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Effect of using the bee pollen as feed additive for suckling Rahmani lambs on immunity response, diseases infection, mortality rate, growth performance and output of weaning weight

Hamed Gaafar[1], Mostafa El-Nahrawy[1], Mohamed F. ElKersh[2], Ghada S. El-Esawy[1]and Reda Abdel-Bary Mesbah[1]

[1]Animal Production Research Institute (APRI), Agriculture Research Center, Dokki, Giza, Egypt.
[2] Animal Health Research Institute (AHRI), Agriculture Research Center, Dokki, Giza, Egypt.
Corresponding email: hamedgafar@gmail.com

ABSTRACT

Forty-five suckling lambs 24 males and 21 females at 7 days after birth with an average live body weight of 5.2 ± 0.06 kg were allocated into three similar groups (8 males and 7 females in each group) according to LBW and sex. All lambs naturally suckled their dams. The 1st group was un-supplemented and served as control (G1), whereas the 2nd and 3rd groups orally supplemented with 0.5 and 1.0 g bee pollen/lamb/day, respectively. The experiment continued for 84 days. Results showed that all types of plasma immunoglobulins were significantly (P<0.05) increased in tested groups (G2 and G3) compared with the control one (G1). The high bee pollen level (G3) showed significantly (P<0.05) the highest values compared to low level in G2. The percentages of induced diseases were decreased significantly (P<0.05) with increase the level of BP additive. Moreover, G3 received the high level of bee pollen revealed the lowest percentages of various diseases followed by G2, whereas control one G1 had the highest percentages (P<0.05). (G1) recorded significantly (P<0.05) the highest mortality rate 20% followed by G2 13.33%, while the G3 recorded the lowest rate 6.67%. Lambs were significantly (P<0.05) heavier in BP additive groups than that of control one, being the heaviest in G3. Also, TWG and ADG were significantly (P<0.05) higher in BP additive in G3 being 13.84 kg and 164.76 g, respectively. Improvements in feed intake, conversion and output of weaning weight increased significantly (P<0.05) with increasing bee pollen from 0.5 to 1.0 g/lamb/day.

Keywords: *Suckling lambs, bee pollen, immunity, diseases, mortality, growth performance.*

INTRODUCTION

Bee Pollen is quite a varied plant product rich in biologically active substances approximately, 200 substances were found in the pollen grains from different plant species. In the group of basic chemical substances, there are proteins, amino acids, carbohydrates, lipids and fatty acids, phenolic compounds, enzymes, and coenzymes as well as vitamins and other beneficial (Campos *et al.*, 2008; 2010). Bee Pollen contains 22.7% protein on average, including 10.4% of essential amino acids such as methionine, lysine, threonine, histidine, leucine, isoleucine, valine, phenylalanine, and tryptophan. These protein elements are vitally essential for life, and the organisms cannot synthesize bio essential compounds. Moreover, in bee pollen, there are significant amounts of nucleic acids, especially the ribonucleic one. Further, digestible carbohydrates were occurred in the BP in amount of 30.8% on average. Reducing sugars, mainly fructose and glucose, are involved in this additive by about 25.7% (Roulston and Cane, 2000, Almeida-Muradian *et al.*, 2005; Kędzia and Hołderna-Kędzia, 2005; 2012).

Among lipids, which are present in the bee pollen in amount of about 5,1where the essential fatty acids (EFAs) are categories the first place. Acids such as linoleic, γ-linoleic and archaic were exist in amount of 0.4%. while phospholipids amount to 1.5%, while phytosterols, especially P-sitosterol, are present in the amount of 1.1% (Szczesna, 2006). Another group was represent phenolic compounds and amounted up to 1.6% on average. This group includes flavonoids, leukotrienes, catechins, and phenolic acids. Among flavonoids that occurring in the BP in (1.4%), there are mainly kaempferol,

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quercetin, and isorhamnetin, while in the group of phenolic acids, (0.2%), mainly chlorogenic acid was contained (Asafova *et al.,* 2001).

Definitely, BP is characterized by a quite significant content of triterpene bonds. The most frequent compounds are oleanolic acids, 3-ursolic acid, and betulin alcohol (Kędzia and Hołderna-Kędzia, 2005; 2012). Moreover, vitamins and other bioelements also belong to valuable substances. Bee pollen is quite a significant source of vitamins either fat-soluble (0.1%), such as provitamin A and vitamins E and D, and water-soluble 0,6%, such as B1, B2, B6, and C, and acids: pantothenic, nicotinic and folic, biotin, rutin, and inositol. Their total amount is equal to 0.7% in the whole product (Campos et al., 2008; 2010).

Total minerals represent about 1.6% and including macro minerals like (calcium, phosphorus, magnesium, sodium, and potassium) and micro minerals (iron, copper, zinc, manganese, silicon, and selenium). The latter one is existing in amount of 0.02% (Kędzia and Hołderna-Kędzia, 2005; 2012). According to the latest National Data, the average content of main ingredients in the air-dried pollen (at the temperature 40 °C) amounts to such values as follows: proteins, 32.8%, including essential amino acids, 11.5%, and reducing sugars, 40.7%, including sucrose, 3,7%, lipids, 12.8%, vitamin C, 0.19%, β-carotene, 0,07%, and bioelements, 4.0% (Komosinska-Vassev *et al.,* 2015).

Bee pollen, the highly nutritive food, is composed of around 45-60% carbohydrates, 15-25% proteins, 5-10% fats and 10-19% fibers beside its valuable content of minerals (manganese, zinc, potassium, magnesium, selenium) and vitamins (Abdelmontaleb et al., 2023). Another very interesting bee product like propolis which has antibiotic properties and therefore may be improve growth performance, feed efficiency and feed intake of animals (Sarker and Yang, 2010). These favorable effects may be due to its content of antioxidants, vitamins, minerals, phenolic constituents and enzymes (El-Hanoun *et al.,* 2007). The immunity responsiveness represented in leukocytes counts and mainly on lymphocytes increased significantly with increasing bee pollen levels. Hematological parameters and liver functions have been improved due to bee pollen additional with rabbit rations (El-Neney and El-Kholy, 2014).

The objective of this study was to investigate the effect of bee pollen additive on the growth performance, immunity response, health status, mortality rate and economic efficiency of suckling Rahmani lambs.

MATERIALS AND METHODS

The current work was carried out at Sakha Animal Production Research Station that belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture. The experiments were performed according to the guidelines of a local ethics committee for animal care and welfare (Number 08/2016 EC).

Experimental animals

Forty-five suckling lambs (24 males and 21 females) at 7 days after birth with average live body weight of 5.2 ± 0.06 kg were allocated into three similar groups (8 males and 7 females in each group) according to live body weight and sex. All lambs naturally suckled their dams. The 1st group was unsupplemented and served as control (G1), whereas the 2nd and 3rd groups orally supplemented with 0.5 and 1.0 g bee pollen/lamb/day for G2 and G3, respectively. The experiment was continued for 84 days during the period from 7 days after birth until weaning at 90 days.

Management procedures

All lambs were housed in semi-shaded open yard for each group and kept under the same managerial and hygienic experimental conditions. Lambs were weighed biweekly from the beginning of experiment until weaning at 90 days. Total weight gain and average daily gain were calculated for each lamb.

Blood Samples

Blood samples were taken at the last week of experiment from the jugular vein of each lamb by clean sterile needle in clean dry glass tubs using heparin as an anticoagulant. Samples were centrifuged for 15 minutes at 4000 rotations per minute to obtain plasma. The concentrations of immunoglobulins IgG, IgM and IgA in the blood plasma samples were measured using the

quantitative ELISA Bovine (IgG, IgM and IgA), ELISA Quantitation Kit, Bethyl laboratories, UK.

Diseases infection and mortality rate

The number of lambs that infected to diseases of diarrhea, respiratory, septicemia, navel and general weakness as well as mortality percentages was recorded during the whole experimental period.

Statistical analysis

Data were analyzed using general linear models procedure adapted by IBM SPSS Statistics (2014) for user's guide with one-way ANOVA. Significant differences in the mean values among dietary treatments were analyzed by Duncan's tests within SPSS program set at the level of significance P<0.05 (Duncan, 1955).

RESULTS AND DISCUSSION

Immunity response

Different types of serum immunoglobulins (IgG, IgM, and IgA) concentrations in plasma of lambs at weaning were affected significantly by BP additive (Table 1). Results showed that all types of plasma immunoglobulins were significantly (P<0.05) increased in tested groups (G2 and G3) compared with the control one (G1). The high bee pollen level (G3) showed significantly (P<0.05) the higher values compared to the low one G2. These results indicated similarity in the beneficial impacts of BP, but their synergetic effect was recorded on the immune response of lambs at weaning. The results here are similar to those obtained by Abdel-Raouf *et al.*, 2018 who worked with early weaning Friesian calves the same author demonstrated that BP additive with calves at levels of 5 and 10 g/head/day significantly increased the concentrations of IgG, IgM and IgA in plasma at 3 and 6 months of age as well as with increasing BP level. Also, BP has been documented as a significant nutritional additive with several therapeutic actions, as an immunomodulatory activator (Khalifa *et al.*, 2021). The inclusion of BP in broiler diets (1.5%) during the 1st three weeks of age could be increased IgM concentration (De Oliveira et al., 2013) and this impact may be attributed to the favorable effect of BP that was found to protect the health of the intestinal tract of broiler and rabbits (Wang et al., 2007; Attia *et al.*, 2011a, b), respectively. The aforementioned increases in serum immunoglobulin (IgG, IgM, and IgA) could be satisfied to B-lymphocytes stimulation. Consequently, the immunoglobulins would be elevating initiating and immune response through helper T-cells, cytotoxic T-cells, and C8 T- cells (El-Gaafrawy *et. al.*, 2000; Tizard, 2004) which may lead to perfect immunocompetent calves.

Table 1: Immunity response of suckling lambs in different experimental groups.

Item	Experimental groups			SE	P-value
	G1	G2	G3		
IgG, g/L	30.50^{c}	37.20^{b}	43.70^{a}	1.92	0.002
IgM, g/L	4.10^{c}	4.50^{b}	4.80^{a}	0.10	0.003
IgA, g/L	2.70^{c}	2.98^{b}	3.20^{a}	0.07	0.001

a, b, c: Values in the same row with different superscripts differ significantly at P<0.05.

Diseases infection

The percentages of infection of suckling lambs with various diseases are presented in Table (2). The percentages of diarrhea, respiratory, septicemia, navel and general weakness diseases of lambs were decreased significantly (P<0.05) with BP additive as well as with increasing the level of additive based on control treatment. These results might be due to the higher concentrations of immunoglobulins in plasma of lambs with BP additive (Table 1) that extremely improving health status. These findings are in accordance with the previous studies; bee pollen appears promising because it protects intestinal tract health of rabbit (Attia *et al.*, 2011a, 2011b). Wang *et al*. (2007) used 1.5% BP in broiler diets and demonstrated that the BP had a trophic effect in the small intestine. Moreover, bee pollen addition in the diet in mammals feed increased

the intestinal absorptive capacity through the longer and thicker villi. BP additive at levels of 5 and 10 g/head/day significantly reduced diarrhea incidence of suckling and post weaning Friesian calves as well as the pest result was occurred with the higher level of BP (Abdel-Raouf *et al.,* 2018).

Table 2: Diseases infection of suckling lambs in different experimental groups.

Item	Experimental groups			SE	P-value
	G1	G2	G3		
Diarrhea, %	22.50[a]	14.30[b]	6.80[c]	2.27	0.002
Respiratory, %	16.40[a]	11.10[b]	5.60[c]	1.56	0.003
Septicemia, %	12.80[a]	8.50[b]	4.20[c]	1.24	0.002
Navel, %	9.20[a]	6.10[b]	3.60[c]	0.81	0.001
General weakness, %	8.10[a]	5.60[b]	3.30[c]	0.69	0.003

a, b, c: Values in the same row with different superscripts differ significantly at P<0.05.

Mortality rate

Result of mortality rate of suckling lambs as affected by BP additive are illustrated by Fig. 1. Mortality rate of suckling lambs revealed similar trend amongst the infection diseases which decreased significantly (P<0.05) with BP additive as well as with increasing the level of additive. Control group (G1) recorded significantly (P<0.05) the highest mortality rate 20% followed by G2 13.33%, but G3 appeared to the lowest mortality rate 6.67%. Natural complements as bee pollen have been largely applied in the overall animal population for well-being and health, and possible therapy for some diseases (Wang et al., 2007). Bee pollen is considering as one of the most useful therapeutic products that adapted by natural medicine scientists because of its possible nutritional and medical applications. It exhibits many impacts such as antimicrobial, immunostimulating, antioxidants and hepatoprotective bioactivities. Furthermore, BP has some useful therapeutic features in numerous pathological situations such as its impact to normalize wound healing (Abdelnour et al., 2019). The average lamb mortality rate between lambing and weaning was 12.5%, ranging from 9.8% to 13.9%, with no significant variations between birth seasons (*Heinzen et al.,* 2023). The total morbidity and mortality in lamb were 27.3% and 32.5%, respectively. Malnutrition was the most common problem in lambs causing up to 31.3% mortality followed by diarrhea 24% and respiratory problems 21.3% (Hadgu *et al.,* 2021). The mean annual birth-to-weaning mortality in the mixed crop-livestock system was reported in the range of 14.9–33.5% in lambs (Tibbo, 2006). Also, he stated that the incidence of lamb mortality was mostly due to diarrhea, pneumonia, septicemia, dehydration, and navel illness.

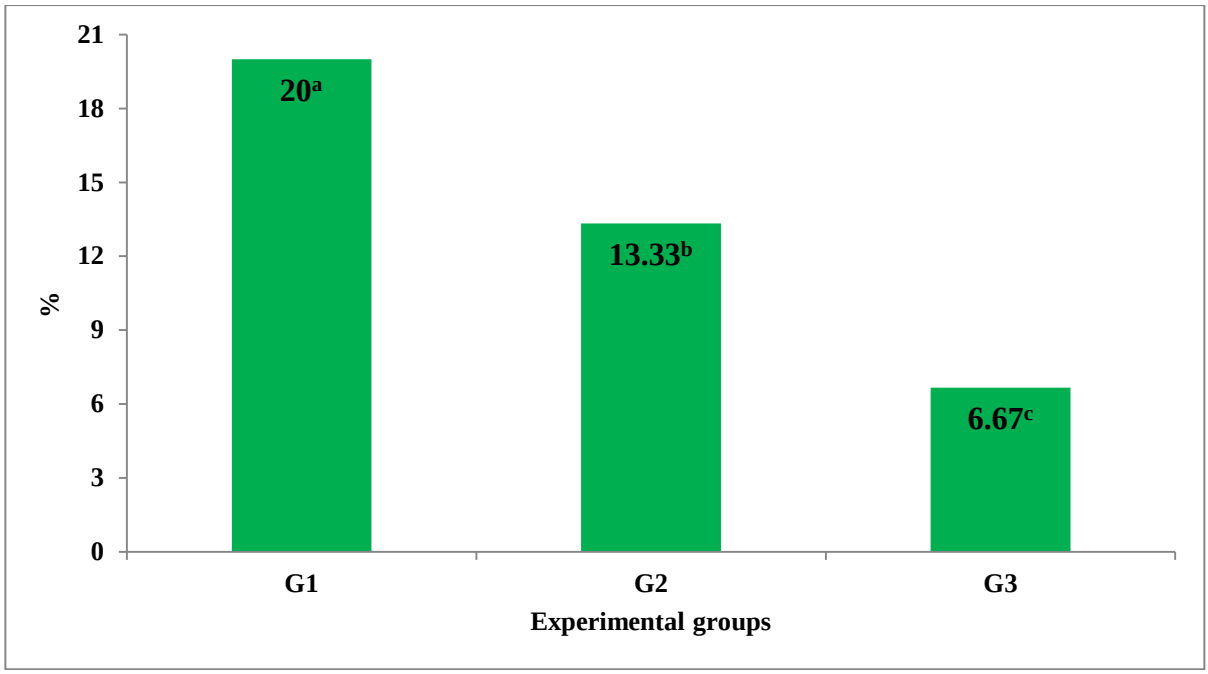

Fig. 1: Mortality rate of suckling lambs in different experimental groups.

Live body weight and weight gain

Based on the equality of the live body weight of lambs at the beginning of experiment amongst all groups, lambs received BP (G2 and G3) tended to perform higher live body weight and daily gains. Lambs were significantly (P<0.05) heavier in BP additive groups than in the control one, being the heaviest in G3. Also, total and daily gain were significantly (P<0.05) higher in BP additive groups than in control group, being significant higher with G3 than those of G2 (Table 3). The weaning weight of lambs in G2 and G3 was higher by about 5.49 and 10.12% relative to the control lambs, respectively. Moreover, total weight gain was increased by about 7.77 and 14.38% in G2 and G3 relative to the control one. The corresponding values regarding average daily gain was about 7.93 and 14.38%. Bee pollen has been comprised of high concentrations of reducing sugars, essential amino acids and unsaturated/saturated fatty acids where half of them are the unsaturated acids like oleic, linoleic (omega-6) and linolenic (omega-3), minerals as Zn, Cu, Fe, Se and high K/Na ratio. Significant quantities of several vitamins in bee pollen like provitamin A, B complex, A, C, D, K3, E (tocopherol), vitamin B2 (riboflavin), niacin, thiamine, folic acid was observed. Biotin has an important role in metabolic processes of proteins, fats and carbohydrates also, has vital participation with vitamins B6, B9 and B12 metabolism which reflected in an enhanced animal performance. It is also a potential source of polyphenols and other healthy compounds (Santos Pereira, *et al.*, 2010, Campos, *et al.*, 2010 and Attia *et.al.*, (2014). The effects of BP can also be attributed to the fact that BP is an energy enhancer, a growth promoter, and a potential replacement of antibiotic (Almaraz-Abarca *et al.*, 2004). The nourishing and medical benefits of BP as an antioxidant, anti-aging substance, and a supplement to improve immune function and growth in animals have been widely appreciated (Almaraz-Abarca *et al.*, 2004 and El-Neney and ElKholy 2014). On the other recent study, BP additive at levels of 5 and 10 g/head/day significantly increased weaning weight, total and average daily gain of pre and post weaning Friesian calves as well as with increasing BP level (Mohsen *et al.*, 2017).

Feed intake and conversion

Results in Table (3) achieved that feed intake increased significantly (P<0.05) with BP supplementation. Feed intake was significantly (P<0.05) higher in G3 compared to G1, with insignificant difference with G2. Moreover, feed

conversion improved significantly (P<0.05) with BP supplementation, which G3 recorded the lower amount of feed per kg live weight compared to G1, with insignificant difference with G2 (P<0.05). These results are in agreement with finding of Hosseini *et al*. (2016) noticed that a significant ($p < 0.05$) increase in average daily feed intake (ADFI) by 15.3% when BP was added to the broiler starter basal diet. At all investigated periods, birds received BP at levels of 0.2%, 0.4% or 0.6% demonstrated an improvement in FCR compared to the control group. Also, the best FCR was obtained in the group fed 0.6% BP for the whole experimental period (Farag and El-Rayes, 2016). Growing rabbits treated with BP significantly (P≤0.05) improved in feed conversion as compared with control group (Zeedan *et al*., 2017). Bee pollen additive at the level of 10 g/head/day for the diet of Friesian calves during suckling and post weaning periods led to an improvement of feed intake and feed conversion (Mohsen *et al*., 2017).

Output of weaning weight

The output of weaning weight in Egyptian pound (LE) is shown in Fig. (2). Bee pollen additive was recorded significant increase (P<0.05) in output of weaning weight compared to that of control one. Also, output of lambs weaning weights increased significantly (P<0.05) with increasing BP pollen from 0.5 to 1.0 g/lamb/day. The output of weaning weight increased by 190 LE (5.49%) in G2 and 350 LE (10.12%) in comparison with that control (G1). On the other words, output of weaning weight was increased by190 L.E (5.49%) in (G2) and 350 L.E (10.12%) in comparison with that of control one (G1). Similar effect was found by (El-Neney and El-Kholy, 2014) who showed higher economic efficiency when added 400 mg BP to the diet of rabbits than that of control group. Also, Mohsen *et al.* (2017) found that total and net revenues of Friesian calves increased significantly with BP additive at the levels of 5 and 10 g/calf/day.

Table 3: Growth performance of suckling lambs in different experimental groups.

Item	Experimental groups			SE	P-value
	G1	G2	G3		
Initial weight, kg	5.20	5.19	5.21	0.02	0.938
Final weight (weaning), kg	17.30^{c}	18.25^{b}	19.05^{a}	0.27	0.002
Total weight gain, kg	12.10^{c}	13.06^{b}	13.84^{a}	0.26	0.001
Average daily gain, g	144.05^{c}	155.48^{b}	164.76^{a}	9.38	0.001
Feed intake, g/day	225.00^{b}	234.40^{ab}	242.60^{a}	2.92	0.014
Feed conversion, kg/kg gain	1.56^{a}	1.51^{ab}	1.47^{b}	0.02	0.029

a, b, c: Values in the same row with different superscripts differ significantly at P<0.05.
Price of 1 kg live weight was 200 LE according to prices of 2024.

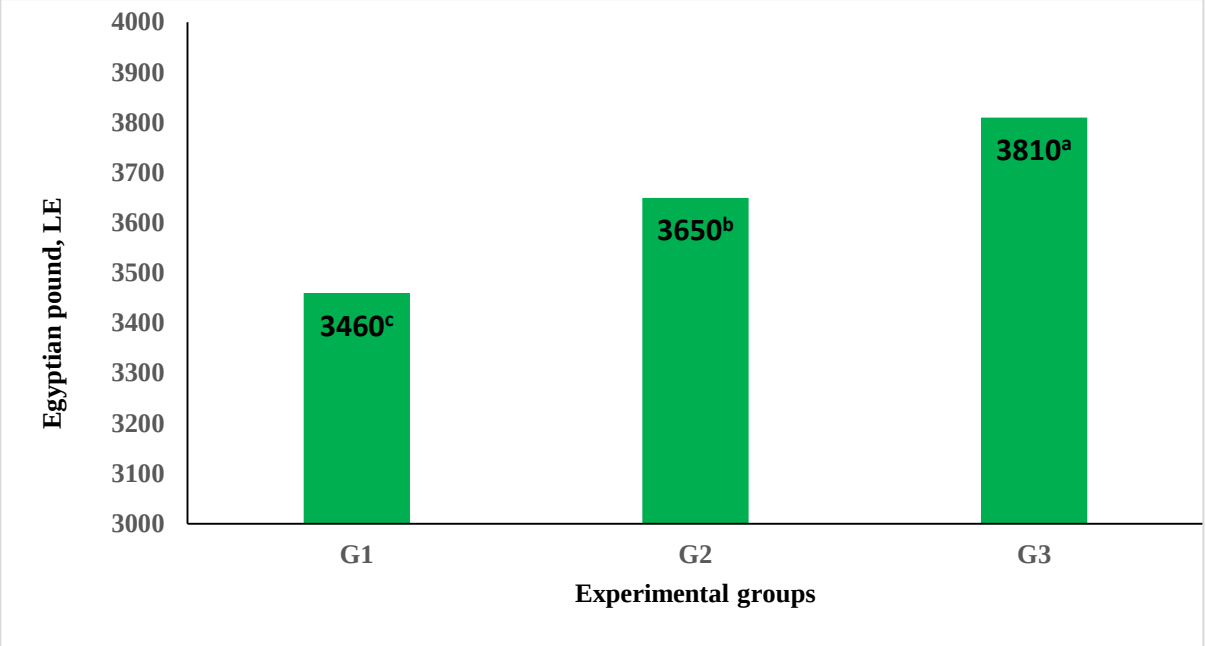

Fig. 2: Output of weaning weight of lambs in different experimental groups.

CONCLUSION

From the present results, it could be concluded that using the bee pollen as feed additive for suckling lambs at the level of 1 g/lamb/day led to an improvement immunity response, reduced diseases infection and mortality rate and increased weaning weight, total and average daily gain and output of weaning weight.

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تأثير استخدام حبوب لقاح النحل كإضافة غذائية للحملان الرحمانى الرضيعة على الاستجابة المناعية والإصابة بالأمراض ومعدل النفوق وأداء النمو وعائد وزن الفطام

حامد محمد جعفر[1]، مصطفى محمد النحراوى[1]، محمد فكرى القرش [2] ، غادة صلاح العيسوى [1]، رضا عبد البارى مصباح[1]

[1]قسم بحوث تغذية الحيوان – معهد بحوث الإنتاج الحيوانى – وزارة الزراعة – الدقى – جيزة – مصر.
[2] معهد بحوث صحة الحيوان - وزارة الزراعة – الدقى – جيزة – مصر.

تم تقسيم خمسة وأربعون حملاً رضيعاً (٢٤ ذكراً و٢١ أنثى) في اليوم السابع بعد الولادة بمتوسط وزن حي ٥.٢ ± ٠.٠٦ كجم إلى ثلاث مجموعات متماثلة (٨ ذكور و٧ إناث كل مجموعة) حسب وزن الجسم الحي والجنس. أرضعت جميع الحملان أمهاتها بشكل طبيعي. المجموعة الأولى بدون اضافة (مجموعة مقارنة G1)، بينما تم إضافة ٠.٥ و١.٠ جرام من حبوب لقاح النحل / حمل / يوم عن طريق الفم إلى المجموعتين الثانية والثالثة على التوالي. استمرت التجربة لمدة ٨٤ يومًا. أظهرت النتائج أن جميع أنواع الجلوبولينات المناعية في بلازما الدم زادت بشكل ملحوظ (P <0.05) في المجموعات المختبرة (G2 وG3) مقارنة (G1). أظهر مستوى حبوب لقاح النحل المرتفع (G3) أعلى القيم بشكل ملحوظ (P <0.05) مقارنة بالمستوى في G2. انخفضت نسب الإصابة بالأمراض بشكل ملحوظ (P <0.05) مع إضافة حبوب لقاح النحل مع زيادة مستوى الاضافة. سجلت مجموعة المقارنة (G1) أعلى معدل نفوق بشكل ملحوظ (P <0.05) ٢٠٪ تليها المجموعة G2 ١٣.٣٣٪، بينما حققت المجموعة G3 ٦.٦٧٪. زيادة وزن الفطام للحملان بشكل ملحوظ (P<0.05) في مجموعات إضافة حبوب لقاح النحل مقارنة بمجموعة المقارنة. سجلت المجموعة الثالثة أعلى زيادة كلية في الوزن ومعدل النمو اليومى حيث بلغت ١٣.٨٤ كجم و١٦٤.٧٦ جم على التوالي. كما حققت اضافة حبوب لقاح النحل تحسن ملحوظ (P<0.05) في كمية المأكول والتحويل الغذائى مع أفضلية في المجموعة الثالثة. كما زاد عائد وزن الفطام بشكل ملحوظ (P<0.05) مع زيادة حبوب لقاح النحل من ٠.٥ إلى ١.٠ جم/حمل/يوم.