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Effects of Substitution of Alfalfa Meal with Dried Tomato Pomace (DTP) in Newzealand Male Rabbits Rations on Semen Characteristics and Some Blood Constituents

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Abstract: This study aimed to investigate the effect of incorporation of dried tomato pomace (DTP) into rabbit bucks rations on semen quality characteristics, libido and some seminal plasma and blood biochemical constituent. A total of twenty New Zealand White male rabbits, 8 months old, were randomly divided into four equal groups (5 bucks for each). Group one served as a control group, which received a basal diet, while, groups 2, 3 and 4 received a diet containing 10, 15 and 20% of DTP for 12 weeks as an experimental period, respectively. There was no effect due to feed rabbit bucks on DTP inclusion diets on live body weight, while, feed consumption was decreased by 9.3, 11.2 and 14.8% for 10, 15 and 20% TP levels, but this decrease was non-significant. Results indicated that 10 or 15% DTP groups showed significant improvement in semen ejaculate volume, sperm cell concentration, sperm motility, total sperm out-put, total motile sperm and total motile normal sperm and the rate of sperm abnormalities was decreased. On the other hand, 20% DTP has positive effect on semen measurements compared to the control however, this improvement was non-significant. Seminal plasma total proteins and albumin appeared remarkably higher for bucks' fed DTP at any studied level especially for 20% DTP than the control group, whereas, calcium concentration was markedly decreased in all DTP groups. Blood hematological parameters (Hb, PCV, RBC and WBC) were increased significantly in DTP feeding groups compared to the control and these finding were more prominent for the bucks put on 10% DTP level. Liver enzymes activities (AST and ALT) were significantly decreased due to added DTP in male rabbits' diets with any level compared to the control group. Testicular parenchyma of 10 and 15% DTP groups showed a better histological characterization with healthy somniferous tubules (ST) compared to the control or the 20% DTP level and 20% group still showed histological characterization better than the control group. It could be concluded that addition of DTP in the mature male rabbit's diets by 10, 15 and 20% levels has positive effect on the semen quantity and quality, as well as, some blood biochemical properties of the white New Zealand breed.

Key words: Rabbits, live body weight, reproduction, semen, tomato pomace

INTRODUCTION

Tomato and tomato-based products contain some phytochemicals that may have health benefits and are important sources of many established nutrients. Lycopene, folate, vitamin C, vitamin A, phenolics and flavonoids are potential bioactive compounds found in tomatoes (Beecher, 1998; Agarwal and Rao, 2000). Lycopene is a major carotenoid present in tomatoes and a highly potent antioxidant that provides protection against cellular damage that caused by reactive oxygen species (Rao and Agarwal, 1999). Dried Tomato Pomace (DTP), a mixture of tomato peels, pulp and crushed seeds, is the remnant of tomato juice processing. According to the report issued by FAO (2011), tomato wastes were estimated to be 11 million

tons of which more than 4 million tons are DTP. Gippert *et al.* (1989) reported that DTP contains more fat, crude fiber and crude protein especially essential amino acids than alfalfa meal of good quality. According to Feedipedia (2011) this by-product is an excellent source of soluble fiber (33-37%), protein (17-22%), fat (up to 20%) and rich in the antioxidant lycopene. Thereby, it has the potential to be utilized on the expense of the conventional grains and forages in rabbit diet processing. It has been reported that the DTP can be utilized efficiently and safely in the diets of rabbits up to 20% without any adverse effect on the performance and carcass traits (Sawal *et al.*, 1996; Sayed and Abdel-Azeem, 2009).

It is of importance to notice that the quantity and quality of buck semen have a reversible effect on the male/female ratio and herd fertility in terms of kindling rate and litter size (Brun *et al.*, 2002). Dietary components are believed to have a significant effect on semen production and quality (Nizza *et al.*, 2003). Addition of DTP to the poultry diet was noticed to improve the sperm cell concentration, motility and viability, while it did not affect the sperm morphology or abnormalities (Sudjarwo *et al.*, 2011).

Fewer data are available regarding the effect of DTP on the male rabbit fertility. The present study was carried out to investigate the effect of adding DTP to rabbit diet on live body weight, feed consumption, semen characteristics, libido and chemical estimates of semen and some blood biochemical measurements, as well as, the histological findings of the testes of mature NewZealand white male rabbits.

MATERIALS AND METHODS

The present study was conducted during spring season from March to May 2011. Table 1 shows the biweekly environmental conditions (temperature and humidity) in cages during the experimental period.

Animal husbandry and formulation of food ration: Twenty healthy mature New Zealand white male rabbits; 8 months of age with average initial body weight 2.97±57 kg; were randomly divided into four equal groups (5 bucks for each). Group one served as a control group, which received a basal diet, while, groups 2, 3 and 4 received a diet containing 10, 15 and 20% of DTP, respectively (Table 3) for 12 weeks. Each buck was kept in a separate cage in a well-ventilated building. Feed and water were provided *ad libitum*.

Wet TP was air-dried to final moisture content in order to obtain DTP (9% moisture) and grounded. The chemical composition of tomato pomace is shown in Table 2. All treated diets were formulated using UFFDA feed formulation package according to the nutrient requirements of adult rabbit (NRC, 1994) and balanced to be iso-nitrogenous and iso-caloric. Ingredients and chemical composition of the experimental diets (% on a dry mater basis) is shown in Table 3.

Data collection:

Semen collection and evaluation: Libido, expressed by the reaction time, (sec.) was determined as the moment of subjecting a doe to the buck until the completion of erection and ejaculating using a stopwatch. Semen from each buck was collected biweekly during the whole experimental period by using an artificial vagina and a teaser doe (Tesh and Tesh, 1971). The volume of each ejaculate was recorded to the nearest 0.1 ml (using a graduated collection tube) after removal of the gel mass. A weak eosin 10% formalin solution was used for

Table 1: Mean of biweekly temperature and relative humidity during the experimental period

Time/weeks	--- Temperature (°C) ---		Relative humidity
	High	Low	
2	25.0	15.3	53.5
4	29.5	16.7	37.5
6	29.0	18.7	45.5
8	29.3	20.7	61.0
10	30.3	21.0	44.0
12	30.7	18.3	48.5

Table 2: Proximate chemical analysis of dried tomato pomace,

DTP Ingredients	Chemical composition (%)
Dry matter (DM)	91.01
Organic matter (OM)	96.76
Crude protein (CP)	20.18
Ether extract (EE)	8.97
Crude fiber (CF)	28.5
Ash	3.24
Non-fat-extract (NFE)	30.12

Table 3: Ingredients and chemical composition of the experimental diets

Items	----- Feeding groups (%) -----			
	Control	10% DTP	15% DTP	20% DTP
Ingredients				
Corn	30.00	30.00	30.00	30.00
Alfalfa hay	30.00	20.00	15.00	10.00
Tomato pomace	0.00	10.00	15.00	20.00
Barley	15.00	15.00	15.00	15.00
Wheat bran	8.00	10.70	12.20	13.70
Soybean meal 44%	12.70	10.00	8.50	7.00
Molasses	3.00	3.00	3.00	3.00
Lime stone	0.20	0.20	0.20	0.20
Premix*	0.50	0.50	0.50	0.50
NaCl salt	0.50	0.50	0.50	0.50
Di-methionin	0.10	0.10	0.10	0.10
Total	100	100	100	100
Chemical composition				
Dry matter	93.44	93.43	93.53	93.6
Organic matter	94.07	94.66	95.31	95.28
Crude fiber	14.30	14.00	13.82	13.59
Either extract	2.19	1.61	1.89	2.34
Crude protein	19.58	19.03	20.02	19.56
Ash	5.93	5.34	4.69	4.72
Nitrogen free extract	58.00	60.02	59.58	59.79

estimating the sperm cell concentration according to Smith and Mayer (1955) by the improved Neubauer haemocytometer slide (GmbH+Co., Brandstwierte 4, 2000 Hamburg 11, Germany). Total sperm output (TSO) was calculated by multiplying semen ejaculate volume and semen concentration. The percentage of motile sperm was estimated by visual examination under low-power magnification (10x) using a phase-contrast microscope with heated stage. Total number of motile sperm (TMS) was calculated by multiplying percentage of motile sperm and TSO. Assessments of percentages of live, dead and abnormal spermatozoa were performed using an eosin nigrosine blue staining mixture (Blom, 1950) and total motile normal sperm (TMNS) was calculated as {[TSO* motility (%)]* normal morphological sperm (%)} (Correa and Zavos, 1996;

Carluccio *et al.*, 2004). Evaluation of seminal initial fructose was carried out immediately after collection according to Mann (1948).

Seminal plasma was obtained by centrifugation of the remaining part of semen samples at 3500 rpm for 20 min at 4°C and the supernatant was stored at -20°C until later analysis. Seminal plasma samples were analyzed for total protein (TP) concentration by the Biuret method according to Henry *et al.* (1974), albumin (A) concentrations was determined by the method of Doumas *et al.* (1977), total lipids was measured according to Fringes *et al.* (1972), calcium concentration was determined according to the method of Gindler and King (1972) and alkaline phosphatase (ALP) enzyme activity was measured according to the method of Belfield Goldberg (1971).

Some blood biochemical constituents: Whole blood samples were collected biweekly, from each buck into heparinized vacutainer tubes by vein puncturing to evaluate hemoglobin (Hb), red blood cell count (RBCs), packed cell volume (PCV) and white blood cell count (WBCs). The remainder of blood samples were centrifuged at 3500 rpm for 20 min to obtain plasma that stored at -20°C. Plasma total proteins concentration was measured by the Biuret method according to Henry *et al.* (1974), albumin concentrations was determined by the method of Doumas *et al.* (1977), globulin, total lipids was measured according to Fringes *et al.* (1972), cholesterol was determined according to the method of Richmond (1973) and calcium concentrations was determined according to the method of Gindler (1972), as well as, liver enzymes activities (AST and ALT) were determined by the method of Reitman and Frankel (1957).

Slaughter traits: At the end of the experimental period, all bucks under each treated group were weighed and slaughtered. The percentage of slaughter weight (slaughter weight x 100/live weight) was estimated. The relative weight of liver, kidneys, testes, epididymis and seminal vesicles were determined. Histo-pathological examinations for tests were done by dissecting testes and fixed in Bouin's staining solution for at least two days and processed for histological evaluation according to Castro *et al.* (2002).

Statistical analysis: Data were tabulated and statistically analyzed, where appropriate, by using the statistical analysis system (SPSS, 2007). Mean±S.E., one way ANOVA and LSD for traits, under investigation, were calculated in relation to the effect of DPT-treatment. Significance of the effects was tested at level $p < 0.05$ (*) with the appropriate F statistic. Duncan's multiple range tests was used to detect any significant differences among the experimental means (Duncan, 1955).

The model was as follow:

$$\text{Two-way analysis: } Y_{ijk} = \mu + T_i + W_j + (TW)_{ij} + e_{ijk}$$

where, Y_{ijk} = observed value of the dependent variable, μ = overall mean, T_i = fixed effect of the i^{th} level of treatment, W_j = fixed effect of the j^{th} level of week, $(TW)_{ij}$ = interaction between treatment and week, e_{ijk} = the random error.

RESULTS AND DISCUSSION

Semen characteristics: Data of semen quality characteristics (Ejaculate volume, Sperm motility, live sperm and dead sperm, abnormal sperm, sperm concentration, total sperm output, total motile normal sperm and libido) are illustrated in Table 4.

Regarding the effect of DTP on libido of bucks (measured by the reaction time, sec) it could be noticed that there was a significant ($p < 0.01$) improvement in libido for bucks fed 10% DTP inclusion diet as they had less time before ejaculation compared to the control group (7.33 vs 8.47 sec) or the other two DTP groups. On the other hand, no significant differences were found between the 15 and 20% DTP treated groups and the control group in reaction time.

Ejaculate volume of bucks, which received DTP rations was increased and this effect was significant ($p < 0.01$) with 10 and 15% DTP inclusion diet only by 21.31% over the control group mean. While, increasing ejaculated volume, in the 20% DTP group did not significantly differ than the control group. This increase was necessary for sperms to provide them by nutritious elements that are needed for their live (Kamel *et al.*, 2009). The increase semen ejaculate volume was correlated with a significant increase in sperm concentration (SCon.) and total sperm out-put (TSO) in all DTP treated groups.

Sperm motility produced from bucks treated with DTP at any levels increased by 30.34, 29.16 and 11.9% over the control group mean for 10, 15 and 20% DTP level, respectively. This effect was significant ($p < 0.01$) with the low and medium DTP levels (10 and 15%) only. Dried TP at any inclusion levels resulted in a significant increase ($p < 0.01$) in live sperm to reach 27.9, 26.88 and 29.2% than the control group. Partial replacement of alfalfa meal with DTP by 10 or 15% produced more normal shape sperm than the control group and this increase was significant ($p < 0.01$), while the 20% DTP did not have any effect. The opposite trend was shown in the dead sperm which showed a significant decrease ($p < 0.01$) with all DTP levels and in abnormal sperm with the low and medium DTP levels (10 and 15%). The beneficial effect of DTP on the previous measurements may be attributed to increased activation of spermatogenesis in seminiferous tubules to produce a complete sperm in response to the bioactive compounds that found in DTP such as Lycopene, folate,

Table 4: Substitution of alfalfa meal with different levels of dried tomato pomace (DTP) in male rabbits' diets and its effects on libido and semen characteristics

Items	Feeding groups			
	Control	10% DTP	15% DTP	20% DTP
Reaction time (sec)	8.47±0.39 ^a	7.33±0.50 ^b	8.78±0.36 ^a	9.56±0.39 ^a
Ejaculated volume (ml)	0.61±0.03 ^b	0.74±0.05 ^c	0.74±0.03 ^a	0.65±0.04 ^{ab}
Sperm motility (%)	46.67±2.14 ^b	60.83±2.30 ^a	60.28±2.37 ^a	52.22±2.60 ^b
Live sperm (%)	55.56±2.08 ^b	71.06±1.58 ^a	70.50±1.51 ^a	71.78±2.09 ^a
Dead sperm (%)	44.44±2.08 ^a	28.94±1.58 ^b	29.50±1.51 ^b	28.22±2.09 ^b
Normal sperm (%)	78.72±1.04 ^b	81.56±0.90 ^a	81.11±0.64 ^a	77.56±0.63 ^b
Abnormal sperm (%)	21.28±1.04 ^a	18.44±0.90 ^b	18.89±0.64 ^b	22.44±0.63 ^a
Sperm concentration (10 ⁶ /ml)	265.56±7.75 ^b	318.67±7.61 ^a	313.33±7.00 ^a	311.31±7.53 ^a
Total sperm output (TSO) (n*10 ⁶)	163.02±1.10 ^b	234.92±2.12 ^a	231.99±1.16 ^a	200.92±1.17 ^{ab}
Total motile sperm (TMS) (n*10 ⁶)	75.14±5.78 ^b	144.05±6.19 ^a	139.96±9.16 ^a	103.61±7.34 ^b
Total motile normal sperm (TMNS) (n*10 ⁶)	59.25±4.67 ^b	117.43±3.06 ^a	113.04±6.98 ^a	80.34±5.74 ^b
Fructose (mg/100 ml)	214.32±1.02	221.79±5.48	231.43±1.04	220.02±5.63

^{abc}Means in the same row having different superscripts are significantly different (p<0.05)

Table 5: Substitution of alfalfa meal with different levels of dried tomato pomace (DTP) in male rabbits' diets and its effects on seminal plasma total protein, albumin, total lipids, calcium concentration and alkaline phosphatase enzyme activity

Traits	Feeding groups			
	Control	10% DTP	15% DTP	20% DTP
Total protein (mg/dl)	1.73±0.13	1.81±0.11	1.79±0.09	2.03±0.16
Albumin (mg/dl)	0.68±0.06 ^b	1.01±0.08 ^a	1.01±0.10 ^a	1.11±0.10 ^a
Total lipids (mg/dl)	94.92±8.98 ^a	78.27±7.10 ^b	74.45±10.87 ^b	92.00±9.15 ^a
Calcium (mg/dl)	12.61±0.38 ^a	10.76±0.37 ^b	10.89±0.33 ^b	11.48±0.31 ^a
ALP (mg/dl)	614.78±3.69	604.94±1.09	588.64±1.26	576.41±3.04

^{abc}Means in the same row having different superscripts are significantly different (p<0.05)

vitamin C, vitamin A, phenolics and flavonoids (Agarwal and Rao, 2000) and its effect on testicular germinal epithelium.

Substitution of alfalfa meal with DTP in rabbit' bucks rations resulted in a significant increase (p<0.01) in sperm concentration at any studied levels to reach 20.0, 17.98 and 17.22% for 10, 15 and 20% DTP levels respectively, over the control group. Also, DTP groups resulted in increased total sperm output (TSO), total motile sperm (TMS) and total motile normal sperm (TMNS) and this increase was significant (p<0.01) with 10 and 15% levels only compared to the control group. Data revealed that the 10% DTP inclusion level had the highest means than the other studied DTP levels (15 and 20% DTP levels). Improvement in percentage of live and motile sperm found in the DTP groups were conducted to a significant increase (p<0.01) in a total motile sperm per ejaculate (TMS) and total motile normal sperm (TMNS) compared to the control male rabbits. Increased TMS and TMNS mean in the DTP groups were due to increased ejaculate volume and sperm concentration. According to Tawfeek *et al.* (1995), substitution of 10% alfalfa meal with TP in feeding buck rations improved their semen characteristics by increasing the ejaculate volume, sperm motility and sperm concentration and decreasing the percentage of abnormal sperm and dead sperm compared to the control group, whereas, the 20% TP did not differ than the control group.

Data of fructose concentration in seminal plasma revealed that there was a slight increase in fructose concentration after feeding rabbit' bucks on different

levels of DTP compared to the control group, but this effect was not significant. Increased semen fructose concentration in the DTP treated groups may be due to increased fructose synthesis by the accessory sex glands to provide the sperm with enough amounts of energy.

Some biochemical analysis of seminal plasma: Data of seminal plasma biochemical measurements (Table 5) illustrated that feeding bucks on DTP at any studied levels resulted in a non-significant increase in seminal plasma total protein concentration. Further, seminal plasma albumin concentration was increased significantly (p<0.01) in all DTP treated groups compared to the control group. Rabbit bucks fed on rations containing DTP until 10 and 15% resulted in a gradual and significant (p<0.05) decrease of total lipids concentration in seminal plasma and this decrease was 17.54 and 21.56% less than the control. The increase seminal plasma total protein and albumin was a reflection of the improvement in the nutritional status of males. Whereas, substituting alfalfa meal with DTP in buck rations provides buck with essential amino acids, which found in DTP more than in alfalfa meal. According to El-Azab *et al.* (1977) it is important to notice that the semen production potential of a male is influenced by the nutritional status of the animal. From the results of Bordowski and Geisman (1980) tomato seeds protein contains approximately 13% more lysine than soybean protein, which would allow it to be used in fortifying low lysine foods. Karadas *et al.* (2006) reported that dried tomato pomace has been considered as a good source

Table 6: Substitution of alfalfa meal with different levels of dried tomato pomace (DTP) in male rabbits' diets and its effects on some blood hematological and biochemical parameters

Traits	Feeding groups			
	Control	10% DTP	15% DTP	20% DTP
Hemoglobin	11.50±0.42 ^c	13.61±0.31 ^a	12.44±0.28 ^b	12.78±0.30 ^{ab}
PCV	33.23±1.49 ^c	40.89±0.90 ^a	37.41±0.88 ^b	37.59±1.17 ^b
RBC count	3.79±0.14 ^c	4.53±0.10 ^a	4.14±0.10 ^b	4.22±0.10 ^{ab}
Total WBCs count	4.21±0.39 ^b	4.61±4.61 ^{ab}	5.14±0.32 ^a	5.03±0.20 ^{ab}
Total Protein (mg/dl)	7.25±0.13	7.59±0.15	7.55±0.21	7.52±0.15
Albumin (mg/dl)	3.23±0.11	3.40±0.10	3.37±0.09	3.23±0.07
Globulin (mg/dl)	3.88±0.17	4.19±0.17	4.31±0.23	4.29±0.18
Total lipid (mg/dl)	219.92±25.65	228.41±23.20	231.82±21.06	246.84±27.09
Cholesterol (mg/dl)	38.56±1.06 ^b	55.55±1.65 ^a	54.40±2.03 ^a	54.34±1.88 ^a
Calcium (mg/dl)	1.69±0.09	1.61±0.09	1.63±0.09	1.67±0.07
ALT (mg/dl)	70.56±1.87 ^a	61.72±2.38 ^{ab}	64.17±2.37 ^a	56.39±1.72 ^c
AST (mg/dl)	85.22±4.29 ^a	75.22±2.69 ^b	74.67±2.14 ^a	69.06±1.78 ^b

^{abc}Means in the same row having different superscripts are significantly different (p<0.05)

Table 7: Substitution of alfalfa meal with different levels of dried tomato pomace (DTP) in male rabbits' diets and its effects on live body weight, relative carcass weight and some organs weight

Traits	Feeding groups			
	Control	10% DTP	15% DTP	20% DTP
Feed Intake (g/day)	114.45±7.94	103.73±8.35	101.55±9.19	97.50±7.60
Live body weight (kg)	3.00±0.53	2.82±0.11	2.83±0.23	3.02±0.14
Slaughter weight (%)	93.49±2.9	98.18±0.14	89.81±0.74	97.58±0.56
Liver (%)	1.83±0.27	2.57±0.50	2.27±0.17	2.01±0.41
Kidney (%)	0.58±0.01 ^a	0.45±0.06 ^{ab}	0.31±0.05 ^b	0.53±0.10 ^{ab}
Testis (%)	0.035±0.004 ^c	0.073±0.007 ^b	0.088±0.001 ^{ab}	0.097±0.008 ^a
Seminal vesicle (%)	0.013±0.002 ^b	0.048±0.001 ^a	0.058±0.001 ^a	0.060±0.015 ^a

^{abc}Means in the same row having different superscripts are significantly different (p<0.05)

of protein for providing amino acids, especially lysine. Increase seminal plasma total protein and albumin were compatible with increase these parameters in blood and this improvement was reflected on improvement semen characteristics of DTP treated groups. Elkomy *et al.* (2008) reported that an increase in seminal plasma total protein and albumin concentrations were showed in high fertile male rabbits compared to low fertile rabbits and this increase was associated with increase their seminal quality measurements. Taha *et al.* (2000) revealed that there was a positive relationship between semen quality and level of seminal plasma total proteins. Similar results were found by Osama and El-Sahn (2006), they found a positive relationship between total proteins and albumin on seminal plasma and total number of sperm output. Alkaline phosphatase enzyme activity in tested groups' boosted a gradual and a non significant decrease than the control group and this effect was DTP level-dependent. Calcium concentration in seminal plasma of DTP groups showed a significant (p<0.01) decrease compared to the control group. It could be noticed that decrease calcium content in seminal plasma of the DTP groups was correlated with increasing sperm concentration. Decreased calcium content in DTP seminal plasma group may be due to that the spermatozoa absorbed more calcium during storage in the Epididymis which is important for their spermatozoa functions: (a) maintain the mobility of the sperm (b) for the flagellar motility and (c) the acrosome

reaction. The previous finding was in agreement with that found by Sorensen *et al.* (1999) who found that estimation of calcium concentration in semen can be of considerable interest as a result of its relationship with sperm motility, metabolism, acrosome reaction and fertilization itself. Also, it could be noticed that there was a relation between the sperm motility (%) and the decrease Ca²⁺ content in seminal plasma. In view of the observation that calcium (Ca²⁺) and magnesium (Mg²⁺) are necessary to maintain motility in the spermatozoa from a number of mammalian species (Morton *et al.*, 1974; Heffner and Storey, 1981). The acrosome reaction can be brought about *in vitro* by prolonged incubation of spermatozoa in a suitable medium, of which an essential component is Ca²⁺ (Yanagimachi and Usui, 1974). Extracellular Ca²⁺ is essential for the flagellar motility of membrane-intact hamster spermatozoa.

Histological examination: Looking through the testicular tissue in control and DTP fed groups, Testicular parenchyma of 10 and 15% DTP groups showed nearly similar architecture characterized by healthy intact seminiferous tubules (ST) and the interstitial spaces were relatively narrower. As well, the numbers of Leydig cells were dense in 10 and 15% DTP groups when compared to the 20% that still showed histological characterization better than the control group. Concomitantly, the lumen of efferent ductules showed accumulation of sperm cells which were clearly correlated with changes occurring in the ST. However,

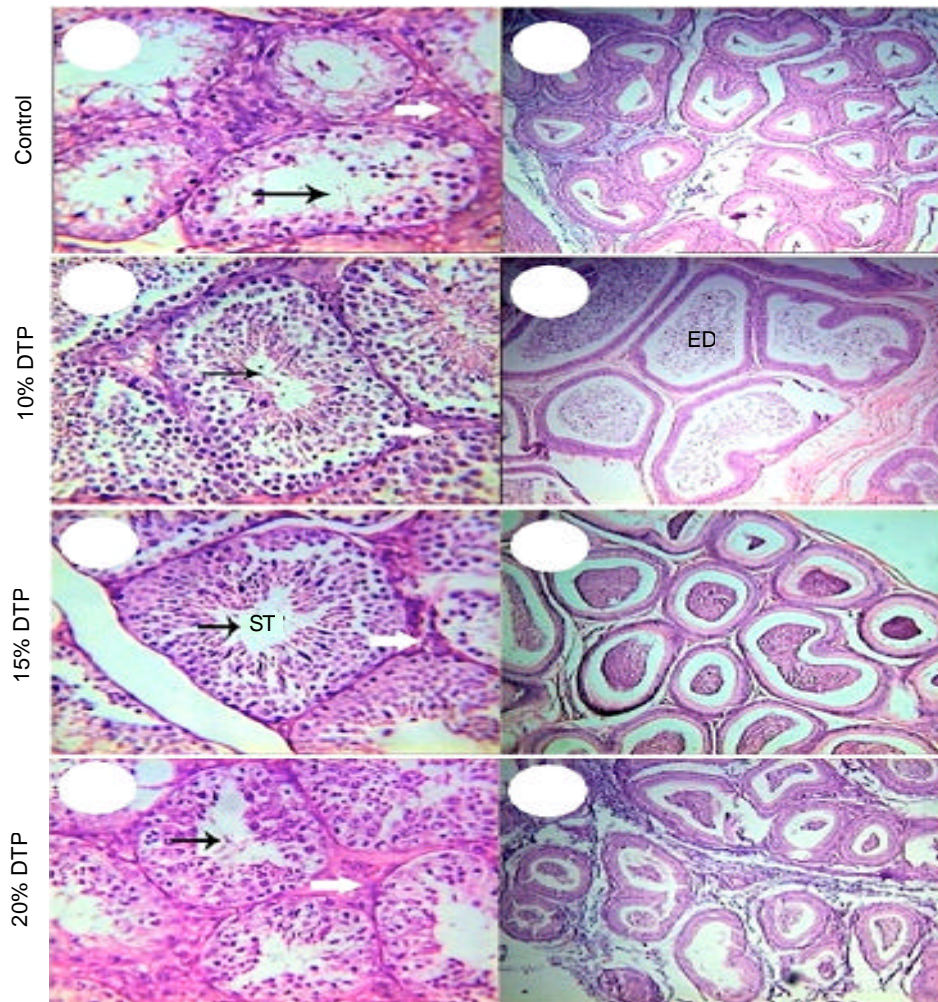


Fig. 1: Histopathological changes in testicular parenchyma in bucks after incorporation of dried tomato pomace (DTP) in ration at 0% (control (1, 2), 10% (3, 4), 15% (5, 6) and 20% (7, 8) levels. Black arrow refers to seminiferous tubules. White arrow indicated the interstitial spaces contained Leydig cells

efferent ductules of 10 and 15% DTP were comparatively the widest in diameter among the groups in this experiment (Fig. 1).

Some blood hematological and biochemical constituents: Data of blood hematological and biochemical measurements are illustrated in Table 6. Blood hematological measurements hemoglobin, PCV, RBC and WBC were increased in bucks that fed diet contains different levels of DTP (10, 15 and 20%) and this effect was significant ($p < 0.01$) compared to the control group. As well, 10% level had the highest values for Hb, PCV and RBC count than the other two DTP levels. WBC increase in bucks that fed diet contains DTP, but, in comparison to the control, just 15% DTP group reach the statistical significance. These results indicated a normal erythrocyte indices, therefore no

apparent indication of health troubles could be noticed in the blood picture with feeding DTP to rabbits up to 20% of their diet. These findings are in agreement with that reported by Khadr and Abdel-Fattah (2008) who mentioned that tomato waste results in a significant increase in the RBCs count, total WBCs count and mean corpuscular hemoglobin in growing rabbits by increasing addition of DTP from 14 to 30% in rabbit diet. El-Azab *et al.* (2011) reported that feeding growing rabbits on diets containing DTP up to 20%, revealed an increase in blood Hb, PCV, RBCs and WBCs at any tested level compared to the control group and this effect was non-significant. Improvement in the blood picture perhaps occurred due to the high vitamin B12, iron and copper contents of DTP, essential for the normal blood cellular formation and hemoglobin (Lopez and Williams, 1981; Haddadin *et al.*, 2001).

Adding DTP to male rabbits' diets with different testing levels resulted in a slight increase in plasma total protein, albumin and globulin concentrations than that recorded by the control group and this effect was non-significant; concerning the globulin, the increase was 8.0, 11.0 and 10.5% above the control value. Present findings are in a good agreement with that of King and Zeidler (2004) and Karadas *et al.* (2006) who reported that TP is rich in protein or amino acids (especially lysine) and natural pigments such as β -carotene and lycopene, as well as, it is a valuable source of α -tocopherol (vit E), which is used as an antioxidant and it is more beneficial for the rabbit compared to alfalfa hay (Onimisi, 2005). Tawfeek *et al.* (1995) mentioned that replacing 10 or 20% dietary alfalfa meal with TP resulted in an insignificant increase in total protein, albumin and globulin values by increasing levels of TP in breeding rations. The same results were found by Abd El-Razik (1996) who mentioned that feeding NZW rabbit on diet contained 0, 5 and 10% level of DTP caused an insignificant increase in total serum protein in group 3 which contained 10% TP than in other groups fed diet contained (0 and 5% TP).

Regarding plasma total lipids and cholesterol values, inclusion of DTP in rabbits' bucks at any studied levels resulted in a slight and gradual increase in plasma total lipids concentration compared to the control group, while, this increase was significant ($p < 0.01$) with cholesterol concentration. As mentioned by Elliott *et al.* (1981), feeding rats on 10% DTP-diet resulted in a high level of serum cholesterol. Similar results were found by El-Azab *et al.* (2011) when replaced alfalfa meal with different levels of DTP in growing rabbits' rations by levels 10, 15 and 20%.

Data of liver enzymes' activities (ALT and AST) showed an improvement in liver enzymes' functions due to feeding bucks on diets containing different levels of DTP, where, the values of ALT and AST were significantly decreased ($p < 0.05$) in all DTP groups compared to the control group. These results suggest an improvement in rabbits' liver function due to incorporation of dried tomato pomace in their diets. Our findings are in accordance with that found by Khadr and Abdel-Fattah (2008) who reported that adding tomato wastes in rabbits' diets had no adverse effect on AST and ALT enzyme activities. According to El-Azab *et al.* (2011) incorporation of DTP in growing rabbits' ration by up to 20% resulted in a gradual decrease in AST and ALT, which was significant with AST only.

The effect of feeding bucks on DTP with different levels (10, 15 and 20%) on plasma calcium concentration revealed a slight decrease in plasma calcium concentration in DTP treated groups compared to the control group and the 10% DTP level had the lowest value then the 15% DTP level. The decreased calcium concentration in DTP plasma groups was accompanied

with decreased concentration in seminal plasma and this effect may be due to supplying sperms with adequate amounts of calcium to maintain their function.

Live body weight, feed intake and some organs weight: Effect of adding DTP on live body weight (LBW), feed intake and some organs weight were illustrated in Table 7.

There was no effect due to feeding rabbit bucks on DTP inclusion diets on live body weight, in spite of the 10 and 15% DTP levels resulted in a slight decrease in live body weight compared to the control group or that fed 20% DTP level. Bucks fed a diet containing different levels of DTP (10, 15 and 20%) consumed less feed than control group by 9.3, 11.2 and 14.8%, but this decrease was non-significant. Liver relative weight showed a non-significant increase in the tested groups compared to the control group, in regard to DTP tested group, there was a non-significant decrease in liver relative weight with increase in DTP inclusion level. Rabbits' bucks fed with tested DTP levels had a gradual and significant increase ($p < 0.05$) in testes and seminal vesicles relative weights compared to the control group and these increases were by 108.5, 151.4 and 177.1% of testes relative weight and 269.2, 346.1 and 361.55% of seminal vesicles relative weight, respectively. In spite of the male rabbits that put on DTP rations consumed feed less than the control group, however, the semen quality measurement showed an improvement in treated groups compared to the control group. This effect may be attributed to the protein quality of DTP was high than that of alfalfa meal and the DTP has several beneficial micronutrients known as antioxidants such as vitamin E, ascorbic acid, β -carotene and lycopene. This finding was in a good agreement with that found by Elliott *et al.* (1981) demonstrated that tomato pomace is a good source of protein but may be limited in energy due to the high fiber content. According to Bordowski and Geisman (1980) tomato seeds protein contains approximately 13% more lysine than soybean protein, which would allow it to be used in fortifying low lysine foods. Gippert *et al.* (1989) found that substitution of 10 and 20% alfalfa meal with TP increased the utilization of nutrients for rabbits. In addition, King and Zeidler (2004) and Karadas *et al.* (2006) reported that dried tomato pomace has been considered as a good source of protein for providing amino acids, especially lysine and natural pigments such as beta-carotene and lycopene as well as a valuable source of alpha-tocopherol which is used as an antioxidant. Aghajanzadeh-Golshani *et al.* (2010) reported that this by-product is a good source of vitamin B1 and a reasonable source of vitamin A and B2. Sawal *et al.* (1996) noticed a decrease in the feed intake of the growing rabbits by increasing the level of DTP in diet. According to Abdel-Baset *et al.* (2009), dried tomato pomace can be introduced in the growing rabbits' diet

up to 20% without any problem on growth performance and it could be allocated in the diet to replace alfalfa (Alicata *et al.*, 1988) or maize grain (Abd El-Razik, 1996). In properly formulated diet, Persia *et al.* (2003) reported that dried tomato pomace inclusion rate could be increased up to 15-30% without any adverse effect on growth performance. Sayed and Abdel-Azeem (2009) reported that there were no significant differences between the experimental groups fed diet containing 10, 20 and 30%DTP compared to the control group in live body weight and feed intake. In regard to the digestibility of DTP, Pier *et al.* (2012) reported that rabbits fed diet with 3 and 6%DTP showed higher apparent digestibility coefficients than the control group. With regard to the live body weight and relative carcass weight, Abd El-Razik (1996) revealed that carcass weight and dressing percentage values of rabbits did not differ significantly among group fed the experimental diets containing 0, 5 and 10% dried tomato pomace. Abdel-Baset *et al.* (2009) reported that a non-significant increase in relative liver weight due to feed growing rabbits on DTO inclusion diets up to 20%. The previous studies of Akboraly *et al.* (2007) and Basu and Imrhan (2007) showed that feeding rabbits on diet supplemented with DTP-up to 20% has no clear deteriorative effect on carcass traits and reproductive organs.

Conclusion: From the present study, it could be concluded that addition DTP in diet of mature bucks with studied levels, seems to have an appreciable effect to improve semen characteristics and some blood hematological and biochemical constituents when compared to those in the control group.

REFERENCES

- Abd El-Razik, W.A., 1996. Effect of substitution of tomato pomace for corn in growing rabbit diets on growth performance and carcass traits. *Egypt. J. Rabbit Sci.*, 6: 79-86.
- Abdel-Baset, N. Sayed and Ali M. Abdel-azeem, 2009. Evaluation of Dried Tomato Pomace as Feedstuff in the Diets of Growing Rabbits. *IJAVMS.*, 3: 13-18.
- Agarwal, S. and A.V. Rao, 2000. Tomato lycopene and its role in human health and chronic diseases. *CMAJ.*, 163: 739-744.
- Aghajanzadeh-Golshani, A., N. Maheri-Sis, A. Mirzaei-Aghsaghali and A. Baradaran-Hasanzadeh, 2010. Comparison of nutritive value of tomato pomace and brewers grain for ruminants using *in vitro* gas production technique. *Asian J. Anim. Vet. Adv.*, 5: 43-51.
- Akboraly, N.T., H. Faure, V. Gourlet, A. Favier and C. Berr, 2007. Plasma carotenoid levels and cognitive performance in an elderly population: results of the EVA Study. *J. Gerontol. A Biol. Sci. Med. Sci.*, 62: 308-316.
- Alicata, M.L., A. Bonanno, P. Giaccone, G. Leto and D. Battaglia, 1988. Use of tomato skins and seeds in the feeding of meat rabbits. *Rivista di Coniglicoltura*, 25: 23-31.
- Elkomy, A.E., M.E. El-Seiby and K.I. Kamel, 2008. Comparative study of semen quality and free amino acid content in seminal plasma between high and low motile sperm rabbit bucks. *Egypt. Poult. Sci.*, 28: 633-649.
- Basu, A. and V. Imrhan, 2007. Tomatoes versus lycopene in oxidative stress and carcinogenesis: conclusions from clinical trials. *Eur. J. Clin. Nutr.*, 61: 295-303.
- Beecher, G.R., 1998. Nutrient content of tomatoes and tomato products. *Proc. Soc. Exp. Biol. Med.*, 218: 98-100.
- Belfield, A. and D.M. Goldberg, 1971. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. *Enzyme*, 12: 561-573.
- Blom, E., 1950. A one-minute live-dead sperm stain by means of eosin-nigrosin. *J. Fertil. Steril.*, 1: 176-177.
- Bordowski, I. and J.R. Geisman, 1980. Protein content and amino acid composition of protein of seeds from tomatoes at various stages of ripeness. *J. Food Sci.*, 45: 228-235.
- Brun, J.M., M. Theau-Clement and G. Bolet, 2002. The relationship between rabbit semen characteristics and reproductive performance after artificial insemination. *Anim. Reprod. Sci.*, 70: 139-149.
- Carluccio, A., D. Robbe, I. DE Amicis, A. Contri, U. Tosi, F. Russo and M. Paoletti, 2004. Artificial insemination in rabbits: laboratory and field trial with three different semen extenders. *World Rabbit Sci.*, 12: 65-79.
- Castro, A.C.S., W.E. Berndtson and F.M. Cardoso, 2002. Plasma and testicular testosterone levels, volume density and number of Leydig cells and spermatogenic efficiency of rabbits Braz J. Med. Biol. Res., 35: 493-498.
- Correa, J.R. and P.M. Zavos, 1996. Preparation and recovery of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies. *Theriogenol.*, 46: 1225-1232.
- Doumas, B.T., W.A. Watson and H.G. Biggs, 1977. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinic. Chem. Acta.*, 31: 87-96.
- Duncan, D.B., 1955. Multiple ranges and multiple F testes. *Biometr.*, 11: 1-42.
- El-Azab, A.I., A.M. Rakha, K. Zaki and A.A. El-Chahidi, 1977. Studies of some factors affecting semen production in buffalo bulls. *Egypt. J. Vet. Sci.*, 1: 115.
- El-Azab, M.A., S.M. Zahran, M.H. Ahmed and A.E. Elkomy, 2011. Productive performance of growing rabbits fed diet containing different levels of tomato pomace. *Benha Vet. Med. J.*, 22: 46-57.

- Elliott, J., E. Mulvihill, C. Duncan, R. Forsythe and D. Kritchevsky, 1981. Effects of tomato pomace and mixed-vegetable pomace on serum and liver cholesterol in rats. *J. Nutr.*, 111: 2203-2211.
- FAO, 2011. FAOSTAT. Food and Agriculture Organization of the United Nations.
- Feedipedia, 2011. Animal Feed resources Information centre (<http://www.trc.zootechnie.fr/node/689>).
- Fringes, C.S., T.W. Fendly, R.T. Dunn and C.A. Queen, 1972. Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction. *Clin. Chem.*, 18: 673-674.
- Gindler, M. and J.D. King, 1972. Chemical method for determination of calcium in serum. *Am. J. Clin. Path.*, 58: 376.
- Gippert, T., S. Lacza and J. Hullar, 1989. Utilization of agricultural byproducts in the nutrition of rabbit. Proceeding of 4th World Rabbit Congress. Budapest, 1: 163-172.
- Haddadin, M.S., I.M. Abu-Reesh, F.A. Haddadin and R.K. Robinson, 2001. Utilisation of tomato pomace as a substrate for the production of vitamin B12-a preliminary appraisal. *Bioresour. Technol.*, 78: 225-230.
- Heffner, I.J. and B.T. Storey, 1981. The role of calcium in maintaining motility in mouse spermatozoa. *J. Exp. Zool.*, 218: 427-434.
- Henry, R.J., D.C. Cannon and W. Winkelman, 1974. *Clinical chemistry principles and techniques*, 11th ed. Happer and Row Publishers, pp: 1629.
- Kamel, K.I., A.E. Elkomy and M.E. El-Sbeiy, 2009. The Androgenic Action of Gibberellic Acid (GA) 3 on Reproductive Performance of New Zealand White Rabbit Bucks. *World J. Agri. Sci.*, 5: 40-48.
- Karadas, F., P. Surai, E. Grammenidis, N.H.C. Sparks and T. Acamovic, 2006. Supplementation of the maternal diet with tomato powder and marigold extract: effects on the antioxidant system of the developing quail. *Br. Poult. Sci.*, 47: 200-208.
- Khadr, N.A. and F.A.I. Abdel-Fattah, 2008. Tomato waste as an unusual feedstuff for rabbit. 1-Response of growing rabbits to diets containing tomato waste. *Zag. Vet. J.*, 36: 29-48.
- King, A.J. and G. Zeidler, 2004. Tomato pomace may be a good source of vitamin E in broiler diets. *Calif. Agric.*, 58: 59.
- Lopez, A. and H.L. Williams, 1981. Essential elements in fresh and canned tomatoes. *J. Food Sci.*, 46: 432-434.
- Mann, T., 1948. Fructose content and fructolysis in semen. Practical application in evaluation of semen quality. *J. Agric. Sci.*, 38: 323-331.
- Morton, B., J. Harrigan-Lum and L. Albagli, 1974. The activation of motility in quiescent hamster sperm from the epididymis by calcium and cyclic nucleotides. *Biochem. Biophys. Res. Commun.*, 56: 372-379.
- Nizza, A., Di C. Meo and S. Taranto, 2003. Effect of collection rhythms and season on rabbit semen production. *Reprod. Dom. Anim.*, 38: 436-439.
- NRC, 1994. National Research Council, Nutrient requirements of Domestic Animals. Nutrient Requirements of Rabbits. Washington, D.C., USA.
- Onimisi, P.A., 2005. Evaluation of Ginger waste meal as energy source in the diets of broiler chicken. M.Sc. Thesis submitted to Dept. Anim. Sci., Ahmadu Bello Univ., Zaria, Nigeria.
- Osama, M.A. and A. El-Sahn, 2006. Effect of crossing on the performance of local strains. 3. Seminal quality, electrophoretic pattern of seminal plasma proteins, fertility and hatchability in Bandara, Gimmizah and their reciprocal crosses. *Egypt. Poult. Sci.*, 26: 123-136.
- Persia, M.E., C.M. Parsons, M. Schang and J. Azcona, 2003. Nutritional evaluation of dried tomato seeds. *Poult. Sci.*, 82: 141-146.
- Pier, G.P., G. Francesco, R. Luca and G. Laura, 2012. Effect of Diets with Increasing Levels of Dried Tomato Pomace on the Performances and Apparent Digestibility of Growing Rabbits. *Asian J. Anim. Vet. Advan.*, 7: 521-527.
- Rao, A.V. and S. Agarwal, 1999. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A Review. *Nutr. Res.*, 19: 305-323.
- Reitman, S. and S.A. Frankel, 1957. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, 28: 56-63.
- Richmond, W., 1973. Colorimetric method for the determination of plasma cholesterol. *Clin. Chem.*, 19: 1350-1356.
- Sawal, R.K., D.R. Bhatia and V. Bhasin, 1996. Incorporation of tomato pomace in the diet of rabbits. *Ind. J. Anim. Nutr.*, 13: 3538.
- Sayed, A.N. and A.M. Abdel-azeem, 2009. Evaluation of Dried Tomato Pomace as Feedstuff in the Diets of Growing Rabbits. *IJAVMS*, 3: 13-18.
- Smith, J.T. and D.T. Mayer, 1955. Evaluation of sperm concentration by the haemocytometer method. *Fertil.*, 6: 271-275.
- Sorensen, M.B., I.A. Bergdahl, N.H.I. Hjollund, J.P.E. Bonde, M. Stottenberg and E. Enst, 1999. Zinc, magnesium and calcium in human seminal fluid: relations to other semen parameters and fertility. *MHR. Basic Sci. Reprod. Med.*, 5: 331-337.
- Spss, 2007. SPSS Users Guide statistics version 16.0. SPSS Inc. Washington, D.C.USA.
- Sudjarwo, E., T.S. Achmanu and E. Widodo, 2011. Drake semen quality improved by supplementation of dietary dried tomato powder. Proceedings of the 3rd International Conference on sustainable animal agriculture for developing countries SAADC 2011 strategies AND challenges for sustainable animal agriculture-crop systems. Nakhon Ratchasima, Thailand, 26-29 July, 2011.

- Taha, T.A., E.I. Abdel-Gawad and M.A. Ayoub, 2000. Monthly variations in some reproductive parameters of barki and awassi rams throughout 1 year under subtropical conditions. 2-Biochemical and enzymatic properties of seminal plasma. *Anim. Sci.*, 71: 325-332.
- Tawfeek, M.I., A.A. Rashwan, H. Ibrahim, Dawlat El-Kerdawy and Soad S. Ahmed, 1995. Reproductive efficiency, semen characteristics, digestibility and some blood constituents of breeding rabbits fed tomato pomace as a partial substitution for alfalfa meal. *Zagazig J. Agric. Res.*, 22: 739-749.
- Tesh, S.A. and J.M. Tesh, 1971. Artificial insemination in the rabbit and its use in routine teratogenic studies. *Excerpta medica (Amsterdam) Int. Congers Series.* 220: 332-336.
- Yanagimachi, R. and N. Usui, 1974. Calcium dependence of the acrosome reaction and activation of guinea pig spermatozoa. *Exp. Cell Res.*, 89: 161-174