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ENHANCING ANTIOXIDANT STATUS, SEMEN QUALITY AND FERTILITY OF V-LINE RABBIT BUCKS FED ON SUPPLEMENTED DIETS WITH PUMPKIN SEED POWDER

H.A. Shahba;[1] Rawia S. Hamed;[1] and B.M. Abou-Shehema [2]

[1] Rabbit, Turkey and Waterfowl Rese. Dep., Anim.Prod. Res. Inst., Agric.Res. Center, Giza, Egypt; [2] Dep. of Poult.Nut., Anim. Prod. Res. Inst.Agric. Res. Center, Giza, Egypt

Corresponding Author: Hossam Shahba Email: Hossam-shahba2009@arc.sci.eg

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ABSTRACT: This study was conducted to evaluate the effect of supplemented pumpkin seed powder (PKSP) in the diet of V-line rabbit bucks in comparison to zinc oxide (ZnO) on blood hematology, serum biochemical parameters, semen quality, and fertility. Thirty-five male V-Line rabbits at 120 days of age were randomly distributed into five dietary treatments (7 bucks each) as follows: control (fed a basic diet), ZnO treatment (fed a basic diet supplemented with 150 mg/kg diet), the third, fourth and fifth treatments were fed a basic diet supplemented with different levels of PKSP (0.5, 1.0, and 2.0g/kg diet) respectively. Results showed that red blood cells (RBC) and lymphocytes were significantly increased ($P \leq 0.05$) in ZnO treatment and groups supplemented with 1.0 or 2.0 g PKSP/kg diet, compared with the control. Bucks fed a diet supplemented with 2.0 g of PKSP/kg significantly increased high-density lipoprotein (HDL) and decreased very low-density lipoprotein (VLDL) compared with other experimental groups. Rabbit bucks in ZnO treatment or 2.0 g PKSP/kg diet significantly increased total antioxidant capacity (TAC) in serum and seminal plasma compared with other experimental groups. Meanwhile, testosterone concentration in both serum and seminal plasma were significantly increased in the group that received 2.0 g PKSP /kg diet compared with others, but not significantly when compared to ZnO treatment. Also, ZnO treatment or 2.0g PKSP/kg diet represented higher values of semen quality and fertility. In conclusion: PKSP favorably affected rabbit hematology, lipid profile, antioxidant status, testosterone level, and semen quality, especially at the level of 2.0 g PKSP/kg diet.

Key words: zinc oxide, pumpkin seeds, antioxidants, rabbit bucks, semen quality

INTRODUCTION

The production of meat from rabbits is very important, especially in developing countries, to increase per capita animal protein. As is known, increasing the production of rabbits depends on the success of the reproductive processes of spermatogenesis in the male and oogenesis in the female Bakeer *et al*. (2021). Spermatogenesis is a continuous and complex process, producing some free radicals that negatively affect spermatogenesis and the spermatozoa membrane Bakeer *et al.* (2021). Antioxidant feed additives improve the antioxidant status of the animal and reproductive performance (Attia *et al*., 2017; Hassan *et al*., 2022). Antioxidant feed additives such as minerals, vitamins, and phytogenic play an important role in spermatogenesis (Jimoh *et al*., 2023).

Zinc (Zn) is a trace micronutrient that plays an important role as an antioxidant that reacts with reactive oxygen species in the seminal plasma and protects the spermatozoa cell membrane from damage (Minisy *et al*., 2017). Likewise, Zn regulates testosterone balance, spermatogenesis, and acrosome reactions (Ma *et al.*, 2023). Zinc is important in body metabolism and is a part or co-factor of many enzymes. In addition, Zn has a positive effect on immunity and hormone function, such as sex hormones. Its deficiency in animals is characterized by poor fertility, decreased feed intake, poor growth, testicular atrophy, and weakened immunity (Ogbuewu and Mbajiorgu, 2023). Rabbit bucks respond positively to 150 mg Zn/kg diets in terms of improving semen quality (Baiomy, 2008).

Pumpkins belong to the family Cucurbitaceae, and the seeds of the plant were used to improve fertility problems in ancient medicine (Gundidza *et al.*, 2009). Pumpkin seeds (PKS) contain oil of more than 40–45%, which includes linolenic acid, palmitic acid, stearic acid, and oleic acid (Majid *et al*., 2020). Also, protein between 25–35%, which includes main essential amino acids such as alanine, phenylalanine, and lysine, and minerals such as Zn, selenium, copper, manganese, phosphorus, and potassium (Rohman, 2020). Hashemi (2013) and Shaban and Sahu (2017) demonstrated that the present of natural antioxidants in pumpkin seed oil (PKSO) and supplementation of vitamin E improved the testosterone level and semen quality. Ragab *et al*. (2016) reported that PKSO with black seed oil improved semen quality, antioxidant activity, and testosterone levels in rabbits. There is little information pertaining to the effect of Pumpkin seeds powder (PKSP) on the reproductive efficiency of male rabbits. This study aims to evaluate the impact of dietary different levels of PKSP in comparison to Zinc oxide on hematological parameters, serum biochemicals, semen quality, and fertility of V-Line rabbit bucks from 120 to 240 days of age.

MATERIALS AND METHODS

This study was conducted at El-Sabahia Poultry Research Station, Animal Production Research Institute, Agriculture Research Center, Egypt.

Pumpkin seed powder preparation

Dried PKS "*Cucurbita moschata*" was purchased from the local market and ground to a fine powder using an electric dry mill; the powders were stored in well-tied black plastic bags at room temperature (~25 °C) until inclusion in the diets of the bucks. Chemical composition of PKSP is presented in Table 1 according to official methods of AOAC (2007). Total phenolic compounds (equivalent to gallic acid) and antioxidant activity (equivalent to ascorbic acid) were determined according to the methods of Fogliano *et al*. (1999) and Viuda-Martos *et al*. (2010), respectively.

Animals and experimental design

A total of 35 V-line rabbit bucks (120-day-old) were used in the present study, and the experiment was continued until 240 days of age. All rabbits were housed individually in a naturally ventilated building, kept in wire galvanized cages measuring 50× 50 ×40 and given 16 hr. of light daily, including 12 h of natural day light and 4 h of supplementary electric light. The batteries were accommodated with automatic stainless-steel nipple drinkers and feeders for pelleted rations. The rabbits were randomly distributed in to five treatment groups with seven replicates per each, the first group served as a control and fed the basal diet without any supplementation. Rabbit bucks in the second group were fed the basic diet supplemented with 150 mg/kg diet of zinc oxide (ZnO). The 3rd, 4th, and 5th groups were fed the basal diets with different levels of PKSP (0.5, 1.0, and 2.0 g/kg diet), respectively. The diet and water offered ad libitum, the diet formulated according NRC (1977) and AOAC (2007). Feed ingredients and the chemical composition of the basal diet are presented in Table 2. All animals were kept under similar managerial and hygienic conditions. The rabbits were weighed at 120 and 240 days of age, and then body weight gain (BWG) was calculated. Feed intake (FI) was calculated in this period from the difference between the weights of the offered feed and the feed remainder.

Blood hematological and biochemical constituents

At 240 days of age, seven blood samples from the marginal ear vein of the bucks of each group were collected in the morning at 8 o'clock before the regular time of feeding. The blood samples were collected in clean tubes with or without heparin. Blood samples with heparin were used to measure hematological variables. The hematological variables were assessed according to Schalm *et al.* (1975). The phagocyte activity (PA) and phagocytic index (PI) were determined according to Kawahara et al. (1991). Blood serum was collected by centrifugation at 860 x g for 20 min at 4°C and stored at -20°C until analysis. Serum glucose concentration, total protein (TP), albumin (Alb), total lipids (TL), triglyceride(TG), total cholesterol (TC), high density lipoproteins (HDL), aspartate transaminase (AST), alanine transaminase (ALT), urea (BU), creatinine (CR), glutathione peroxidase (GPX), glutathione reductase activity (GR), total antioxidant capacity (TAC), malondialdehyde (MDA) were determined using specific kits obtained from sentinel CH Milano, Italy, CAL-TECH Diagnostics, Inc., Chino, CA, USA, by means of spectrophotometer (Beckman DU-530, Hanau, Germany), Diagnostic Products Corporation, Los Angeles, USA, or Reactivos GPL, Barcelona, Spain, according to kits manufacturers recommendations. Serum globulin (Glb) level was calculated by the difference between TP and Alb. Serum low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were determine according to Friedewald *et al*. (1972). Also, serum testosterone concentrations were determined by radioimmunoassay (RIA) in duplicate 100 μl aliquots using a commercial kit (Diagnostic Product Company, LOS Angeles, CA). Assay sensitivity was 0.1 ng/ml with a coefficient of variation of < 8 %. Seminal plasma was collected by centrifugation at 860xg for 20 min at 4°C and stored at -20°C until analysis and determined GPX, GR, TAC, MDA and seminal plasma testosterone concentration according to the methods mentioned above in the blood serum.

Semen quality and reproductive performance

Semen was collected once monthly after 60 days of experimental initiation three times (at 180,210 and 240 days of age). Ejaculates were collected using an artificial vagina maintained at 45-46°C and a teaser doe. Reaction time (RT), ejaculate volume (EV), sperm concentration (SC), total sperm output (TSO), advanced motility (AM), dead sperm (DS), abnormal sperm (AbS), live sperm (LS) were measured according to (Attia and Kamel, 2012).

Fifty females were distributed to five homogeneous groups and fed a basal diet without any supplementation to be ready for natural mating with the males of the experimental five groups. The reproductive performance and fertility rate (FR) assessments of bucks have been carried out according to Attia *et al*., 2017 during the period between 180 to 240 days of age. Briefly, at 8:00 a.m. bucks of each group were mated to ten receptive nulliparous female rabbits. The mating was done randomly so that the male in any treatment have similar chances to mate with any female in the population. Every doe was transferred to the buck's cage for mating and returned to its cage after copulation. Each doe was subjected to two insemination services within 30 min by the same buck. Total litter size at birth (TLSB) and litter size at weaning (LSW) were recorded per each doe and the average value was calculated per each buck. The fertility rate was measured by dividing the number of kindled does by the number of mated does per each buck ×100.

Statistical analysis

Data were statistically analyzed using the General Linear Model (GLM) procedure of the statistical analysis system of ("SAS Institute," 2000) using one-way analysis of variance according to the following formula: Yij = μ + Ti + eij Where: Yij= The observation of the statistical measured, μ= The general overall mean, Ti= The effect of treatment, eij= The experimental random error. The least square mean (LSM) + standard errors were calculated and tested for significance using the "t" test Steel and Torrie (1980).

RESULTS AND DISCUSSION

Body weight, body weight gain, and feed intake

The data in Table 3 represent the effect of dietary supplementation with ZnO or different levels of PKSP on the final body weight (FBW), BWG, and FI of V-line rabbit bucks. The data showed that groups supplemented with 1.0 and 2.0 g of PKSP/kg diets significantly increased FBW compared with the control group. Meanwhile, no significant difference was observed in FBW between groups supplemented with ZnO and 2.0 g of PKSP/kg diet. Groups supplemented with 1.0 and 2.0 PKSP/kg diets represented significantly increased BWG compared with those for groups supplemented with ZnO and control. Also, supplementing the rabbit bucks with ZnO has no significant effect on the BWG compared to the control. Bucks treated with ZnO and different levels of PKSP did not affect feed consumption compared with the control group. Regarding ZnO results reported by Selim *et al.* (2012) indicated that FBW was improved at dose of 100 mg ZnO/kg diet, compared to 400 mg of ZnO/kg diet, and explaining these results by Zaghari *et al*. (2015) to the ability of ZnO to react with free radicals in body cells, it plays an important role in the immune system as well as improving enzyme function. Also, Abdel-Wareth *et al*. *(*2022) revealed that the addition of nano ZnO to the diet improved FBW and BWG in growing rabbits at different dose administrations (0, 20, 40, 60, or 80 mg/kg diets). In agreement with our results regarding the effect of ZnO Dosoky *et al.* (2022) reported that broilers receiving different levels of nano ZnO (0, 5, 10, 20, 40, and 80 mg/kg diet) had no significant effect on BW or BWG. Similarly, Jahanian and Rasouli (2015) found that ZnO did not affect FI compared to the control group in the boiler study. On the other hand, Abd El-Hack *et al.* (2018) found that laying hens receiving organic Zn significantly increased their daily FI compared to the control. Supporting our results, Wafar *et al*. (2017) reported that the substitution of pumpkin instead of soybean meal in the diet at different levels (0, 5, 15, and 20%) significantly improved BW and BWG, of broiler chickens' dependent on the percentage of PKSP increase. The increase in BW and BWG in our study might be related to the active substances found in PKSP, such as total phenolic compounds and antioxidant activity, which are presented in Table 1 and correspond to the results of Rohman (2020); Hussain *et al*. (2021), and Kulaitiene *et al*. (2018) who reported that the oil found in PKSP has an antioxidant effect related to the content of phenolic compounds. Keeping with our results reported by Ragab *et al.*(2016) who found that a mixture of 2.5 g/kg of both PKSO and black seed oils improves growth performance. Meanwhile, Martínez *et al.* (2010) reported that 10% PKSP in a broiler diet did not affect growth performance. In our results regarding the non-significant effect of PKSP on feed consumption in rabbit bucks, Wafar *et al*. (2017) reported similar results where 5.0%, 10%, 15%, and 20% PKSP did not affect feed consumption in broiler chickens compared to the control. Also, Martínez *et al*. (2010) reported that 10% pumpkin seed meal in a broiler diet did not affect FI.

Hematology parameters and immunological indices

The effects of ZnO and PKSP levels on hematological parameters, white blood cell differentiation, and immune indices of rabbit bucks are shown in Table 4. Groups supplemented with ZnO alongside 1.0 and 2.0g PKSP/kg diet represented a significant increase in red blood cells (RBC) compared with the control. While ZnO group and all supplemented doses of PKSP did not represent any statistical change compared to the control with respect to white blood cells (WBC), hemoglobin (Hgb), packed cells volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The diet for rabbit bucks with ZnO and different levels of PKSP did not represent any statistical change compared to the control with respect to neutrophils, eosinophils, basophils, phagocytic activity, or phagocytic index. Only lymphocytes were significantly increased in groups treated with 150 mg ZnO, and 1.0, 2.0 g PKSP /kg diet when compared with the control. Meanwhile, monocyte percentage was significantly decreased for bucks' groups supplemented with 1.0 and 2.0 g of PKSP/kg diet compared with those for control without any significant change with those supplemented with ZnO and 0.5g PKSP/kg diet. The neutrophils/lymphocytes ratio significantly decreased in all supplemented groups compared to the control. The improvement of RBC count in the current study could be due to the effect of ZnO as explained by Konomi and Yokoi (2005) who mentioned that Zn plays a critical role in RBC maturation and has an effect on erythroid in the bone marrow and erythropoietin found in the plasma. Agree with our results regarding the effect of ZnO on MCV, MCH, and MCHC Srivastav *et al.* (2016) reported that in female rats receiving 300 mg/kg nano ZnO, there was no significant change in MCV, MCH, or MCHC. Furthermore, Elgayed *et al.* (2022) showed that ZnO significantly increased RBC count and hemoglobin concentration compared to the control group. Meanwhile, Dosoky *et al.* (2022) found no significant effect on hematological parameters in broiler diets supplemented with different levels of nano ZnO. The increase in lymphocyte percentage due to using ZnO in the diet was cleared by El Hendy *et al*. (2001), who stated that Zn protects the immune cells from free radical damage in the study on rats. Supporting our results, Hafez *et al.* (2020) report that broiler chicken supplemented with 40 mg or 80 mg ZnO nanoparticles/kg diet significantly increased lymphocyte count, phagocytic activity, and phagocytic index compared to the control group. Similarly, Dosoky *et al.* (2022) found that receiving broilers with nano ZnO in the diet at different levels significantly increased PA and PI. Contrary to our study Mahmood *et al*. (2023) showed that supplemented broiler diet with different sources of Zn under heat stress WBC was significantly increased compared to the control and explained that this was due to the immunomodulatory effect of Zn which improves the function of the immune response and increases interferon production. Supporting our results regarding the improvement in hematology parameters due to supplementation of PKSP was reported by Abdelnour *et al*. (2023) who found that 2 ml of PKSO/kg diet for growing rabbits improved all hematological parameters related to the antioxidant properties of PKS that protect red blood cells from lysis by free radicals. Regarding WBC differentiation Ragab *et al*. (2013) disagree with our results, since they reported that no different effect for growing rabbit treated with PKSO. Meanwhile, Mathewos *et al*. (2019) found that in the broiler study, which supplemented 1%

PKSP, WBC significantly decreased compared to the control.

Serum biochemical parameters

Table 5 presents the effect of ZnO and PKSP on serum biochemical parameters of V-line rabbit bucks. Supplementing the diets with ZnO and PKSP levels for V-line rabbit bucks did not significantly affect serum glucose, Alb, or Glb concentrations. Total protein was significantly increased in the group supplemented with ZnO compared to the group supplemented with 2.0 g PKSP/kg diet, but this increase in serum total protein wasn't significant compared to the control group. Total lipid significantly decreased in groups supplemented with ZnO or PKSP at levels of 1.0 or 2.0 g/kg diet compared to the control. Moreover, ZnO and all levels of PKSP significantly decreased blood TG, TC, and VLDL compared with the control. This significant decrease was observed for PKSP levels compared with those for the ZnO group in TC value. Also, HDL was significantly increased in all supplemented groups compared with the control, and a highly significant increase was seen in the group supplemented with 2.0 g PKSP/kg diet compared with other experimental groups. Regarding liver function, AST and ALT enzymes significantly decreased with supplementation of different levels of PKSP and ZnO compared with the control. The same trend of significant decreases with dietary supplementation was detected for serum BU. While there were no significant differences among groups concerning CR.

Our results are in harmony with those previously reported by Payahoo *et al.* (2013) who found that Zn did not affect blood glucose. Meanwhile, Rogalska *et al.* (2009) reported that Zn significantly decreased blood glucose by improving the sensitivity of the insulin receptor as well as its effect on the metabolism of carbohydrates and lipids. On the other hand, Al-Daraji and Amen (2011) found a significant increase in TP for broiler chickens receiving 75 and 100 mg of pure Zn per kg of diet. Keeping with our results regarding the impact of ZnO on TP, Alb, and Glb reported by Mishra *et al.* (2014) on laying hens supplemented with different types and levels of Zn, there was no significant effect on serum Alb and Glb values compared to the control group. Similar to our results regarding TG Payahoo *et al.* (2013) reported that Zn decreased TG in human studies. Also, Abd El-Hack *et al.* (2018) in a study of laying hens, reported that Zn methionine significantly decreased LDL compared to the control group. Contradiction results regarding the decrease of serum total cholesterol in this study by Al-Daraji and Amen (2011) found that total cholesterol significantly increased at different levels of 50, 75, and 100 mg of pure zinc per kg of diet. Supporting our results concerning the impact of ZnO on liver enzymes reported by Abdel-Wareth *et al.* (2022), who used different levels of Zn in nano ZnO on growing rabbits under high ambient temperatures and found that groups treated with nano ZnO decreased AST and ALT. These improvements may be related to the anti-oxidant properties that maintain the body cells and play an important role in regenerating the cells and cell division Mahmood *et al.* (2023). Regarding the impact of Zn on renal function Abdel-Wareth *et al.* (2022) mentioned that nano-ZnO on fattening rabbits under heat stress conditions significantly decreased BU and CR compared to the control group. Regarding PKSP Abdelnour *et al.* (2023) found that plasma glucose increased in growing rabbits administered PKSO at levels of 2 ml/kg diet. In contrast, Mukherjee *et al.* (2022), who found that PKS increased insulin secretion and decreased blood glucose. Regarding TP Abdelnour *et al.*(2023) mentioned that PKSO supplementation increased serum total protein for growing rabbits compared to control. In harmony with our results regarding no significant changes in serum Alb and Glb which were reported by Abbas and Al-shaheen (2016) in Japanese quail supplementing different levels of PKSO in their diets. Also, Majid *et al.*(2020) reported that 1000 mg of PKSO per day reduced LDL and increased HDL in humans. These results were interpreted by Mukherjee *et al*. (2022) who revealed that active substances found in PKS decreased intestinal absorption of cholesterol. Keeping with our results regarding the values of AST and ALT as a result of treatment with PKSP Abdelnour *et al*.(2023) showed that under high ambient temperatures, groups treated with a 2 ml/kg diet of PKSO significantly decreased AST, ALT, BU and CR compared to the control group. The improvement of liver and kidney function might be related to the antioxidant properties of total phenols and B-carotene content in PKSP as well as high amounts of potassium and phosphorus, which may improve kidney function (Ramadan *et al*., 2016).

Serum and seminal plasma antioxidant status and male hormone

The effect of feeding ZnO and PKSP on serum and seminal plasma antioxidant status and male hormone in V-line rabbit bucks are shown in Table 6. It can be observed that all supplemented groups of PKSP and ZnO significantly increased serum GPX concentration compared with the control group. Meanwhile, groups supplemented with ZnO or 2.0 g PKSP/kg diet had the highest significant increase in serum GPX value compared with other experimental groups. The group that received 2.0 g of PKSP/kg had the highest significantly increased GR value compared with other experimental groups except for the group supplemented with ZnO, which there were no significant difference was detected between them. Serum TAC was significantly increased in the same superior groups (ZnO and 2.0 g PKSP/kg diet) compared with other groups. Serum MDA concentration significantly responded and significantly decreased for all bucks' groups supplemented with PKSP or ZnO compared with control and found that the group receiving 0.5 g PKSP/kg diet recorded a high value of MDA when compared with other supplemented groups. Supplementing the diets for bucks with ZnO or 2.0 g PKSP/kg diet significantly increased serum testosterone hormone compared with the control group. It is clear that supplementing the diet for rabbit bucks with ZnO or 2.0 g PKSP/kg diet significantly increased seminal plasma GPX, GR, and TAC concentrations compared with those for control. However, seminal plasma MDA did not represent any statistical change among the experimental groups due to the experimental supplementation. In addition, the seminal plasma testosterone concentration of rabbit bucks was significantly increased for all supplementing groups with PKSP and ZnO compared with control. Also, a highly significant increase was observed when comparing groups treated with ZnO and 2.0g PKSP/kg with other supplemented groups. Supporting our results regarding TAC in the ZnO group, Ma *et al*. (2011) reported that Zn at level 120 mg/kg on a broiler diet increased plasma total antioxidant capacity and decreased MDA compared to the control group. Likewise, Zeweil *et al.* (2017) reported that male rabbits receiving 100 or 200 mg Zn sulfate /kg diet, significantly increased TAC compared with other experimental groups. The increase in testosterone hormone in the current study due to supplementation of the diet with 150 mg of ZnO/kg is related to Zn had an important role of Zn in testosterone synthesis and is also required by all the steroid hormone receptors to maintain their secondary structure and function and to normalize the concentration of testosterone, LH, and FSH (Heidari *et al*., 2019). Osadchuk *et al*. (2021) reported that in human studies, Zn is a very essential element required in seminal plasma to maintain the function of spermatozoa, seminal antioxidant status, and reserve for successful spermatogenesis. The increase in antioxidants here is in keeping with those previously reported by Bakeer (2021) who mentioned that supplementing PKSO in rabbit diets showed a significant increase in serum antioxidant enzymes and decreased MDA activity. These results were cleared by Kulaitiene et al. (2018) who reported that PKSP contain active compounds such as Zn, vitamin E, and phenolic compounds, which have antioxidant properties against free radicals and protect the organ from damage. Similar to our results, Hashemi (2013) found that a combination of pumpkin seed oils and vitamin E significantly increased serum levels of testosterone and explained these results by Tsai *et al*. (2006) who reported that reported pumpkin seeds contain squalene, which inhibits converting testosterone into dihydrotestosterone, increasing testosterone levels and improving the libido.

Semen quality and reproductive performance

Data concerning the effects of ZnO and PKSP on fresh semen characteristics, litter size, and FR of rabbit bucks are summarized in Table 7. Results showed that the groups supplemented with ZnO or 2.0 g PKSP/kg diet had a significant improvement in EV, SC, TSO, and AM compared with the control. A decrease in DS percent was also observed in the group supplemented with 2.0 g PKSP/kg compared with the rest groups. Abnormal sperm percent was significantly decreased in the ZnO group and 2.0 g PKSP/kg diet compared with those for control, while all supplemented groups represented significant improvements in LS percent compared to control. Total litter size at birth was significantly increased for bucks in groups supplemented with 1.0 g PKSP/kg compared with the control group, with non-statistical changes compared with the ZnO group and the group supplemented with 2.0 g PKSP/kg diet. The bucks' groups supplemented with ZnO, 1.0g and 2.0g PKSP /kg diet showed numerical improvement in LSW compared with the control group. Also, a numerical improvement in FR was detected for groups of 150 mg ZnO/kg diet and 2.0 g PKSP/kg diet compared with the others. Concerning our results on ZnO, Baiomy (2008) reported that 150 ppm of ZnO in the diets of New Zealand white rabbit bucks is recommended for improving semen quality. Likewise, E buehi and Akande (2009) found that ZnO was able to stimulate testosterone synthesis, which plays an important role in the number of sperm through its effect on the work of enzymes, improving FR. The vital role of Zn in decreasing sperm abnormalities and increasing sperm production is explained by Aporvari *et al*., (2018) who reported that Zn protects the cell membrane of spermatozoa from damage by free radicals as well as its ability to improve cell division by affecting the activity of RNA polymerase. Also, Ma *et al*. (2023) reported that Zn able to improve semen quality in the group treated with the high-fat diet. Similarly, Bakeer et al. (2021) reported that Zinc ion is required by all the steroid hormone receptors to maintain their secondary structure and function and to normalize the concentrations of testosterone, LH, and FSH. Regarding our results concerning PKSP on semen characteristics reported by Minisy *et al.* (2017) who found that PKSO improves testes function and sperm quality in rats by reducing lipid peroxidation, it is related to the high antioxidant properties of substances in pumpkin seed oils, which contain vitamins and minerals that can return serum testosterone levels to normal levels. Ragab *et al.* (2016) reported that reproductive performance such as puberty and semen quality improved in the treated groups of 5g/kg PKSO, 5g/kg black seed oils, or a mixture of 2.5 g/kg of both PKSO and black seed oils. These results are in harmony with Rochmi *et al.* (2019) and Rochmi and Pertiwi (2020), who found that PKSP (*Cucurbita moschata*) improved semen quality, which increased sperm motility and decreased AbS in rooster studies under heat stress condition.

IN CONCLUSION,

dietary supplementation of PKSP has a favorable effect on the blood hematology, lipid profile, antioxidant status, liver and kidney function, testosterone level, semen quality, and FR of rabbit bucks, especially at 2.0 g/kg diet.

Table (1): chemical composition of Pumpkin Seeds Powder (PKSP).

Component	**%**
Dry matter	95.02
Organic matter	88.80
Crude protein	28.37
Crude fibre	11.49
Ether extract	34.56
Nitrogen-free extract	14.38
Ash	6.22
Total polyphenols	0.387
Antioxidant activity	0.698

Table (2): feed ingredients and chemical determined composition of the basal diet

Ingredients	(kg/ton)	Chemical composition (%)	Basal diet
Yellow corn	100.0	Dry matter	90.32
Barley	125.0	Organic matter	80.48
Molasses	30.0	Crude protein	17.24
Clover hay	400.0	Crude fibre	13.46
Wheat bran	145.0	Ether extract	2.800
Soybean meal	180.0	Nitrogen-free extract	56.98
Dicalcium phosphate	8.0	Ash	9.520
Limestone	5.0	Digestible energy (kcal/kg)[1]	2440
Sodium chloride	3.0	Zn (mg/kg)[2]	66.4
Vitamin and minerals mixture*	3.0		
DL-methionine	1.0		

*Provides per kg of diet: Vit.A,1200 IU; Vit.D3, 2500 IU; Vit. E, 10 mg; Vit. K3, 3mg; Vit.B1, 1mg; Vit.B2, 4mg; Pantothenic acid, 10 mg; Nicotinic acid, 20 mg; Folic acid, 1 mg; Biotin, 0.05mg; Niacin, 40 mg; Vit.B6, 3 mg; Vit. B12, 20 mcg; Choline Chloride, 400 mg; Mn, 62 mg; Fe,44 mg; Zn, 56 mg; I, 1 mg; Cu, 5 mg and Se, 0.01 mg. (1) Digestible energy (kcal/kg) was calculated according to Fekete and Gippert, 1986. (2) Calculated on the basis of the ingredients composition.

Table (3): Effect of zinc oxide (ZnO) and Pumpkin seeds powder (PKSP) on body weight, body weight gain, and feed intake of V-line rabbit bucks

Item	**Control**	**ZnO mg /kg diet**	**PKSP g/kg diet**			**SEM**	**P value**
		150	**0.5**	**1.0**	**2.0**		
IBW, g	2749	2773	2739	2779	2754	31.63	0.107
FBW, g	3008^{c}	3043^{bc}	3032^{c}	3101^{a}	3087^{ab}	45.69	0.003
BWG, g	259.4^{c}	269.1^{c}	292.7^{bc}	320.9^{ab}	333.3^{ab}	25.53	0.0001
FI, g/d	142.4	145.0	144.4	144.1	141.4	3.44	0.285

^{a,b,c} means having different superscripts in the same row are significantly different ($P \leq 0.05$). SEM: standard error of means; P value: probability level; IBW: initial body weight; FBW: final body weight; BWG: body weight gain; FI: feed intake.

Table (4): Effect of zinc oxide (ZnO) and Pumpkin seeds powder (PKSP) on hematology, white blood cell differentiation, and immune indices of V-Line rabbit bucks

Item	Control	ZnO mg /kg diet	PKSP g/kg diet			SEM	P value
		150	0.5	1.0	2.0		
Hematological parameters							
RBCs(10^6/mm^3)	6.62^b	6.98^a	6.83^{ab}	6.92^a	6.93^a	0.1971	0.029
WBC(10^3/mm^3)	6.24	6.50	6.30	6.40	6.27	0.205	0.198
Hgb (g/dl)	11.17	11.83	11.33	11.50	11.67	0.688	0.490
PCV (%)	43.17	45.17	44.50	44.00	44.67	1.631	0.298
MCV(fL)	65.22	64.74	65.15	63.62	64.45	2.273	0.743
MCH (pg)	16.87	16.95	16.61	16.63	16.84	1.076	0.974
MCHC(g/dL)	25.85	26.19	25.48	26.14	26.11	1.077	0.772
White blood cell differentiation							
Lymph. (%)	39.00^c	42.17^{ab}	40.67^{bc}	41.33^{ab}	43.00^a	1.806	0.0089
Mono. (%)	14.67^a	13.67^{ab}	12.67^{ab}	11.83^b	12.33^b	1.699	0.056
Neut. (%)	31.33	30.33	32.83	32.50	33.00	3.831	0.716
Eosin. (%)	14.33	13.67	13.33	13.83	11.33	2.385	0.259
Bas. (%)	0.67	0.17	0.50	0.50	0.33	0.510	0.517
Neut. / Lymph.	0.377^a	0.325^b	0.311^b	0.286^b	0.287^b	0.042	0.006
PA (%)	19.83	21.17	20.83	19.67	20.67	1.251	0.199
PI (%)	1.47	1.57	1.57	1.62	1.45	0.223	0.652

^{a,b,c} means having different superscripts in the same row are significantly different (P≤0.05). SEM: standard error of means; P value: probability level; RBC: red blood cells; WBC: white blood cells; Hgb: hemoglobin; PCV: packed cells volume; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; Lymph.: lymphocytes; Mono.: monocytes; Neut.: neutrophils; Eosin.: eosinophils; Bas.: basophils; PA: phagocytic activity; PI: phagocytic index.

Table (5): Effect of zinc oxide (ZnO) and Pumpkin seeds powder (PKSP) on serum biochemical parameters of V-line rabbit bucks

Item	Control	ZnO mg /kg diet	PKSP g/kg diet			SEM	P value
		150	0.5	1.0	2.0		
Glucose and protein constituents							
Glucose (mg/dl)	145.7	155.7	153.0	160.0	138.67	15.373	0.157
TP (g/dl)	6.83^{ab}	6.86^{a}	6.58^{bc}	6.86^{a}	6.56^{c}	0.223	0.0498
Alb (g/dl)	3.77	3.84	3.70	3.82	3.50	0.223	0.102
Glb (g/dl)	3.06	3.02	2.88	3.04	3.06	0.253	0.691
Lipid profile							
TL (mg/dl)	636.0^{a}	602.5^{b}	617.2^{ab}	608.8^{b}	595.5^{b}	21.70	0.033
TG (mg/dl)	268.0^{a}	186.33^{b}	190.33^{b}	152.00^{c}	117.67^{d}	27.39	<0.0001
TC (mg/dl)	178.00^{a}	170.66^{b}	166.00^{c}	166.33^{c}	167.0^{c}	2.487	<0.0001
HDL(mg/dl)	47.07^{d}	54.07^{bc}	50.43^{dc}	60.77^{b}	71.97^{a}	5.802	<0.0001
LDL(mg/dl)	77.33^{a}	79.33^{a}	77.50^{a}	75.17^{ab}	71.50^{b}	4.510	0.0569
VLDL(mg/dl)	53.6^{a}	37.26^{b}	38.07^{b}	30.40^{c}	23.53^{d}	5.478	<0.0001
Liver and kidney function							
AST, (U/L)	212.0^{a}	101.67^{dc}	156.67^{b}	122.33^{c}	97.33^{d}	19.378	<0.0001
ALT, (U/L)	68.00^{a}	45.50^{c}	53.00^{b}	47.00^{bc}	41.00^{c}	5.519	<0.0001
BU (mg/dl)	31.67^{a}	27.00^{b}	26.00^{bc}	25.33^{bc}	24.67^{c}	1.918	<0.0001
CR (mg/dl)	0.86	0.88	0.84	0.90	0.89	0.048	0.248

^{a,b,c} means having different superscripts in the same row are significantly different ($P \leq 0.05$). SEM: standard error of means; P value: probability level; TP: total protein; Alb: albumin; Glb: globulin; TL: total lipid TG: triglycerides; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BU: blood urea; CR: creatinine.

Table (6): Effect of zinc oxide (ZnO) and Pumpkin seeds powder (PKSP) on serum and seminal plasma antioxidant status and male hormone of V-line rabbit bucks

Item	Control	ZnO mg /kg diet	PKSP g/kg diet			SEM	P value
		150	0.5	1.0	2.0		
Serum antioxidant status and male hormone							
GPX(mg/dl)	1.027[d]	1.352[a]	1.183[c]	1.267[b]	1.343[ab]	0.0681	<0.0001
GR (mg/dl)	1.050[c]	1.420[a]	1.133[c]	1.237[b]	1.413[a]	0.0847	<0.0001
TAC mmol/l	1.11[c]	1.43[a]	1.27[b]	1.17[bc]	1.46[a]	0.0814	<0.0001
MDA nmol/ml	4.187[a]	3.432[c]	3.893[b]	3.570[c]	3.392[c]	0.2072	<0.0001
Testosterone (ng/dl)	5.38[c]	6.45[ab]	5.40[c]	5.73[bc]	6.66[a]	0.621	0.0025
Seminal plasma antioxidant status and male hormone							
GPX (mg/dl)	0.963[c]	1.08[a]	0.998[c]	1.01[bc]	1.07[ab]	0.060	0.009
GR (mg/dl)	1.09[c]	1.29[ab]	1.15[c]	1.18[bc]	1.34[a]	0.111	0.0031
TAC mmol/l	0.89[b]	1.08[a]	0.87[b]	0.93[b]	1.17[a]	0.111	0.0002
MDA nmol/ml	3.86	3.56	3.58	3.46	3.30	0.407	0.234
Testosterone (ng/dl)	2.33[c]	3.29[a]	2.70[b]	2.96[b]	3.29[a]	0.225	<0.0001

[a,b,c] means having different superscripts in the same row are significantly different (P≤0.05). SEM: standard error of means; P value: probability level; GPX: glutathione peroxidase; GR: glutathione reductase; TAC: total antioxidant capacity; MDA: malondialdehyde.

Table (7): Effect of zinc oxide (ZnO) and Pumpkin seeds powder (PKSP) on fresh semen characteristics, litter size and fertility

Item	Control	ZnO mg /kg diet	PKSP g/kg diet			SEM	P value
		150	0.5	1.0	2.0		
Semen characteristics							
RT (sec)	5.26	5.00	5.00	4.80	4.73	1.255	0.798
EV (ml)	0.59[b]	0.68[a]	0.62[b]	0.64[ab]	0.68[a]	0.073	0.0039
SC (10^6/ml)	342.7[b]	390.1[a]	347.3[b]	374.7[ab]	406.0[a]	50.75	0.0036
TSO (10^6)	204.9[b]	265.5[a]	217.6[b]	241.8[ab]	277.2[a]	53.48	0.0015
AM (%)	73.67[c]	78.67[ab]	76.33[bc]	76.33[bc]	80.00[a]	4.845	0.008
DS (%)	4.67	4.33	4.20	4.13	3.67	1.134	0.2037
AbS (%)	8.20[a]	6.20[b]	7.13[ab]	7.13[ab]	6.80[b]	1.807	0.056
LS (%)	87.13[b]	89.47[a]	88.67[a]	88.73[a]	89.53[a]	2.096	0.018
TLSB (N)	7.69[c]	8.19[ab]	7.75[bc]	8.25[a]	8.19[ab]	0.676	0.0457
LSW(N)	7.06	7.56	7.25	7.63	7.50	0.727	0.1608
FR (%)	76.29	84.57	78.71	77.42	85.71	13.04	0.558

[a,b,c] means having different superscripts in the same row are significantly different (P≤0.05). SEM: standard error of means; P value: probability level; RT: reaction time; EV: ejaculate volume; SC: sperm concentration; TSO: total sperm output; AM: advanced motility; DS: dead sperm; AbS: abnormal sperm; LS: live sperm; TLSB: total litter size at birth; LSW: litter size at weaning; FR: fertility rate.

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الملخص العربي

تعزيز حالة مضادات الاكسدة و جودة السائل المنوي والخصوبة لذكور أرانب الفي لاين المغذاة علي علائق مضاف اليها مسحوق بذور اليقطين

حسام عبد المنعم شهبه[1] ، راوية صادق حامد[1] ، بهاء محمد السيد أبو شحيمة[2]

[1]-قسم بحوث الارانب و الرومي والطيور المائية – معهد بحوث الإنتاج الحيواني – مركز البحوث الزراعية
2- قسم بحوث تغذية الدواجن – معهد بحوث الإنتاج الحيواني – مركز البحوث الزراعية

أجريت هذه الدراسة لتقييم مسحوق بذور اليقطين المضافة الي علائق ذكور الارانب مقارنة بأكسيد الزنك علي الهيماتولوجي وقياسات سيرم الدم البيوكيميائية وصفات السائل المنوي والخصوبة. تم توزيع خمسة وثلاثين ذكر من سلالة الفي لاين عمر 120 يوماً توزيعاً عشوائياً على خمس معاملات غذائية (7 ذكور بكل معاملة وسكنت في أقفاص فرديا) على النحو التالي: المجموعة الاولي تتغذي علي العليقة الأساسية بدون إضافات واستخدمت كمجموعة مقارنة أما المجموعة الثانية تتغذي علي العليقة الأساسية مضافا لها 150مللجم زنك لكل كجم عليقة المجاميع من الثالثة حتي الخامسة تتغذي علي العليقة الأساسية مضافا لها مسحوق بذور اليقطين بمستوي 0.5و1.0و2.0 جم لكل كيلوجرام عليقة علي الترتيب. وأظهرت النتائج أن عدد كرات الدم الحمراء و خلايا الليمفوسيت المناعية ذادت معنويا في المعاملة بالزنك وكذلك المعاملتين من بذور اليقطين عند مستوي 1.0 و 2.0جم/كجم بالمقارنة بالمجموعة الضابطة. كما أدت المعاملة ب 2.0جم/كجم من بذور اليقطين الي زيادة النوع من الكولسترول عالي الكثافة معنويا وانخفاض النوع من الكولسترول منخفض الكثافة جدا معنويا بالمقارنة بباقي المجاميع التجريبية. كما أظهرت نفس المعاملة (2.0جم بذور اليقطين/كجم عليقة) تحسن معنويا في مضادات الاكسدة الكلية سواء في الدم او بلازما السائل المنوي بالمقارنة بباقي المجاميع التجريبية. أيضا أظهرت المعاملة ب 2.0جم من بذور اليقطين /كجم علف تحسن في مستوي الهرمون الذكري (التستستيرون) سواء في سيرم الدم أو في بلازما السائل المنوي عند المقارنة بباقي المعاملات ما عدا المعاملة بالزنك لم تختلف عنها معنويا. كما أظهرت كلا المعاملتين سواء بالزنك (150ملجم /كجم) أو 2.0جم/كجم عليقة من بذور اليقطين تحسن معنوي في معظم قياسات جودة السائل المنوي وكذا نسبة الخصوبة بالمقارنة بباقي المعاملات.

الخلاصة: اضافة مسحوق بذور اليقطين لعلائق ذكور الارانب بمعدل 2.0جم /كجم حسن معظم صفات الدم الهيماتولوجية وكذلك الدهون العالية والمنخفضة الكثافة وحالة مضادات الاكسدة وهرمون التستسترون في سيرم الدم وبلازما السائل المنوى بالاضافة الي تحسن صفات جودة السائل المنوي والخصوبة.