تأثير إضافة حبوب اللقاح على جودة السائل المنوي في ذكور الأرانب

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الملخص العربي :

أجريت هذه الدراسة لتقييم تأثير إضافة حبوب اللقاح (Bee pollen) بمستويات مختلفة كإضافة غذائية على جودة السائل المنوي والحد من تأثير أكسدة الليبيدات على الحيوانات المنوية لذكور الأرانب النيوزيلندية ، حيث استخدم عدد 28 ذكراً ناضجاً بعمر 8-9 أشهر مع ثبات الخصوبة وكان متوسط وزن الجسم الحي 3.15 ±0.24 كجم ، قسمت ذكور الأرانب إلى أربع مجموعات متجانسة ووضعت هذه الذكور تحت اضاءة 16 ساعة و8 ساعات ظلام، استخدمت أربع علائق تجريبية لتمثيل أربعة إضافات غذائية حيث أعطيت الذكور في المجموعة الأولى السيطرة (control group) عليقة الشاهد بدون مكملات وأعطيت المجموعة الثانية والثالثة والرابعة مكملات حبوب اللقاح بنسبة 0.2 ، 0.4 ، 0.6 % على التوالي.

أظهرت النتائج أن إضافة 0.6 % من حبوب اللقاح كان له تأثير معنوي على خفض تركيز أيون الهيدروجين pH في السائل المنوي مقارنة بمجموعة السيطرة والمعاملات الأخرى، إضافة حبوب اللقاح كان له تأثير معنوي على زيادة حجم القذفة وتركيز الحيوانات المنوية مقارنة بمجموعة السيطرة ، وإضافة 0.6 % حبوب اللقاح كان له تأثيراً معنوياً على زيادة نسبة حركة الحيوانات المنوية ونشاطها بنسبة 23.46 و 17.84 % على التوالي. بلغت نسبة الزيادة المعنوية في حجم الحيوانات المنوية 22.96 % بسبب إضافة 0.6 % حبوب اللقاح .

من ناحية أخرى لوحظ أن إضافة حبوب اللقاح حسن معنوياً من تركيز هرمون التستستيرون ونسبة حيوية الجسم الطرفي (الاكروسوم) مقارنة بالسيطرة ، كما أن هناك انخفاضاً معنوياً في(MDA) وعلى العكس من ذلك كان هناك زيادة معنوية في (TAC) في بلازما السائل المنوي نتيجة إضافة حبوب اللقاح مقارنة بالسيطرة ،

نستنتج من هذه الدراسة أن إضافة حبوب اللقاح أدت لتحسين جودة السائل المنوي ومضادات الأكسدة في ذكور الأرانب.

Effect of Bee Pollen Supplementation on Semen Quality of Rabbit Males

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ABSTRACT: This study was designed to evaluate the effect of using bee pollen (BP) at different levels as a natural promoter on semen quality and reducing the effect of lipid peroxidation of New Zealand rabbit buck semen. A total number of 28 mature New Zealand white (NZW) 8-9 months rabbit bucks was classified into four homogeneous treatment groups (7 bucks each). These bucks were selected of proven fertility with an average of 3.15±0.24 kg living body weight. Bucks were kept under a continuous 16 h light/ 8 h dark photo period. Four experimental diets were formulated to represent four dietary treatments. The bucks of the first treatment group (the control group) were given the basal diet without supplementation, whereas the bucks on the second,the third and the fourth groups were given amounts of 0.2, 0.4 and 0.6% supplementation of bee pollen respectively. The results show that the addition of 0.6% bee pollen did not produce any significant decrease in semen (pH) comparing with the control group and the other experimental feed additives. It is also shown that the addition of bee pollen significantly increased the ejaculate volume and the individual sperm concentration as compared with the control group.Moreover, the administration of 0.6% bee pollen significantly increased the live sperm and the mass activity by 23.46% and 17.84 % respectively. The results also revealed that

the 22.96% increase of packed sperm volume (PSV) was due to the administration of 0.6% bee pollen. This factor also improved the testosterone concentration and acrosome action in comparison with the control group. There was an increase in seminal plasma MDA concentration, but there was not any increase in seminal plasma TAC due to bee pollen supplementation in comparison with the control group. In conclusion, semen quality and antioxidant status of rabbit bucks can be significantly improved by bee pollen.

Key words: Bee pollen, Rabbits, Semen quality, Antioxidant status.

INTRODUCTION

Bee pollen considers as one of the natural feed supplements which largely used in animal and human. The bee pollen forms an important food rich in protein, carbohydrates and fats with a large number of minerals and vitamins. This food is very useful for animals and humans [1]. Pollen a fine powder collected by bees from flowering plants and considered as the male gametophytes and an important source of bee foods [2]. The bee pollen was very useful to increase the blood hemoglobin and regulated the level of cholesterol of blood [3]. The histological changes of fish testis treated with bee pollen and Propolis showed an increase in the spermatogonia at the lumen of the somniferous tubules, while the walls of the tubules appeared thinner thanof the untreated *Oreochromisniloticus* [4]. In tropical and sub-tropical areas rabbits are faced with many problems that related to hot climate; particularly heat stress.This stress had asignificant adverse effect on testosterone concentration, ejaculate volume, sperm motility, sperm concentration and semen

fructose concentration [5]. High environmental temperature not only has adverse effects on rabbits' performance, but also causes an increase in oxidative stress [6]. Bee pollen contains at least 22 amino acids, 18 vitamins, 25 minerals, 59 trace elements, 11 enzymes or co- enzymes, 14 fatty acids, 11 carbohydrates (35-61% glucose, fructose and sucrose) and approximately 25% protein. Bee pollen is extremely rich in carotenes, which are metabolic precursors of vitamin A. It is also high in B complex and vitamins C, D, E and Lecithin [7]. Bee pollen has recently received an increased attention for its antibacterial and anti-fungicidal effects [8]. Relevant is also the quantity of polyphenolic components, mainly flavonoids [9], that prevent the negative effect of free radicals [10].Bee pollen appears promising because it enhances the immune function of poultry, promotes animal growth, protects intestinal tract health and improves the quality and safety of animal products [11], [12]. Bee pollen is confirmed as an interesting supplement in rabbits abe to improve productive and reproductive performance [12] and also under heat stress conditions [11]. These effects may be due to the content of antioxidants, vitamins, minerals, phenolic constituents and enzymes [13]**.**

Therefore, this study aims to verify the effect of using bee pollen on semen quality and reducing the effect of lipid peroxidation of New Zealand rabbit buck semen.

MATERIALS AND METHODS

The present study was carried out at the Rabbit Research Laboratory, Department of Animal Production, Faculty of Veterinary Science, Agriculture, Zawia University during the period from December 2017 to February 2018.

A total number of 28 mature New Zealand (NZW) 8-9 months rabbit bucks of proven fertility with an average initial living body weight of 3.15±0.24 kg were classified into four homogeneous treatment groups of 7 bucks. Bucks were kept under a continuous 16 h light/8 h dark photoperiod and the ambient temperature ranged from 18.3 to 22.3 °C.Animals were housed individually in flat-deck cages and had been trained earlier for semen collection using an artificial vagina. Four experimental diets were formulated to represent four dietary treatments. Bucks in the first treatment group were given the basal diet without supplementation; while the second, third and fourth groups included bucks with supplementation of 0.2,0.4 and 0.6 % bee pollen in their diets, respectively. All the experimental diets were formulated in such a way to ensure they were both isonitrogenous and isocaloric. The composition and calculated analysis of the basal experimental diet is presented in **Table 1**. Pellets of the experimental diets were made as follow; pelleting was initiated by molasses addition, asa binding material, and then all diet ingredients were pressed at 70C^{o} after that pellets were cooled. The basal experimental diet was formulated tocover all essential nutrient requirements for adult male rabbits according to [14]. Bucks were allowed to become accustomed to treatment for a preliminary period of 30 days during December. Semen collection occurred weekly over the 8 weeks from junrary to February. Feed and water were offered ad libitum throughout the whole experiment. All bucks were kept under similar managerial and environmental conditions. Bucks were housed individually in galvanized batteries (50Lx50Wx40Hcm) provided with feeders and automatic drinkers in a windowed rabbitry.

Semen samples were collected weekly over the 8 weeks using an artificial vagina and the samples of each week were subjected to chemical analysis. Semen collection and handling were carried out and evaluated according to the international guidelines of [15]. Ejaculation volume was measured to the nearest 0.01 ml. The volume of each ejaculate was recorded after removal of the gel mass. Immediately after collection, semen was maintained at 37°C in a water bath for evaluation. Semen mass motility was given an arbitrary score from 0 to 3 based on the following assessment and the following variables were estimated: 0= No current, (0.5) =Very few slow current, 1 = Few slow current, 1.5= Many moderate waves, 2=Many sweeping waves, 2.5=Numerous vigorous waves, 3= Numerous rapid and vigorous waves, as described by [16]. A weak eosin–formalin (10% formalin) solution was used at a rate of 1: 99 before counting the cells for evaluation of sperm concentration by the improved Neubauer hemocytometer slide method as described by [17].Individual sperm motility was estimated at 400× magnification [18]. Assessment of live and abnormal spermatozoa was performed using an eosin–nigrosin blue-staining mixture [19]. Semen pH was determined just after collection using a pH cooperative paper ranging from 0 to 14 with 1 grade (Merck KgaA, 64271 Darmstadt, Germany). Serum testosterone was determined by enzyme immunoassay using commercial kits purchased from Biosource. Packed sperm volume (PSV) was recorded using Micro-AID® microhematocrit tubes and microhematocrit-centrifuge which were centrifuged for 5 min at 4000 rpm. Evaluation of seminal initial fructose was carried out immediately after collection according to [20]. The

acrosome reaction of spermatozoa was analyzed by microscopy in bright-field illumination after staining the smear preparations with naphthol yellow S and erythrosin according to [21] with a minor modification. After air-drying, the slides were dipped twice in distilled water to remove the seminal vesicle proteins obscuring the visual assessment of the acrosome reaction. With this method, the acrosomal caps in intact spermatozoa stained cherry-pink, whereas sperm heads devoid of acrosomes stained pale yellow. A total of 400 spermatozoa were counted from each slide and the percentages of acrosome reaction-positive (acrosome-reacted) and negative spermatozoa were determined. The acrosome reaction was also checked by phase-contrast microscopy and by transmission electron microscopy (TEM). In phase-contrast microscopy (1000, oil immersion), the spermatozoa with an intact acrosome displayed sharp margins of the head, and after the acrosome reaction the apical regions became fuzzy and the total loss of acrosomes was readily distinguished [22].

Seminal plasma was separated by centrifugation at 3000 RPM for 20 minutes and was stored at-20^{o}Cin Eppendorftubes for further analysis of total antioxidants capacity and malondialdehyde were determined in seminal plasma calorimetrically using commercial kits obtained from (BIO-DIAGNOSTICS, Egypt) according to the procedure outlined by the manufacturer. The data were assessed by analysis of variance using the [23]. Test of significance for the difference between different treatments was done by Duncan's multiple range test [24].

Table (1): Composition and calculated analysis of basal experimental diet

Ingredients	Experimental diet %
Barley	20.00
Yellow corn	15.00
Wheat bran	14.10
Clover hay	23.00
Soybean meal (44%)	22.00
Molasses	3.00
Limestone	1.50
DI- calcium phosphate	0.30
Salt	0.50
Vitamin and minerals mixture*	0.30
Lysine	0.15
Methionine	0.15
Total	
Chemical Analysis (as fed)**	
Crude protein, %	17.10
Ether extract, %	2.86
Crude fiber, %	13.74
Nitrogen free extract, %	55.10
Ash, %	7.90
Methionine, %	0.20
Cystine, %	0.22
DE Kcal/kg	2685

*Vitamin/trace mineral premix provides the following (per kg of diet): vitamin A, 1.800 mg retinol; vitamin E, 6.67 mg D-a-tocopherol; menadione, 2.5 mg; vitamin D3, 50mg cholecalciferol; riboflavin, 2.5 mg; Ca pantothenate, 10 mg; nicotinic acid, 12 mg; choline chloride, 300 mg; vitamin B12, 4 mg; vitamin B6, 5 mg; thiamine, 3 mg; folic acid, 0.50 mg; biotin, 0.02 mg; Mn, 80 mg; Fe, 40 mg; Cu, 4 mg; Se, 0.10 mg.

** Calculated according to NRC (1977).

RESULTS AND DISCUSSIONS

Results illustrated in **Table 2** show the effect of bee pollen on semen quality.Results revealed that the addition of 0.6% bee pollen resulted in a significant decrease in semen hydrogen ion concentration (pH) as compared with the control and the other Experimental feed additives. Ejaculate volume of rabbit bucks was significantly increased by the addition of 0.2, 0.4 and 0.6% bee pollen and increase surpassed the control by 6.9, 13.8 and 18%. The addition of 0.2, 0.4 and 0.6%bee pollen significantly ($p \leq 0.05$) increased individual sperm motility while, addition of 0.2, 0.4 and 0.6% bee pollen significantly increased sperm concentration, [25] reported that sperm density in male Cobb broiler breeders increased in the group given 0.5g/kg MOS in comparison with control. However, there were no differences in proportion of live sperm between the two groups. The results of sperm concentration are in agreement with a previous report in which breeders were fed diets supplemented with a yeast culture [26]. The observed improvement in sperm concentration in the MOS-fed males might have been due to enhanced availability of nutrients facilitated by more efficient nutrient absorption at the level of the gastrointestinal tract. Furthermore, several workers

have reported higher antioxidant activity in chickens and piglets fed MOS-supplemented diets [27], [28]. From this perspective, a key aspect that should be deliberated upon is a possible improvement in the activity of antioxidants such as glutathione peroxidase (GSH-Px) and improving total antioxidant capacity in MOS-fed bucks and its importance in spermatozoa production and maturation. High levels of GSH-Px are found in the testes, and it acts as a powerful antioxidant in the developing spermatids and spermatozoa [29]. Spermatozoa are subject to the damaging effects of high concentrations of peroxides in the testes, semen, and utero-vaginal sperm host glands [30], [31]. In organs such as testes that have high metabolic rates, levels of antioxidants required to ensure survival of spermatozoa in those aerobic environments are high. Thus, high density of spermatozoa recorded in MOS-fed bucks in this trial might have been due to its influence on the antioxidant activity.

Table 2 shows that the addition of 0.2, and 0.4% bee pollen resulted in a significant increase in live sperm % by 9.42 and18.42%, respectively. The aforementioned treatments significantly increase mass activity by 6.57 and 13.6%, respectively, in comparison with the control group free of feed additives. On the other hand, administration of 0.6% bee pollen significantly ($p \leq 0.05$) increased live sperm percentage and mass activity by 23.46 and 17.84%, respectively, as compared with control. In this respect, [32] reported that there was an increase in sperm counts and daily sperm production of rats fed with pollen of Raphanusspp and Cistus spp. Although the increases were not significantly different in statistical terms from the control, however, the authors suggested that pollen caused an increase in

these parameters. Generally, there is no literature available on pollen effects on sperm counts.

A significant increase in PSV percentage amounted to 22.96 % due to administration of 0.6% bee pollen was observed. It was recorded that bee pollen improved testosterone concentration and acrosome action percentage in comparison with the control group.

Table (2): Effect of bee pollen on semen quality of New Zealand rabbit bucks

Traits	Control	Feed additives bee pollen %			P.Value
		0.2	0.4	0.6	
Semen quality:					
Hydrogen ion (pH)	7.69±0.02^{a}	7.49±0.03^{b}	7.37±0.07^{c}	7.28±0.02^{d}	0.0001
Ejaculate volume (ml)	0.72±0.007^{d}	0.77±0.003^{c}	0.82±0.004^{b}	0.85±0.003^{a}	0.0001
Individual motility %	67.05±0.22^{d}	75.05±0.34^{c}	76.82±0.37^{b}	78.37±0.22^{a}	0.0001
Sperm concentration (10^{6}/ml)	137.26±0.67^{d}	207.52±0.60^{c}	233.45±0.59^{b}	249.86±0.96^{a}	0.0001
Live sperm%	57.1±0.46^{d}	62.37±0.37^{c}	67.44±0.32^{b}	70.50±0.34^{a}	0.0001
Mass activity (1-3)	2.13±0.003^{d}	2.27±0.002^{c}	2.42±0.003^{b}	2.51±0.16^{a}	0.0001
Initial fructose	255.12±1.38^{d}	262.13±1.32^{c}	267.37±1.35^{b}	275.25±1.25^{a}	0.0001
	13.28±0.25^{d}	14.51±0.15^{c}	15.23±0.03^{b}	16.33±0.11^{a}	0.0001
	2.57±0.01^{d}	2.93±0.01^{c}	3.11±0.03^{b}	3.34±0.04^{a}	00001
	52.75±0.45^{d}	60.45±0.4	62.12±0.3	65.11±0.65^{a}	

(mm/dl)		6[c]	5[b]		
*PSV %	1.04±0.004[d]			1.44±0.003[a]	
Testosterone (ng/dl)	6.65±0.01[a]	1.27±0.006[c]	1.38±0.004[b]	5.23±0.01[d]	
Acrosome action %		5.85±0.02[b]	5.43±0.01[c]		
Seminal lipid peroxidation:					
**TAC (mm/l)					
***MDA (nmol/ml)					

[a-b-c-d] Means within a row having different superscripts are significantly different ($P \leq 0.05$).

*PSV= packed sperm volume.

**TAC= total antioxidant capacity.

***MDA= malondialdehyde.

[32] found that there was an increase in testosterone levels of rats fed with pollen of RaphanussppandCistus spp. Although the increases were not significantly different in statistical terms from the control, however, the authors showed that pollen caused an increase in this parameter. There is rare, literature available on pollen effects on testosterone levels.

Data for the effect of bee pollen on the seminal lipid peroxidation are presented in **Table 2** Lipid peroxidation, measured as malondialdehyde (MDA), is a free-radical mediated propagation of oxidative insult to polyunsaturated fatty acids

involving several types of free radicals [33]. Results show a significant decrease in seminal plasma MDA concentration and asignificant increase in seminal plasma TAC due to different supplementation used in the present study in comparison with the control group. [34], [35] reported that the hepatic content of MDA was decreased and GSH content was increased by the administration of some flavonoids (quercetin and rutin found in citrus) to rats fed on a high - cholesterol diet. A reduction of these enzymeactivities is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes when they are present in high concentrations, free radicals are able to interact with the enzymes and inactivate them [36]. In the study of [37], testicular MDA levels were elevated and in diabetic rat group animals and the antioxidant parameters were reduced (SOD and Catalase). Oxidative stress-mediated damage to the sperm plasma membrane, integrity of DNA and on germ cell leads to deterioration of sperm quality [38], [39].

[40] found that the addition of 100 and 200 mg /kg diet of bee pollen to growing rabbits diet decreased the level of blood serum TAC through summer season in comparison with the control rabbits fed diet free of feed additives. This may refer to the strong antioxidant activity of bee pollen. Bee pollen possesses a noticeable source of compounds with health protective potential and antioxidant activity. Results obtained by [41] reported that the addition of bee pollen caused a significant increase of total antioxidants capacity as compared to control rabbits.

Vitamin C and bee pollen have been defined as an important component of an antioxidant network that prevents membrane

damage from oxidation [42], [43], [44], [45]**.** Several investigations on bee pollen have showed that flavonoids concentrated in bee pollen are powerful antioxidants which are capable to scavenge free radicals [13]. It was recorded that antioxidant constituents in bee pollen (including flavonoids and polyphenols) have been reported to increase glutathione content in the liver of laboratory animals [46]. The elevation of this enzyme by flavonoids may also be responsible for the observed protection against radiation-induced damag [47]. By increasing the activities of antioxidant enzymes, flavonoids from bee pollen reduces the number of free radicals or ROS generated and increases the production of molecules protecting against oxidative stress. The increase of antioxidant enzyme activities such as SOD, CAT and GSH may be considered as a protective mechanism against heat-induced free radical production and lipid peroxidation [48].

Conclusion

The results indicated that bee pollen improved semen quality and antioxidant status of rabbit bucks.

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