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The Role of Propolis to Enhance Reproductive Performance of Male Rabbits

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دور البروبوليس (الدنج) في تحسين الاداء التناسلي لذكور الأرانب

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Abstract:
The natural resinous derivative known as propolis is synthesized by honey bees through the integration of their salivary enzymes with botanical secretions harvested from leaf buds and cortical fissures. Due to its potent therapeutic potential, propolis has gained significant traction as a functional ingredient in nutritional supplements and health-oriented beverages aimed at disease prevention and systemic well-being. Its pharmacological profile is characterized by a diverse array of bioactive properties, most notably its antioxidant, antimicrobial, fungicidal, and anti-inflammatory activities, which have been linked to protective effects against chronic conditions such as cardiovascular disease, diabetes, and various malignancies. The objective of the current investigation was to determine the physiological impact of propolis supplementation on the reproductive performance of male New Zealand White rabbits. Subjects were administered a dosage of 40 mg/kg body weight (BW) on an alternating-day schedule over a 12-week experimental period. Empirical evidence from this study indicates that propolis treatment significantly enhanced ($P < 0.05$) several key fertility parameters. Notably, there was a marked increase in the concentration and total output of spermatozoa, as well as an improvement in packed sperm volume (PSV) and the total count of motile sperm per ejaculate (TMS). Furthermore, the treatment appeared to positively influence sexual behavior (libido), evidenced by a reduction in reaction time, while simultaneously elevating the proportions of live and morphologically normal spermatozoa.

Keywords: Rabbits; Propolis; Testes; Fertility.

الملخص:
البروبوليس مادة راتنجية طبيعية يتم استخراجها من شقوق لحاء الاشجار وبراعم الاوراق وتعزز بإنزيمات لعاب نحل العسل وقد أصبح يستخدم على نطاق واسع في الاطعمة والمشروبات والوجبات الغذائية لتعزيز الصحة والوقاية من الأمراض مثل الالتهابات، لما له من خصائص بيولوجية متعددة بما في ذلك خصائص مضادة للالتهابات، ومضاد للسرطان ومضادات الأكسدة، ومضادات البكتيريا، مما يجعله مفيدا في علاج السرطان والسكري وامراض القلب. تشمل اثاره ايضا النشاط المضاد للفطريات، وقد اظهرت الأبحاث التي اجريت على البر وبوليس أن فعاليته تستمر لمدة يوم واحد تقريبا بعد تناوله .وتحسنت القدرات التناسلية لدى الذكور الجدد بعد 12 أسبوعا من العلاج بالبر وبوليس. حيث زاد إجمالي عدد الحيوانات المنوية المتحركة في كل قذفة (TMS).) (PSV وتركيز المصل وإجمالي انتاج الحيوانات المنوية. وحجم

الحيوانات المنوية والرغبة الجنسية وذلك من خلال زيادة سرعة ردة الفعل كما لوحظ تحسن في عدد الحيوانات المنوية الحية والطبيعية.

الكلمات الافتتاحية: الأرانب، بروبوليس، الخصيتين، الخصوبة.

Introduction:

Honeybees (*Apis mellifera*) synthesize the resinous substance known as propolis by gathering balsamic exudates from a diverse range of botanical sources, including bark, leaf buds, floral structures, and plant shells. Through the metabolic integration of beeswax and specialized salivary enzymes, these insects transform raw materials into a multifunctional sealant (1). Within the hive ecosystem, this sticky compound serves critical structural and defensive roles, including the insulation of crevices, moisture regulation, and protection against predatory intrusion. Historically, propolis has occupied a significant place in traditional pharmacopeia, frequently utilized either in its pure form or as a synergetic component in natural wound-healing ointments (2).

Biochemically, propolis is a complex matrix rich in essential micronutrients and bioactive enzymes. Its nutritional profile encompasses an array of fatty acids, essential vitamins (B1, B2, B6, C, and E), and a comprehensive mineral suite including, but not limited to, Mg, Ca, Fe, Zn, and Cu. Furthermore, it serves as a vehicle for vital enzymatic catalysts such as glucose-6-phosphatase, acid phosphatase, and succinic dehydrogenase (3).

From a physiological perspective, propolis exerts a formidable protective influence over the male reproductive system. This cytoprotective capacity is largely attributed to its high concentration of phenolic compounds and flavonoids, which mitigate the oxidative stress induced by environmental toxins like aluminum chloride, a known disruptor of testicular integrity and androgenic synthesis (4). Prior empirical investigations have substantiated these benefits, demonstrating that propolis supplementation in leporine models fosters an increase in systemic testosterone concentrations and reproductive organ mass. Additionally, the literature suggests that its administration optimizes semen quality by enhancing sperm motility and morphology, stabilizing seminal plasma enzymes, and suppressing the proliferation of free radicals and lactate dehydrogenase (5).

Materials and Methods:

The propolis utilized in this investigation was sourced from California Health Products, Inc. (Los Angeles, CA, USA). The experimental cohort comprised adult male New Zealand White rabbits, aged six months at the onset of the study, with an initial mean body mass of 3064 /pm 29.8 g. Over the 12-week experimental duration, subjects were individually housed in metabolic cages to facilitate controlled observation. Environmental conditions were maintained with *ad libitum* access to both water and a standardized basal diet, with body weight fluctuations monitored via weekly assessments.

The dietary regimen for the experimental subjects was centered on a specialized pelletized formulation. This basal ration was meticulously engineered from a diverse matrix of raw ingredients, which included 30% berseem hay (Trifolium alexandrinum), 26.2% wheat bran, 14% soybean meal and 25% yellow maize. To ensure metabolic optimization and palatability, the diet was further fortified with a selection of vital additives. Specifically, the chemical profile was augmented through the inclusion of 3% molasses, 1% CaCl2, and 0.4% NaCl. Final nutritional stabilization was achieved via the integration of a 0.3% concentrated vitamin and mineral premix, supplemented by 0.1% methionine to ensure a balanced amino acid profile. The micronutrient profile per kilogram of the premix (Holland Feed Inter. Co.) provided the following concentrations: Vitamin A (4,000,000 IU), Vitamin D3 (5,000,000 IU), Vitamin E (16.7 g), and Vitamin K (0.67 g). The complex also integrated B-vitamins (B1, B2, and B6 at 0.67 g each; B12 at 0.004 g; and B5 at 16.7 g), alongside 6.67 g of pantothenic acid, 0.07 g of biotin, 1.67 g of folic acid, and 400 g of choline chloride. Trace mineral fortification included 133.4 g of Mg, 0.25g of I ,25g of Fe, 10g of Mn, 1.67 g of Cu ,0.033g of Se and 23.3g of Zn

Based on the laboratory evaluation of the dietary pellets (6), the chemical profile exhibited a high concentration of organic matter, accounting for 92.9% of the dry matter (DM) content. Furthermore, proximate analysis substantiated the nutritional density of the feed, identifying a crude protein level of 15.8% and a crude fiber fraction of 11.3%. The remaining lipid and mineral components were quantified at 3.7% for ether extract and 7.2% for ash, respectively, while the carbohydrate portion, represented by nitrogen-free extract, was determined to be 62.4%.

Over a consecutive 12-week period, propolis was administered via oral gavage at a standardized dosage of 50 mg/kg body weight (BW) (7). To ensure longitudinal accuracy, individual body mass was recorded at weekly intervals throughout the trial. These anthropometric measurements were consistently conducted during the morning hours, prior to the provision of food or water, to mitigate potential postprandial variance. Upon completion of the 12-week treatment phase, the subjects were

euthanized for organ harvesting. Specifically, the testes were weighed and promptly cryopreserved at -20°C to maintain biochemical stability until the subsequent assays were performed.

During the final six weeks of the study, a total of 60 ejaculates were harvested per treatment group through weekly collection cycles. Samples were obtained using an artificial vagina in the presence of a teaser doe. Following the excision of the gel mass, ejaculate volume was quantified via graduated collection tubes. To determine spermatozoa concentration, a diluted eosin solution was utilized in conjunction with an improved Neubauer hemocytometer (GmbH + Co., Hamburg, Germany) (8). The total sperm output (TSO) was quantified by integrating the ejaculate volume with the corresponding spermatozoa concentration. Subsequent to the collection phase, immediate physiological assessments were performed to characterize the seminal environment. Specifically, the initial fructose concentration within the plasma was determined promptly (9). In parallel, the acidity or alkalinity of the samples was evaluated; this hydrogen ion concentration (pH) was established through the application of high-precision indicator strips (Merck KgaA, Darmstadt, Germany).

Microscopic evaluation included the assessment of motility and morphology. Using a light microscope at 10x magnification, the percentage of motile sperm was visually estimated. These data allowed for the calculation of the total motile sperm count (TMS), derived from the product of the motile fraction and the TSO. Additionally, the ratio of viable and morphologically normal spermatozoa was identified using an eosin-nigrosine blue staining protocol (10). The Total Functional Sperm Fraction (TFSF) was subsequently modeled by integrating TSO, motility percentage, and normal morphology (%) (11). Behavioral assessments included the measurement of libido, defined by the "reaction time" (in seconds), the latency between the introduction of the doe and the completion of the erection.

Quantitative data were processed using Minitab software (version 17). Following the confirmation of normal distribution across the datasets, the statistical significance of treatment effects was evaluated using a one-way analysis of variance (ANOVA). Post-hoc differentiation between means was achieved through the Tukey multiple comparison test, with the threshold for critical significance established at $P < 0.05$.

Results:

As delineated in Table 1, the administration of propolis elicited a significant elevation ($P < 0.05$) in both absolute body weight (BW) and the relative mass of the testes when compared to the control cohort. The empirical data further revealed that propolis supplementation exerted a profound positive influence ($P < 0.05$) on a comprehensive suite of semen quality markers. Specifically, treated subjects exhibited marked increases in sperm concentration, total spermatozoa output, and motility percentages. Furthermore, enhancements were observed in packed sperm volume (PSV), the total functional sperm fraction (TFSF), and the concentrations of initial seminal fructose. Propolis treatment also appeared to modulate reproductive behavior, evidenced by a significant improvement in libido, which was quantified via a reduction in reaction time.

Observations detailed in Table 2 highlight a significant attenuation ($P < 0.05$) in the initial hydrogen ion concentration (pH) following propolis administration. Moreover, the treatment effectively reduced the prevalence of both non-viable and morphologically abnormal spermatozoa. Conversely, the proportions of live and structurally normal sperm were significantly bolstered, suggesting a cytoprotective effect on the germ cells. These findings collectively indicate that propolis serves as a potent agent in optimizing the leporine reproductive profile by improving both the biochemical environment and the physical characteristics of the semen.

Table (1): Longitudinal effects of propolis administration on total body mass and relative testicular weight in male rabbits (Data expressed as Mean ± SE).

Parameters	Allocation of Subjects	
	Control	Propolis
Body Weight (g)	3059 ± 29.8^{a}	3245 ± 37.4^{a}
Relative Testicular Weight (g/100 g BW)	8.4 ± 0.586^{b}	15.25 ± 0.633^{a}

Means within the same row followed by distinct superscript letters {a, b, c} indicate statistically significant differences at the $P < 0.05$ level.

Table (2): Quantitative assessment of seminal parameters in male rabbits following propolis administration (Data expressed as Mean ± SE).

Parameters	Allocation of Subjects	
	Control	Propolis
Ejaculate volume (ml)	0.64 ± 0.017^{c}	0.92 ± 0.018^{a}
PH	7.58 ± 0.022^{b}	7.44 ± 0.038^{c}
Reaction time (s)	3.55 ± 0.099^{c}	2.98 ± 0.145^{d}
Packed sperm volume (%)	15.1 ± 0.16^{b}	18.1 ± 0.38^{a}
Sperm concentration ($\times 10^{6}$ ml^{-1})	264 ± 6.5^{b}	319 ± 7.2^{a}
Total sperm output ($\times 10^{6}$)	172 ± 5.9^{c}	299 ± 10.3^{a}
Sperm motility (%)	67.8 ± 0.7^{b}	73.5 ± 0.9^{a}
Total motile sperm ($\times 10^{6}$)	118 ± 4.3^{c}	220 ± 8.8^{a}
Live sperm (%)	72.9 ± 0.8^{b}	82.2 ± 1.1^{a}
Dead sperm (%)	26.3 ± 0.82^{b}	17.9 ± 1.07^{c}
Normal sperm (%)	82 ± 0.3^{b}	86 ± 0.4^{a}
Abnormal (%)	18 ± 0.3^{c}	14 ± 0.4^{d}
Total functional sperm fraction ($\times 10^{6}$)	94 ± 3.6^{b}	184 ± 8.3^{a}
Initial fructose (mg/dl)	258 ± 3.9^{b}	275 ± 4.0^{a}

Means within the same row followed by distinct superscript letters {a, b, c, d} indicate statistically significant differences at the $P < 0.05$ level

Discussion:

The empirical data from the present study indicate that propolis administration significantly enhanced both body weight (BW) and testicular weight (TW) (Table 1). These observations align with the findings of (12), who reported that propolis supplementation not only augmented relative testicular mass but also mitigated the deleterious effects of chlorpyrifos. Similarly, (5) noted substantial gains in both total body mass and reproductive organ weight following propolis treatment. Such increases in testicular weight are particularly noteworthy, as TW serves as a reliable surrogate marker for spermatogenic capacity due to its robust correlation with sperm reserves in both the testes and the epididymis (13).

The observed elevations in ejaculate volume (EV), sperm concentration, and total sperm output (TSO) in propolis-treated cohorts are consistent with research by (14), who examined the protective role of propolis against aluminum chloride toxicity in male models. Their evidence suggests that propolis administration effectively counters the sharp declines in sperm density typically induced by environmental toxins. This physiological enhancement is largely attributed to the diverse bioactive profile of propolis, specifically its high concentration of flavonoids, caffeic acid derivatives (15), and phenolic constituents (16), which function as potent antioxidants and free radical scavengers.

Furthermore, (17) proposed that the efficacy of propolis stems from its ability to suppress membrane lipid peroxidation and inhibit the proliferation of free radicals. Consequently, it is hypothesized that propolis serves as a pivotal mediator in optimizing reproductive success in rabbits. Sperm motility remains a cornerstone of this success, as it is indispensable for the ascent of spermatozoa through the female reproductive tract and the eventual achievement of fertilization (18).

High-quality semen is traditionally characterized by elevated glycolytic and fructolytic rates, which sustain a higher proportion of vigorous, motile spermatozoa. This kinetic energy is vital not only for reaching the fertilization site but also for the critical penetration of the oocyte's protective layers, namely the zona pellucida and cumulus oophorus. Given its central role in the fertilization process, sperm motility has been established as a primary diagnostic benchmark in the clinical assessment of male infertility (18).

Consistent with (5), our findings demonstrate that propolis supplementation significantly reduces the prevalence of non-viable and morphologically abnormal sperm while simultaneously bolstering systemic testosterone concentrations. In summary, the oral administration of propolis appears to catalyze various physiometabolic improvements, particularly regarding semen characteristics and overall growth performance. The enhanced reproductive and productive capacity of male rabbits can be definitively linked to the fundamental antioxidant properties of propolis, which safeguard cellular integrity against oxidative stress.

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