21, IgM titers were high in response to vaccination at 7 and 14 days of age (Di Fábio & Rossini, 2008).

When evaluating the inclusion of 1, 0.5 and 0.1% BP in the diets of broilers vaccinated against Newcastle disease (ND), Wang *et al*. (2005a) observed that the lowest antibody titers against ND were obtained in the control group, with no BP, indicating that BP improves the immune response to vaccines or challenges up to 28 days of age, but thereafter, antibody titers were similar in all treatments. Evaluating the same BP feed inclusion levels (1, 0.5, and 0.1%) in broilers vaccinated against ND, Wang *et al*. (2005b) also reported that there was no influence of dietary BP inclusion on the humoral response of birds at 42 days of age.

BP inclusion did not influence (p>0.05) absolute or relative weights of the spleen and bursa on days 21 and 42, and of thymus on day 21; however, there was a linear increase (p<0.01) in thymus weight determined when birds were 42 days old (Table 3).

Table 3 – Weights of lymphoid organs of broilers fed diets containing different bee pollen levels

	Bee pollen level (%)				CV[1]
Weights	0.0	0.5	1.0	1.5	(%)
21 days of age					
Spleen (g)	0.97	1.21	1.19	1.03	4.15
Spleen (%)	0.04	0.05	0.05	0.05	4.30
Thymus (g)	5.76	6.75	6.17	6.52	4.72
Thymus (%)	0.23	0.28	0.26	0.29	3.92
Bursa of Fabricius (g)	1.48	2.22	2.28	1.67	3.82
Bursa of Fabricius (%)	0.06	0.09	0.10	0.07	5.60
42 days of age					
Spleen (g)	2.58	2.95	2.57	2.70	5.55
Spleen (%)	0.10	0.12	0.11	0.12	5.69
Thymus (g)[2]	14.37	14.56	20.75	19.37	5.00
Thymus (%)[3]	0.58	0.61	0.87	0.85	5.80
Bursa of Fabricius (g)	2.60	3.61	3.09	2.75	4.53
Bursa of Fabricius (%)	0.10	0.15	0.13	0.12	4.96

[1]Coefficient of variation.
[2]Linear effect ($\hat{Y}$ = 11.96 + 2.12x, r^2 = 0.43).
[3]Linear effect ($\hat{Y}$ = 0.45 + 0.11x, r^2 = 0.53).

BP is rich in many nutrients that stimulate immune cell proliferation and differentiation. According to Wang *et al.* (2005b), selenium (Se) increases thymus cortical thickness, vitamin C is important to maintain thymus reticular cells, polysaccharides in BP promote T lymphocytes production, cell proliferation and activity in the spleen, as well as in the thymus and bursa, which produce T and B lymphocytes, respectively. In addition, BP contains superoxide dismutase , an antioxidant enzyme that prevents cell senescence in these organs. All of these effects result in a better immune status in animals.

Lymphoid tissues play an important role in the body defense against microorganisms. Broilers have central (thymus and bursa) and peripheral (spleen and all the lymphoid tissue associated to the intestinal mucosa) lymphoid tissues (Akter *et al*., 2006). BP is rich in nutrients, such as proteins, amino acids, vitamins, and trace elements, among others, that promote faster cell proliferation and differentiation in the immune system of birds. shownAccording to Wang *et al*. (2005a) that Se addition to rat diets increased the thickness of the thymus cortex; vitamin C supplementation aid the maintenance of reticular thymus cells, and BP polysaccharides may stimulate the T lymphocytes formation, as well as the division, proliferation and activity of thymus cells .

Studying the inclusion of 1, 5 and 10 g of BE/kg in broiler diets fed between 7 and 42 days of age, Wang *et al*. (2005a) found that the thymus, spleen and bursa were heavier in broilers fed the diets with 10 and 5 g BP/kg, compared with those fed 1 g and the control diet, with no BP, demonstrating the trophic effects of BP on these organs. Consistent results were obtained in a subsequent study (Wang *et al*., 2005b), evaluating the effects of 1.5% BP dietary addition on broiler spleen, thymus and bursa. The authors reported that, at 21 and 42 days of age, the birds fed diets with BP presented heavier spleen and thymus. Considering organs relative weight, only the bursae were heavier in 42-day-old birds receiving BP in the diet . Cheng (2009), however, showed that 0.5, 1 and 1.5% BP dietary inclusion did not increase the relative weight of these organs in 42-day-old broilers.

BP inclusion did not affect (p>0.05) the evaluated bone characteristics. It was expected that the bones of birds fed diets containing BP would have higher calcium content, but this did not occur (Table 4).

BP contains vitamin D (Zuo & Xu, 2003), which improves calcium absorption in the small intestine and, according to Wang *et al*. (2007), BP increases intestinal absorption surface, enhancing calcium absorption and, subsequently, its deposition in the bones. Yamaguchi *et al*. (2006) reported that rats orally fed BP (5 or 10 mg/mL/100 g body weight) presented higher calcium and DNA content and higher alkaline phosphatase activity in the femur than those in the control group. Alkaline phosphatase is an enzyme that participates in bone mineralization and the DNA content in the bone tissue is an indication of cell numbers. In addition, according to Yamaguchi *et al*. (2007a), BP can inhibit osteoclastogenic activity in the bone.

Table 4 – Tibial weight, morphometric measures, weight/length index (WLI) and mineralization in broilers fed diets containing different bee pollen levels.

	Bee pollen level (%)				CV[1]
	0.0	0.5	1.0	1.5	(%)
	21 days of age				
Weight (g)	4.88	4.58	4.24	4.81	5.93
Length (mm)	67.30	68.00	67.10	66.20	3.94
Diameter (mm)	6.10	6.10	5.80	6.30	5.88
WLI (mg/mm)	72.61	67.36	63.23	72.71	5.68
Ashes (%)	48.90	47.17	49.54	50.62	5.79
Ca on DM basis (%)	14.72	13.99	14.30	13.56	5.42
Ca on ash basis (%)	30.20	29.70	29.09	27.04	4.69
P on DM basis (%)	7.15	7.29	7.18	7.12	5.03
P on ash basis (%)	13.18	13.49	13.19	12.98	5.77
	42 days of age				
Weight (g)	15.07	13.52	12.78	13.84	4.10
Length (mm)	100.10	98.30	96.60	99.90	4.63
Diameter (mm)	10.00	9.10	9.30	9.80	4.89
WLI (mg/mm)	151.00	137.45	131.35	138.26	3.33
Ashes (%)	47.09	47.07	46.51	47.34	3.92
Ca on DM basis (%)	30.02	31.36	27.15	27.59	5.76
Ca on ash basis (%)	14.51	15.95	13.90	14.17	4.67
P on DM basis (%)	14.65	14.75	14.27	13.41	5.98
P on ash basis (%)	7.25	7.30	7.10	7.14	3.68

[1]coefficiet of variation.

CONCLUSION

Bee pollen can be included in broiler diets at 1.5% until 21 days of age to increase IgM levels.

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