



## Bee pollen supplementation in diets for rabbit does and growing rabbits

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**ABSTRACT.** It was evaluated the effects of bee pollen (BP) on the doe and kits productivity and on the carcass and organs of the rabbits. Twenty White New Zealand does and their kits were used in a randomized block design, with four treatments and five blocks, in a factorial arrangement 2 x 2 with two supplementation levels for the doe and for the kits after the weaning. BP supplementation for the does did not influence ( $p > 0.05$ ) the doe and kit productivity during the lactation, except by the kits survival rate ( $p < 0.003$ ), the total ( $p < 0.002$ ) and daily ( $p < 0.001$ ) milk production that increased in supplemented doe. BP supplementation for the doe and/or kits did not affect ( $p > 0.05$ ) the rabbit performance from the weaning until the slaughter age, the slaughter weight, carcass characteristics, except by the spleen and small intestine weights, higher in supplemented rabbits. It was not recommended that BP supplementation for does and/or rabbits for not improving the rabbit productive performance.

**Keywords:** alimentary additive, rabbit nutrition, bee product.

### Suplementação com pólen apícola em dietas para coelhas e coelhos em crescimento

**RESUMO.** Os efeitos do pólen apícola (PA) sobre a produtividade de coelhas e lãparos e sobre a carcaça e órgãos dos coelhos foram avaliados. Foram utilizadas 20 coelhas Nova Zelândia Branco e seus lãparos em delineamento em blocos ao acaso, com quatro tratamentos e cinco blocos, em arranjo fatorial 2 x 2 com dois níveis de suplementação para a coelha e para os lãparos após o desmame. A suplementação com PA para as coelhas não influenciou ( $p > 0,05$ ) a produtividade da coelha e dos lãparos durante a lactação, exceto pela taxa de sobrevivência dos lãparos ( $p < 0,003$ ) e a produção total ( $p < 0,002$ ) e diária ( $p < 0,001$ ) de leite que aumentou nas coelhas suplementadas. A suplementação para as coelhas e/ou lãparos não afetou ( $p > 0,05$ ) o desempenho dos coelhos do desmame até a idade ao abate, o peso ao abate, características da carcaça, exceto pelos pesos do baço e intestino delgado, maiores em coelhos suplementados. Não foi recomendada a suplementação com PA para coelhas e/ou lãparos por não melhorar o desempenho produtivo.

**Palavras-chave:** aditivo alimentar, nutrição de coelhos, produto apícola.

### Introduction

Bee pollen (BP) production started modestly in Brazil at the end of the 1980s. Nowadays, the favorable market for natural products, complementary to the diets or with therapeutics effects, is stimulating and promoting the improvement of the bee productive chain (BARRETO et al., 2005).

BP is an agglomerate of flower pollen collected from several plant sources by the bees and mixed with nectar and secretions from the hypopharyngeal glands (CARPES et al., 2008) and is a rich source of proteins, essential amino acids, containing more than 51% polyunsaturated fatty acids, and 39% are

linolenic acid, 20% palmitic acid, and 13% linoleic acid; more than 12 vitamins, 28 minerals, 11 enzymes or coenzymes, 11 carbohydrates, mainly glucose, fructose and sucrose, besides flavonoids, carotenoids and phytosterols (ATTIA et al., 2011a).

Amino acids can be found in its composition such as histidine, leucine, isoleucine, tryptophan, valine, lysine, and others) and vitamins of the B complex, A, C, D and K<sub>3</sub>. BP also contains antibiotic substances, active on the coli bacilli species and on certain species of the *Proteus* and *Salmonella* genera, and antioxidant substances (BASTOS et al., 2003).

Amino acids, vitamins and trace elements of BP are nutritionally beneficial for improving intestinal

absorption due the stimulus to the development, proliferation and differentiation of intestinal cells and because they improve the environmental conditions for the intestinal microbial ecosystem. BP can also improve the cell immune response, the antibody production speed, and reinforce the immunological system (SONG et al., 2005).

The BP composition is very variable and depends on the local harvest and plant source and consists of 75.9 – 98.31% dry matter, 97.9% organic matter, 1.5 – 4.8% ash, 15.04 – 27.69% crude protein, 3.6 – 14% fats, and 68.1% total carbohydrates (BASTOS et al., 2003; FUNARI et al., 2005; CARPES et al., 2009; MODRO et al., 2009). The BP mineral composition (mg 100 g<sup>-1</sup>) is comprised of P (346 – 710), Ca (85 – 260), Na (93 – 358) K (465 – 670), Mg (77 – 82) (BELL et al., 1983; FUNARI et al., 2005; CARPES et al., 2009), along with Se (2100 ppm), Fe (114 ppm), Zn (88 ppm), Cu (15 ppm), and Mn (32 ppm) (FUNARI et al., 2005).

However, its use as an additive in animal feeding is only incipient and reports in the literature are scarce. In studies with swine, Zeng et al. (2004) noted that BP included in the diets improved the amino acid profile of the pig meat. Wang and Cheng (2005) evaluated the inclusion of BP (1, 2, 3, 4 and 5%) in diets for growing and finishing pigs and reported that the 4 and 5% levels improved the daily weight gain and the feed: gain ratio of the animals.

Evaluating 1.5% BP inclusion in diets for broilers, Song et al. (2005) reported that the intestinal cells were longer in the three small intestine segments until the three first weeks of life, and there was no difference after this period. Wang et al. (2007) used 1.5% BP in broiler diets and demonstrated that the BP had a trophic effect in the small intestine and, consequently, promoted the growth of the birds.

Attia et al. (2011a) evaluated the use of 100, 200 and 300 mg BP kg<sup>-1</sup> body weight (BW) for rabbit does and verified that the dose of 200 mg kg<sup>-1</sup> resulted in a higher conception rate, a lower number of service conception rates, a higher litter size, and higher milk production compared to the control group.

Thus, the present study was carried out to evaluate the effects of BP administration to does and/or rabbits, on the doe nursing efficiency, the productive performance, and on the carcass characteristics in slaughtered rabbits.

## Material and methods

Twenty multiparous 2.6 years old New Zealand White does were individually allocated in cages (0.8 x 0.75 x 0.67 m, length, width and height, respectively), with ceramic feeder and drinker. The mean weight of the does was 4048 ± 118 g and all of them had seven to nine kits. The does fed *ad libitum* a commercial pellet feed (17% crude protein, 15% crude fiber, 1.11% calcium, 0.77% phosphorus, 0.90% lysine, 0.63% methionine + cysteine, and 2300 kcal kg<sup>-1</sup> digestible energy). The determined composition of the BP used was, in %, 3.83 moisture, 22.97 crude protein, 0.39 calcium, 0.99 phosphorus, 3.14 mineral matter and 1.71 fats, in addition to 3953 kcal kg<sup>-1</sup> crude energy and pH 4.68.

The experimental design before the weaning was in randomized blocks with two treatments and 10 replications, with two supplementation levels for the rabbit does (0 and 1 g) and, after weaning, a randomized block design in a 2 x 2 factorial arrangement was used with two supplementation levels for the rabbit does (0 and 1 g) and two supplementation levels for the kits after weaning (0 and 1 g), with four treatments and five replications. In both cases, the replications were carried out at different times of the year, from October 2011 to February 2012. The treatments consisted of BP supplementation levels for the does and kits as a water suspension. The BP was provided from one week before until one week after the mating for the does, once a day during the two weeks, and later twice a week throughout the lactation period. BP was provided twice a week for the kits from the weaning up to the slaughter age. The BP was given by insulin syringe in the oral cavity.

The rabbit does were mated with non-treated adult New Zealand White male rabbits 20 days after kindling using mother-litter separation. Each doe was subjected to two services within 30 minutes by the same buck, in the morning and in the afternoon. Each doe was transferred to the buck's cage for mating and after copulation; she was returned to her cage. The rabbit does that failed to become pregnant were discarded from the experiment. Pregnancy was diagnosed by abdominal palpation at day 11 after service.

On the next day postpartum, the doe was separated from the litter and she was taken to the nest only once a day, in the morning, for 15 – 20 minutes, to nurse the kits. At this moment, the doe was weighed before and after the nursing to determine, by difference, the total milk production (ATTIA et al., 2011a).

The following traits were measured: daily feed intake for the does throughout the lactation period and for the kits from the birth to weaning. The total, daily and by kit milk yield, the litter size at birth, kit survival rate until weaning, kit body weight at birth and 35 days of age, weight gain at 35 days, feed conversion ratio of kits from birth until 35 days of age. The total milk production was divided by the number of kits of each doe to determine the milk yield per kit. The kit feed conversion ratio was calculated considering the total feed intake and the milk intake of each kit. The kits were weaned at 35 days.

In the performance trial, eighty rabbits from treated and non-treated does, with an initial weight of  $753 \pm 51.40$  g were used. The animals (two males and two females) were placed in galvanized cages (0.77 x 0.60 x 0.39 m, length x width x height) with an external feeder and three nipple drinkers. The rabbits were fed *ad libitum* a commercial pellet feed (cited above).

The kits were weighed at weaning and at 82 days (slaughter age) and the following traits were determined: weaning weight, final weight and daily feed intake, daily weight gain, feed conversion rate and survival rate from weaning until the slaughter age.

At 82 days, the rabbits were food fasted for 8 hours and weighed in order to obtain the slaughter weight. Later, the animals were slaughtered and the carcasses, with no head, feet and viscera, were weighed. Edible viscera, spleen and small intestine were also weighed. The carcass yield and organ relative weight were determined considering the slaughter weight and carcass weight, respectively. The small intestine length was measured and the intestinal density was calculated dividing the intestine weight (g) by its length (m) (ABDEL-FATTAH et al., 2008).

The statistical analysis of the characteristics related to the does and their kits followed the mathematic model comprised by one factor (BP supplementation for the does) and total and daily milk productions were analyzed using the litter size as a co-variable. The other characteristics, after weaning, followed the mathematic model in a 2 x 2 factorial arrangement, as described above. The analyses were performed using the SAEG software and the means were compared by the F test, at 5% probability level.

## Results and Discussion

BP supplementation for the does during the

mating and lactation period affected ( $p > 0.05$ ) the doe nursing efficiency (Table 1) and the kit growth performance from birth until weaning (Table 2) due to the higher total ( $p < 0.002$ ) and daily ( $p < 0.002$ ) milk production in the does supplemented with BP and higher ( $p < 0.003$ ) kits survival rate.

**Table 1.** Nursery efficiency of does supplemented or not with bee pollen in the mating and lactation periods.

Traits	Bee pollen level (g)		CV <sup>1</sup> (%)
	0	1	
Daily feed intake (g day <sup>-1</sup> )	312	353	3.41
Litter size	7.40	8.20	3.23
Kits body weight at the birth (g)	55.40	51.70	2.11
Kits survival rate (%)	84.38b	94.66a	1.82
Total milk production (g)	5451b	6224a	1.30
Daily milk production (g day <sup>-1</sup> )	159.82b	182.58a	2.20
Milk production/kit (g)	681.45	791.86	3.53

<sup>1</sup>CV = coefficient of variation. Means followed by different letters, in the lines, are different by F test.

**Table 2.** Growth performance of the kits from does supplemented or not with bee pollen in the mating and lactation periods.

Traits	Bee pollen level (g)		CV <sup>1</sup> (%)
	0	1	
Weaning weight (g)	667.40	763.00	2.41
Weight gain (g)	615.70	707.60	2.60
Feed intake (g)	282.40	300.40	5.50
Feed conversion rate (ration + milk)	1.43	1.53	5.42

<sup>1</sup>CV = coefficient of variation.

The improvement on the total and daily milk production was due to the presence of a high micronutrient content (polyunsaturated fatty acids, mineral, vitamins, amino acids, etc.) and protective agents, such as flavonoids, carotenoids, and phenolic constituents in the BP (LEJA et al., 2007; SARIC et al., 2009). Attia et al. (2011a) also reported a higher milk production in does supplemented with 200 mg BP kg<sup>-1</sup> BW. This effect is important because, up to weaning, milk is the main food for young rabbits, even if the intake of solid food begins from 18 to 21 days of age (BOVERA et al., 2009). A higher milk supply for the kits resulted in a better survival rate during the lactation period.

Although does supplemented with BP produced more milk, this effect was not reflected in the body weight, weight gain, feed intake and feed conversion rate of the kits from birth to weaning.

These results are different than those reported by Attia et al. (2011a) that evaluated the effects of BP supplementation of 100, 200 and 300 mg kg<sup>-1</sup> BW for does and noted the does had a lower feed intake up to the 200 mg kg<sup>-1</sup> level. The authors also verified that the litter size and kit body weight at 7, 14, 21 and 28 days of age were higher for the does supplemented with 200 mg BP kg<sup>-1</sup> BW.

BP administration for does and/or weaned rabbits did not affect ( $p > 0.05$ ) the productive performance (Table 3) and the carcass characteristics (Table 4), except for the spleen and small intestine weights, that were higher in supplemented rabbits.

**Table 3.** Performance of growing rabbits supplemented or not with bee pollen (BP) from weaning to slaughter age.

Traits	Does treated	Rabbits treated with BP		CV	
	with BP	No	Yes		Mean (%)
Body weight (g)	Yes	2310	2410	2360	2.53
	No	2360	2274	2317	
	Mean	2335	2342		
Daily weight gain (g day <sup>-1</sup> )	Yes	33.68	34.33	34.00	2.92
	No	33.45	33.31	33.38	
	Mean	33.56	33.82		
Daily feed intake (g day <sup>-1</sup> )	Yes	104.18	95.59	99.89	2.73
	No	100.98	96.67	98.83	
	Mean	102.58	96.13		
Feed conversion rate	Yes	3.12	2.78	2.95	3.29
	No	3.02	2.90	2.96	
	Mean	3.06	2.84		
Survival rate (%)	Yes	91.56	100.00	95.78	
	No	91.87	95.28	93.57	
	Mean	91.71	97.63	4.60	

CV = coefficient of variation.

Although the BP is a substance rich in carbohydrates, proteins, amino acids, fats (60-91% are polyunsaturated fatty acids) (WANG et al., 2005), vitamins A, B, C, D, E (MARCHINI et al., 2006) and B complex, minerals, carotenoids and flavonoids (WANG et al., 2005), BP supplementation for the does and/or growing rabbits was not sufficient to improve performance from weaning up to slaughter age. According to Bell et al. (1983), BP is rich in proteins and has a favorable amino acid profile, but their digestibilities are relatively low, that is a limiting factor for its use as a food for humans and monogastrics.

Different results were reported by Attia et al. (2011a) in which kits born of does supplemented with BP showed higher growth rates and lower feed intake from 4 to 8, 9 to 12 and 4 to 12 weeks of age, compared to the control group. As a consequence, the feed conversion rate was also better.

Attia et al. (2011b) observed that the feed efficiency was improved in growing rabbits due to the supplementation of 200 - 500 mg BP kg<sup>-1</sup> BW compared to the unsupplemented control group, during moderate and hot seasons, from weaning to mature age.

**Table 4.** Slaughter, carcass and organ weights, small intestine length and density of rabbits supplemented or not with bee pollen (BP) from weaning until slaughter age.

Traits	Does treated	Rabbits treated with BP		CV	
	with BP	Yes	No		Mean (%)
Slaughter weight (g)	Yes	2324	2246	2285	
	No	2221	2273	2247	
	Mean	2272	2259	3.99	
Carcass weight (g)	Yes	1252	1214	1233	
	No	1131	1225	1178	
	Mean	1191	1219	4.77	
Kidney weight (g)	Yes	7.04	6.93	6.98	
	No	6.66	7.03	6.85	
	Mean	6.85	6.98	2.55	
Liver weight (g)	Yes	52.28	50.20	51.24	
	No	48.78	56.66	52.72	
	Mean	50.53	53.43	4.93	
Heart weight (g)	Yes	4.91	4.80	4.86	
	No	4.64	4.51	4.57	
	Mean	4.77	4.65	2.13	
Spleen weight (g)	Yes	1.03	0.80	0.92	
	No	1.07	0.76	0.92	
	Mean	1.05a	0.78b	2.95	
Small intestine weight (g)	Yes	48.35	47.40	47.88	
	No	50.49	43.19	46.84	
	Mean	49.42a	45.29b	3.80	
Small intestine length (m)	Yes	2.83	2.74	2.83	
	No	3.03	2.75	2.78	
	Mean	2.93	2.74	2.65	
Small intestine density (g m <sup>-3</sup> )	Yes	17.23	17.43	17.34	
	No	16.65	15.74	16.19	
	Mean	16.94	16.59	3.12	

CV = coefficient of variation. Means followed by different letters, in the lines, are different by F test.

Reports about BP effects on the carcass and organ characteristics in rabbits are scarce. Song et al. (2005) and Wang et al. (2007) evaluated the BP effects on the liver and pancreas weights in broilers and noted that their weights were higher in treated broilers, however, Ke et al. (2009), studying the use of 0.2% BP polysaccharides in broiler diets, reported that BP did not influence the carcass yield of the birds.

Spleen weight was higher in rabbits treated with BP from weaning to slaughter age. According to Wang et al. (2005), BP also has nucleic acids that stimulate the natural killer cells and T lymphocyte activities, such as vitamin A and E and microelements, such as Fe, Se, Zn and Mn, which promote the proliferation and differentiation of immune system cells. These results are in agreement with Wang et al. (2005) who included 1.5% BP in broiler feed and noted that the spleen absolute weight increased due to the BP, but not the relative weight. Cheng (2009) studied the effect of BP inclusion (0, 0.5, 1 and 1.5%) in broiler diets and verified that the spleen weight was higher from the 7<sup>th</sup> up to 42<sup>nd</sup> day of age.

The small intestine weight increased due to the BP supplementation because its cells require amino acids, vitamins, trace elements for their development, proliferation and differentiation and these constituents are also beneficial to the intestinal microbiota. Even though the intestine

weight was higher in supplemented rabbits, the intestinal density was not influenced by the BP. This higher weight in the same length means there was an improvement in the intestinal surface for the nutrient absorption (SONG et al., 2005; WANG et al., 2005). Wang et al. (2007) reported that the 1.5% BP in broiler diets did not influence the small intestine length but the authors also verified that the villi and intestinal glands were more developed in treated birds, demonstrating the BP trophic effect on the small intestine.

The small intestine density is an indirect measure of the intestinal mass and function (CROOM et al., 2006), villi dimension (PALO et al., 1995), and intestinal wall thickness (TREVISI et al., 2009). Lower intestinal density might mean lower villi height and width, which results in lower intestinal digestive and absorptive capacities (CORLESS; SELL, 1999).

### Conclusion

Bee pollen supplementation for does improved milk production and kit survival rate, but is not recommended for rabbits after weaning due to the lack of positive effect on their productive performance.

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