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Nutrição *in ovo* usando mel: efeitos sobre eclodibilidade, desempenho e rendimento de carcaça em frangos de corte

In ovo nutrition using honey: effects on hatchability, performance and carcass yields in broilers

Nutrición *in ovo* con miel: efectos sobre la incubabilidad, el rendimiento y el rendimiento de la canal en pollos de engorde

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Resumo

O objetivo deste estudo foi determinar se a inoculação de mel de *Apis mellifera* em ovos de frangos de corte (nutrição *in ovo*) durante a incubação melhoraria a eclodibilidade e o desempenho. Inicialmente, quatrocentos ovos foram incubados; aos 12 dias de incubação, foi realizada ovoscopia para selecionar os ovos férteis e dividi-los em três grupos: controle, solução salina e mel. No 17º dia de incubação, inoculamos 0,2 mL de cada solução diretamente na cavidade amniótica. Após a eclosão, os pintos foram alojados em um galpão experimental até os 28 dias de idade, quando duas aves por unidade experimental foram submetidas a euthanasia para avaliar o rendimento de carcaça. A eclodibilidade não foi afetada pela inoculação de soro fisiológico ou mel ($p>0,05$). No entanto, a mortalidade foi maior nos grupos mel e soro fisiológico do que no grupo controle ($p<0,05$). As aves que receberam nutrição *in ovo* com mel de abelha apresentaram um peso corporal 11% maior que 28 dias em comparação ao controle. O grupo mel apresentou melhor conversão alimentar e maior peso cardíaco aos 28 dias ($p<0,05$). As variáveis mortalidade, rendimento de carcaça e intestino não diferiram entre os tratamentos ($p>0,05$). Esses resultados mostram que a nutrição in ovo com mel de abelha resultou em menores custos de produção, ou seja, as aves consumiram menos alimento (ração) e tiveram o mesmo ganho de peso que os demais tratamentos.

Palavras-chave: Carboidratos; Desempenho; Eclodibilidade; Incubação; Nutrientes.

Abstract

The objective of this study was to determine whether inoculating honey from *Apis mellifera* into broiler chick eggs (*in ovo* nutrition) during incubation would improve hatchability and performance. Initially, four hundred eggs were incubated; at 12 days of incubation, ovoscopy was performed to select the fertile eggs and divide them into three groups: control, saline and honey groups. On the 17th day of incubation we inoculated 0.2 mL of each solution directly into the amniotic cavity. After hatching, the chicks were housed in an experimental house until 28 days of age, when two birds per experimental unit were sacrificed to evaluate carcass yield. The hatchability was not affected by inoculation of saline or honey ($p>0.05$). However, mortality was higher in the honey and saline groups than in the control group ($p<0.05$). The birds that received *in ovo* nutrition with bee honey had a body weight 11% higher than 28 days compared to the control. The honey group showed better feed conversion and greater heart weight at 28 days ($p<0.05$). Mortality, carcass yield and bowel variables did not differ between treatments ($p>0.05$). These results show that the *in ovo* nutrition with bee honey resulted in lower production costs, that is, the birds consumed less food (feed) and had the same weight gain as other treatments.

Keywords: Carbohydrates; Performance; Hatchability; Incubation; Nutrients.

Resumen

El objetivo de este estudio fue determinar si la inoculación de miel de Apis mellifera en huevos de pollo de engorde (nutrición in ovo) durante la incubación mejoraría la incubabilidad y el rendimiento. Inicialmente, cuatrocientos huevos fueron incubados; a los 12 días de incubación, se realizó una ovoscopia para seleccionar los huevos fértiles y dividirlos en tres grupos: grupos de control, salinos y de miel. En el día 17 de incubación, inoculamos 0,2 ml de cada solución directamente en la cavidad amniótica. Después de la eclosión, los polluelos fueron alojados en una casa experimental hasta los 28 días de edad, cuando se sacrificaron das aves por unidad experimental para evaluar el rendimiento de la canal. La incubabilidad no se vio afectada por la inoculación de solución salina o miel ($p>0,05$). Sin embargo, la mortalidad fue mayor en los grupos de miel y solución salina que en el grupo control ($p<0,05$). Las aves que recibieron nutrición in ovo con miel de abeja tenían un peso corporal 11% mayor que 28 días en comparación con el control. El grupo de miel mostró una mejor conversión alimenticia y un mayor peso cardíaco a los 28 días ($p<0,05$). La mortalidad, el rendimiento en canal y las variables intestinales no fueron diferentes entre los tratamientos ($p>0,05$). Estos resultados muestran que la nutrición in ovo con miel de abeja resultó en

menores costos de producción, es decir, las aves consumieron menos alimento (alimento) y tuvieron el mismo aumento de peso que otros tratamientos.

Palabras clave: Carbohidratos; Actuación; Incubabilidad; Incubación; Nutrientes.

1. Introduction

Because commercial chicken strains are selected for high weight gain and rapid development of the pectoral muscle, there is increased demand for energy and protein. Consequently, there is an imbalance between demand and reserves in the eggs themselves. In ovo nutrition via amniotic fluid injection can mitigate this deficit and boost chick development (Campos et al., 2010). Bird embryos digest and absorb nutrients before hatching; the development of the gut occurs throughout incubation (Uni et al., 2005); however, functional skills only occur after oral consumption of amniotic fluid between the 17-19-day-old embryo (Campos et al., 2010). The inoculation of an *in ovo* substance into amniotic fluid causes the embryo to naturally consume supplemental nutrients, orally, before hatching; this supplementation can accelerate enteric development and the ability to digest nutrients (Vieira, 2005; Campos et al., 2010). In so doing, it promotes the development of healthy intestinal flora, an essential condition for a good initial development (Ferket, 2013).

The *in ovo* nutrition technique aims to provide nutrients (primarily glucose) to the embryo, avoiding gluconeogenesis of endogenous proteins (Uni and Ferket, 2004; Ferket et al., 2005), because most glycogen reserves are used during hatching. Subsequently, the chick must replenish glycogen reserves through gluconeogenesis of body proteins (mostly the pectoral muscle) to support thermoregulation and survival until beginning to consume and use dietary nutrients (Moran, 1985). Because the intestine is the organ that provides nutrients, the sooner it reaches its functional capacity, the faster the bird will be able to use the nutrients in the diet and grow efficiently, expressing its genetic potential and resistance to infectious agents and metabolic diseases (Uni & Ferket, 2004).

Carbohydrates are widely used in *in ovo* nutrition because they increase the level of glucose available to the embryo (Uni et al., 2005). It is important to note that the carbohydrate concentration of the egg is only 1% of the total, compared to the other nutrients (Sugino et al., 1997), with blood glucose and glycogen reserves maintained by the gluconeogenesis of amino acids, glycerol and other gluconeogenic components (Sunny and Bequette, 2010), reducing the availability of these nutrients for muscle growth. These explanations are used to justify *in ovo* nutrition, as seen in injections with glucose, fructose, sucrose, dextrin and maltose (Leitão

et al., 2008), carbohydrates (Zhai et al., 2011), amino acids (Al-Murrani, 1982), among others. According to Leitão et al. (2008), glucose-based solutions for *in ovo* nutrition for the period close to hatching are important energy sources during this phase, reserving proteins and lipids for other specific functions.

Bee honey is a suitable substance for *in ovo* nutrition because it has considerable amounts of reducing sugars (fructose and glucose) (Gois et al., 2015), with α- and β-glucose and fructose being the most abundant (Campo et al., 2015). Because honey is composed mostly of glucose and fructose, this monosaccharide would not need enzymes to be digested and would be readily available for use. In addition to its nutritional and energetic capacity, honey has antimicrobial and antioxidant effects, and it promotes wound healing (Silva et al., 2006). For these reasons, the objective of this study was to determine whether egg nutrition with injections of honey solution would improve embryonic development, performance and broiler carcass yield.

2. Materials and Methods

This research had an exploratory and quantitative nature (Pereira et al., 2018). To achieve the objectives of this study, we used a completely randomized design, as detailed below.

This project was approved by the Committee for the Use of Animals in Research (CEUA) of the Universidade do Estado de Santa Catarina (UDESC) under protocol number 7561300617. It complies with the rules issued by the National Council for Control of Animal Experimentation (CONCEA).

Four hundred COBB® eggs were incubated in four automatic incubators, model Premium (Ecológica EP160), with automatic turning every 2 hours. Before starting the incubation process, the incubators were washed and disinfected with sodium hypochlorite, and had their temperature and humidity adjusted to 37.5 °C and 60%, respectively. The four hundred eggs were weighed in trays with a capacity of 24 eggs to determine the average egg weight, later disinfected with 70% alcohol and distributed at random in the incubators. The design used was randomized blocks, with four blocks (incubators) and three treatments (types of inoculation or control).

The solutions used to nutrition the eggs were prepared on the same day as the inoculation. Two solutions were used for nutrition, according to each treatment, based on 0.5% saline solution prepared with 0.5 g of NaCl dissolved in 100 mL of sterile distilled water

(Saline group). Honey from commercial polyfloral origins was included as a nutritional component in the saline solution at 20% (Honey group). Both solutions were autoclaved at 120 ºC for 15 minutes to ensure the sterility of the material to be inoculated.

After sterilization, the honey solution was analyzed for the presence of hydroxymethylfurfural, because this component is considered toxic if present above 60 mg/kg; both saline and honey solutions were analyzed for the presence or absence of microorganisms (IAL, 2008). After these tests, the solutions were considered suitable for *in ovo* nutrition.

At 12 days of incubation, ovoscopy was performed to identify fertile eggs and determine the mortality rate using embryo diagnosis, determining fertile eggs, infertile eggs, early embryonic mortality (1 to 7 days) and intermediate embryonic mortality (8 to 14 days) (AviagenTM). The eggs considered viable were distributed in three treatments as follows: 27 eggs for the Control group (without inoculation/nutrition), 117 eggs for the Saline group (inoculation of 0.2 mL of a saline solution via amniotic fluid) and 147 eggs for the Honey group (inoculation of 0.2 mL 20% honey-saline solution via amniotic fluid = *in ovo* nutrition). Because amniotic fluid ingestion starts from the 17th day of incubation and ends on the 19th day (Bohorquez, 2010), we decided to perform inoculations on the 17th day of incubation. Innoculations were carried out at the Microbiology and Immunology Laboratory (Labmin) of UDESC - CEO, in a room previously heated at 30 ºC. Before *in ovo* nutrition, the equipment used was placed in a sterile chamber with ultraviolet light to ensure sterility. The eggs were cleaned with 70% alcohol and subjected to ovoscopy for identification and delimitation of the inoculation site (Foye et al., 2006). The inoculation process was carried out in a sterile continuous flow hood. After ovoscopy, the shell was perforated in the region of the air chamber using a portable micro-artificial with a 2-mm diameter drill, avoiding the perforation of the inner membrane of the egg shell. The inoculum was delivered directly into the amniotic cavity with a 7 x 2.5 mm syringe and, immediately after inoculation, the hole was sealed with PVA glue. After the procedure, the eggs were individually packed in filo bags and returned to the incubator.

After 20 days of incubation, the temperature and humidity of the incubators were adjusted to 36°C and 62%, respectively, and the turning feature was turned off. Hatching was attended without interference; during this process we checked the activity and vigor of the chicks. At the end of the 22 days of incubation, the eggs that did not hatch were opened for analysis using embryo diagnosis, determining mortality after *in ovo* nutrition.

At 24 hours after hatching, the chicks were housed in the aviary of the poultry section of UDESC-CEO; the experimental shed consists of 16 boxes with a 1.70 x 0.88 m (1.49 m²) masonry wall, containing a nipple drinking fountain and a suspended feeder. The experimental unit considered a single box, six boxes in the Honey group (22 birds/box), five boxes for the Saline group (17 birds/box), and two boxes for the Control group (11 birds/box). To minimize the variation between the experimental units and ensure the same condition of equal weight for the treatments, at the time of accommodation, all birds were weighed individually and divided into five weight ranges (<37.5 g, 37.5 g at 40 g, 40 g to 42.5 g, 42.5 g to 45 g and > 45 g). The number of available birds per weight range was divided by the number of experimental units, depending on the treatments. The birds were then distributed according to the weight ranges, such that the experimental units had the same initial average weight (Sakomura and Rostagno, 2007).

The feed provided throughout the experimental period had 22.4% crude protein, based on soybean meal and corn meal (Table 1). Feed consumption was recorded weekly with the weight of the feeder using analytical balance.

Weighing the animals was performed at the time of housing and at 28 days. We weighed the birds per experimental unit so as to calculate performance indexes. The performance variables obtained by period were as follows: weight gain, average weight, feed intake, feed conversion and feed efficiency. Feed conversion was determined as the ratio of feed consumption in a given period/weight gain during the period.

Table 1: Ingredients and calculated chemical composition of the basal diet used in our study.

Ingredients (kg)	Age 1 – 21	Age 22–28
Corn	58.5	62.2
Soybean meal	35.8	30.6
Soy oil	1.13	3.40
Bicalcium phosphate	1.92	1.67
Calcitic limestone	0.86	0.79
Iodized salt	0.45	0.42
DL-Methionine	0.38	0.25
Lysine	0.43	0.25
Threonine	0.17	0.06
Kaolin (inert)	0.04	0.04
Premix of vitamins[1]	0.10	0.10
Premix of minerals[2]	0.10	0.10
Calculated chemical composition		
Energy (Mcal/kg)	29.6	31.5
Crude protein (g/kg)	221	197
Calcium (g/kg)	9.42	8.37
Available phosphorus (g/kg)	4.71	4.18
Digestible lysine (g/kg)	13.6	10.9
Chloride (g/kg)	2.00	1.83
Digestible methionine + cysteine (g/kg)	9.68	7.91
Digestible threonine (g/kg)	8.86	7.14
Digestible tryptophan (g/kg)	2.41	2.13
Sodium (g/kg)	2.24	1.87
Linoleic acid (g/kg)	10.8	10.5

Note:
[1] Minimal vitamin levels per kg of food: vitamin A (5000000 IU); vitamin D3 (1.000.000 IU); vitamin E (15000 IU); vitamin K3 (1,500 mg); vitamin B1 (1,500 mg); vitamin B2 (3,000 mg); vitamin B6 (2,000 mg); vitamin B12 (7,000 mcg); folic acid (500 mg); nicotinic acid (15 g); pantothenic acid (7,000 mcg); choline (80 g); biotin (100 mg); minimum humidity (40 g); maximum mineral matter (500 g).
[2] Minimal mineral levels per kg of food: copper (10 g); iron (50 g); iodine (1.000 mcg); manganese (80 g); selenium (300 mg); zinc (70 g); minimum humidity (20 g); maximum mineral matter (980 g).
Source: Authors.

At 28 days of age, two birds per experimental unit were sacrificed (one male and one female), weighing equal to or close to the average weight of the brood. The birds were kept under an 8-hour water fast for later sacrifice. We weighed and sacrificed them using cervical dislocation, a method that kills bird by cutting the spinal cord and stretching the main blood vessels (Ludtke et al., 2010). According to Concea (2015) this method can be used to sacrifice birds up to 3 kg. After this procedure, bleeding was carried out and the birds were weighed again. Then they were scalded and plucked.

The carcasses were eviscerated, discarding non-edible viscera. The weights of the cold carcasses and their cuts (chest, wing, thigh and drumstick, and back) and edible viscera (heart,

liver, and gizzard) were obtained using an analytical scale. The small and large intestines were separated, separating the duodenum, jejunum and ileum (small intestine), and the cecum, colon, and rectum (large intestine). Each intestinal portion was weighed on an analytical scale and measured using a millimeter ruler.

The data obtained were analyzed for normality; subsequently, the analysis of variance was performed using the PROC GLM of the SAS. When there was a significant difference, values were compared using the Tukey test at 5% significance level.

3. Results and Discussion

The parameters related to incubation were displayed in Table 2. The nutritional increase during the incubation period can affect the animals' weight gain after hatching; however, the weight gain result varies according to the type of nutrient inoculated. In our study, there was no significant difference ($P>0.05$) in final weight at 28 days, average daily gain (ADG) and mortality between treatments (Table 2); but the birds that received *in ovo* nutrition with honey had a bodyweight 11% higher than 28 days compared to the control.

The *in ovo* nutrition with honey did not interfere with the hatchability index, obtaining 81.48% of chicks born for the Control group, 81.63% for the Honey group, and 89.74% for the Saline group. However, post-inoculation mortality rate was greater (16.32% for the Honey group and 9.4% for the Saline group). According to Plano et al. (2005), the increase of the internal humidity of the egg during the final days of incubation causes increased embryonic mortality, with the embryos adhering to the internal membrane, making hatching difficult. Pedroso et al. (2006a) attributed this increase to what they considered an excessive volume of the inoculation (0.5 mL). Pedroso et al. (2006b) inoculated 0.2 mL volumes and found no differences between the control group and the group that received the inoculum, differently from the results observed in the present study.

Table 2: Index of hatchability and embryonic mortality.

Variable	Treatment		
	Honey	**Saline**	**Control**
	Hatchability		
Incubated eggs (n)	147	117	27
Chicks born (n)	120	105	22
Percent hatchability (%)	81.63	89.74	81.48
	Mortality (after egg nutrition)		
After 17 days (%)	10.2	8.55	0
Beak (%)	2.04	0.85	0
Total (%)	16.32	9.4	0

Note: Mortality assessed in relation to 400 eggs incubated at the beginning of the experiment
Source: Authors.

Neves et al. (2016) inoculated various concentrations of glycerol and obtained low incubation values (51.5% in treatments 25 and 50 nmol/mL, as well as 72.1% for treatment without *in ovo* injection). The post-inoculation mortality rate reported by Neves et al. (2016) was also high, ranging from 32.3% in the 37.5 nmol glycerol/mL treatment to 48.5% in the 25 and 50 nmol glycerol/mL treatments. Campos et al. (2011) found hatchability of 93.57% for eggs without inoculation, 84.64% for eggs that received saline and 82.5% for eggs that received glucose + sucrose; they also observed higher embryonic mortality in the post-peck phase, different from what we observed in the present study, that is, the highest mortality occurred after the inoculation performed on the 17th day. Late mortality was observed only for the chicks that received some type of inoculation. When comparing the results obtained from the Saline and Honey groups to Control group, we noted that the increased post-inoculation mortality may have been related to the inoculation process, which was done manually, and may not have been performed with precision; factors such as the speed of injection, location and volume injected, even the needle used can interfere with the results of *in ovo* injection (Neves et al, 2016).

There was no significant difference ($p>0.05$) for live weight at 28 days (Table 3) and for carcass cuts (Table 4). There was an effect of the treatment, that is, those that passed through the inoculation with honey had less feed conversion; as well as greater heart weight (Table 3 and 4).

Table 3: Final weight, average daily gain (ADG), feed conversion (FC) and mortality according to each treatment within 28 days.

Variables	Honey	Saline	Control	P-value	CV
Final weight (kg)	0.973	0.916	0.865	0.479	12.15
ADG (kg)	0.035	0.030	0.030	0.354	18.31
FC	1.322[a]	1.394[a]	1.585[b]	**0.005***	5.43
Mortality (%)	14.792	16.542	17.425	0.921	58.04

ADG - average daily weight gain; FC - feed conversion.
*Different letters on the same line, differ by Tukey's test at 5% significance.
Source: Authors.

Table 4: Carcass yield of birds slaughtered at 28 days of age.

	Treatment			
Variable	**Honey**	**Sal**	**Control**	**P-value**
Live weight (g)	1103.50	1097.40	1110.50	0.9769
Hot carcass weight (g)	1087.83	1007.64	1031.00	0.2381
Eviscerated cold carcass weight (g)	763.75	686.80	714.00	0.1500
Carcass yield (%)	70.14	68.10	69.22	0.3635
Breast (%)	35.96	34.79	35.43	0.5845
Wing (%)	11.52	10.93	11.89	0.1003
Leg + thigh (%)	30.91	29.96	30.09	0.2762
Back (%)	21.47[b]	24.11[a]	22.04[b]	**0.0313**
Heart (g)	8.50[a]	7.20[ab]	6.00[b]	**0.0306***
Liver (g)	29.83	27.40	25.00	0.2052
Gizzard (g)	30.50	31.60	34.00	0.5263
Small intestine + pancreas (g)	35.58	32.20	31.00	0.313
Small intestine length (cm)	92.50	82.80	87.00	0.0702
Large intestine (g)	22.67	15.00	20.00	0.0884
Large intestine length (cm)	68.92	63.90	66.75	0.2888

Note: Different lowercase letters on the same line differ from each other by the Tukey test (P<0.05).
Source: Authors..

Pedroso et al. (2006) also found no difference in the performance of birds that received glucose during the embryonic period; however, Uni et al. (2005) found better performance at 10 days of age in birds that received maltose, sucrose and dextrin during the embryonic period. According to these authors, the greatest performance at 10 days of age was due to increased hepatic glycogen reserves, caused by inoculation of disaccharides. Al-

Murrani (1982) also observed greater weight gain at 20 days of age in birds when the birds received exogenous supplementation *in ovo,* compared to those that did not receive any type of supplementation.

Campos et al. (2011) observed changes in feed conversion and an increase in weight gain of chickens that received a nutrient solution (2.5% glucose + 3% sucrose) compared to the control group. In our study, there was a significant difference ($p<0.05$) between treatments for the feed conversion index (CA), with birds in the honey and saline group having lower feed conversion compared to the control group. Campos et al. (2011), found an increase in feed consumption in chickens that received nutrition *in ovo*, however, there was no difference in feed conversion.

According to the literature, carcass yield can be affected by *in ovo* nutrition, depending on the nutrient added; studies that used *in ovo* nutrition sucrose together with another carbohydrate such as glucose (Campos et al., 2011) or maltose and dextrin (Uni et al., 2005) found better breast yield in chickens. Most honey contains glucose and fructose (Alvarez-Suarez, 2013); for this reason, it is possible that the presence of lower amounts of sucrose did not allow an increase in the carcass yield of broilers in the honey group compared to the control group.

Authors reported increased weight and yield of breast to bone weight (5.07%) and breast fillet (5.47%) in the group that received 2.5% glucose + 3% sucrose, compared to the control group (Campos et al. 2011). Uni et al. (2005) also reported an increase of 8.7% and 8.3% in breast yield at 25 days in broilers of the Cobb and Ross lineage by inoculating carbohydrate-based solution at 17.5 days of incubation.

It is possible that the composition of the honey-based nutrient solution was insufficient (i.e., 20%) to cause the expected effect of increased chick performance. Ipek et al. (2004) inoculated low glucose levels (5, 10 and 15 mg) and did not observe an increase in embryonic mortality or improvement in broiler performance, concluding that low glucose levels do not contribute as an energy supplement for a bird during embryonic development. Tako et al. (2004) pointed out that the use of disaccharides such as maltose and sucrose in egg supplementation provided better results due to the stimulus of the synthesis of enzymes and greater absorption of nutrients. It is noteworthy that honey contains 70% monosaccharides and between 10 to 15% disaccharides (including maltose, sucrose, maltulose, turanose, isomaltose, laminaribiose, nigerose, kojibiose, gentiobiose and ß-trehalose) and oligosaccharides (maltotriose, erlose, melezitose, centose 3-α5 isomaltosylglucose, l-kestose, isomaltotriose, panose, psopanose and theanderose) (Bogdanov et al., 2004). Low glucose

levels (100, 200 and 300 mg) provide 0.34, 0.69 and 1.02 kcal of metabolizable energy, respectively; this is insufficient for the process of breaking the inner and outer membranes of the eggshell (Longo et al., 2005). Honey has 82 g of carbohydrates in 100 g that provide 304 calories; the amount of honey diluted in saline may have been insufficient to provide the energy needed to improve performance and hatchability.

The hearts in the group that received honey were heavier at the end of the experimental period (28 days) ($p<0.05$) than those of the control group. Few studies show increased heart weights attributable to addition of nutrients in the egg; Neves et al. (2016) found greater heart weights in chickens that received glycerol *in ovo* and related this increase to increased blood volume. We found no significant difference ($p>0.05$) for liver or gizzard weights, or for small and large intestine variables. Neves et al. (2016) evaluated various levels of glycerol in *in ovo* nutrition and did not observe significant differences in the viscera weights, except for the spleen, heart and gizzard.

4. Final Considerations

In ovo nutrition is a technique used to increase hatchability and improve the viability of newborn chicks. Therefore, we hypothesize that the inoculation of honey *in ovo* is a viable alternative, as this is a highly energetic food.

The inoculation of the 20% bee honey solution *in ovo* to nutrition does not alter hatchability, as well as the weight and carcass yield of chickens at 28 days. However, it positively influenced dietary conversion and heart weight. Nevertheless, some issues remain to be clarified, including the level of honey content to be used, the efficiency of the nutrition method, and its benefits for the production indexes in a complete production cycle.

Conflict of Interest

The authors declare that they have no conflict of interest.

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