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EFFECT OF DIETARY SUPPLEMENTATION OF AMLA FRUIT POWDER ON SEMEN QUALITY, FERTILITY, AND ANTIOXIDANT STATUS OF RABBIT BUCKS

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ABSTRACT

This study was conducted to evaluate the effect of dietary addition of amla fruits powder (AFP) on blood hematology, serum biochemical parameters, semen quality, fertility and antioxidant status of V-line rabbit bucks during 21-42 weeks of age. A total number of twenty-eight male V-Line rabbits at 21 weeks of age were randomly distributed into four homogeneous groups of seven replicates with one rabbits per each (7 bucks/group). The first group was fed a basal diet without any additives and assigned as a control group. While, the second, third, and fourth groups were fed a basal diet enhanced with varying levels of AFP: 0.25, 0.5, and 1.0%, respectively.

***Results indicated that** bucks fed basal diet supplemented with 0.5 and 1.0% AFP had a significant improvement in red blood cells, hemoglobin, lymphocytes, phagocytic index, immunoglobin type G, serum lipid profile, blood urea, and alanine transaminase compared with those for the rest groups. Reaction time, ejaculate volume, sperm concentration, total sperm output, total litter size at birth, and litter size at weaning for bucks fed a basal diet with different levels of AFP were significantly enhanced compared with the control group.Moreover, bucks fed basic diet with 0.5 and 1.0% AFP showed a significant increase in advanced motility and serum testosterone compared with other groups. The fertility rate for groups supplied with 0.5 and 1.0% AFP was numerically improved compared with the control or group fortified with 0.25% AFP. In addition, antioxidant parameters of blood serum or seminal plasma for rabbit bucks fed a basal diet with 0.5 and 1.0% AFP were substantially increased compared with either group treated with 0.25% AFP or the control.*

***In conclusion,** AFP has a beneficial effect on hematology, immunity, lipid profile, semen quality, fertility rate, testosterone hormone and antioxidant status of*

rabbit bucks, and 0.5% level is recommended as a better value in rabbit farming.

Keywords: Amla Fruit Powder, Rabbit Bucks, Immunity, Semen Quality, Antioxidants

INTRODUCTION

Rabbit spermatogenesis is a complex process it requires ideal conditions to be carried out efficiently in terms of the availability of appropriate nutrition as well as sperm membrane is sensitive to free radicals because it contains large amounts of polyunsaturated fatty acids, leading to deterioration of semen quality and the consequent low fertility and low productivity of rabbits (Attia *et al.*, 2017; Shahba *et al.*, 2023). Phytogenic feed additives, such as different parts of plants are considered sources of natural antioxidants that are healthier than artificial antioxidants (Kumar *et al.*, 2023).

Some plants contain many active substances that play a significant role in solving these problems, thus improving fertility (Attia *et al.*, 2017). Among potential phytogenic feed additives is amla (*Emblica officinalis)*, and the most important part of amla is the fruit (Khan, 2009). Amla fruit powder (AFP) contains phenolic compounds such as gallic and ellagic acids, in addition, essential amino acids and fatty acids, as well as an appropriate amount of vitamins such as vitamin A and α-tocopherol, thiamine, riboflavin, niacin, pyridoxine, and a high amount of vitamin C (Shrivastava *et al.*, 2022). Also, AFP contains high amounts of minerals such as calcium, copper, zinc, and iron, and these compounds are responsible for their anti-oxidative activity (Shrivastava *et al.*, 2022).

Furthermore, Abo Ghanima *et al.*(2023) found that broiler-fed diets with different levels of AFP significantly increased lymphocytes compared with the control group. Also, Begum *et al.* (2019) reported that supplementing a broiler diet with 0.5% AFP significantly decreased serum cholesterol and triglycerides, as well as considerably improved superoxide dismutase and glutathione peroxidase levels. Several studies have supported that amla fruits have antimicrobial, antioxidant and anti-inflammatory properties reported by (Chatterjee *et al.*, 2011; Golechha *et al.*, 2011). As well as, AFP improves semen characteristics of broiler breeder cocks (Manju *et al.*, 2010). Moreover, ellagic acid found in AFP has antioxidant effects and, repairs acrosome damage and forbids gene mutations (Priya *et al.*, 2012). Furthermore, Arun *et al.*(2018) showed that leaf extract of the amla plant has a protective effect on testicular damage in rats. To our knowledge, no study has been conducted on the effects of using AFP as an antioxidant in rabbit buck diets on reproductive performance. So, this study aims to evaluate the effect of different levels of dietary AFP on hematology, immunity, lipid profile, semen quality, fertility rate, testosterone hormone and antioxidant status of V-Line rabbit bucks.

MATERIALS AND METHODS

The current study was done at El-Bostan Farm, Faculty of Agriculture, Damanhour University, and the laboratory analysis was conducted at the Animal Production Research Institute, Agriculture Research Center, Egypt.

Amla fruit powder preparation

Dried amla fruits *(Emblica officinalis*) were 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) (Begum *et al*., 2019; Dalal *et al*., 2018). The chemical composition of amla fruit powder (AFP) is presented in Table 1 according to the official methods (AOAC, 1995). 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.

Table (1): Chemical composition of amla fruit powder (AFP)

Chemical composition	**%**
Dry matter	92.81
Organic matter	82.98
Crude protein	7.73
Ether extract	2.88
Nitrogen-free extract	56.86
Crude fibre	15.52
Ash	9.83
Total polyphenols	0.420
Antioxidant activity	0.742

Animals and experimental design

A total of 28 male V-line rabbits at 21 weeks of age were used (average initial body weight of 3074 ± 77 g) were used in this study, and the experiment was continued until 42 weeks of age. All rabbits were housed individually in a naturally ventilated building, kept in wire galvanized cages measured 50× 50 ×40, and given 16 hr. of light daily, including 12 h of natural daylight 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 into four homogeneous groups, seven replicates with one rabbits per each (7 bucks/group). The first group fed the basal diet without any supplementation and served as a control. The 2nd, 3rd, and 4th groups were fed the basal diets with different levels of AFP (0.25, 0.50, and 1.0%), respectively. The groups were fed the same basal diet, formulated to meet rabbit bucks

nutrient requirements according to NRC (1977) The ingredients and nutrient composition of pelleted diets fed during the experimental period are illustrated in Table 2. Feed and water were provided *ad libitum* throughout the experimental period. All animals were kept under similar managerial and hygienic conditions.

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	56.76
Dicalcium phosphate	8.0	Ash	9.720
Limestone	5.0	Digestible energy (kcal/kg)[1]	2440
Sodium chloride	3.0		
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).

Blood hematological and biochemical constituents

At the end of the experimental period, 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 feeding time. The blood was collected in clean tubes with or without heparin. Blood samples with heparin were used to measure white blood cells (WBCs) count, red blood cells (RBCs) count, hemoglobin (Hgb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte count were determined according to (Feldman *et al.*, 2000).

The phagocyte activity and phagocytic index 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 immunoglobulins (IgG and IgM) were determined using ELISA technique. The IgG ELISA kit determined immunoglobulins' type G value (Catalog No: MBS043814). Immunoglobulin type M value was determined by IgM ELISA

kit (Catalog No: MBS700823). Serum glucose concentration, total protein (TP), albumin (Alb), total lipids (TL), triglyceride (TG), total cholesterol (TC), high-density lipoproteins (HDL), blood urea (BU), creatinine(CR), aspartate transaminase (AST), alanine transaminase (ALT), Alkaline phosphatase (ALP) superoxide dismutase (SOD), glutathione dehydrogenase (GSH) glutathione peroxidase (GPX), 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 a spectrophotometer (Beckman DU-530, Hanau, Germany), Diagnostic Products Corporation, Los Angeles, USA, or Reactivos GPL, Barcelona, Spain, according to kits manufacturers recommendations. The serum globulin (Glb) level was calculated using the difference between TP and Alb. Serum low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were determined 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 of the semen at 860xg for 20 min at 4°C and stored at -20°C until analysis and determination of SOD, GSH, GPX, TAC, and MDA according to the methods mentioned above in the blood serum.

Semen quality and reproductive performance

Semen was collected after 7 weeks of experimental initiation once biweekly. Ejaculates were collected using an artificial vagina maintained at 45-46°C and a teaser doe. Reaction time (RT), ejaculate volume (EV), dead sperm (DS), live sperm (LS), abnormal sperm (AbS), sperm concentration (SC), Advanced motility (AM), total sperm output (TSO), were measured according to (Attia and Kamel, 2012).

Forty females were distributed to four homogeneous groups and fed a basal diet without any supplementation to be ready for natural mating with the males of the experimental four groups.The reproductive performance and fertility rate (FR) assessment of bucks have been carried out according to Attia et al. (2017) between 28 to 42 weeks of age. 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 males 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. The same buck was subjected to two insemination services within 30 minutes. 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 (FR) was measured by

dividing the number of kindled does by the number of mated does per 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: $Yij = \mu + Ti + eij$ Where: Yij= The observation of the statistical measured, μ= The general overall mean, Ti= The effect of treatment, eij= The experimental random error.

The significance of the variations in treatment means was examined according to Duncan (Duncan, 1955).

RESULTS AND DISCUSSION

Hematological parameters and differentiation of leukocytes

Table 3 shows the effect of different dietary levels of AFP on hematological parameters and differentiation of leukocytes of V-line rabbit bucks. White blood cells, RBC, Hgb, and PCV were significantly increased in rabbit bucks fed diets supplemented with AFP compared with the control, especially groups supplemented with 0.50 and 1.0%, which significantly increased RBC and Hgb compared with group supplemented with 0.25% AFP.

On the other hand, MCV decreased considerably in supplemented groups, except for the group fed basal diet enhanced with 0.50% AFP diet, which had no significant change compared with the control. Supplemented basal diet with 1.0% AFP significantly increased MCH compared with other supplemented groups while increasing MCHC compared with the other groups except the group fed basal diet with 0.5% AFP. Rabbit bucks fed basal diet with 0.5 or 1.0% AFP significantly increased lymphocytes, while neutrophils and the ratio of neutrophils to lymphocytes decreased compared with the group fed basal diet with 0.25% AFP or the control. Moreover, monocytes were significantly reduced in the group fed basal diet with 1.0% AFP compared to the other experimental groups. Meanwhile, bucks treated with 0.50 or 1.0% AFP significantly decreased eosinophils compared with the rest groups. Non-significant changes in basophils were detected among the experimental groups. These results are in disagreement with the results of many other studies Mekala *et al.,* (2014), who found that a broiler-fed diet supplemented with AFP did not influence hematological parameters. Also, Dalal et al. (2018), who showed no significant effect on hematological parameters of different levels of AFP (0.25, 0.5, 0.75, and 1.0%) of the broiler diet compared with the control.

Moreover, Islam *et al.,*(2020) reported that AFP in broiler diet had no significant effects on the hematological measurements (RBC, WBC, PCV, and

Hgb). Meanwhile, our results were supported by those of Kamil et al. (2021), who reported a significant improvement in RBC, PCV, and Hgb values in AFP extracts compared with the control in Japanese quail. This improvement in hematological parameters could be related to their content of iron and vitamin C, which play an essential role in improving hematological parameters Abo Ghanima *et al.,* (2023). Regarding the effect of AFP on the differentiation of leukocytes, Dalal *et al.,* (2018) reported that supplementing AFP in the broiler diet at different levels of 0.25, 0.5, 0.75, and 1.0% significantly increased lymphocytes while significantly decreasing heterophil and heterophil-lymphocyte ratios compared with the control. Likewise, Abo Ghanima *et al.*, (2023) reported that broilers supplemented with 3 g/kg of extract significantly increased lymphocytes and heterophils while decreasing eosinophils.

Table (3): Effect of different dietary levels of amla fruit powder (AFP) on hematological parameters and differentiation of leukocytes of V-line rabbit bucks

Items	Control	AFP			SEM	P-Value
		0.25%	0.50%	1.0%		
WBC's (10^3/mm^3)	5.83^{b}	6.53^{a}	6.67^{a}	6.63^{a}	0.250	0.0001
RBC,s (10^6/mm^3)	5.68^{c}	6.14^{b}	6.27^{a}	6.28^{a}	0.086	0.0001
Hgb (g/dl)	10.41^{c}	11.77^{b}	12.57^{a}	13.00^{a}	0.542	0.0001
PCV (%)	30.93^{c}	32.03^{b}	33.33^{a}	32.50^{ab}	0.805	0.0005
MCV (fL)	54.43^{a}	52.15^{b}	53.14^{ab}	51.77^{b}	1.150	0.0033
MCH (pg)	18.31^{c}	19.15^{bc}	20.04^{b}	20.72^{a}	0.894	0.0009
MCHC (g/dL)	33.69^{c}	36.76^{b}	37.70^{ab}	40.01^{a}	1.953	0.0002
Differentiation of leukocytes						
Lymphocytes (%)	36.0^{c}	36.7^{c}	39.3^{b}	41.3^{a}	1.095	0.0001
Monocytes (%)	14.0^{a}	14.3^{a}	13.7^{a}	12.7^{b}	0.632	0.0012
Basophils (%)	0.67	0.67	0.33	0.33	0.516	0.4913
Eosinophils (%)	14.3^{a}	14.7^{a}	13.3^{b}	13.3^{b}	0.516	0.0002
Neutrophils (%)	35.0^{a}	33.7^{b}	33.3^{bc}	32.3^{c}	1.00	0.0017
Neutro / Lymph	0.97^{a}	0.92^{b}	0.85^{c}	0.78^{d}	0.045	0.0001

^{a,b,c,d} Means having different superscripts in the same row are significantly different (P≤0.05). SEM: standard error of means; P value: probability level; WBC: white blood cells; RBC: Red blood cells; Hgb: hemoglobin; PCV: Packed cells volume; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration. Neutro / Lymph: Neutrophils/ Lymphocytes.

These improvements are related to iron, ascorbic acid, riboflavin, folic acid, and other necessary amino acids, as well as phenolic compounds found in AFP, which play a vital role in the differentiation of leukocytes and proliferation of lymphocytes (Abo Ghanima *et al.*, 2023).

Immune indices

Immune indices of V-line rabbit bucks supplemented with different levels of AFP are represented in Table 4. It can be observed that phagocytic activity and phagocytic index for group fed basal diet with 0.5% AFP were significantly increased as compared with the rest groups except for the group fed a basal diet plus 1.0% AFP, which did not demonstrate any significant difference between them. Rabbit bucks fed a basal diet with 0.5 or 1.0% AFP had a significant increase in IgG when compared with the other groups.

Furthermore, IgM for bucks fed basal diet supplied with 0.5% AFP significantly improved compared with the other treated groups. Regarding the positive effect of AFP on immunity parameters,Sai Ram *et al.,* (2002) reported that AFP improves spleen weight, which plays a vital role in producing antibodies and phagocytes. Also, Mandal *et al.,* (2017) reported that AFP at 0.2% in a broiler diet was beneficial to improving the cell-mediated immune response. These improvements may be related to ascorbic acid and phenolic compounds like gallic and ellagic acid found in AFP, which play a vital role in immune system activation in macrophages and natural killer cells (Kumari *et al.,* 2019; Maheshwari *et al.,* 2022; Zhao *et al.,* 2015). Furthermore, Abo Ghanima *et al.,* (2023) reported that broilers supplemented with 3 g/kg AFP extract significantly increased phagocytic activity and phagocytic index. In the same line , Nguse *et al.,* (2022) reported that AFP improves serum IgA, IgG, and IgM at low doses (5g per day) compared to high doses (10 g/day) in dairy calves at preweaning period.

Table (4): Effect of different dietary levels of amla fruit powder (AFP) on immune indices of V-line rabbit bucks

Items	Control	AFP			SEM	P-Value
		0.25%	0.50%	1.0%		
Immune indices						
PA (%)	16.50^{b}	16.58^{b}	17.16^{a}	16.97^{ab}	0.401	0.0302
PI (%)	1.10^{b}	1.07^{b}	1.27^{a}	1.25^{a}	0.104	0.0040
IgG (mg/ml)	900^{b}	917^{b}	980^{a}	970^{a}	20.37	0.0001
IgM (mg/ml)	235^{c}	246^{b}	260^{a}	240^{bc}	5.66	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; PA: phagocytic activity; PI: phagocytic index; IgG: immunoglobin type G; IgM: immunoglobin type M.

Serum biochemical parameters

Data of Table 5 illustrated that values of total protein and globulin for bucks fed basal diet with 0.5 and 1.0% AFP were significantly increased compared with the control group. On the other hand, values of albumin for group fed basal diet plus 1.0% AFP were substantially decreased compared with the rest group. Moreover, bucks fed different levels of AFP had a significant ($P \leq 0.05$) decrease in total lipid, triglycerides, total cholesterol, LDL and VLDL compared with the control group, with the best values associated with the groups fed diets enriched with 0.5 and 1.0% AFP compared with the other experimental groups. Meanwhile, HDL levels for bucks fed diets enhanced with 0.5 and 1.0% AFP were significantly decreased compared with other treated groups.

Otherwise, serum glucose did not show any significant change among the experimental groups. Bucks fed a basal diet supplied with 0.5 or 1.0% AFP showed a significant decrease in BU, ALT, and ALP in comparison with bucks fed 0.25% AFP in the diet or the control group. Meanwhile, CR and AST were significantly decreased in all supplemented groups compared with the control group. The positive effect of AFP on the lipid profile may be related to flavonoid compounds that inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which plays an important role in cholesterol synthesis and reduces cholesterol absorption (Gotto, 2002). Another explanation of the improvement in serum lipid profile may be due to the active tannoid principles of AFP, which benefit serum lipids (Sairam *et al.*, 2003). The impact of AFP juice extract on cholesterol-fed rats decreases serum total lipid, LDL, and total cholesterol levels due to the flavonoids found in the fruit, which can improve the lipid profile (Kim *et al.*, 2005). Our results were in agreement with Dalal *et al.*, (2018), who found that broilers supplemented with 0.75% or 1.0% AFP of the diet significantly decreased TC and LDL, while HDL increased dramatically compared with other low levels or the control group. The present results were confirmed by Begum *et al.*, (2019), who found that supplemented 0.5% of AFP to broiler diet significantly decreased serum cholesterol and triglycerides. Similar conclusions were drawn by Kamil *et al.*, (2021) who showed that TG, TC, significantly reduced as a result of being treated with AFP extracts compared with the control. The decrease in LDL and TG by the addition AFP to the diet is related to the high content of polyphenol and phenolic compounds (Shrivastava *et al.*,2022). Also, Kazal *et al.*, (2023) reported that AFP in a broiler diet significantly decreased TC, TG, and LDL values compared with the control group, meanwhile HDL increased without any statistical changes.

Likewise, Abo Ghanima *et al.*, (2023), who found that serum total lipid, triglycerides, and cholesterol were significantly decreased in broiler diet receiving 3g/kg AFP extract. Supporting our results, Abo Ghanima *et al.*, (2023)

reported that AFP at 3g/kg in broiler diet significantly decreased BU and CR. Likewise, Gupta (2006), Goswami (2008), and Tiwari (2008) reported significantly decreased AST and ALT activity in the groups treated with AFP in the broiler diet compared with the control group.

The results of the present study are in agreement with the results of Rahman *et al*. (2020) reported that rats treated with AFP ethanoic extract significantly improved serum biochemical parameters such as ALT, AST, ALP, BU, and CR and explained these results due to the antioxidant properties of AFP, which have a beneficial effect against liver and renal damage. Also, Abo Ghanima *et al.*, (2023) reported that 3g/kg diet of AFP extract significantly decreased serum AST and ALT values. However, Begum *et al.*, (2019), reported that AFP in the broiler diet did not affect liver enzymes.

Table (5): Effect of different dietary levels of amla fruit powder (AFP) on blood serum biochemical parameters of V-line rabbit bucks

Items	Control	AFP			SEM	P-Value
		0.25%	0.50%	1.0%		
Glucose (mg/dl)	123.7	124.3	119.3	123.3	6.088	0.494
Protein profile						
Total protein (g/dl)	6.08^{b}	6.42^{a}	6.50^{a}	6.37^{a}	0.128	0.0001
Albumin (g/dl)	3.16^{a}	3.27^{a}	3.03^{a}	2.67^{b}	0.250	0.0028
Globulin (g/dl)	2.92^{b}	3.15^{b}	3.47^{a}	3.70^{a}	0.242	0.0001
Lipid profile						
Total lipid (mg/dl)	270.3^{a}	246.7^{b}	236.3^{c}	238.3^{c}	4.058	0.0001
Triglycerides (mg/dl)	119.33^{a}	107.67^{b}	105.33^{b}	106.67^{b}	5.733	0.0014
TC (mg/ dl)	86.33^{a}	80.00^{b}	79.00^{bc}	77.67^{c}	1.712	0.0001
HDL(mg/ dl)	19.63^{b}	20.17^{b}	23.34^{a}	22.97^{a}	0.797	0.0001
LDL(mg/ dl)	42.83^{a}	38.30^{b}	34.59^{c}	33.36^{c}	2.881	0.0001
VLDL(mg/ dl)	23.87^{a}	21.53^{b}	21.07^{b}	21.33^{b}	1.147	0.0014
Kidney and liver function						
BU (mg/dl)	23.50^{a}	24.20^{a}	22.07^{b}	21.67^{b}	0.596	0.0001
CR (mg/dl)	1.23^{a}	1.02^{b}	0.92^{bc}	0.88^{c}	0.095	0.0001
AST(U/L)	22.27^{a}	18.10^{b}	17.70^{b}	18.43^{b}	0.944	0.0001
ALT (U/L)	30.47^{a}	29.87^{a}	26.20^{b}	27.00^{b}	2.052	0.0035
ALT/AST	1.37^{c}	1.65^{a}	1.48^{b}	1.47^{bc}	0.086	0.0002
ALP (IU/I)	30.33^{a}	31.00^{a}	29.67^{ab}	28.00^{b}	1.390	0.0085

^{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; TC: total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein; BU: blood Urea; CR: Creatinine; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase.

Semen quality and fertility rate

Table 6 reveals the effect of feeding AFP on the semen quality and fertility rate of V-line rabbit bucks. Results showed that dietary addition of AFP significantly enhanced RT, EV, and SC. At the same time, EV and TSO increased dramatically in the group supplemented with 1.0% AFP in the diet compared with the group supplemented with 0.25% AFP without significant differences when compared to the group supplemented with 0.5% AFP in the diet.

On the other hand, non-significant changes in DS, LS, and AbS were detected among the experimental groups. Total litter size at birth and LSW were significantly improved by treatments with AFP, while there were no significant differences between 0.5 or 1.0% AFP in the diet. The fertility rate was numerically improved in the group supplemented with 0.5 and 1.0% AFP compared with the control or group supplemented with 0.25% AFP. Regarding semen quality Charaborty and Verma, (2009) found that AFP aqueous extract is able to recover the bad effect of ochratoxin and improve SC, motility, and FR. The aqueous extract of AFP contains phenolic compounds that have antioxidant properties, reduce the harmful impact of free radicals on sex organs, and enhance sperm production and quality (Khan, 2009).

Meanwhile, Manju *et al.,* (2010) reported that the combination of amla and grape seed, each at a 0.5 percent level of the broiler breeder cocks diet, significantly improved the EV, SC, AM, LS, and AbS. Similar results were obtained by Ali *et al.,* (2011) reported that AFP aqueous extract at 150 mg /kg body weight eliminates the harmful effect of endosulfan in mice which improves testosterone, sperm count, and sperm motility. Also, Dutta and Sahu (2013) reported that aqueous extract of AFP improves sperm motility testosterone levels in rat exposed to the toxicity of chlorpyrifos. These improvements in semen quality are associated with the improvement in testosterone hormone, which plays an essential role in spermatogenesis. Additionally, AFP contains minerals, vitamins, and total phenols, which protect cells and maintain their efficiency. Moreover, AFP contains phytoestrogens that stimulate sexual hormones and improve reproductive traits in stressed animals (Liu *et al.,* 2014). Confirmed our results Mais *et al.,* (2018) who found that the germinal layer area and thickness of the semenifeurs tubules in the testis were significantly ($P \leq 0.05$) increased in male Japanese quail at 2 g/kg diet of AFP. This advantage of AFP on testis cells may be related to the antioxidant properties of AFP, which contain mainly ascorbic acid and phenolic compounds which protect the cells from damage by free radicals (Lakhani *et al.,* 2017). Furthermore, Kumar *et al.,* (2023) found that supplemented AFP ethanoic extract at 0.6% to the semen freezing extender of goat bucks improves semen quality, such as motility and sperm abnormalities.

Table (6): Effect of different dietary levels of amla fruit powder (AFP)on semen quality and fertility of V-line rabbit bucks

Items	Control	AFP			SEM	P-Value
		0.25%	0.50%	1.0%		
RT (sec)	6.86[a]	6.00[b]	5.86[b]	5.62[b]	1.26	0.012
EV (ml)	0.575[c]	0.650[b]	0.702[ab]	0.743[a]	0.089	0.0001
DS (%)	6.24	5.90	5.71	5.62	1.409	0.503
LS (%)	82.71	83.33	83.05	83.10	2.544	0.889
AbS (%)	11.05	10.76	11.24	11.29	2.170	0.860
SC (10^6/ml)	491.43[b]	528.10[a]	540.00[a]	554.29[a]	46.94	0.0003
AM (%)	75.71[b]	75.71[b]	79.52[a]	81.19[a]	4.77	0.0003
TSO (10^6)	281.5[c]	344.1[b]	380.8[ab]	412.4[a]	61.99	0.0001
TLSB (N)	7.77[c]	8.36[b]	8.55[ab]	9.00[a]	0.876	0.0002
LSW (N)	7.27[c]	7.82[b]	8.09[b]	8.59[ab]	0.777	0.0001
FR (%)	78.7	81.14	85.86	87.00	7.01	0.117

[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; DS: dead sperm; LS: live sperm; AbS: abnormal sperm, SC: sperm concentration; AM: advanced motility; TSO: total sperm output; TLSB: total litter size at birth; LSW: litter size at weaning; FR: fertility rate.

Blood serum testosterone and antioxidant parameters in serum and seminal plasma

The effects of dietary supplementation with AFP on blood serum testosterone and antioxidant parameters in serum and seminal plasma are summarized in Table 7.

Results revealed that feeding bucks on basal diet supplemented with graded levels of AFP significantly ($P\leq0.05$) improved serum testosterone hormone compared with the control group. Additionally, testosterone hormone was significantly increased for groups fed basal diet with 0.5 or 1.0% AFP compared with the rest groups. Bucks in all supplemented groups with AFP significantly increased serum SOD and GSH compared with the control, while groups fed diets with 0.5 or 1.0% AFP significantly increased serum GPX and TAC compared with the control or group fed diets with 0.25% AFP. Furthermore, serum MDA significantly declined in all supplemented groups compared with the control. Moreover, groups fed basal diet enriched with 0.5 and 1.0% AFP significantly recorded the best values of serum MDA compared with those fed basal diet supplemented with 0.25% AFP and control group. Dietary supplementation with 0.5 and 1.0% AFP significantly increased seminal plasma antioxidant parameters such as SOD, GSH, GPX, and TAC compared with the group treated with 0.25% AFP or the control group. Meanwhile, seminal plasma MDA significantly decreased in the group supplemented with 0.5 or 1.0% AFP compared with the control or 0.25% AFP group. The improvement in testosterone hormone may be due to steroid compounds found in AFP that can enhance testosterone levels and

Table (7): Effect of different dietary levels of amla fruit powder (AFP) on blood serum testosterone and antioxidant parameters in serum and seminal plasma

Items	Control	AFP			SEM	P-Value
		0.25%	0.50%	1.0%		
Blood serum						
Testosterone (ng/ml)	2.09[c]	2.17[b]	2.49[a]	2.45[a]	0.054	0.0001
SOD (U/mL)	2.25[b]	2.34[a]	2.39[a]	2.40[a]	0.049	0.0001
GSH(nmol/mL)	35.99[b]	38.93[a]	38.41[a]	38.81[a]	1.159	0.0007
GPX(mg/dl)	1.13[b]	1.14[b]	1.24[a]	1.26[a]	0.0014	0.058
TAC(mmol/l)	1.52[c]	1.77[b]	1.99[a]	1.96[a]	0.095	0.0001
MDA(nmol/ml)	3.20[a]	2.98[b]	2.76[c]	2.77[c]	0.057	0.0001
Seminal plasma						
SOD(U/mL)	1.39[b]	1.39[b]	1.53[a]	1.51[a]	0.054	0.0001
GSH(nmol/mL)	18.54[b]	18.64[b]	19.31[a]	19.28[a]	0.197	0.0001
GPX(mg/dl)	0.600[b]	0.606[b]	0.713[a]	0.733[a]	0.041	0.0001
TAC(mmol/l)	0.733[b]	0.760[b]	0.867[a]	0.860[a]	0.077	0.012
MDA(nmol/ml)	3.39[a]	3.38[a]	3.23[b]	3.21[b]	0.039	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; SOD: superoxide dismutase; GSH: glutathione dehydrogenase; GPX: Glutathione peroxidase; TAC: total antioxidant capacity; MDA: Malondialdehyde.

function (Gupta, 2006). The present results agree with the finding of Dutta and Sahu (2013) reported that 20 mg juice of AFP /kg body weight improves testosterone levels in rats.These results were interpreted by the same researcher who mentioned earlier that the AFP contains minerals, vitamins, and phenolic compounds that benefit the interstitial Leydig cells responsible for testosterone secretion. Additionally, the improvements in antioxidant parameters are related to the active components found in AFP, which can stimulate natural antioxidant enzymes Rajak *et al.*, (2004) and are confirmed in our study Table 1.

Encouraging the present results Begum *et al.,* (2019) who reported that AFP in a broiler diet significantly decreased serum MDA and increased GSH in a broiler diet at 0.5%. Our findings were in agreement with Manju *et al.*,(2011) who showed that AFP and grape seed powders, each at a 0.5 percent level, significantly increased SOD, catalase, and GSH activities in the seminal plasma of broiler breeder cocks. Similar results were reported by Begum et al. (2019), who found that SOD and GPx levels were significantly (P<0.05) improved in groups supplemented with AFP.

In conclusion, dietary supplementation with AFP especially at 0.5% dose has a beneficial effect on V-line rabbit hematology, immunity status, lipid profile, semen quality and fertility.

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تأثير إضافة مسحوق ثمرة الأملا إلى العلائق لتحسين جودة السائل المنوي والخصوبة و حالة مضادات الأكسدة في ذكور الارانب

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تم اجراء هذه الدراسة بهدف تقيم تأثير إضافة مسحوق ثمرة نبات الاملا علي قياسات الدم الهيماتولوجية والبيوكيميائية وجودة السائل المنوي والخصوبة والهرمون الذكري وحالة مضادات الاكسدة علي ذكور أرانب الفيلاين تم استخدام ٢٨ ذكر(متوسط وزن الجسم ٣٠٧٤ ± ٧٧ جم) من خط الفيلاين عمر ٢١ أسبوع حتي ٤٢أسبوع من العمر بواقع ٤ معاملات تجريبية كل معاملة تحتوى علي ٧ مكررات (ذكر واحد لكل مكررة). المجموعة الاولي مجموعة ضابطة تتغذي علي العليقة الأساسية والمجموعات من الثانية حتي الرابعة تم تغذيتها علي العليقة الاساسية مضافا اليها مسحوق ثمرة الاملا بمستويات ٠.٢٥% و٠.٥٠ % ١.٠% علي التوالي و أظهرت النتائج تحسن معظم الصفات الهيماتولوجية و المناعية والدهون الكلية و أنواعها سواء منخفضة وعالية الكثافة بسيرم الدم كما لوحظ ان هناك تحسن معنوي في صفات السائل المنوي ومستوي الهرمون الذكري بسيرم الدم و كذالك معظم قياسات مضادات الاكسدة في سيرم الدم او بلازما السائل المنوي وكان التفوق للذكور المغذاه علي علائق تحتوي ٠.٥% أو ١.٠% من مسحوق ثمرة نبات الاملا مقارنة بباقي المعاملات التجريبية.

أدت إضافة مسحوق ثمرة الاملا الي علائق ذكور الارانب الي تحسن الصفات الهيماتولوجية والمناعية ومعظم قياسات الدم البيوكيميائية المختلفة وجودة السائل المنوي والخصوبة والهرمون الذكري وحالة مضادات الاكسدة وقد تميزت المجموعات المعاملة بمستوي ٠.٥% و ١.٠%

التوصية: ينصح باستخدام المستوي ٠.٥% من علائق ذكور الارانب حيث لم يختلف معنويا عن المستوي ١.٠% ويعتبر أفضل من الناحية الاقتصادية.