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Effect of Using Propolis on Productive Performance and Cecum Microbial Activity of Postweaning Growing Male Rabbits

[1]Walaa H. Khalifa, [1]Nagwa A. Maghraby and [2]Fatma Abou-Hashim

[1]Animal Production Department, Agricultural and Biology Research Institute, National Research Centre, Dokki, Giza, Egypt
[2]Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt

Abstract: Rabbits can get intestinal illnesses, particularly after they've been weaned and switched from milk to solid food. Propolis (PR) is a mixture of proteins, carbohydrates and phenolic compounds that can increase the level of serum immunoglobulin, improve the antioxidant state of the animals and lessen the severity of clinical symptoms and death. So this study was designed to evaluate the effect of using (PR) on productive performance, cecum microbial activity of postweaning growing male rabbits. Twenty New Zealand White (NZW) male rabbits, 5 weeks old were randomly divided into two equal groups, with 695.75± 10.01 g BW. In the 1st group, rabbits were fed without any supplementation and considered as the control group. While, those in the 2nd group were orally given (PR) at 500 mg/kg BW 3days /week for 10 weeks. Results revealed that growing rabbits treated with (PR) show a decrease in pH value and *E. coli* count as compared with the control group.in addition, using (PR) causing significantly increased in plasma total protein, albumin and globulin concentrations in treated than the control group. Conclusively, from the obtained results it could be concluded that the supplementation of(PR) had positive effects on rabbit's performance as well as, cecal microbial activity of growing rabbits.

Key words: Propolis · Productive performance · Cecal microbes · Post weaning · Rabbits

INTRODUCTION

It has been suggested that producing rabbits could serve as an option to address the growing scarcity of meat in developing nations because of its brief production cycle [1]. However, due to their complex digestive physiology, rabbits are vulnerable to intestinal diseases, particularly during the post-weaning stage [2]. In order to increase productive performance and lower mortality from digestive diseases [3], antibiotics are commonly fed to growing and fattening rabbits. Unfortunately, due to the overuse of antibiotics, there has been a global rapid rise of microbial resistance to these medications, endangering both human and animal health [4].

Propolis (PR) is a mixture of flower pollen grains collected by bees with nectar and secretions from the hypopharyngeal glands of bees [5, 6]. According to Thakur and Nanda [7], PR contains carbohydrates, proteins, lipids, fiber, minerals and phenolic compounds. Flavonoid and phenolic compounds in propolis is responsible for many biological and pharmacological activities including anticancer, anti-inflammatory, anti-bacterial, antifungal, antiviral, antioxidant, hepato-protective and immuno-stimulating activities [8]. PR supplementation has been shown to enhance cecum morphology and decrease Salmonella spp. and *Escherichia coli* levels in the cecum of growing rabbits [9]. Furthermore, the addition of PR to rabbit diets has been shown to enhance the animals' antioxidant status and raise the content of serum immunoglobulin [10]. According to other studies, PR supplementation in rabbits has been shown to decrease the severity of clinical symptoms and mortality brought on by *Pasteurella multocida* while having no adverse effects [11]. Similarly, Sierra-Galicia *et al*. [12] and Attia *et al*. [13] found that growing rabbits supplemented with PR at 250 and 500 mg/kg BW significantly increased growth and survival rates from weaning till mature age. Better rabbit

Corresponding Author: Walaa Kalifa, Department of Animal Production, Agriculture and Biological Research Institute, National Research Centre, Dokki, Giza, Egypt.

performance, BWG, oxidative state, immune response, decreased feed intake and increased FCR were reported by El-Neney and El-Kholy [14] and Zeedan and El-Neney [15]. The curresent investigation aimed to relieve weaning shock and alleviate oxidative stress in addition to reducing mortality and morbidity of post weaning rabbits.

MATERIALS AND METHODS

Preparation and Analysis of Propolis: Propolis was collected from an apiary located at the Faculty of Agriculture, Cairo University (Giza province, Egypt). The collected propolis was kept in a clean, dark bottle at 4°C until use in the experiment [15]. the nutritional composition of the PR was 91.4% dry matter, 3.12 % crude protein, 8.76% ether extract and 0.95% ash. The total phenolic compound content of the PR extract was determined using the colorimetric method described by Singleton [16]. For this, gallic acid was used as a standard and the absorbance was measured at 765 nm using a UV-VIS spectrophotometer (Aquamate Plus UV-Vis model, Thermo Fisher Scientific TM, Waltham, MA, USA). Similarly, the total flavonoid content of the PR extract was obtained following the colorimetric method using aluminum chloride, as previously described by Chang *et al*. [17]. In this case, quercetin was used as a standard and the absorbance was measured at 415 nm using a UV-VIS spectrophotometer (Aquamate Plus UV-Vis, Thermo Fisher Scientific TM, Waltham, MA, USA). The total content of phenolic compounds of the PR was 22.97 mg gallic acid equivalent per g dry weight of the extract. Likewise, the total flavonoid content of the PR was 9.53 mg quercetin equivalent per g dry weight of the extract.

Experimental Design: A total number of 24 Healthy New Zealand white (NZW) male rabbits, with 695.75 ± 10.01 g BW, 5 weeks of age was raised under normal managerial and hygienic conditions throughout experimental period from 5 to 15 weeks of age. Rabbits were individually raised in wire galvanized battery cages supplied with feeders and automatic stainless-steel nipples. Fresh drinking tap water was automatically available all the time in each cage. All rabbits were randomly divided into two equal groups. In the 1st group, rabbits were fed standard ration without any supplementation and served as the control group, while the 2nd group were treated with Propolis as the water suspension at 500 mg/kg BW [add reference] 3 days / week for 10 weeks. All rabbits were fed *ad-libitum* on the commercial pellets ration (Table A) covering their daily nutritional requirements [18]. The rabbits in all groups were individually weighed, while weight gain (BWG) was calculated by subtracting the initial live body weights from final ones of each period. Feed consumption was weekly recorded, while feed conversion ratio (FCR) was calculated as g feed/g gain.

Table A: The basal diet formulation for rabbits

Ingredients% content		chemical analysis %	
Barley	30.28	OM%	90.33
Clover hay (6%)	27.05	CP%	17.76
Wheat bran	18.40	CF%	11.5
Soybean meal (44% CP)	18.10	EE%	2.5
Molasses	3.00	NFE%	57.43
Di calcium phosphate	2.20	Ash%	9.66
NaCl	0.30	DE (kcal/kg)	2560
Premix (Vit. Min) *	0.30	Calcium	1.11
Limestone	0.22	Total phosphorus	0.85
DL-Methionine	0.10	Methionine+ cyst.	0.65
Anticoccidia	0.05	Lysine	0.91
Total	100		

Slaughter Procedures and Blood Sampling: At the end of experimental period, (week 15) five rabbits per group with a weight close to the average of the group were selected and slaughtered in a commercial slaughterhouse. The carcass was weighed and expressed as a percentage of slaughter weight (SW). The weight of heart, liver, Cecum and dressing were recorded for each carcass

Blood samples were taken from five animals in each group after slaughtering in heparin test tubes then centrifugated at 4000 rpm for 10 minutes then plasma was harvested and kept at -20°C until the clinical analysis. Total proteins, albumin, Globulin, Glucose, cholesterol, Total lipids and Triglycerides were measured using commercial colorimetric kits (Bio-Diagnostics, Egypt).

Cecum Activity and Cecum Microbial Counts: Five rabbits in each group were allowed to give individual samples of their cecum contents, which were then extracted after the rabbits were killed and filtered to determine the pH and microflora of the cecum. According to Collins *et al*. [19], the total number of *E. coli* bacteria was estimated. In accordance with Ahmed [20] and AOAC [21], respectively, the ammonia-nitrogen and total volatile fatty acid (VFA) contents in cecum samples were determined.

Statistical Analysis: Data were analyzed by one-way ANOVA using SPSS 20.0 [22]. If a significant difference ($P < 0.05$) was detected, Duncan's test was performed for multiple comparisons [23].

RESULTS AND DISCUSSION

Data presented in Table 1, showed that the rabbits in propolis group have significantly (P<0.05) increased body weight and weight gain, decreased feed intake and improved feed conversion rate (FCR)compared to the control group. In addition, rabbits in propolis group had significantly(P<0.05) lower mortality rate than others in the control group. These results are in agreement with Hashem *et al*. [24] who found that feed conversion was improved and live body weights and weight gain of rabbits were higher in the groups received diets contained 150 and 300mg/kg. propolis compared to the control. Also, Attia *et al.* [25] reported that all natural growth promoters including propolis improving productive and reproductive performance, significant lowering of feed intake and improved feed conversion of rabbit does. On the other hand, Coloni [26] and Piza *et al*. [27] reported that inclusion of crude propolis in growing rabbit diets did not increase the weight gain. The current results may be attributed to the action of propolis on promoting intestinal health by increase the levels of beneficial bacteria and decrease the pathogenic types [28]. Additionally, propolis is an alternative source to antibiotics in diet [29]. Which may improve growth performance and feed efficiency of animals [30]. Also, propolis has antimicrobial, anti-inflammatory and immunomodulatory properties [31] which allowing for better utilization of nutrients. Moreover, propolis stimulates the activities of saccharase, amylase and phosphatase by progress nutrient digestibility and absorption [32]. Generally, the improvement in growth performance of rabbits supplemented with propolis could be reflecting better absorption of amino acids or/and due to antibacterial properties and enzymes or coenzymes of propolis.

The decreased mortality rate may have been due to the decreased pH, NH3–N; pathogenic bacteria count in the cecum (Table 3) leading to decreasing diarrhea of rabbits. Also, increased survival rate in treated groups could be due to enhancing immune functions, promote the growth of an animal, protect the intestinal tract health and consequently improve animal products quality and security [33, 34]. Also, propolis itself has been improve of immune system and was, showed to exhibit antifungal, anti-inflammatory antibacterial activity and/or an antimicrobial agent [35, 36].

Data presented in Table 2 showed that rabbits treated with PR had the heaviest mean carcass characteristics than the control group and the weight of heart, liver and cecum in rabbits received a Propolis were insignificantly increased than that of the control These findings agree with those of El-Neney *et al.* [13] and Dias and Pereira [37]. In contrast, the findings of Attia *et al*. [38] showed that the carcass yield of rabbits was not influenced by PR supplementation. The significant (P<0.05) increase in carcass traits for treated groups may be mainly related to the increase in growth performance and digestibility of treated rabbits, which was used as an indicator for good health status of rabbits.

Data presented in Table 3 indicate that cecal pH value and *E. coli* count for treated group were significantly (P<0.05) decreased than that of the control group. Referring to cecal VFAs concentrations, these findings indicated that the cecal VFAs concentrations in the treated groups increased in comparison with the control group, that point to greater cecal microbial activity. The total VFA concentration in rabbit received PR significantly (P<0.05) increased than that of the control group. The increased total VFA could be attributed to the decreased pH, NH3–N (Table 3). These findings agree with those of Zeedan *et al.* [14], who found that the addition PR in growing rabbits increasing TVFAs as well as decreasing pH and NH3-N. When absorbed, VFAs produced in the cecum can cover about 40% of rabbit maintenance requirement so; higher VFAs production could be beneficial with regard to better energy supply and better body weight gain as a consequence Additionally, VFA provide the main metabolic fuel for the mucosa of the large intestine [39].

Results of cecum activity indicated that supplementation of PR improved the microbial fermentation by increasing utilizing ammonia nitrogen and FVAs and reducing pH.

Data presented in Table 4 indicated that plasma total protein, albumin and globulin concentrations in treated rabbits increased significantly (P<0.05) than that of the control group. The increased blood proteins in rabbits received PR may be associated with improvement of crude protein digestibility as well as to the high level and good quality of protein contents in PR. These results are in agreement with the findings of Attia *et al.* [25] whereas they noted that the supplementation of propolis for rabbits has a positive effect on blood proteins. Referring to glucose level, these findings showed that plasma glucose concentration increased significantly (P<0.05) for treated group than that of the control group. Also, Wei *et al.* [40] stated that the increased plasma glucose in the treated group may be reflecting the increasing energy availability (sugars) for the physiological and biochemical functions.

Table 1: Effect of Propolis on growth performance of growing rabbits

Parameters	Control (C)	Propolis (PR)
Initial live body weight (g)	698±10.54	695±10.01
Final live body weight (g)	2175±12.42[b]	2844±12.32 [a]
Average daily gain (ADG), g/d	35.5±4.76[b]	38.9±5.68[a]
Daily feed intake (DFI), g/d	109.7±3.56[a]	96.8±7.32[b]
Feed conversion rate (FCR)	3.1±003[a]	2.48±005[b]
Mortality rate%	13.33%	0.00%

Data were expressed as Mean ± SE. Means with different superscripts (a , b) within row differ significantly at P<0.05

Table 2: Effect of propolis on some carcass traits of growing rabbit

Traits	Control	Propolis (PR)
BW at slaughter (gm)	1980±0.38[b]	2140±0.35[a]
Carcass weight (gm)	1265±0.25[b]	1540±0.27[a]
Dressing (gm)	102±0.11	107±0.09
Heart (gm)	6.8±0.10	7.3±0.11
Liver (gm)	62±0.12	65±0.13
Cecum(gm)	90.5±0.08	92.8±0.10

Table 3: Effect of propolis on cecum traits of growing rabbits

Parameters	Control	Propolis
Cecum pH	7.23±0.01[a]	5.12±0.03[b]
Bacteria count E. coli × 104 CFU/g	15.27±0.06[a]	4.14±0.07[b]
NH3-N (mmol/l)	10.07±0.06[a]	4.03±0.09[b]
Total VFA (mmol/l)	55.32±3.50[b]	82.01±3.66[a]

Data were expressed as Mean ± SE. Means with different superscripts (a, b) within row differ significantly at P<0.05. 1 Number of bacterial cells / gm of cecum content (total count × 105)

Table 4: Effect of Propolis on some blood constituent of rabbit does

Parameters	Control	Propolis (PR)
Total protein (mg/ 100ml)	6.13±0.03[b]	7.52±0.05[a]
Albumin (mg/100ml)	2.83±0.01[b]	3.67±0.03[a]
Globulin (mg/100ml)	3.40±0.03[b]	3.95±0.04[a]
Glucose (mg/dl)	97.5±0.65[b]	139.7±0.75[a]
Total lipids (mg/dl)	466.9 ±14.30[a]	370.2±13.50[d]
Cholesterol (mg/dl)	212.1±0.20 [a]	190.1±0.10 [c]
Triglycerides (mg/dl)	187.7±0.26[a]	135.5±0.24 [d]

Data were expressed as Mean ± SE. Means with different superscripts (a, b) within row differ significantly at P<0

CONCLUSION

This study shown that administering propolis (PR) reduce weaning shock, lower the death and morbidity rates and improve the productive performance of post-weaning rabbits.

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