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POSGRADO EN PRODUCCIÓN ANIMAL

DESEMPEÑO PRODUCTIVO, INMUNIDAD E INCIDENCIA DE ENFERMEDADES RESPIRATORIAS Y PARASITARIAS EN CONEJOS SUPLEMENTADOS CON POLEN APÍCOLA Y PROPÓLEO

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DEDICATORIAS

Es momento de ver el dibujo número uno y conocer a la serpiente boa.

Para Aleli e Itai, las amaré eternamente ♥

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RESUMEN GENERAL

DESEMPEÑO PRODUCTIVO, INMUNIDAD E INCIDENCIA DE ENFERMEDADES RESPIRATORIAS Y PARASITARIAS EN CONEJOS SUPLEMENTADOS CON POLEN APÍCOLA Y PROPÓLEO

El uso de aditivos en ganadería está muy extendido y los suplementos alimenticios se pueden utilizar de manera segura en la producción de conejos para mejorar su rendimiento. En el primer estudio, se realizó una evaluación de los efectos de la suplementación con polen apícola (PA) y propóleo (PRO) en el rendimiento productivo y los metabolitos séricos de conejos a través de un metaanálisis. Dieciséis publicaciones revisadas por pares se incluyeron en el conjunto de datos analizados. La suplementación con PA disminuyó el índice de conversión alimenticia (CA) y aumentó la ganancia diaria de peso (GDP) y el rendimiento de canal caliente (RCC). La suplementación con PRO redujo la CA y aumentó el RCC. En el suero sanguíneo, la suplementación con PA aumentó la capacidad antioxidante total (TAC) y disminuyó la concentración de creatinina. En el segundo estudio se evaluó el efecto de la suplementación con PA y PRO en el comportamiento productivo, conteo de ooquistes de *Eimeria* en heces, los metabolitos sanguíneos y la calidad de la carne de conejos en crecimiento. El tratamiento de conejos suplementados con PRO50 (50 µL/kg PV) obtuvo mayor GDP y menor CA. La suplementación con PRO50 y PA + PRO (500 mg/kg PV de polen y 50 µL/kg PV de propóleo) redujo la cantidad de ooquistes de *Eimeria* por gramo de heces. En conclusión, la suplementación con PA y PRO se puede utilizar como promotor de crecimiento en conejos, sin afectar el estado de salud renal o hepático y la calidad de la carne.

Palabras clave: promotores de crecimiento, productos de abejas, metabolitos séricos, coccidiosis.

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[1] Tesis de Doctorado en Ciencias en Innovación Ganadera, Posgrado en Producción Animal, Universidad Autónoma Chapingo

Autor: María Inés Sierra Galicia

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GENERAL ABSTRACT

PRODUCTIVE PERFORMANCE, IMMUNITY AND INCIDENCE OF RESPIRATORY AND PARASITIC DISEASES IN RABBITS SUPPLEMENTED WITH BEE POLLEN AND PROPOLIS

The use of additives in livestock is widespread and feed supplements can be used safely in rabbit production to improve performance. The first study evaluated the effects of bee pollen (PA) and propolis (PRO) supplementation on rabbit´s productive performance and serum metabolites through a meta-analysis. Sixteen peer-reviewed publications were included in the analyzed data set. PA supplementation decreased the feed conversion ratio (FCR) and increased daily weight gain (DWG) and hot carcass yield (HCY). PRO supplementation reduced FCR and increased HCY. In blood serum, PA supplementation increased total antioxidant capacity (TAC) and decreased serum creatinine concentration. The second study evaluated the effect of PA and PRO supplementation on productive performance, *Eimeria* oocyst count in feces, blood metabolites, and growing rabbits' meat quality. The treatment of rabbits supplemented with PRO50 (50 µL/kg LW) obtained higher DWG and lower FCR. In addition, supplementation with PRO50 and BP + PRO (500 mg/kg LW of pollen and 50 µL/kg LW of propolis) reduced the number of *Eimeria* oocysts per gram of feces. In conclusion, BP and PRO supplementation can be used as a growth promoter in rabbits, without affecting renal or hepatic health status and meat quality.

Keywords: growth promoters, bee products, serum metabolites, coccidiosis

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[2] Doctoral Thesis in Livestock Innovation, Graduate Program in Animal Production, Universidad Autónoma Chapingo

Author: María Inés Sierra Galicia

Advisor: Raymundo Rodríguez De Lara

1 INTRODUCCIÓN GENERAL

Attia et al. (2019) mencionan que la producción de conejo podría ser una alternativa para solucionar la creciente demanda de carne en países en desarrollo, debido a que su ciclo de producción es corto. Sin embargo, para obtener granjas de producción cunícola rentables es necesario reducir la mortalidad y aumentar la productividad durante el periodo de crecimiento (Hashem, El-Hady, & Hassan, 2017). Por lo anterior, en varios países es común la inclusión dietética de antibióticos para reducir la incidencia de enfermedades y aumentar la tasa de crecimiento de los conejos (Attia et al., 2013). Desafortunadamente, el uso descontrolado de antibióticos ha generado la aparición de cepas microbianas con resistencia a los efectos de estos medicamentos, lo cual representa una amenaza para la salud de humanos y animales a nivel mundial (Holmes et al., 2016). En consecuencia, en años recientes ha incrementado el interés por la búsqueda y validación de productos naturales que puedan mejorar la salud y el comportamiento productivo de los conejos (Hashem, Hassanein, & Simal-Gandara, 2021). Entre los productos naturales más prometedores se encuentran los productos derivados de las abejas (*Apis mellifera),* tales como el polen apícola (PA) y el propóleo (PRO), los cuales contienen una amplia variedad de compuestos nutricionales y metabolitos bioactivos, algunos de ellos con propiedades farmacéuticas (Abdelnour, El-Hack, Alagawany, Farag, & Elnesr, 2019; Hashem et al., 2021).

De acuerdo con Martinello y Mutinello (2021), el PA es una mezcla de néctar, secreciones salivales de las abejas y granos de polen recolectados de las flores, el cual contiene proteínas (5-60 %), azúcares (13-55 %), fibra cruda (0.3-20 %), lípidos (4-7 %), minerales y compuestos fenólicos, principalmente flavonoides (3-8 %). Abdelnour et al. (2019) han reportado que la suplementación dietética con

PA tiene algunos efectos terapéuticos en los animales, tales como antioxidante, antimicrobiano, antiinflamatorio e inmunomodulador. En conejos en crecimiento, la suplementación con PA mejora la digestibilidad de los nutrientes, la producción de ácidos grasos volátiles en el ciego y la actividad de las enzimas digestivas (Zeedan, El-Neney, Aboughaba, & El-Kholy, 2017). En conejos en crecimiento, se ha reportado que la suplementación con PA aumenta los niveles séricos de factor de crecimiento semejante a insulina tipo I (Abdel-Hamid, & El Tarabany, 2019) y mejora la morfología de las vellosidades intestinales (Attia et al., 2013).

El PRO es una mezcla compleja de sustancias resinosas, gomosas y balsámicas recolectadas por las abejas de brotes, flores y exudados de plantas (Al-Kahtani, Alaqil, & Abbas, 2022). Bhargava et al. (2021) detectaron que el PRO contiene aproximadamente 180 compuestos volátiles, de los cuales la mayoría son flavonoides y ácidos fenólicos que tienen propiedades inmunoestimulantes, antioxidantes y antimicrobianas (Hashem et al., 2021). Al-Homidan et al. (2022) reportaron que la suplementación con PRO disminuyó la presencia de bacterias *Escherichia coli* y *Salmonella* spp. en el ciego de conejos en crecimiento. Por otro lado, Hashem et al. (2017) observaron mayor concentración sérica de inmunoglobulinas y enzimas antioxidantes en conejos en crecimiento suplementados con PRO. Además, se ha demostrado que la suplementación con PRO no tiene efectos tóxicos en conejos (Nassar, Mohamed, Soufy, Nasr, & Mahran, 2012).

La investigación acerca del uso de productos apícolas en la producción de conejos y su impacto en la salud aún es escasa, por ello, el presente estudio considera el efecto del uso del PA y PRO como suplemento alimenticio durante el período de crecimiento.

En el **Capítulo 2** se presenta una revisión sistemática (metaanálisis) que sintetiza cuantitativamente la información publicada de enero de 2000 a noviembre de 2022 en revistas revisadas por pares, donde se incluye información del rendimiento productivo, calidad de la canal, estado antioxidante del suero sanguíneo y metabolitos séricos de conejos en crecimiento suplementados con PA y PRO.

En el **Capítulo 3** se presentan los resultados obtenidos del trabajo experimental con conejos California × Nueva Zelanda Blanco, donde se evaluaron los efectos del polen apícola, el propóleo y la combinación de ambos productos en el comportamiento productivo, el conteo de ooquistes de *Eimeria*, parámetros sanguíneos y calidad de la carne.

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2 EFFECTS OF SUPPLEMENTATION WITH BEE POLLEN AND PROPOLIS ON GROWTH PERFORMANCE, AND SERUM METABOLITES OF RABBITS: A META-ANALYSIS

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animals

MDPI

Article

Effects of Supplementation with Bee Pollen and Propolis on Growth Performance and Serum Metabolites of Rabbits: A Meta-Analysis

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Simple Summary: Bee pollen and propolis have been used successfully to improve performance and serum metabolites in poultry; however, their effects in rabbits have been inconsistent. Therefore, the objective of this study was to evaluate the supplementation with bee pollen and propolis on animal performance and serum metabolites of rabbits through a meta-analysis. In rabbits, supplementation with bee pollen and propolis has been shown to reduce the feed conversion rate; however, it can also increase weight gain and total antioxidant capacity in blood serum. These results suggest that bee pollen and propolis could be used as natural growth promoters and to improve rabbits' antioxidant status.

Abstract: The objective of this study was to evaluate the effects of bee pollen (BP) and propolis (PRO) supplementation on rabbits' productive performance and serum metabolites through a meta-analysis. Sixteen peer-reviewed publications were included in the data set. The rabbit strains used in the studies included in the data set were New Zealand White, V-line, Rex, and V-line crosses. Weighted mean differences (WMD) between treatments supplemented with BP or PRO and control treatments were used to assess the magnitude of the effect. BP supplementation decreased ($p < 0.001$) daily feed intake (DFI) and feed conversion ratio (FCR); however, increased ($p < 0.001$) average daily gain (ADG) and hot carcass yield (HCY). PRO supplementation reduced DFI ($p = 0.041$) and FCR ($p < 0.001$), and increased ADG ($p < 0.001$) and HCY ($p = 0.005$). In blood serum, BP supplementation increased total antioxidant capacity (TAC; $p = 0.002$) and decreased serum creatinine concentration ($p = 0.049$). Likewise, decreased serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and malondialdehyde (MDA) were detected in response to BP supplementation ($p < 0.05$). PRO supplementation increased the TAC in blood serum ($p = 0.018$); however, decreased serum concentrations of AST, ALT, and MDA were observed ($p < 0.05$). In conclusion, BP or PRO supplementation can be used as a natural growth promoter in rabbits, and both can also improve rabbits' antioxidant status. However, BP or PRO supplementation does not affect rabbits' renal or hepatic health status.

Keywords: growth promoters; serum metabolites; honeybee products; antioxidant status

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1. Introduction

It is necessary to increase the number and productivity of weaned rabbits and reduce mortality during the growth period to improve profitability in rabbit farms [1]. Therefore, in diets for growing rabbits, it is common to include antibiotics (for example, zinc bacitracin) that reduce the incidence of diseases and act as growth promoters [2]. However, the indiscriminate use of antibiotics contributes to the increase in the appearance of bacteria resistant to their effects, representing a significant threat to the health of animals and

humans [3]. Consequently, the use of antibiotics as growth promoters has been prohibited in several countries, representing a major challenge for rabbit meat producers [4]. For these reasons, in recent years, the interest of researchers in the search and development of new natural alternatives to antibiotics and synthetic antioxidants has increased [4]. Among the natural alternatives currently available are products derived from bees (*Apis mellifera*), such as bee pollen (BP) and propolis (PRO). These products contain various bioactive metabolites with pharmaceutical properties [5].

BP is a mixture of nectar, salivary secretions from bees, and pollen grains collected from flowers [6]. According to Martinello and Mutinello [7], BP is composed of proteins (5–60%), sugars (13–55%), crude fiber (0.3–20%), lipids (4–7%), minerals, and phenolic compounds, mainly flavonoids (3–8%). BP has been reported to have various therapeutic properties, including antioxidant, anti-inflammatory, immunomodulatory, and antimicrobial activity [6]. On the other hand, PRO is a resinous substance that bees produce by mixing salivary gland secretions with beeswax and plant exudates [5]. PRO is mainly composed of flavonoids and phenolic acids (40–70%), waxes (20–35%), essential oils (1–3%), and approximately 5% of other organic substances [8]. In addition, PRO has been reported to have antioxidant, anti-inflammatory, antifungal, and antimicrobial properties [5]. In animal science, the effects of BP and PRO supplementation have been evaluated primarily in poultry [9,10]. However, the information available on the effects of BP and PRO supplementation in rabbits is still limited. In several species of domestic animals (for example, sheep and broilers, among others), it has been reported that supplementation with BP or PRO improves the immune response [11], increases feed digestibility [12], reduces oxidative stress [13], and improves animal performance [9].

Particularly in rabbits, studies have been conducted to evaluate the effects of BP and PRO supplementation on productive performance [2,14], carcass yield [15,16], antioxidant status in blood [1,17], and blood biochemistry [18,19]. In addition, some studies have reported that BP or PRO supplementation can effectively replace zinc bacitracin (the main antibiotic used in rabbits) in rabbit diets without affecting performance, mortality, or economic profitability. [4,20]. However, the results obtained to date have yet to be homogeneous or conclusive. The variability in doses, experimental periods, supplementation methods, and age of the animals are associated with the heterogeneity of the results observed in rabbits supplemented with BP and PRO [19]. These sources of variability must be identified and controlled to develop products containing BP and PRO that can be used as food supplements to improve rabbits' productive performance and health.

A few review articles have been published [5,6,10], mentioning that BP or PRO supplementation can be used to improve productive and reproductive performance and the health of mammals and poultry. However, none of these review articles focused only on rabbits, nor did they use meta-analytic methods. Meta-analysis (MA) is a statistical tool that estimates the average effect of a given intervention through the combination and quantitative synthesis of results previously published in different studies [21]. Additionally, the MA makes it possible to identify sources of heterogeneity between studies [22]. Although the use of MA in research related to animal nutrition is proliferating, in rabbit nutrition, the use of MA is still limited [23]. The present study hypothesizes that supplementation with BP or PRO will benefit the productive performance of rabbits without affecting their health. Therefore, the objective of this meta-analysis was to evaluate the effects of BP and PRO supplementation on animal performance, carcass yield, oxidative status, and serum metabolites of rabbits.

2. Materials and Methods

2.1. Literature Search and Study Selection

A meta-analysis was performed to assess the effects of BP and PRO supplementation on rabbits' productive performance and serum metabolites. For this, an exhaustive and structured search of scientific articles focused on evaluating the effects of supplementation with BP or PRO was carried out, following the PRISMA guidelines [24] in the identification,

selection, choice, and inclusion of studies (Figure A1). The Scopus, PubMed, Web of Science, and ScienceDirect databases were used for the search process. The keywords used in the four databases were the following: rabbit, bee pollen, propolis, growth performance, carcass yield, and blood metabolites. Search results were restricted to studies published between January 2000 and November 2022, and 307 scientific publications were identified (Figure A1). Duplicate publications were excluded from the database. The remaining publications were subjected to a two-step selection process, as previously reported by other authors [25–27].

For this process, the titles and abstracts of each publication were first reviewed. Based on this information, all studies that were not conducted in rabbits, those that used experimentally infected rabbits, those that did not measure any of the variables of interest, and review articles were excluded. In the second step of the selection process, the articles analyzed had to meet some inclusion criteria to be considered in the final database. The inclusion criteria applied in the present meta-analysis were similar to those previously reported by other authors [23,26,28]: (1) studies using rabbits housed in cages (total confinement); (2) data on productive performance or serum metabolites; (3) studies using control and experimental treatments fed similarly, except for BP or PRO supplementation; (4) studies that reported the doses of BP or PRO used, or that contained sufficient information to estimate the doses of BP or PRO given to rabbits; (5) studies published in peer-reviewed scientific journals and written in English; and (6) studies reporting the treatment means (control and experimental), the standard error or standard deviation, and the number of replicates.

2.2. Data Extraction

Considering the inclusion criteria previously described in the database used for the meta-analysis, only 16 articles were included (Table A1). Furthermore, of the articles included in the final database, only data for response variables reported in at least three studies were extracted [25,27,28]. Consequently, in the present meta-analysis, variables of animal performance (weight gain, daily feed intake, and feed conversion rate) and hot carcass yield were included. In addition, the serum concentration of urea, creatinine, cholesterol, albumin, globulin, total protein, liver enzymes (aspartate aminotransferase and alanine aminotransferase), malondialdehyde, and total antioxidant capacity in blood serum were included.

For each of the variables mentioned, the means of the control (without supplementation) and experimental treatments (supplemented with BP or PRO), the standard deviations (SD), and the number of repetitions (n) were extracted. When an article did not report the SD, it was calculated using the following equation [29]: $SD = SEM \times \sqrt{n}$, where SEM = standard error of the treatment means. Additionally, from each of the selected publications (n = 16), the following complementary information was obtained: (1) author and year of publication, (2) country where the study was conducted, (3) nutritional composition of the experimental diets (g/kg DM), (4) duration of BP or PRO supplementation period (days), (5) dose of BP or PRO used (mg/kg BW), (6) age of rabbits, and (7) sex and rabbit strain.

2.3. Calculations and Statistical Analysis

Statistical analyses of meta-analysis, analysis of heterogeneity, publication bias, meta-regression, and subgroup analysis were performed using the 'metafor' [30] package of R statistical software version 4.1.2 (R Core Team, Vienna, Austria). The effects of BP or PRO supplementation in rabbits were determined using the weighted mean differences (WMD) between the experimental treatments (rabbits supplemented with BP or PRO) and control treatments (rabbits not supplemented with BP or PRO). In the present study, the WMD was used because it allows the interpretation of the results obtained in the original units of measurement [31]. The treatment means for all the evaluated variables were weighted by the inverse of the variance, according to the method previously proposed by Der-Simonian and Laird [32] for random effects models.

Descriptive statistical values were obtained for the nutritional composition of the diets used using the PROC MEANS procedure of the SAS statistical software [33]. The SAS PROC MIXED procedure was used to determine the differences in the nutritional composition of the diets used in the treatments supplemented with BP or PRO and the control treatments. For this, the different studies were included as a random effect, and the Tukey test was used to detect possible statistical differences ($p \leq 0.05$) between the treatments, as previously described by other authors [26,27].

2.4. Heterogeneity and Publication Bias

In the present meta-analysis, the heterogeneity of the effect of the treatments (variability between studies) was determined using the statistical tests of chi-square (Q) and I^2 (percentage of variation) [22]. For the Q test, a significance level of $p \leq 0.10$ was used, as its power has been reported to be relatively low in detecting heterogeneity among a small number of comparisons [34]. On the other hand, the I^2 statistical test was used to measure heterogeneity as a percentage [35]. In the I^2 test, the values are between 0 and 100%; values less than 25, between 25 and 50, and greater than 50% indicate low, moderate, and high heterogeneity, respectively [21,22].

Egger's linear regression asymmetry test was used to assess the presence of publication bias [36]. This test was considered statistically significant when $p \leq 0.05$ was obtained. Additionally, when a significant bias was detected ($p \leq 0.05$) with Egger's test, the "trim and fill" method of Duval and Tweedie [37] was applied to determine the number of missing observations.

2.5. Meta-regression and Subgroup Analysis

Meta-regression analyses were performed to investigate potential sources of heterogeneity in the response variables tested. The variables had to meet the following meta-regression criteria: (1) variables reported in at least ten different studies [38]; (2) p-value ≤ 0.10 for the Q or I^2 test greater than 50% [21,35]; and (3) p-value ≥ 0.05 for the Egger regression asymmetry test [37]. For the meta-regression, the methods of Der-Simonian and Laird [32] were followed since these procedures are well established to estimate the between-study variance. In cases where any covariate was significant with a p-value ≤ 0.05, a subgroup analysis was applied to the WMD. First, the supplementation method, the rabbits' age, sex, and rabbit strain were used as categorical covariates. Next, the length of the experimental period (days) and doses (mg/kg BW) were used as continuous covariates. Subsequently, the statistically significant covariates ($p \leq 0.05$) were evaluated by subgroup analysis [25–27]. The supplementation method covariate was divided into the following subgroups: (1) oral aqueous solution with a syringe, (2) capsules taken orally, and (3) orally through drinking water. The covariate rabbit sex was divided into three subgroups: (1) male rabbits, (2) female rabbits, and (3) mixed male and female rabbits (50% of each). The covariate rabbit strain was divided into four subgroups: (1) New Zealand White, (2) Rex, (3) V-line, and (4) V-line crosses. In addition, the covariate age of the rabbits was divided into two subgroups: (1) $\leq$ 15 weeks and (2) > 15 weeks. The continuous covariates that were significant in the meta-regression were evaluated using the following subgroups: supplementation period ($\leq$ 70 and > 70 days) and dose used ($\leq$ 350 and > 350 mg/kg BW). The reference values of the covariates were established based on the median values obtained with the descriptive statistical analysis performed on each covariate. For example, the age and the experimental period median were 15 weeks and 70 days, respectively. In the case of the dose, the median was 335 mg/kg BW, but we decided to close the amount to 350 mg/kg BW.

3. Results

3.1. Study Attributes and Excluded Studies

Table 1 shows no statistical differences ($p > 0.05$) between the control treatment and the one supplemented with BP for the nutrient content of the diet. Similarly, no differences ($p > 0.05$) were detected between the control treatment and the PRO-supplemented treat-

ment for any of the dietary components (Table A2). These results suggest that, for our data set, it is possible to exclude the effects of dietary nutrients on the response of rabbits to BP or PRO supplementation.

Table 1. Descriptive statistics of the complete data set for the effect of BP supplementation on rabbits' diets.

Parameter		Mean		Median		Minimum		Maximum		SD	
Dietary features	NC	Control	BP	Control	BP	Control	BP	Control	BP	Control	BP
DM, g/kg DM	9	901.0	901.1	903.2	903.2	874.7	874.7	917.1	917.1	11.81	11.81
CP, g/kg DM	27	177.8	177.8	180.0	180.0	170.0	170.0	184.0	184.0	5.49	5.49
EE, g/kg DM	12	29.93	29.93	27.75	27.75	26.20	26.20	51.40	51.40	6.89	6.89
NDF, g/kg DM	5	324.1	324.1	326.9	326.9	316.4	316.4	331.1	331.1	7.21	7.21
ADF, g/kg DM	5	154.2	154.2	149.2	149.2	148.1	148.1	163.4	163.4	7.36	7.36
CF, g/kg DM	24	131.8	131.8	130.0	130.0	126.0	126.0	150.0	150.0	6.39	6.39
Ash, g/kg DM	7	94.43	94.43	95.20	95.20	74.8	74.8	103.6	103.6	10.30	10.30
Ca, g/kg DM	3	0.95	0.95	0.87	0.87	0.87	0.87	1.11	1.11	0.13	0.13
P, g/kg DM	3	0.53	0.53	0.41	0.41	0.41	0.41	0.77	0.77	0.20	0.20
DE, MJ/kg DM	26	10.60	10.60	10.47	10.47	9.2	9.2	12.15	12.15	0.69	0.69
BP, mg/kg BW	27	-	374.0	-	335.0	-	100	-	1000	-	195.9
Duration, days		85.0		70.0		28.0		140.0		37.68	

NC = number of comparisons; BP = bee pollen; SD = standard deviation; DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; CF = crude fiber; Ca = calcium; P = phosphorus; DE: digestible energy. In the same column, means followed by different letters differ significantly by the Tukey test ($p \leq 0.05$).

The studies included in the present meta-analysis were conducted in only four countries. In summary, studies evaluating BP were conducted in Egypt (81.8%), Brazil (9.1%), and Mexico (9.1%). Similarly, studies evaluating PRO were conducted in Egypt (62.5%), Saudi Arabia (12.5%), Mexico (12.5%), and Brazil (12.5%). Table 1 shows that the doses of BP used varied between 100 and 1000 mg/kg BW. The doses of PRO used were between 30 and 846 mg/kg BW (Table A2). The experimental periods of the studies using BP ranged from 28 to 140 days (Table 1). Table A2 shows that the studies that evaluated PRO used experimental periods of 32 to 140 days. In most treatments that evaluated BP, the rabbits used were old (70.3%) and only 29.7% of the treatments used rabbits that were ≤15 weeks old. The treatments that evaluated PRO mainly used rabbits that were ≤15 weeks of age (80.0%), and only 20.0% of the treatments used rabbits that were >15 weeks of age. Regarding the supplementation method, most treatments (67.7%) supplied the BP in an aqueous solution using an oral syringe. Likewise, 25.9% of the treatments supplied the BP orally through drinking water, and the remaining treatments (7.4%) supplemented the BP in capsules. In the treatments that evaluated PRO, this product was supplemented mixed with the basal diet (66.7%) by oral aqueous solution with a syringe (20.0%) and using capsules (13.3%). Most studies (50%) used male rabbits, 18.7% used female rabbits, 18.7% used mixtures of male and female rabbits (50% of each), and 12.6% of the studies did not report the sex of the rabbits used. Regarding the rabbit strain, most studies (62.5%) used New Zealand White rabbits, 18.7% used V-line rabbits, 12.5% used V-line rabbit crosses, and 6.3% of the studies used Rex rabbits.

3.2. Growth Performance

Table 2 shows that average daily gain (ADG) and hot carcass yield (HCY) increased in response to BP supplementation ($p < 0.001$). In contrast, a lower feed conversion ratio (FCR) and daily feed intake (DFI) were observed in rabbits supplemented with BP ($p < 0.001$). On the other hand, Table 3 shows that PRO supplementation increased ADG ($p < 0.001$) and HCY ($p = 0.005$); however, FCR and DFI decreased ($p < 0.001$).

Table 2. Growth performance of rabbits supplemented with bee pollen.

Item	N (NC)				Heterogeneity		Egger Test [1]
		Control means (SD)	WMD (95 % CI)	*p*-Value	*p*-Value	I^2 (%)	*p*-Value
ADG, g/d	11 (27)	21.38 (7.33)	1.309 (0.802; 1.816)	<0.001	<0.001	87.91	0.182
DFI, g/d	10 (25)	149.4 (62.9)	−0.935 (−1.343; −0.527)	<0.001	<0.001	81.96	0.061
FCR, DMI/ADG	8 (19)	4.49 (1.00)	−0.708 (−1.021; −0.395)	<0.001	<0.001	99.45	0.353
HCY, %	4 (7)	53.68 (4.53)	2.723 (1.155; 4.290)	<0.001	<0.001	93.10	NA

N: number of studies; NC: number of comparisons; SD: standard deviation; WMD: weighted mean differences between control and treatments supplemented with bee pollen; CI: confidence interval of SMD; p-Value for the χ^2 test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; ADG: average daily gain; DFI: daily feed intake; FCR: feed conversion ratio; HCY: hot carcass yield.

Table 3. Growth performance of rabbits supplemented with propolis.

Item	N (NC)				Heterogeneity		Egger Test [1]
		Control means (SD)	WMD (95 % CI)	*p*-Value	*p*-Value	I^2 (%)	*p*-Value
ADG, g/d	8 (15)	29.53 (6.06)	1.035 (0.441; 1.628)	<0.001	<0.001	76.27	0.117
DFI, g/d	8 (15)	109.44 (26.25)	−0.427 (−0.837; −0.018)	0.041	0.004	55.94	0.196
FCR, DMI/ADG	7 (14)	3.15 (0.87)	−0.442 (−0.560; −0.324)	<0.001	<0.001	81.03	0.095
HCY, %	5 (8)	55.55 (5.63)	3.504 (1.052; 5.957)	0.005	<0.001	90.63	NA

N: number of studies; NC: number of comparisons; SD: standard deviation; WMD: weighted mean differences between control and treatments supplemented with propolis; CI: confidence interval of SMD; *p*-value for the χ^2 test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; ADG: average daily gain; DFI: daily feed intake; FCR: feed conversion ratio; HCY: hot carcass yield.

3.3. Serum Metabolites

Table 4 shows that BP supplementation reduced ($p < 0.05$) the serum concentration of urea, creatinine, cholesterol, total lipids, aspartate aminotransferase (AST), alanine aminotransferase (ALA), and malondialdehyde (MDA). In contrast, higher ($p < 0.05$) serum concentrations of glucose, albumin, globulin, total protein, and higher total antioxidant capacity (TAC) were observed in response to BP supplementation. On the other hand, Table 5 shows that PRO supplementation did not affect ($p > 0.05$) the serum concentration of urea, creatinine, and glucose. However, lower ($p < 0.05$) serum concentrations of cholesterol, total lipids, AST, ALA, and MDA were observed in response to PRO supplementation. In contrast, PRO supplementation increased ($p < 0.05$) the serum concentration of albumin, globulin, total protein, and TAC (Table 4).

Table 4. Serum metabolites of rabbits supplemented with bee pollen.

Item	N (NC)				Heterogeneity		Egger Test [1]
		Control means (SD)	WMD (95 % CI)	*p*-Value	*p*-Value	I^2 (%)	*p*-Value
Urea, mg/dL	9 (20)	31.38 (8.94)	−4.023 (−6.827; −1.219)	0.005	<0.001	99.64	0.266
Creatinine, mg/dL	9 (18)	1.17 (0.33)	−0.152 (−0.303; −0.001)	0.049	<0.001	98.95	0.968
Glucose, mg/dL	10 (20)	88.28 (19.24)	13.759 (7.641; 19.876)	<0.001	<0.001	99.69	0.121
Cholesterol, mg/dL	9 (18)	118.80 (56.1)	−11.607 (−13.347; −9.868)	<0.001	<0.001	99.95	0.063
Albumin, mg/dL	10 (20)	3.08 (0.46)	0.268 (0.138; 0.397)	<0.001	<0.001	97.68	0.480
Globulin, mg/dL	10 (20)	2.79 (0.67)	0.196 (0.039; 0.353)	0.015	<0.001	94.78	0.728
Total protein, mg/dL	10 (20)	5.87 (0.92)	0.490 (0.238; 0.742)	<0.001	<0.001	96.58	0.567
AST, UI/dL	8 (17)	50.88 (12.26)	−6.074 (−8.068; −4.080)	<0.001	<0.001	84.99	0.083
ALT, UI/dL	7 (16)	61.91 (13.29)	−6.429 (−8.505; −4.353)	<0.001	<0.001	90.94	0.460
TAC, mmol/L	4 (7)	3.88 (1.22)	0.716 (0.273; 1.159)	0.002	<0.001	99.74	NA
MDA, nmol/mL	3 (6)	6.33 (3.70)	−0.774 (−1.368; −0.180)	0.011	<0.001	89.17	NA

N: number of studies; NC: number of comparisons; SD: standard deviation; WMD: weighted mean differences between control and treatments supplemented with bee pollen; CI: confidence interval of WMD; p-value for the χ^2 test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; NA: variables with n < 10 observations, the test does not apply; AST: aspartate aminotransferase; ALT: alanine aminotransferase; MDA: malondialdehyde; TAC: total antioxidant capacity.

Table 5. Serum metabolites of rabbits supplemented with propolis.

Item	N (NC)				Heterogeneity		Egger Test [1]
		Control means (SD)	WMD (95 % CI)	*p*-Value	*p*-Value	I^2 (%)	*p*-Value
Urea, mg/dL	3 (5)	30.20 (7.59)	−0.842 (−13.942; 12.259)	0.900	<0.001	99.89	NA
Creatinine, mg/dL	3 (5)	0.88 (0.28)	0.151 (−0.121; 0.424)	0.277	<0.001	92.66	NA
Glucose, mg/dL	5 (7)	95.07 (24.56)	7.905 (−5.451; 21.262)	0.246	<0.001	98.16	NA
Cholesterol, mg/dL	5 (7)	107.60 (39.6)	−8.012 (−14.000; −2.024)	0.009	0.030	59.51	NA
Albumin, mg/dL	6 (9)	3.15 (0.52)	0.202 (0.001; 0.404)	0.049	<0.001	82.99	NA
Globulin, mg/dL	6 (9)	2.63 (0.86)	0.275 (0.077; 0.473)	0.006	<0.001	72.13	NA
Total protein, mg/dL	6 (9)	5.78 (1.05)	0.419 (0.072; 0.766)	0.018	<0.001	88.62	NA
AST, UI/dL	5 (7)	50.31 (16.13)	−5.539 (−9.246; 1.543)	0.006	<0.001	81.39	NA
ALT, UI/dL	4 (6)	57.32 (17.71)	−5.571 (−10.333; −0.810)	0.022	<0.001	93.32	NA
TAC, mmol/L	5 (8)	2.47 (1.62)	0.210 (0.036; 0.385)	0.018	<0.001	90.74	NA
MDA, nmol/mL	4 (7)	1.62 (0.58)	−0.213 (−0.294; −0.132)	<0.001	0.045	55.4	NA

N: number of studies; NC: number of comparisons; SD: standard deviation; WMD: weighted mean differences between control and treatments supplemented with propolis; CI: confidence interval of WMD; p-value for the χ^2 test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; NA: variables with n < 10 observations, the test does not apply; AST: aspartate aminotransferase; ALT: alanine aminotransferase; MDA: malondialdehyde; TAC: total antioxidant capacity.

3.4. Publication Bias and Meta-Regression

Tables 2–5 show that the Egger asymmetry regression test was not significant ($p > 0.05$) for any of the evaluated variables, indicating no publication bias. On the other hand, Tables 2 and 3 show that there was significant heterogeneity (Q) ($p \leq 0.10$) for ADG, DFI, FCR, and HCY. Similarly, Tables 4 and 5 show significant Q for the serum concentration of urea, creatinine, glucose, cholesterol, total lipids, albumin, globulin, total protein, AST,

ALA, MDA, and TAC. However, meta-regression analyses should only be used to obtain reliable results when the variable of interest was reported in at least ten different studies [38]. Therefore, in the present meta-analysis, the meta-regression was only applied to the variables: ADG and DFI (Table 2), glucose, albumin, globulin, and total protein (Table 4) of rabbits supplemented with BP.

Table 6 shows that ADG and serum glucose concentration had no significant relationship ($p > 0.05$) with any of the covariates used. BP dose explained ($p = 0.026$) 27.46% of the observed heterogeneity for serum albumin concentration. The supplementation period explained ($p < 0.05$) 27.57, 30.01, and 45.80% of the heterogeneity observed for the serum concentration of globulin, albumin, and total protein, respectively. Likewise, the age of rabbits explained ($p = 0.036$) 21.83% of the heterogeneity observed for the serum albumin concentration. BP supplementation method explained ($p < 0.05$) 19.70 and 20.38% of the observed heterogeneity for serum albumin concentration and DMI, respectively (Table 6). The covariates sex and rabbit strain had no significant relationship ($p > 0.05$) with ADG, DFI, glucose, albumin, globulin, or total protein.

Table 6. Meta-regression comparing the associations between covariates and measured outcomes.

Parameter	Covariates	QM	Df	*p*-Value	R^2 (%)
Average daily gain (ADG)	Bee pollen dose	0.351	1	0.554	0.0
	Supplementation period	0.259	1	0.611	0.0
	Rabbit´s age	1.287	1	0.257	0.0
	Supplementation method	2.919	2	0.232	0.0
	Sex	4.093	3	0.252	0.0
	Rabbit strain	1.327	2	0.515	0.0
Daily feed intake (DFI)	Bee pollen dose	0.419	1	0.517	0.0
	Supplementation period	0.005	1	0.942	0.0
	Rabbit´s age	0.136	1	0.713	0.0
	Supplementation method	7.729	2	0.021	20.38
	Sex	6.102	3	0.107	0.0
	Rabbit strain	4.295	2	0.117	5.27
Glucose	Bee pollen dose	0.001	1	0.975	0.0
	Supplementation period	0.502	1	0.479	0.0
	Rabbit´s age	0.036	1	0.850	0.0
	Supplementation method	1.961	2	0.375	0.86
	Sex	2.824	2	0.244	6.72
	Rabbit strain	0.147	2	0.929	2.40
Albumin	Bee pollen dose	4.971	1	0.026	27.46
	Supplementation period	8.465	1	0.004	30.01
	Rabbit´s age	13.467	1	0.036	21.83
	Supplementation method	7.471	2	0.024	19.70
	Sex	7.205	2	0.127	0.0
	Rabbit strain	0.538	2	0.764	0.0
Globulin	Bee pollen dose	0.420	1	0.517	0.0
	Supplementation period	5.680	1	0.017	27.57
	Rabbit´s age	0.008	1	0.928	0.0
	Supplementation method	3.086	2	0.214	7.46
	Sex	6.056	2	0.114	2.18
	Rabbit strain	0.150	2	0.928	0.0
Total protein	Bee pollen dose	2.345	1	0.126	5.49
	Supplementation period	10.458	1	0.001	45.80
	Rabbit´s age	0.003	1	0.960	0.0
	Supplementation method	5.909	2	0.062	10.74
	Sex	6.269	2	0.061	5.76
	Rabbit strain	0.291	2	0.865	0.0

QM: coefficient of moderators; QM is considered significant at $p \leq 0.05$; df: degree of freedom; R^2: the amount of heterogeneity in the meta-analysis.

3.5. Subgroup Analysis

Figure 1a shows that DFI decreased ($p < 0.05$), regardless of how BP was supplemented. However, the effect was greater (WMD = –2.763 g/d; $p = 0.004$) when BP was administered orally via drinking water than when BP was supplemented via oral cap-

sules (WMD = – 0.901 g/d; $p = 0.015$) or with BP in aqueous solution using an oral syringe (WMD = –0.516 g/d; $p = 0.002$). In contrast, a higher ($p < 0.001$) serum albumin concentration was observed in rabbits when BP was administered orally via drinking water (WMD = 0.319 mg/dL) and via capsules (WMD = 0.780 mg/dL; Figure 1b). However, serum albumin concentration was not affected with BP in aqueous solution using an oral syringe (WMD = 0.145 mg/dL; $p = 0.216$).

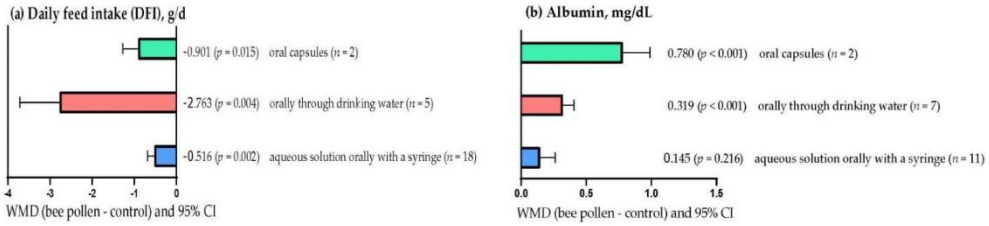

Figure 1. Subgroup analysis (subgroup = supplementation method) of the effect of bee pollen supplementation in rabbits, WMD = weighted mean differences between bee pollen treatments and control.

Figure 2a shows that the serum albumin concentration increased ($p < 0.05$), regardless of the supplementation period used; however, the effect was greater (WMD = 0.410 mg/dL) when BP was supplemented for more than 70 days than periods of up to 70 days (WMD = 0.166 mg/dL). On the other hand, the serum globulin concentration increased (WMD = 0.394 mg/dL; $p = 0.030$) when BP supplementation was longer than 70 days (Figure 2b). However, serum globulin concentration was not affected (WMD = 0.100 mg/dL; $p = 0.301$) when BP supplementation lasted up to 70 days. Figure 2c shows that serum total protein concentration increased when rabbits were supplemented with BP for more than 70 days (WMD = 0.917 mg/dL; $p < 0.001$). However, BP supplementation for up to 70 days did not affect serum total protein concentration (WMD = 0.241 mg/dL; $p = 0.163$).

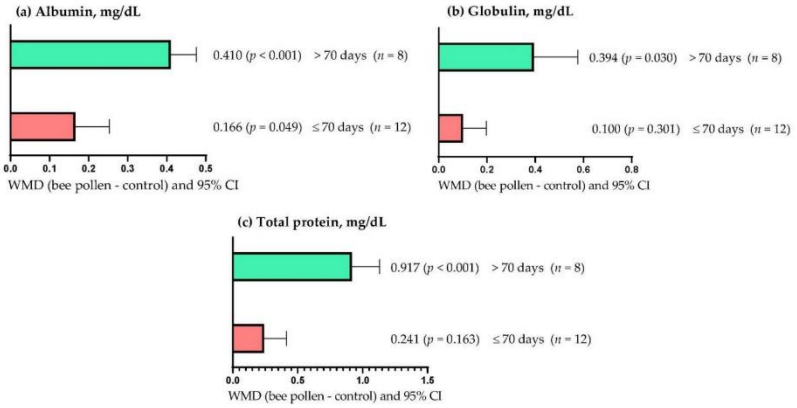

Figure 2. Subgroup analysis (subgroup = supplementation period (days)) of the effect of bee pollen supplementation in rabbits, WMD = weighted mean differences between bee pollen treatments and control.

Figure 3a shows that serum albumin concentration increased when BP doses greater than 350 mg/kg BW were used (WMD = 0.434 mg/dL; $p < 0.001$). However, low doses

(≤ 350 mg/kg DM) of BP did not affect serum albumin concentration (WMD = 0.072 mg/dL; $p = 0.384$). On the other hand, Figure 3b shows that the serum albumin concentration increased when BP was administered to rabbits older than 15 weeks of age (WMD = 0.434 mg/dL; $p < 0.001$). However, in rabbits up to 15 weeks of age, BP supplementation did not affect serum albumin concentration (WMD = 0.132 mg/dL; $p = 0.186$).

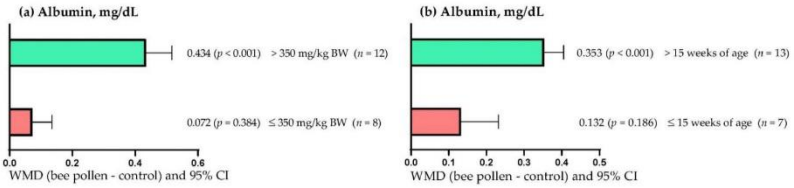

Figure 3. Subgroup analysis (subgroups = bee pollen dose (mg/kg of BW), and age of rabbits (weeks)) of the effect of bee pollen supplementation in rabbits, WMD = weighted mean differences between bee pollen treatments and control.

4. Discussion

4.1. Growth Performance

Some previously published review articles [6,39] have mentioned that dietary inclusion of BP or PRO could improve the taste of livestock foods. In addition, BP and PRO contain several bioactive compounds (e.g., flavonoids and phenolic acids) with antimicrobial and antioxidant properties [40,41], which could improve feed quality and palatability and lead to higher DFI. However, in the present meta-analysis, lower DFI was observed in response to BP and PRO supplementation. Similar to our results, a meta-analysis conducted by Sadarman et al. [9] reported that PRO supplementation decreased DFI in broilers. The mechanism of action of BP, PRO, and their bioactive metabolites on DFI regulation has not been studied in rabbits. However, recent studies [42,43] have shown that supplementation with FLAs (one of the primary bioactive metabolites of BP and PRO) increases gene expression of bitter taste receptors (TAS2R) in the epithelium of the bovine digestive tract. Activation of TAS2R receptors triggers the release of some anorexigenic molecules (cholecystokinin and peptide YY) [44,45]. Therefore, similar effects of the consumption of BP, PRO, and their flavonoids in the present study partially explain the reduction observed for DFI. On the other hand, BP and PRO contain water-soluble vitamins and minerals [40,41], which according to Attia et al. [14], accelerate nutrient metabolism in rabbits and increase metabolic energy availability. This effect results in lower DFI because in rabbits, as energy availability increases, DFI decreases [46].

In growing rabbits, supplementation with moderate BP doses (500 mg/kg BW) increases the cecal concentration of volatile fatty acids by up to 22% [15]. This effect could result in increased metabolic energy availability and lead to increased ADG since volatile fatty acids provide about 40% of the energy required for maintenance in rabbits [47]. On the other hand, Abdel-Hamid et al. [48] detected increased serum insulin-like growth factor-1 (IGF-1) concentration in rabbits supplemented with BP (250 mg/kg BW). This effect could result in increased ADG since IGF-1 serum levels have been positively correlated with ADG in rabbits [49]. In the present study, BP and PRO supplementation reduced MDA and increased TAC in blood serum. Al-Homidan et al. [18] observed a 21% higher serum concentration of total immunoglobulins (IgM + IgY) in rabbits supplemented with low doses of PRO (250 mg/kg DM). Likewise, it has been reported that supplementation with BP and PRO decreases between 30 and 100% the cecal bacterial count of *Escherichia coli* and *Salmonella spp.* in rabbits [15,18]. These effects could result in better health status of the rabbits and lead to higher ADG. Moreover, in rabbits, flavonoid supplementation increases the serum concentration of growth hormone [50] and the relative cecal abundance of bacterial families (*Peptococcaceae*, *Eubacteriaceae*, and *Syntrophomonadaceae*) that have a

positive correlation with weight gain [51]. Similar effects of the consumption of BP, PRO, and their flavonoids in the present meta-analysis would explain the increases observed for ADG.

In rabbits, BP supplementation increases the activity of digestive enzymes (protease, amylase, and lipase) in the intestinal contents and the digestibility of crude fiber, crude protein, and ether extract [15]. Likewise, Waly et al. [16] reported increased digestibility of crude protein and organic matter in rabbits supplemented with low doses (200 mg/kg DM) of PRO. On the other hand, it has been documented that supplementation with BP or PRO increases between 39 and 90% the length of intestinal villi in rabbits [15], which could result in increased nutrient absorption. Additionally, in growing rabbits, North et al. [51] reported that dietary supplementation with flavonoids increases the relative abundance of cecal bacteria (*Clostridiaceae*, *Haloplasmataceae*, and *Erysipelotrichaceae*), which have a negative correlation (r between -0.61 and -0.68) with FCR in rabbits. Similar effects of the consumption of BP, PRO, and their flavonoids in the present meta-analysis partially explain the observed reduction in FCR.

Most studies used New Zealand White rabbits in the present meta-analysis. Therefore, the positive effects of BP and PRO on ADG and FCR should be carefully interpreted, as they may only occur in New Zealand White rabbits. In addition, although the mixture of BP and PRO was not evaluated in this meta-analysis, this combination could act synergistically since the effect of the high flavonoid content of PRO could be potentiated by the high levels of vitamins and minerals provided by BP. Consequently, combining BP and PRO could have a greater positive impact on animal health and performance in rabbits than the individual use of BP or PRO.

4.2. Serum Metabolites

According to Hokamp and Nabity [52], serum urea and creatinine concentrations can be used as biomarkers of renal function. For example, high serum urea and creatinine levels indicate loss of nephron function and renal failure [53]. In the present meta-analysis, BP supplementation decreased serum urea and creatinine levels. However, serum urea and creatinine levels in rabbits supplemented with BP or PRO were within the normal ranges (urea: 20–45 mg/dL; creatinine: 0.5–2.5 mg/dL) reported in the literature for healthy rabbits [54]. These results suggest that BP and PRO do not affect the renal health of rabbits. Furthermore, in rabbits, deficiency of any essential amino acid increases catabolism of the remaining amino acids, increases hepatic urea production, and leads to higher serum urea levels [55]. BP contains essential amino acids (methionine, lysine, and threonine, among others) that improve the amino acid balance of rabbits [40], which would explain the lower serum urea concentration observed in response to BP supplementation.

Rabbits supplemented with BP or PRO had serum glucose levels within the normal range (75–155 mg/dL) [54]; however, serum cholesterol concentrations in rabbits supplemented with BP or PRO were above the normal range (10-80 mg/dL) reported in the literature for healthy rabbits [54]. Khalifa et al. [40] mention that BP contains about 30% carbohydrates, mainly glucose and fructose, which partially explains the increase in serum glucose observed in rabbits supplemented with BP. On the other hand, BP and PRO have a wide variety of flavonoids [56,57]. According to Zeka [58], flavonoids can decrease serum cholesterol concentration because they increase the expression of low-density lipoprotein receptors, decrease intestinal cholesterol absorption, and inhibit hepatic cholesterol synthesis. Consequently, the lower serum cholesterol concentration observed in rabbits supplemented with BP and PRO could be related to the flavonoid content of these two products. In addition, BP contains polyunsaturated fatty acids [40], which reduce serum cholesterol levels by inducing the expression of the enzyme cholesterol 7-hydroxylase and increasing receptors for low-density lipoproteins [59].

Serum albumin, globulin, and total protein concentrations in rabbits supplemented with BP or PRO were within normal ranges (albumin: 2.7–5.0 mg/dL; globulin: 1.5–2.7 mg/dL; total protein: 5.4–7.5 mg/dL) reported in the literature for healthy rabbits [54]. In the present

study, the higher serum total protein concentration observed in response to BP and PRO supplementation could be related to increased serum albumin and globulin levels. Serum albumin levels are decreased in animals with internal parasitism and when hepatic protein synthesis is low [60]. The present meta-analysis showed a higher serum albumin concentration in response to BP and PRO supplementation. This effect could be related to flavonoids in BP and PRO since flavonoids increase hepatic protein synthesis [61] and decrease internal parasites in rabbits [62]. In addition, BP contains approximately 23% protein [40], which could be related to the higher serum total protein, albumin, and globulin concentrations in BP-supplemented rabbits.

Serum concentrations of aminotransferases such as AST and ALT are used as indicators of hepatocellular damage [63]. For example, AST and ALT levels increase in response to almost all liver diseases, such as fatty liver, cirrhosis, hepatic necrosis, and hepatitis [64]. The present meta-analysis showed lower serum AST and ALT concentrations in response to BP and PRO supplementation. However, serum AST and ALT concentrations in rabbits supplemented with BP or PRO were within the normal ranges (AST: 10–78 UI/dL; ALT: 27.4–72.2 UI/dL) reported in the literature for healthy rabbits [65]. These results indicate that BP and PRO do not affect the liver health of rabbits.

According to Ghiselli [66], TAC is an integrated parameter that considers the cumulative action of all blood serum antioxidants. Moreover, MDA is frequently used as an indicator of lipid peroxidation [67]. In the present meta-analysis, higher TAC and lower MDA were observed in response to BP and PRO supplementation, suggesting that BP and PRO intake decreases lipid peroxidation and improves total antioxidant status in rabbits. Although little information exists on the antioxidant mechanisms of BP and PRO in rabbits, it has been reported that BP and PRO contain polyphenols (flavonoids and phenolic acids) that are absorbed in the intestinal tract of rodents [56]. Subsequently, these polyphenols can be transferred to the bloodstream, acting directly as exogenous antioxidants and activating transcription factors that increase serum levels of antioxidant enzymes (e.g., catalase) [57,68]. Similar effects of the consumption of BP, PRO, and their polyphenols in the present meta-analysis would explain the increase and reduction observed for TAC and MDA, respectively.

5. Conclusions

The present meta-analysis results indicate that bee pollen and propolis reduce feed consumption. Likewise, the results of the subgroup analysis indicated that, for bee pollen, the greatest reduction in feed consumption is obtained when this product is supplemented orally through drinking water. However, bee pollen and propolis can be used as natural growth promoters in rabbits since they increase weight gain and, at the same time, reduce the feed conversion ratio. In addition, bee pollen and propolis supplementation improve antioxidant status in rabbit blood serum.

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Appendix A

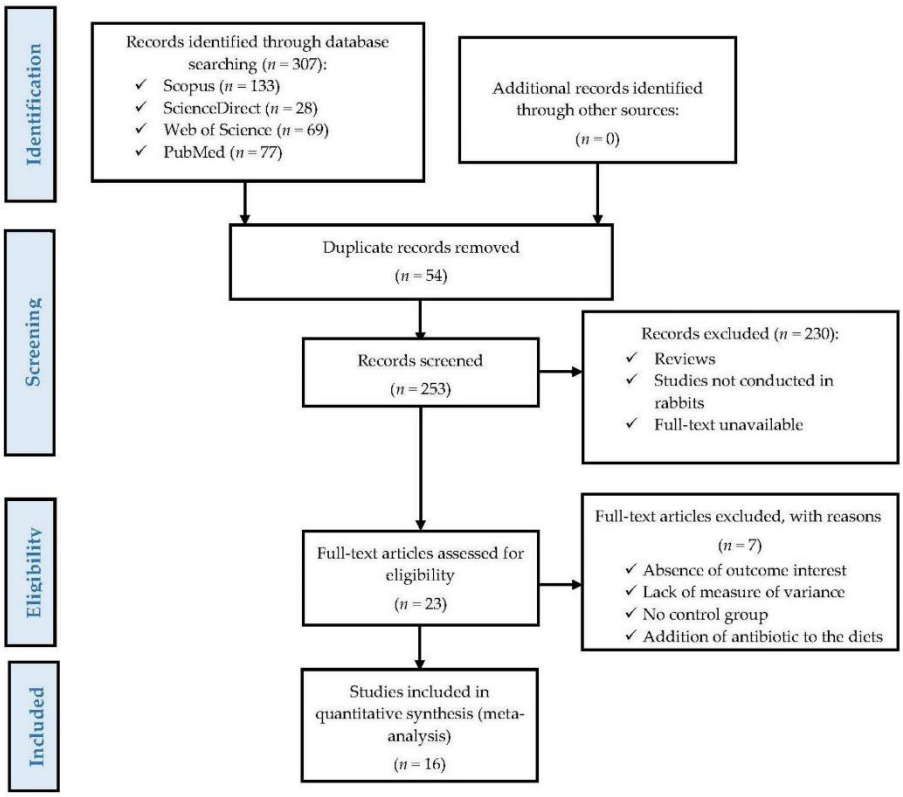

Figure A1. A PRISMA flow diagram detailing the literature search strategy and study selection for the meta-analysis.

Table A1. Summary of the studies included in the meta-analysis.

Author	Country	Product	Duration, d	Age [1]	Method of Supplementation	Dose, mg/kg BW
Abdel-Hamid et al. [48]	Egypt	BP	28	≤15	Aqueous solution orally with a syringe	268, 321
Al-Homidan et al. [18]	Egypt	PRO	42	≤15	Mixed with a basal diet	250, 500
Attia et al. [69]	Egypt	BP	70	>15	Aqueous solution orally with a syringe	54, 120, 171, 309, 600, 904
Attia et al. [70]	Egypt	BP	140	>15	Aqueous solution orally with a syringe	52, 114, 156, 335, 674, 1002
Attia et al. [2]	Egypt	BP, PRO	56	≤15	Aqueous solution orally with a syringe	100, 93
Attia et al. [14]	Egypt	BP, PRO	140	>15	Aqueous solution orally with a syringe	737, 735
Attia et al. [20]	Egypt	BP, PRO	280	>15	Oral capsules	423, 846, 423, 846
Dias et al. [71]	Brazil	BP	82	≤15	Aqueous solution orally with a syringe	1000
El-Hammady et al. [72]	Egypt	BP	56	>15	Orally through drinking water	500, 1000
Hashem et al. [73]	Egypt	PRO	70	>15	Mixed with a basal diet	30
Hashem et al. [1]	Egypt	PRO	35	≤15	Mixed with a basal diet	30, 60
Hassan et al. [17]	Egypt	BP	84	>15	Orally through drinking water	636, 1280
Piza et al. [74]	Brazil	PRO	32	≤15	Mixed with a basal diet	47, 93, 139
Sierra-Galicia et al. [19]	Mexico	BP, PRO	42	≤15	Orally through drinking water	500, 50
Waly et al. [16]	Egypt	PRO	56	≤15	Mixed with a basal diet	100, 150, 200
Zeedan et al. [15]	Egypt	BP	70	≤15	Orally through drinking water	140, 348, 487

BW: body weight; d: days; [1]: age in weeks; BP: bee pollen; PRO: propolis.

Table A2. Descriptive statistics of the complete data set for the effect of PRO supplementation to rabbits' diets.

Parameter		Mean		Median		Minimum		Maximum		SD	
Dietary features	NC	Control	PRO	Control	PRO	Control	PRO	Control	PRO	Control	PRO
DM, g/kg DM	11	888.4	888.4	878.0	878.0	874.7	874.7	917.1	917.1	16.66	16.66
CP, g/kg DM	14	171.2	171.6	172.8	172.8	160.0	160.0	185.0	185.0	7.91	7.76
EE, g/kg DM	10	44.97	44.97	28.80	28.80	26.20	26.60	78.00	78.00	7.59	7.59
NDF, g/kg DM	8	320.3	320.3	316.4	316.4	314.2	314.2	331.1	331.1	7.56	7.56
ADF, g/kg DM	8	171.8	171.8	162.2	162.2	148.1	148.1	201.2	201.2	24.97	24.97
CF, g/kg DM	12	133.1	133.1	133.5	133.5	126.7	126.7	138.5	138.5	4.24	4.24
Ash, g/kg DM	5	94.12	94.12	100.7	100.7	74.8	74.8	103.6	103.6	12.60	12.60
Ca, g/kg DM	6	6.25	6.25	6.30	6.30	5.90	5.90	6.60	6.60	0.39	0.39
P, g/kg DM	6	3.78	3.78	3.75	3.75	3.50	3.50	4.10	4.10	0.31	0.31
DE, MJ/kg DM	13	11.00	11.00	11.22	11.22	9.40	9.40	11.22	11.22	0.51	0.51
PRO, mg/kg BW	15	-	248	-	139	-	30	-	846	-	259.7
Duration, days		50		42		32		140.0		27.54	

NC = number of comparisons; PRO = propolis; SD = standard deviation; DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; CF = crude fiber; Ca = calcium; P = phosphorus; DE: digestible energy. In the same column, means followed by different letters differ significantly by the Tukey test ($p \leq 0.05$).

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3 SUPPLYING BEE POLLEN AND PROPOLIS TO GROWING RABBITS: EFFECTS ON GROWTH PERFORMANCE, BLOOD METABOLITES, AND MEAT QUALITY

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Supplying Bee Pollen and Propolis to Growing Rabbits: Effects on Growth Performance, Blood Metabolites, and Meat Quality

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Abstract: The objective of this study was to evaluate the effect of supplementation with bee pollen (BP) and propolis (PRO) on productive performance, *Eimeria* oocyst counts in feces, blood metabolites, and the meat quality of growing rabbits. A total of 160 hybrid rabbits (California × New Zealand) of 30 days of age and 643 ± 8.0 g body weight (BW) were assigned to four treatments with 10 replicates each (four rabbits/replicate). The treatments were as follows: (1) CON: rabbits fed basal diet and not supplemented with BP or PRO; (2) BP500: CON + BP (500 mg/kg BW); (3) PRO50: CON + PRO (50 µL/kg BW); and (4) BP + PRO: CON + BP (500 mg/kg BW) + PRO (50 µL/kg BW). Higher daily weight gain ($p = 0.04$) and lower feed conversion rate ($p = 0.03$) were observed in rabbits supplemented with PRO50. In addition, supplementation with PRO50 and BP + PRO reduced the amount of *Eimeria* oocysts per gram of feces ($p < 0.05$). Most hematological and serum biochemical parameters were similar in rabbits of all treatments. Protein content, collagen, and meat color were similar between treatments. In conclusion, propolis supplementation (50 µL/kg BW) can prevent coccidiosis and act as a natural growth promoter in rabbits without affecting animal health and meat quality.

Keywords: honeybee products; coccidiosis; blood biochemistry; hematological profile

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1. Introduction

It has been discussed that, due to its short production cycle, rabbit production could be used as an alternative to solve the growing meat shortage in developing countries [1]. However, due to their complex digestive physiology, rabbits are susceptible to enteric diseases, mainly in the post-weaning period [2]. Therefore, antibiotics (e.g., zinc bacitracin) are frequently used in feeding growing and fattening rabbits to improve productive performance and reduce mortality caused by digestive disorders [3]. Unfortunately, due to the uncontrolled use of antibiotics, a rapid increase in microbial resistance to these drugs has been reported worldwide, compromising the health of humans and animals [4]. Consequently, in recent years the search for and development of new natural products that can improve animal performance and health has attracted the attention of researchers [5]. Some bee (*Apis mellifera)* products, such as bee pollen (BP) and propolis (PRO), could be used for this purpose because they contain various nutritional compounds and some bioactive metabolites with pharmaceutical properties [5,6].

BP is a mixture of flower pollen grains collected by bees with nectar and secretions from the hypopharyngeal glands of bees [6,7]. According to Thakur and Nanda [8], BP contains carbohydrates, proteins, lipids, fiber, minerals, and phenolic compounds (on

average 30.59 mg gallic acid/g DM) with anti-inflammatory, antioxidant, and hepatoprotective activity. Some researchers have mentioned that dietary inclusion of BP can be used to improve productive and reproductive performance and intestinal health in domestic animals [6]. Particularly in growing rabbits, supplementation with BP increases nutrient digestibility, volatile fatty acid production in the cecum, and digestive enzyme activity [9]. On the other hand, in growing rabbits, supplementation with BP improves intestinal morphology [10] and increases the serum concentration of insulin-like growth factor type 1 [11]. Previous studies [1,2,11] have evaluated the effects of BP supplementation in rabbits, mainly using low doses (≤350 mg/kg BW); however, information on the effects of high doses (>350 mg/kg BW) of BP is limited. For example, Abdel-Hamid et al. [11] and Attia et al. [2] reported that, in growing rabbits, supplementation with BP doses lower than 350 mg/kg body weight did not improve productive performance. In contrast, Zeedan et al. [9] reported a lower mortality rate and higher hot carcass yield in rabbits supplemented with BP doses greater than 350 mg/kg BW.

On the other hand, PRO is a complex mixture of resinous, gummy, and balsamic substances collected by bees from plant buds, flowers, and exudates [12–15]. It has been reported that PRO contains more than 180 volatile compounds, mainly polyphenols [16]. The primary polyphenols in PRO are flavonoids and phenolic acids [17], which have antioxidant, immunostimulant, and antimicrobial properties [16,18]. It has been reported that PRO supplementation improves cecum morphology and reduces the presence of *Escherichia coli* and *Salmonella* spp. in the cecum of growing rabbits [13]. In addition, when PRO is added to rabbit diets, serum immunoglobulin concentration is increased, and the antioxidant status of the animals is improved [18]. Likewise, it has been reported that PRO supplementation in rabbits has no toxic effects and helps to reduce the severity of clinical signs and mortality caused by *Pasteurella multocida* [19]. The effects of PRO supplementation in rabbits have been investigated by supplying PRO in capsules [1,2] or by mixing PRO into the basal diet [13,18]. However, to our knowledge, there is no previously published information on the effects of PRO supplementation in drinking water for growing rabbits. Although the most feasible method of administering an additive to rabbits is through feed, it has been reported that PRO supplementation through the basal diet generally has no positive effect on rabbit performance [13,18]. Furthermore, the information available in the literature on PRO supplementation in rabbits remains limited, especially regarding the effects of PRO on meat quality and parasite load in the animals.

Due to the positive effects of BP and PRO, this work hypothesizes that supplementation with BP and PRO will benefit the productive performance of rabbits without affecting health and meat quality. Furthermore, the combination of BP and PRO could act synergistically due to their chemical composition and mechanisms of action. Therefore, the objective of this study was to evaluate the effects of supplementation with bee pollen, propolis, and the combination of both products on productive performance, *Eimeria* oocyst count per gram of feces, blood biometry, blood biochemistry, and meat quality in growing rabbits.

2. Materials and Methods

2.1. Experimental Location

The experiment was conducted during April and May 2022 at the "Conejos" Centro de Investigación Científica del Estado de México A.C. (COCICEMAC). This site is located in San Miguel Coatlinchán, State of Mexico, Mexico (latitude 19°26′56″ N and longitude 98°52′20″ W). San Miguel Coatlinchán is 2240 m above sea level, the mean annual temperature is 15 °C, and the mean annual precipitation is 645 mm [20]. The Research Ethics and Bioethics Committee approved the experimental procedures for handling the rabbits used in the study of the Universidad Autónoma del Estado de México (Protocol #24-03-2022).

2.2. Collection of Bee Pollen and Propolis

BP was collected in hives located in the municipality of Contla de Juan Cuamatzi, State of Tlaxcala, Mexico, located at 2320 masl (latitude 19°20′00″ N and longitude 98°10′00″ W).

The BP collection was carried out from September to November 2021 using intermediate pollen traps placed in the hives' inner part [21]. For proper preservation, the BP was dried at 40 °C and stored in amber jars at room temperature [22].

The PRO was collected in the village Charco de Pantoja, in the municipality of Valle de Santiago, Guanajuato State, Mexico, located at 1709 masl (latitude 20°23′34″ N and longitude 101°11′29″ W). PRO collection was carried out from October 2020 to February 2021 using the plastic net mesh technique [23]. Subsequently, for proper preservation, PRO was cleaned, crushed, and stored in amber jars under refrigerated conditions at −20 °C until further processing [24].

2.3. Chemical Composition of Bee Pollen and Propolis, and Preparation and Analysis of Propolis Extract

BP and PRO samples were analyzed in the laboratory to determine the content of dry matter (method 967.03), crude protein (method 981.10), ether extract (method 920.29), and ash (method 942.05), following the procedures previously described by AOAC [25]. The nutritional composition of the BP was: 91.82% dry matter, 20.14% crude protein, 3.95% ether extract, and 2.93% ash. On the other hand, the nutritional composition of the PRO was 90.26% dry matter, 2.55% crude protein, 9.31% ether extract, and 0.85% ash. Therefore, the methodology previously described by Cevk et al. [26] was followed to prepare the PRO extract. Briefly, 100 g of frozen crude PRO was ground using a 1 mm sieve in a Wiley mill (model 4, Arthur Thomas Co., Philadelphia, PA, USA) and mixed with 50 mL of 70% ethanol. Then, the mixture of PRO and ethanol was placed in a hermetically sealed glass container for 48 h (under stirring every 12 h) at room temperature (25 °C). The solution was filtered through Whatman filter paper no. 4 to remove impurities. Finally, the resulting PRO extract solution was stored at −20 °C until further analysis and use (when it was used as a supplement for rabbits) [27].

The total phenolic compound content of the PRO extract was determined using the colorimetric method described by Folin–Ciocalteu [28]. 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™, Waltham, MA, USA). Similarly, the total flavonoid content of the PRO extract was obtained following the colorimetric method using aluminum chloride, as previously described by Chang et al. [29]. 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™, Waltham, MA, USA). The total content of phenolic compounds of the PRO was 23.67 mg gallic acid equivalent per g dry weight of the extract. Likewise, the total flavonoid content of the PRO was 9.21 mg quercetin equivalent per g dry weight of the extract.

2.4. Animals, Experimental Design, Management, and Diet Composition

All rabbits used in the present study were clinically healthy at the start of the experiment. One hundred sixty freshly weaned California × New Zealand hybrid rabbits (80 males and 80 females), 30 days of age and with an average body weight (BW) of 643 ± 8.0 g, were used. The rabbits were weighed to form four homogeneous groups and were entirely randomized for one of four treatments. Each treatment had 10 replicates (cages), and in each replicate there were four rabbits with similar initial weights and of both sexes (two males and two females). The treatments used were as follows: (1) rabbits fed a basal diet and without BP or PRO in the drinking water (CON); (2) BP500: CON + BP (500 mg/kg BW); (3) PRO50: CON + PRO (50 µL/kg BW); and (4) BP + PRO: CON + BP (500 mg/kg BW) + PRO (50 µL/kg BW). The BP dose (500 mg/kg BW) was chosen based on a previous report in which supplementation with this dose improved the digestibility of ingested nutrients in growing rabbits [9]. The PRO dose (50 µL/kg BW) was chosen based on a previously published study [19] in which the investigators reported that supplementation with this dose of PRO improved immune response and reduced mortality in rabbits.

Treatments were administered five times per week (Monday through Friday) throughout the experimental period. Based on a previous experiment carried out by our work team (unpublished data), we verified that the doses of BP and PRO were well dissolved in the drinking water and that the rabbits consumed this water adequately. Therefore, the BP500, PRO50, or BP + PRO treatments were first applied to 200 mL of drinking water in each of the cages using automatic drinkers, as other authors previously used [30–33] to supply additives in the drinking water of growing rabbits. After the rabbits in each cage consumed the water supplied with BP500, PRO50, or BP + PRO, the animals had *ad libitum* access to drinking water without treatment. The animals of the CON treatment had the same conditions as the experimental groups but without applying BP or PRO to the drinking water. Instead, drinking water was provided through nipple drinkers. The BP500, PRO50, or BP + PRO treatments were adjusted each week based on the body weights obtained. Oral administration of the treatments was performed in the morning (09:00 h).

Environmental, hygienic, and handling conditions were the same for all rabbits. Also, the health status of the rabbits was monitored throughout the experimental phase. The rabbits were housed in a module with natural ventilation system and thermal insulation on the walls but not on the ceiling. The rabbits were kept in galvanized metal cages 78 cm long, 56 cm wide, and 30 cm high (four rabbits/cage) arranged in a flat-deck system at 45 cm above the floor level. Each cage had a galvanized English-type hopper feeder (15 cm long, 10 cm wide, and 22 cm high) with a capacity of 1.5 kg and a nipple-type automatic drinker. During the entire experimental period (April and May), the average daylight was 13 h.

All rabbits were fed a commercial pelleted basal diet (diameter: 3.5 mm; length: 10 mm) for growing rabbits (Conejo plus, Unión Tepexpan®, Texcoco, Mexico), which came from the same production lot. According to the labeled feed package, antibiotics are not included, but 1 g of Diclazuril per ton as coccidiostat is added to prevent *Eimeria* spp. Each week, feed samples were taken and analyzed to determine the chemical composition of the feed following the procedures described by AOAC [25]. The nutritional composition of the basal diet was: 91.71% dry matter, 17.61% crude protein, 5.14% ether extract, 7.48% ash, 32.69% neutral detergent fiber, and 14.81% acid detergent fiber. Water and feed were available *ad libitum* during the 42 days of the experimental phase. Figure 1 shows the experimental design used in this study and the samples taken during the experimental period.

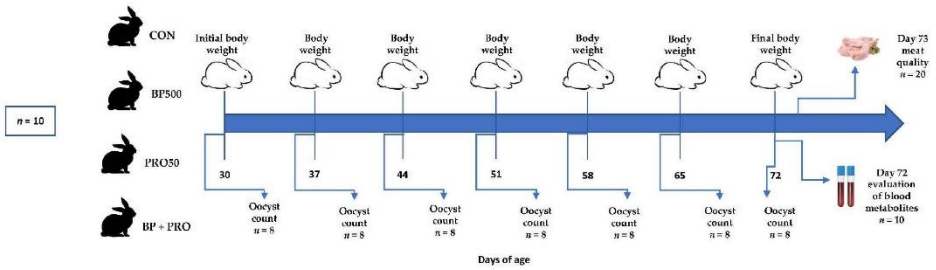

Figure 1. Diagram of the experimental design showing the samples taken during the experiment. CON: basal diet without supplementation with bee pollen (BP) or propolis (PRO); BP500: CON + BP (500 mg/kg BW); PRO50: CON + PRO (50 µL/kg BW); and BP + PRO: CON + BP (500 mg/kg BW) + PRO (50 µL/kg BW).

2.5. Meteorological Variables and Temperature Humidity Index (THI) Estimation

Ambient temperature and relative humidity were recorded daily using a Traceable® brand hygro-thermometer (model 4040CC, Control Company, Webster, TX, USA) located inside the rabbit facility. With the temperature and relative humidity data, the temperature and humidity index (THI) was estimated using the following equation [34]: $THI = T - [(0.31 - 0.31(RH/100)] \times [(T - 14.4)]$, where T is the dry bulb temperature in degrees Celsius (°C), and RH is the

percent relative humidity. As a result, the mean values of ambient temperature, relative humidity, and temperature and humidity index (THI) registered during the experimental period were 30.56 ± 0.59 °C, 22.02 ± 0.51%, and 26.62 ± 0.51, respectively.

2.6. Growth Performance and Oocyst Count

The body weight (BW) of the rabbits was recorded before morning feeding at the beginning (day 0) of the experimental phase, using a TORREY® brand digital scale (model PCR-40, TORREY Electronics Inc, Houston, TX, USA), with an accuracy of ± 0.5 g. Subsequently, the rabbits were weighed weekly on days 7, 14, 21, 28, 35, and 42 of the experimental periods. The feed intake of the rabbits was recorded each week and divided by the number of days of the period to estimate daily feed intake (DFI, g/d). Likewise, average daily gain (ADG, g/d) was calculated using the weekly BW data. In addition, the rabbits' feed conversion ratio (FCR) was calculated using the equation: FCR = DFI/ADG.

To evaluate the parasite load of *Eimeria* spp. in rabbits, freshly voided feces samples were collected from eight replicates (cages) of each treatment each week (days 0, 7, 14, 21, 28, 35, and 42). The feces were collected at the same time (9:00 h) on each sampling and were used to perform oocyst counts using the McMaster counting technique [35]. The results were calculated as a number of oocysts per gram of feces (OPG) with the formula [36]: OPG = oocyst count × dilution factor × (sample volume/counting chamber volume). Finally, comparing the number of dead animals to those that started the experimental phase, the overall mortality of the rabbits was calculated, as previously reported by Martínez et al. [37].

2.7. Blood Metabolites

To determine hematological and biochemical parameters, at the end of the experimental phase (day 42), blood samples were randomly taken from the ear vein of 10 rabbits (five females and five males) from each treatment. Two 2.5 mL blood samples were taken from each rabbit. Tubes with BD Vacutainer® K2 EDTA anticoagulant (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were used to collect the first sample, which was stored at 4 °C, for subsequent determination of the complete blood count and differential leukocyte count using an EasyVet® hematology analyzer (QS Kontrolab, Hamburg, Germany), as previously reported by several authors [38–40]. BD Vacutainer® (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) anticoagulant-free tubes were used to collect the second blood sample. These blood samples were centrifuged at 3500 rpm for 20 min to obtain blood serum using a refrigerated centrifuge (Sigma 2–16 k, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The blood serum was stored in Eppendorf tubes and frozen at −20 °C. Finally, for the analysis of the blood serum samples, an EasyVet® autoanalyzer (QS Kontrolab, Hamburg, Germany) and Spinreact kits (Barcelona, Spain) were used to determine the contents of glucose (kit 41011), cholesterol (kit 41021), albumin (kit 1001020), globulin (kit 1001032), total protein (kit 1001291), urea (kit 41041), uric acid (kit 41001), bilirubin (kit 1001046), creatinine (kit 1001113), liver enzymes (alkaline phosphatase (kit MX41233), lactate dehydrogenase (kit MX41274), and aspartate aminotransferase (kit MX41264)), calcium (kit 1001060), and phosphorus (kit 1001155), as described by other authors [38,41].

2.8. Carcass Yield and Meat Quality

After obtaining the final body weight (day 42 of the experiment), all rabbits were kept fasting for 12 h. Subsequently, 20 rabbits (ten males and ten females) from each treatment were sacrificed following the procedures of the Mexican Official Standard (NOM-033 SAG/ZOO-2014). After completing the slaughter and bleeding of the rabbits, all internal organs, skin, feet, and heads were separated from the carcass, and the hot carcass weight (HCW) was recorded. The hot carcass yield (HCY) was estimated by the equation: HCY = (HCW / final BW) × 100. Subsequently, the leg muscles (HLM) and *Longissimus dorsi* muscles (LDM) were removed from the hot carcass, following procedures similar to those described and recommended by

Blasco and Ouhayoun [42]. Finally, HLM and LDM muscles were stored in a freezer at −20 °C until further analysis.

Before meat quality analyses, samples were thawed at 4 °C for 24 h. Then, meat pH was determined using the procedures previously described by Orzuna-Orzuna et al. [43]. For this, 3 g of muscle was weighed in triplicate from the HLM sample of each rabbit. Subsequently, using a Waring 51BL32 blender (model 700, Torrington, CT, USA), each portion of muscle was homogenized with 20 mL of deionized water. Finally, each sample was measured in triplicate for pH with a Hanna® brand pH meter (Model HI 98127, Waterproof Tester, Woonsocket, RI, USA).

Meat color parameters were measured in triplicate in LDM samples from each rabbit using the procedures described by Miltenburg [44]. For this, lightness (L*), redness (a*), and yellowness (b*) were measured in triplicate with a Minolta CM-2006d spectrophotometer (Konica model, Minolta Holdings Inc., Osaka, Japan). In addition, cooking loss (CL) was evaluated in LDM samples (one sample per rabbit), as previously reported by Manchetti et al. [45]. Therefore, 2.5 cm thick steaks were grilled on an electric grill (Toastmaster cool-edge grill, Macon, MO, USA). The internal temperature of the meat was monitored with a Taylor® brand thermometer (model 99878, Seattle, WA, USA), and when it reached 70 °C, the steaks were removed from the grill and allowed to cool for 1 h at room temperature (20–25 °C). The percentage of CL was estimated with the equation [46]: CL, % = ((Wr − Wc)/Wr) × 100, where Wr is the raw weight and Wc is the cooked weight of the meat samples used.

HLM samples from each rabbit were separately ground and homogenized for 5 min using a Ship to Shore brand meat grinder (Model 99598, Camarillo, CA, USA) [47]. Subsequently, a FOSS FoodScan™ near-infrared spectrophotometer was used to determine in triplicate the content (g/100) of moisture, protein, fat, and collagen, following the procedures described by Anderson [48].

2.9. Statistical Analysis

All data were analyzed by analysis of variance (ANOVA) using SAS statistical software [49]. Before statistical analysis, the normality of the data was evaluated with the Shapiro–Wilk test using the UNIVARIATE procedure. Likewise, the productive performance and OPG data were analyzed using a completely randomized experimental design with repeated measures over time. The MIXED procedure was used, and each cage was considered the experimental unit. Finally, different variance–covariance structures were tested to fit the statistical model, and the compound symmetry structure was chosen for the productive performance and OPG variables because it showed the best fit following the criteria of lowest AIC and BIC values [50]. The structure of the statistical model used was:

$$Y_{ijk} = \mu + T_i + W_j + (T \times W)_{ij} + R_k + e_{ijk} \quad (1)$$

In this model, Y_{ijk} represents the value observed in treatment i in week j for rabbit k; μ represents the overall mean; T_i represents the fixed effect of the i-th treatment (i = 1 (CON), 2 (BP500), 3 (PRO50), 4 (BP + PRO)); W_j represents the fixed effect of the j-th week of the experimental period (j = 1, 2, . . . , 6,); $(T \times W)_{ij}$ represents the fixed effect of the interaction between the j-th week and the i-th treatment; R_k represents the random effect of a cage (repetition) within treatment (k = 1, 2, 3, . . . , 40); and e_{ijk} represents the random error.

The variables of blood biometry, blood biochemistry, meat quality, and HCY were analyzed using a completely randomized design. For this, the GLM procedure of SAS [44] was used, and each rabbit was considered the experimental unit. The structure of the final statistical model used was as follows:

$$Y_{ij} = \mu + T_i + e_{ij} \quad (2)$$

In this model, Y_{ij} represents the observations, μ represents the overall mean, T_i represents the fixed effect of the i-th treatment, and e_{ij} is the random error. For all variables analyzed, treatment means were compared using Tukey's test. The mortality rate was

analyzed as a percentage using chi-square analysis [1]. A statistically significant effect was considered to be present when $p \leq 0.05$. In addition, $p > 0.05$ and ≤ 0.10 was considered as tending to be significant.

3. Results

3.1. Growth Performance and Oocyst Count

Table 1 shows that at the end of the experimental phase, rabbits assigned to the PRO50 treatment were 6.1% heavier ($p < 0.05$) than rabbits assigned to the CON treatment. ADG was 8.8% greater ($p = 0.04$) in the PRO50-group rabbits than in the CON-treatment rabbits. FCR was 7.1% lower ($p = 0.03$) in PRO50-treatment rabbits than in CON-treatment rabbits. Compared to the CON treatment, rabbits in the PRO50 and BP + PRO treatments had 50 and 55% lower OPG ($p < 0.05$), respectively. On the other hand, mortality was higher ($p = 0.05$) in the BP500-treatment rabbits than in the rabbits assigned to the other treatments (Table 1).

Table 1. Growth performance and mortality of rabbits supplemented with bee pollen and propolis in a trial from 30 to 72 days of age.

Parameters	Treatments				SEM	*p*-Value		
	CON	BP500	PRO50	BP + PRO		Treatment	Week	Treatment × Week
Cages (*n*)	10	10	10	10				
Rabbits (*n*)	40	40	40	40				
Initial body weight, g	639	633	642	641	23.21	0.20	-	-
Final body weight, g	2168 ^{b}	2197 ^{ab}	2306 ^{a}	2180 ^{ab}	66.31	0.03	<0.0001	0.12
Average daily gain (ADG), g/d	36.4 ^{b}	37.3 ^{ab}	39.6 ^{a}	36.6 ^{ab}	1.52	0.04	<0.0001	0.11
Daily feed intake (DFI), g/d	107.6	112.2	108.9	104.9	3.75	0.06	<0.0001	0.80
Feed conversion ratio (FCR), DFI/ADG	2.95 ^{a}	3.00 ^{ab}	2.74 ^{b}	2.86 ^{ab}	0.28	0.03	<0.0001	0.28
Initial oocyst/g feces (OPG)	7.1	0.0	0.0	0.0	3.37	0.14	-	-
Oocyst/g feces (OPG)	6159.8 ^{a}	5546.0 ^{ab}	3037.5 ^{bc}	2770.8 ^{c}	1004.15	0.02	0.07	0.08
Mortality, %	22.5 ^{b}	40.0 ^{a}	22.5 ^{b}	17.5 ^{b}	8.16	0.05	-	-

CON: basal diet without supplementation with bee pollen (BP) or propolis (PRO); BP500: CON + BP (500 mg/kg BW); PRO50: CON + PRO (50 µL/kg BW); and BP + PRO: CON + BP (500 mg/kg BW) + PRO (50 µL/kg BW). SEM—standard error of the treatment means; ^{a,b,c}—means within a row with different subscripts differ when $p \leq 0.05$.

3.2. Hematological Parameters

Higher ($p = 0.02$) percent hematocrit was observed in BP500-supplemented rabbits than in CON rabbits (Table 2). Compared to CON- and BP500-treatment rabbits, rabbits supplemented with PRO50 and BP + PRO had lower blood hemoglobin concentration ($p = 0.009$). Mean corpuscular volume was lower ($p = 0.02$) in PRO50-treatment rabbits than in CON rabbits. Lower ($p = 0.001$) blood concentration of monocytes was observed in BP + PRO-treatment rabbits than in rabbits assigned to the other treatments. Rabbits in the PRO50 treatment had higher ($p < 0.05$) blood concentrations of band neutrophils and eosinophils than in the CON, BP500, and BP + PRO treatments.

3.3. Blood Biochemistry

Rabbits supplemented with BP500 had lower ($p = 0.001$) serum urea concentration compared to rabbits in the CON, BP500, and BP + PRO treatments. In addition, rabbits supplemented with BP500 had a higher albumin/globulin ratio ($p = 0.04$) and lower serum total protein and globulin concentration ($p < 0.05$) than CON-treatment rabbits. Serum aspartate aminotransferase concentration tended to be lower in BP500-supplemented rabbits than in CON-treatment rabbits ($p = 0.09$; Table 3).

Table 2. Hematological profile of rabbits supplemented with bee pollen and propolis in a trial from 30 to 72 days of age.

Parameter	Treatment				SEM	*p*-Value
	CON	BP500	PRO50	BP + PRO		
Rabbits (*n*)	10	10	10	10		
Hematocrit, %	37.20 ^{b}	38.79 ^{a}	37.14 ^{b}	37.04 ^{b}	0.509	0.02
Hemoglobin, g/dL	12.84 ^{a}	12.92 ^{a}	12.29 ^{b}	12.23 ^{b}	0.177	0.009
Red blood cells, 10^6/mL	6.52 ^{ab}	6.77 ^{a}	6.38 ^{ab}	6.29 ^{b}	0.141	0.02
Mean corpuscular volume, fL	60.12 ^{a}	59.00 ^{ab}	58.45 ^{b}	60.40 ^{a}	0.560	0.02
Mean corpuscular hemoglobin, pg	19.68	19.65	19.12	19.42	0.222	0.08
Mean corpuscular hemoglobin concentration, g/dL	33.01	33.19	33.22	33.86	0.553	0.28
Platelets, 10^3/mL	256.60	253.40	247.70	247.40	8.083	0.42
Leukocytes, 10^3/mL	14.63	7.68	7.70	7.45	3.477	0.15
Lymphocytes, 10^3/mL	12.80	14.10	12.50	12.90	1.236	0.36
Monocytes, 10^3/mL	10.00 ^{a}	8.40 ^{a}	9.70 ^{a}	6.10 ^{b}	0.812	0.001
Segmented neutrophils, 10^3/mL	75.90	76.80	73.50	73.40	1.799	0.09
Band neutrophils, 10^3/mL	0.40 ^{b}	0.40 ^{b}	1.90 ^{a}	1.10 ^{b}	0.374	0.02
Eosinophils, 10^3/mL	1.00 ^{c}	3.00 ^{b}	4.80 ^{a}	2.10 ^{bc}	0.593	<0.0001
Basophils, 10^3/mL	0	0	0	0	0	0
Plasma protein, g/dL	7.82	7.81	7.62	7.66	0.164	0.39

CON: basal diet without supplementation with bee pollen (BP) or propolis (PRO); BP500: CON + BP (500 mg/kg BW); PRO50: CON + PRO (50 µL/kg BW); and BP + PRO: CON + BP (500 mg/kg BW) + PRO (50 µL/kg BW). SEM—standard error of the treatment means; ^{a,b,c}—means within a row with different subscripts differ when $p \leq 0.05$.

Table 3. Blood serum biochemistry of rabbits supplemented with bee pollen and propolis in a trial from 30 to 72 days of age.

Parameter	Treatment				SEM	*p*-Value
	CON	BP500	PRO50	BP + PRO		
Rabbits (*n*)	10	10	10	10		
Glucose, mg/dL	119.20	111.80	119.10	116.90	4.805	0.28
Urea, mg/dL	39.50 ^{a}	32.60 ^{b}	38.20 ^{a}	37.50 ^{a}	1.423	0.001
Cholesterol, mg/dL	63.30	60.30	66.10	62.50	5.326	0.44
Total protein, g/dL	7.94 ^{a}	7.09 ^{b}	7.42 ^{ab}	7.52 ^{ab}	0.258	0.02
Albumin, g/dL	3.23 ^{ab}	3.18 ^{b}	3.30 ^{a}	3.25 ^{ab}	0.041	0.05
Globulin, g/dL	4.70 ^{a}	3.91 ^{b}	4.12 ^{ab}	4.23 ^{ab}	0.242	0.03
Albumin/globulin	0.73 ^{b}	0.81 ^{a}	0.79 ^{ab}	0.76 ^{ab}	0.026	0.04
Bilirubin, mg/dL	0.33	0.30	0.38	0.28	0.051	0.13
Uric acid, mg/dL	0.58	0.30	0.57	0.46	0.116	0.09
Creatinine, mg/dL	1.09	1.01	1.21	1.20	0.080	0.08
Alkaline phosphatase, UI/dL	297.30	349.00	313.80	363.40	25.30	0.07
Lactate dehydrogenase, UI/dL	472.90	334.40	526.80	499.10	68.39	0.06
Aspartate aminotransferase, UI/dL	62.70	47.10	64.10	48.20	8.63	0.09
Calcium, mg/dL	14.35	14.29	14.58	14.27	0.30	0.46
Phosphorus, mg/dL	5.84	5.94	6.32	5.81	0.23	0.12

CON: basal diet without supplementation with bee pollen (BP) or propolis (PRO); BP500: CON + BP (500 mg/kg BW); PRO50: CON + PRO (50 µL/kg BW); and BP + PRO: CON + BP (500 mg/kg BW) + PRO (50 µL/kg BW). SEM—standard error of the treatment means; ^{a,b}—means within a row with different subscripts differ when $p \leq 0.05$.

3.4. Carcass Yield and Meat Quality

Higher ($p = 0.002$) HCY was observed in rabbits supplemented with BP500 and PRO50 than in rabbits assigned to the CON and BP + PRO treatments (Table 4). The pH was higher ($p = 0.001$) in meat from rabbits supplemented with BP + PRO than in meat from rabbits in

the other treatments. PRO50 supplementation reduced ($p < 0.05$) the CL of meat compared to the other treatments. However, lower fat content ($p = 0.05$) and higher moisture content ($p = 0.03$) were observed in meat from rabbits supplemented with BP500 than in meat from CON rabbits (Table 4).

Table 4. Carcass yield and meat quality of rabbits supplemented with bee pollen and propolis for 42 days and slaughtered at 72 days of age.

Parameter		Treatment				SEM	*p*-Value
		CON	BP500	PRO50	BP + PRO		
Rabbits (*n*)	Muscle used	20	20	20	20		
Hot carcass yield (HCY), %		54.78 ^{b}	57.41 ^{a}	57.59 ^{a}	55.04 ^{b}	0.91	0.002
Meat pH	Hind leg muscle	5.96 ^{b}	5.99 ^{b}	5.98 ^{b}	6.04 ^{a}	0.02	0.001
Lightness (L*)	*Longissimus dorsi* muscle	53.49	52.67	54.08	52.44	0.86	0.11
Redness, (a*)	*Longissimus dorsi* muscle	1.90	1.82	2.01	1.69	0.22	0.18
Yellowness, (b*)	*Longissimus dorsi* muscle	7.18	7.09	7.39	7.34	0.18	0.11
Cooking loss (CL), %	*Longissimus dorsi* muscle	33.24 ^{a}	35.31 ^{a}	28.97 ^{b}	37.05 ^{a}	1.92	0.0006
Protein, g 100 g^{-1}	Hind leg muscle	21.42	21.70	21.38	21.53	0.17	0.25
Fat, g 100 g^{-1}	Hind leg muscle	2.98 ^{a}	1.93 ^{b}	2.70 ^{ab}	2.62 ^{ab}	0.39	0.05
Moisture, g 100 g^{-1}	Hind leg muscle	74.63 ^{b}	75.38 ^{a}	74.89 ^{ab}	74.85 ^{ab}	0.35	0.03
Collagen, g 100 g^{-1}	Hind leg muscle	0.97	0.99	1.03	1.00	0.02	0.17

CON: basal diet without supplementation with bee pollen (BP) or propolis (PRO); BP500: CON + BP (500 mg/kg BW); PRO50: CON + PRO (50 µL/kg BW); and BP + PRO: CON + BP (500 mg/kg BW) + PRO (50 µL/kg BW). SEM—standard error of the treatment means; ^{a,b}—means within a row with different subscripts differ when $p \leq 0.05$.

4. Discussion

4.1. Growth Performance and Oocyst Count

In some review articles [6,51], it has been mentioned that dietary supplementation with BP or PRO may improve the taste of the feed offered, which could result in higher DFI. However, in the present study, supplementing with an aqueous solution of BP, PRO, and BP + PRO did not affect DFI. Similar responses were previously reported by Piza et al. [52] in rabbits supplemented with increasing doses of PRO (0, 500, 1000, and 1500 mg/kg DM); and by El-Hammady et al. [53] in adult rabbits supplemented with 500 or 1000 mg/d of BP. Moreover, Attia et al. [1,10] did not observe significant changes in the DFI of growing rabbits supplemented with capsules containing increasing doses (150, 200, and 300 mg/kg BW) of BP, PRO, or the combination of both products. These results suggest that supplementation with BP, PRO, or BP + PRO does not affect DFI, regardless of the dose and route of administration used.

Zeedan et al. [9] observed that in rabbits, supplementation with BP increases the production of volatile fatty acids in the cecum and the activity of amylase, lipase, and protease in the intestinal contents. In addition, BP has been reported to increase intestinal villus length by more than 50% [10]. Consequently, rabbits supplemented with BP would be expected to have higher ADG and final BW; however, in the present study, ADG and final BW were unaffected by supplementation with either BP500 or BP + PRO. On the other hand, in the present study, higher ADG and final BW were observed in rabbits on PRO50 treatment. This result could be related to the lower *Eimeria* OPG count observed in rabbits supplemented with PRO50 because there is a strong negative correlation ($r = -0.91$) between BW and *Eimeria* spp. OPG count [54]. Furthermore, these results suggest that PRO supplementation could replace some antibiotics (e.g., zinc bacitracin) commonly used as growth promoters in rabbits [10]. Previous studies [13,18] have reported that, in growing rabbits, dietary supplementation with PRO (150, 250, 300, and 500 mg/kg DM) improves the total antioxidant capacity and increases between 8 and 22% the serum concentration of immunoglobulins (IgM, IgY, and IgG). These effects could improve the health of rabbits and

result in higher ADG and final BW. Furthermore, North et al. [55] reported that, in the cecum of rabbits, supplementation with quercetin (a typical flavonoid of propolis) increases the relative abundance of microorganisms (*Eubacteriaceae*, *Peptococcaceae*, and *Natranaerobiaceae*) that are positively correlated with ADG. Likewise, the addition of quercetin in diets for rabbits increases up to 7.6% the serum concentration of growth hormone [56]. Similar effects of the consumption of PRO and its flavonoids in the present study partially explain the observed increases in ADG and final BW.

FCR was not affected by supplementation with BP500 and BP + PR, suggesting that the doses of BP and BP + PR used in the present study do not improve feed efficiency in growing rabbits. In similar studies, Attia et al. [1,10] also did not observe significant changes in the FCR of growing rabbits supplemented with various doses of BP or BP + PRO.

On the other hand, FCR was lower in rabbits supplemented with PRO50. PRO supplementation has been reported to improve rabbits' digestibility of organic matter and ingested crude protein [57]. North et al. [55] observed that flavonoid (quercetin) supplementation increases the relative abundance of microbial families (*Erysipelotrichaceae* and *Haloplasmataceae*) that are negatively correlated with FCR. Consequently, similar PRO and flavonoid consumption effects observed in our study could partially explain the lower FCR in rabbits on PRO50 treatment. The lower FCR observed in rabbits supplemented with PRO50 suggests that PRO could improve the profitability of rabbit production systems since FCR is a crucial indicator for judging an animal production system [11].

Coccidiosis is an infection caused by *Eimeria* protozoa, which in rabbits causes growth retardation and high mortality [58]. In the present study, the OPG count of *Eimeria* decreased in rabbits supplemented with PRO50 and BP + PRO. This result suggests that these products could be used as natural coccidiostats for growing rabbits. This hypothesis is supported by the average OPG values observed in rabbits supplemented with PRO50 and BP + PRO, which were lower than the range (4000–5000 OPG) at which the application of prophylactic treatment against *Eimeria* protozoa is required [59].

On the other hand, although mortality was higher in rabbits supplemented with BP500, the mortality observed in the present study was high (between 17.5 and 40%) for all treatments. In the present study, a seasonal effect and climatic conditions might have an essential role in the high mortality rates observed [60], particularly if considering that the average ambient temperature during the experimental period was high (30.6 $\pm$ 0.59 °C).

4.2. Hematological Parameters and Blood Biochemistry

When evaluating a new feed additive for domestic animals, it is essential to analyze the effects of the consumption of the additive on animal health [31]. According to Diaz Cano et al. [61], knowledge of reference values for blood metabolites in rabbits provides valuable information on the health status of the animals. In particular, hematological parameters provide information on rabbits' visceral organ infections, inflammation, and necrosis [62]. Except for leukocytes, monocytes, and eosinophils, in the present study the hematological parameters of rabbits from all treatments were within the normal range reported in the literature for healthy rabbits [63–65]. These results suggest that supplementation with BP500, PRO50, and BP + PRO does not affect the hematological system of growing rabbits.

The blood concentration of leukocytes was similar between treatments. However, the blood concentration of leukocytes in CON treatment rabbits was higher than the normal range reported for healthy rabbits [63]. In addition, compared with BP + PRO treatment rabbits, the percentage of monocytes in CON treatment rabbits was significantly higher and above the normal range (3.17–4.67 $\times$ 10^3/mL) [63]. These results could be associated with the higher OPG observed in CON treatment rabbits because the blood concentration of leukocytes and monocytes increases in rabbits infected with *Eimeria* parasites [66]. Furthermore, it has been reported that the blood concentration of leukocytes increases in animals with intoxication (endogenous or exogenous) or infectious diseases [67]. On the other hand, the blood concentration of monocytes increases in rabbits with chronic infection and inflammation [66]. Consequently, the results observed in the present study for

leukocytes and monocytes suggest that supplementation with BP500, PRO50, or BP + PRO did not negatively affect the health of growing rabbits.

Serum glucose concentration and lipid metabolites are indicators of rabbits' energy status [68]. In the present study, serum glucose and cholesterol concentrations were similar between treatments and were within the range reported in the literature for healthy rabbits [62]. This result suggests that supplementation with BP500, PRO50, and BP + PRO did not affect the energy status of growing rabbits.

Supplementation with BP500 reduced serum globulin and total protein levels. However, rabbits from all treatments had serum urea, albumin, globulin, and total protein concentrations within the reference range considered normal for healthy rabbits [62]. This result suggests that the doses used of BP, PRO, and BP + PRO have no negative effects on protein catabolism and no adverse effects on the nutritional status of growing rabbits.

If analyzed at appropriate reference intervals, serum uric acid and creatinine concentrations can be used as biomarkers of renal function status in rabbits [61]. For example, serum creatinine levels increase above the reference interval when animals have chronic and acute renal failure [69]. In the present study, serum creatinine and uric acid concentrations were similar between treatments, and serum levels of these metabolites were within the range reported in the literature for healthy rabbits [65]. These results suggest that supplementation with BP or PRO does not affect the renal health of rabbits.

Serum levels of hepatic enzymes are often used as markers of liver disease [70]. In addition, alkaline phosphatase is associated with other disorders, such as intestinal and generalized tissue damage [71]. In the present study, rabbits from all treatments had serum levels of alkaline phosphatase, lactate dehydrogenase, and aspartate aminotransferase higher than the normal range reported for healthy rabbits [62,64]; however, there were no differences between treatments. This result suggests that supplementation with BP or PRO does not affect growing rabbits' hepatic or intestinal health.

It has been mentioned that serum calcium and phosphorus levels can be used as good indicators of nutritional status in domestic animals because their variability is low [72,73]. In the present study, serum calcium and phosphorus concentrations were similar between treatments. In addition, rabbits from all treatments had serum values for calcium and phosphorus within the range reported for healthy rabbits [63]. This result suggests that supplementation with BP or PRO does not affect growing rabbits' mineral and nutritional status.

4.3. Carcass Yield and Meat Quality

In the present study, supplementation with BP500 and PRO50 increased HCY. In a similar study, Zeedan et al. [9] also observed increased HCY in rabbits supplemented with increasing doses of BP (0, 200, 500, and 700 mg/kg BW for 70 days). Similarly, Waly et al. [58] reported higher HCY in growing rabbits supplemented with increasing doses of PRO (0, 100, 150, and 200 mg/kg DM for eight weeks).

The pH is considered one of the most critical parameters determining rabbit meat quality because it is related to its color, flavor, water-holding capacity (WHC), tenderness, and shelf life [74]. For example, when the pH of meat is higher than 6, the development of proteolytic microorganisms increases [75]. In the present study, only supplementation with BP + PRO increased meat pH. This result suggests that when administered individually, BP and PRO do not significantly alter the physicochemical properties of meat. However, simultaneous administration of BP and PRO could reduce rabbit meat's quality and shelf life.

Previous studies have reported that meat color can be modified by changes in fat content and pH [76,77]. In the present study, lower fat content and higher pH were observed in meat from rabbits supplemented with BP500 and BP + PRO, respectively. However, meat colors (L*, a*, and b*) were similar among rabbits of all treatments, indicating that supplementation with BP or PRO does not affect the appearance of rabbit meat. Based on the literature consulted, there is no previously published information on the effects of supplementation with BP, PRO, or the combination of BP and PRO on rabbit meat color.

However, Prakatur et al. [78] also did not observe significant changes in L*, a*, and b* of broiler meat supplemented with increasing doses (0, 200, 250, 500, 1000, and 2000 mg/kg DM for 42 days) of BP, PRO, or the combination of BP and PRO.

In the present study, PRO50 supplementation reduced the CL of meat. This result suggests that PRO supplementation can improve the WHC of rabbit meat because CL and WHC are negatively correlated [76]. Limited information on the effects of BP and PRO supplementation on the CL of rabbit meat makes it difficult to explain the results observed in the present study. However, it has been reported that CL increases when there is oxidative damage to the meat [79]. The PRO used in the present study contained flavonoids, which improve the oxidative stability of meat [80,81]. This effect partially explains the lower CL observed in rabbits supplemented with PRO50.

The chemical composition of rabbit meat from all treatments had values within the normal range [82]. This result suggests that supplementation with BP500, PRO50, or BP + PRO did not affect the nutritional quality of rabbit meat.

5. Conclusions

The results of this study indicate that propolis supplementation (50 µL/kg BW) can be used to prevent coccidiosis and as a natural growth promoter in rabbits without affecting animal health and meat quality. However, more research is needed to understand and explain the impact of bee pollen and propolis supplementation on productivity, parasite load, health status, and the meat quality of growing rabbits.

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CONCLUSIONES GENERALES

Los resultados del presente estudio indican que el PA y PRO reducen el consumo de alimento y la conversión alimenticia de conejos en período de crecimiento, al mismo tiempo que aumentan la ganancia de peso. Además, la suplementación con ambos productos también mejora el estado antioxidante en el suero sanguíneo de los conejos. Por otro lado, la suplementación combinada de los productos reduce la cantidad de ooquistes de *Eimeria* por gramo de heces.

Es importante destacar que la suplementación con PA y PRO no afecta la biometría hemática ni la química sanguínea de los conejos, lo cual indica que no tienen efectos negativos en la salud de los animales. Los resultados también muestran que la suplementación con PA y PRO no afectan la calidad de la carne.

El PA y PRO se pueden utilizar como promotores naturales de crecimiento en conejos, ya que mejoran los parámetros productivos sin afectar la salud y la calidad de la carne. Sin embargo, se requiere de más estudios enfocados a la investigación de los diferentes tipos de polen y propóleos, ya que su diversidad en cuanto a composición química y de metabolitos bioactivos, los hacen únicos para cada región y sus efectos aún no han sido estudiados.