

EFFECTS OF SUPPLEMENTAL FEEDING WITH SOME MEDICINAL PLANTS ON SEMEN CHARACTERISTICS, BLOOD METABOLITES AND HISTOLOGY OF SELECTED ORGANS OF NEW ZELAND MALE RABBITS IN NORTH SINAI

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Twenty five New Zealand White (NZW) rabbit bucks, (aged 9 months and weighed 3.28 ± 0.1 kg) were divided randomly into five comparable groups (5 each). Rabbits in the 1st group were fed pelleted control diet without medicinal plants (CO), while those in the 2nd, 3rd, 4th and 5th groups were fed the pelleted diet containing 3% rosemary (RS), 3% marjoram (MR), 1.5% ginger root (ZR) and 2.5% mixture (MX) of these medicinal plants, respectively.

***Results showed that** rabbit bucks of the ZR group had lower ($P < 0.05$) blood glucose level compared with the control, but did not differ significantly from the other treatments. Serum total protein increased ($P < 0.05$) in RS, MR and MX when compared with control group, but ZR did not differ significantly from the control. Mean concentrations of serum albumin (AL) and AL/GL ratio did not differ significantly due to treatments. Rabbit fed diets containing rosemary, marjoram, ginger or their mixture showed lower ($P < 0.05$) concentrations of plasma triglycerides than those fed the control diet. Rabbit fed ZR and MX had the lowest ($P < 0.05$) level of cholesterol concentration, compared with for CO, RS and MR groups. Serum testosterone levels were significantly ($P \leq 0.05$) higher in rabbit bucks of ZR and MX groups compared with CO, RS and MR, respectively. Semen Ejaculate volume was significantly higher in MR, ZR and MX groups compared with the control but RS group did not differ significantly from the control. Means of mass motility were higher ($P \leq 0.05$) in treatment groups of RS, ZR and MX than the control but MR group did not differ from the control. The effect of dietary treatment on sperm cell concentration was significant. The ZR group had the highest mean followed by MX, RS, CO and MR groups. The histological examination*

of testicular sections showed that the elongated spermatids were more apparent in the ZR group and Leyding cells were well-developed in the MX group. The histological examination of liver sections showed marked increase in the size and number of bile ducts and many bi-nucleated hepatocytes and sinusoidal dilatation appeared in RS group. MR group showed an increase in the number of bile ducts, mononuclear phagocytic cells and sinusoid dilatation was observed. ZR group showed a lymphocytic infiltration was surrounding the bile duct and increase in the number of Kupffer cells. In MX group no magnificent changes were observed except that dilatation of the bile duct also no change in the sinusoidal size was observed. The histological examination of kidney sections of different experimental groups showed a congestion of some blood vessels in the MR, ZR and MX groups.

Conclusively, *the medicinal plants, Rosemary (*Rosmarinus officinalis*), Marjoram (*Origanum majorana*), Ginger root (*Zingiber officinale*) and their mixture could be added to NZW rabbit diets within the limits recorded in this study to improve semen characteristics and reproductive performance of NZW rabbit bucks without any detrimental effects on their health.*

Keywords: Rabbits, medicinal plants, Rosemary, Marjoram, Ginger, blood, semen characteristics, reproduction, hormones, testosterone, histology, testis, liver, kidney, Sinai.

Compared to other larger domestic species, rabbits have many advantages; including high reproductive rates, rapid growth rates, use of non-competitive feeds and simple housing requirements (Cheeke, 1987). Therefore, rabbits can be raised in Egypt to fulfil part of our requirements of animal protein. Non-traditional feed sources such as medicinal plants, crop residues and agro-industrial by-products must be searched in order to decrease the gap between the available and the requirements of animal feedstuffs. It is important to use natural substances such as medicinal plants that have no adverse effects on animal and human health (Abdel-Malek *et al.*, 1995; Abaza and El-Said, 2005). Medicinal plants can be used as growth promoters and could improve the reproductive performance of rabbits. Medicinal plants and herbs can also increase resistance to disease via optimizing the function of the immune system (Amakye-Anim *et al.*, 2000 and Al-Beitawi *et al.*, 2010), and antioxidant activity (Mericali, 1990 and Tipu, *et al.*, 2006).

Therefore, this study was carried out to evaluate the effects of some dried medicinal plants such as Rosemary (*Rosmarinus officinalis* L.), Marjoram (*Origanum majorana* L.) and Ginger root (*Zingiber officinale* L.) on semen characteristics, blood metabolites and histology of some organs of New Zealand White rabbit males under North Sinai conditions.

MATERIALS AND METHODS

The present study was carried out at the Rabbitry Farm, Department of Animal and Poultry Production, Faculty of Environmental Agricultural Sciences, Al-Arish University, North Sinai, Egypt. Experiments were conducted in Summer and Fall seasons of 2014 (July through November).

A total of 25 New Zealand white (NZW) male rabbits (9 month. old, 3.28 kg) was used in this study. Three medicinal plants from North Sinai spices markets were used in this study as follows: Rosemary (*Rosmarinus officinalis* L.), Marjoram (*Origanum majorana* L.), Ginger root (*Zingiber officinale* L.). These medicinal plants were grinded and then mixed with the diet. Five experimental diets were formulated to cover the nutrient requirements for rabbits according to NRC (1977). At the beginning of the experiment, male rabbits were randomly allotted into five equal groups (5 rabbits each). Rabbits in the control group (CO) were fed the control diet without medicinal plants. The 2nd, 3rd, 4th and 5th groups were fed pelleted diet containing 3% rosemary, 3% marjoram, 1.5% ginger root and 2.5% mixture (1.5% rosemary, 1.5 % marjoram, 0.5% ginger root) of these medicinal plants, respectively.

Rabbits were clinically examined and found to be healthy and free of external and internal parasites. They were kept under the same managerial and hygienic conditions. The rabbits were housed in galvanized wire cages in a well-ventilated houses and were fed a pelleted diet ad lib during the whole experimental period. The ingredients and the approximate analysis of the pelleted diet are shown in Table 1. Chemical analysis was carried out for medicinal plants and for diet samples according to A.O.A.C. methods (1995). Chemical analysis of medicinal plants and experimental diets are presented in Table 2. All animals received fresh water containing 705 ppm total dissolved solids (TDS) which was available automatically all the time by stainless steel nipples.

At the end of the experimental period (12 month. of age), three rabbit bucks from each treatment group were chosen randomly, kept off feed overnight and were slaughtered next morning. Blood samples were collected into centrifuge tubes and allowed to clot at room temperature for 30 min.

Table 1. Ingredients of experimental diets used in this study.

Ingredients	Control diet
Alfalfa hay	30.00
Wheat bran	29.00
Yellow corn	12.50
Soybean meal (44%)	14.00
Barley grain	10.00
Molasses	2.00
Calcium carbonate (lime stone)	0.60
Sodium chloride (salt)	0.30
Vitamins & Mineral Premix*	0.30
DL-Methionine	0.10
Di-Calcium phosphate	1.20
Total	100.00
Calculated chemical composition	
Crude protein (CP)	18.128
Ether extract (EE)	2.936
Crude fiber (CF)	12.17
Digestible energy (Kcal/Kg) *	2768.3

* **One kilogram of premix contain:** Vit. A 12000 000 IU, Vit.D₃ 2200 00 IU, Vit .E 1000 mg, Vit K₃ 2000 mg, Vit B₁ 1000 mg, Vit B₂ 4000 mg, Vit B₆ 100 mg, Vit B₁₂ 10 mg, Pantothenic acid 3.33 g, Biotin 33 mg, Folic acid 0.83 g, Choline chloride 200 g, Zn 11.79 g, Mn 5 g, Fe 12.5 g, Cu 0.5 g, I 33.3 mg, Se 16.6 mg and Mg 66.7 g.

* DE (kcal/kg) =5.29 (DCP, g/kg) +9.51 (DEE, g/kg)+ 4.2 (DCF+DNFE, g/kg) + 0.30

Table 2. Chemical analysis (%) of medicinal plants and the experimental diets.

Items	DM	% DM basis					
		OM	CP	EE	CF	NFE	ASH
Rosemary	91.69	94.59	4.13	11.64	13.37	65.45	5.41
Marjoram	91.41	84.22	14.18	4.14	15.22	50.68	15.78
Ginger root	90.12	94.34	9.38	4.04	11.28	69.64	5.66
Chemical composition of the experimental diets fed to NZW rabbits.							
CO	91.89	89.42	18.21	3.04	12.05	56.12	10.58
RS	92.34	89.58	17.79	3.29	12.09	56.39	10.43
MR	92.33	89.26	18.09	3.07	12.15	55.96	10.74
ZR	90.95	89.65	17.81	3.01	11.86	55.48	10.35
MX	91.87	89.75	17.45	3.05	11.74	54.59	10.25

CO; control, pelleted diet.RS; pelleted diet containing 3% rosemary.

MR; pelleted diet containing 3% marjoram.ZR; pelleted diet containing 1.5 % ginger roots.

MX; Mixture, pelleted diet containing 1% rosemary +1% marjoram + 0.5% ginger root

Serum was then separated by centrifugation at 3000 rpm for 15 min and was subsequently decanted into glass vials and stored at -20°C until it was analyzed (Shetaawi, 1998). Concentration of serum total protein (Henry and Cannon, 1974), albumin, glucose, and cholesterol (Deutsche Veterinärmedizinische Gesellschaft, 1976) were assessed. Activity of aspartate transaminases (AST) and alanine transaminases (ALT) were determined (Reitman and Franke, 1957). Creatinine, and urea nitrogen (UN) were analyzed using assay commercial kits supplied by biomed (Germany), spinreact (Spain) and randox (England). Serum globulin was obtained by difference (Total protein minus albumin) and albumin/ globulin (alb/glob) ratio was also calculated. Blood serum testosterone concentration was assessed using RIA technique utilizing Coat-Ab-Count Kits (Diagnostic Production Corporation, Los Angeles, USA) according to Abraham (1977).

At 10 months, semen was collected twice-weekly using artificial vagina. Physical semen characteristics were evaluated in the fresh collected semen. Ejaculate volume (ml) and Initial mass motility of raw semen were estimated as described by Melrose and Loing (1970). Assessment of live/dead spermatozoa percentage was performed according to (Blom, 1950). Percentage of abnormal spermatozoa was determined in smear prepared for live/dead sperm test. A weak eosin solution (Smith and Mayer, 1955) was used for evaluation of sperm cell concentration. Spermatozoa were counted microscopically by the improved Neubauer Haemocytometer slide (GmbH and co., Brandstwierte 4, 200 Hamburg 11, Germany). Total sperm output (TSO) per ejaculate ($\times 10^6/\text{ejaculate}$) was calculated by multiplying semen ejaculate volume and semen concentration/ml. Live normal sperm output ($\times 10^6$). Live normal sperm output/ejaculate (LNSO) was calculated according to the following equation: $\text{LNSO/ejaculate} (\times 10^6) = \text{TSO} \times \text{live spermatozoa} (\%) \times \text{normal spermatozoa} (\%)$.

Buck rabbits were slaughtered at 13 months of age and specimens of liver, kidney and testes were taken and prepared for histological examination according to Bancroft and Stevens (1990).

Data were analyzed by Least – Squares Analysis Of Variance using the General linear model (GLM) procedure of SAS (2004) according to Steel and Torrie (1980). Semen characteristics were analyzed by repeated measures analysis of variance (split-plot; Gill, 1978).

RESULTS AND DISCUSSION

Serum metabolites and hormones.

Rabbits of the ZR group had lower ($P < 0.05$) serum glucose level compared to the control (CO) but did not differ significantly from the other

treatment groups (Table3). Ginger is supposed to be a hypoglycemic substance since ginger supplementation decreased serum glucose and increased insulin (Ghlyssi *et al.*, 2012 and Hafez, 2010). The extracts of ginger reduced blood glucose in rabbits (Mascolo, 1989). The important active components of the ginger root are phenol compounds which have hypoglycemic activity (Zancan *et al.*, 2002). In rabbits, Bakirel *et al.*, (2008) demonstrated that the *Rosmarinus officinalis* extract exerts remarkable hypoglycaemic and antihyperglycaemic activity due to its possible multiple effects involving both pancreatic and extra-pancreatic mechanism. Ibrahim (2010) found that feeding diets containing ginger and thyme to growing NZW rabbits decreased ($P<0.05$) serum glucose.

Results showed that serum total protein (TP) increased ($P<0.05$) in RS, MR and MX when compared with control group (Table3). The importance of increasing total TP is due to the fact that it generates a colloid osmotic pressure, which control the flow of water between blood and interstitial fluids (Harper *et al.*, 1977). Also, Haider *et al.*, (2010) and El-khashab *et al.*, (2011) reported that proteins are playing a role as intracellular buffers within body tissues to provide a reserve buffering capacity. Mean concentrations of serum albumin (AL) and AL/GL ratio did not differ significantly due to treatments. Albumin, rather than total protein or globulin, has been shown to be the most sensitive indicator of protein status (Kirsch *et al.*, 1968; Sykes and Field, 1973 and Shetaewi and Ross, 1991, Shetaewi, 1998). There was a tendency of higher concentrations of serum GL in rabbits fed RS, MR, ZR and MX than those fed CO (Table3). Ibrahim (2010) used some medicinal plants and found that, feeding diets containing ginger and thyme to growing NZW rabbits increased ($P<0.05$) total protein, albumin and globulin in blood.

Rabbit fed diets containing rosemary (RS), marjoram (MR), ginger (ZR) or their mixture (MX) showed lower ($P<0.05$) concentrations of serum triglycerides than those fed the control diet (Table3). Rabbit fed ZR and MX had the lowest ($P<0.05$) level of cholesterol concentration (60.7 and 53.0 mg/dl), compared with 101.2, 78.0 and 95.3 mg/dl for CO, RS and MR, respectively. Many authors reported that *Zingiber officinale* rhizomes have anti-hyperlipidemia and anti-hypocholesterolemic (Suekawa *et al.*, 1984; Young *et al.*, 2005; Ghayur and Gilani, 2005 and Goyal and Kadnur, 2006).

The hypo-lipidemic and hypo-cholesterolemic effects of Marjoram, Rosemary and Ginger are probably due to a decrease in dietary cholesterol absorption or to an increase in bile acid synthesis and/or through its fecal losses. Peschel *et al.*, (2007) suggested that Rosemary can inhibit lipid oxidation and scavenge superoxide radicals. Nakatani (2003), also reported that phenolic diterpenes from rosemary are particularly anti-oxidative.

Table 3: Serum metabolites and Testosterone concentrations of NZW rabbit bucks as affected by supplemental feeding with medicinal plants (LS-means \pm SE).

Serum Constituent	Treatment groups					\pm SE
	CO	RS	MR	ZR	MX	
Glucose, mg/dl	120.3 ^a	97.7 ^{ab}	108.3 ^{ab}	91.3 ^b	108.0 ^{ab}	7.74
Total protein, g/dl	6.07 ^b	8.12 ^a	7.70 ^a	7.25 ^{ab}	7.63 ^a	0.39
Albumin, g/dl	3.83	4.23	3.90	4.03	4.07	0.31
Globulin, g/dl	3.17	3.89	3.87	3.22	3.57	0.37
AL/GL ratio	1.21	1.16	1.01	1.26	1.14	0.16
Triglyceride, mg/dl	192.3 ^a	136.0 ^b	136.7 ^b	101.0 ^{bc}	70.3 ^c	13.3
Total cholesterol, mg/dl	101.2 ^a	78.0 ^{ab}	95.3 ^a	60.67 ^b	53.0 ^b	9.91
Urea-N, mg/dl	22.3	20.3	22.7	20.3	21.0	1.3
Creatinine, mg/dl	1.01	0.91	0.99	0.85	0.76	0.09
AST, IU/dl	28	28.7	28.7	31.7	29.7	3.69
ALT, IU/dl	13.3	15.7	16.0	19.3	16.0	3.09
AST/ALT ratio	2.11	1.84	1.79	1.65	1.87	0.48
Testosterone free, ng/ml	1.25 ^c	1.77 ^{bc}	1.52 ^c	2.48 ^{ab}	2.71 ^a	0.28

^{a, b and c}: Means within the same row with different superscripts differ ($P < 0.05$),

SE= standard error.

CO= Control; RS=Rosemary; MR= Marjoram; ZR= Ginger; MX= mixture of RS, MR and ZR.

Feeding NZW rabbit bucks on rosemary (RS), marjoram (MR), ginger (ZR) and their mixture (MX) did not affect serum urea-N and creatinine significantly compared with the control (Table 3). Similar results were obtained by Ibrahim (2010) feeding diets containing ginger and thyme to growing NZW rabbits, but Chiofalo *et al.* (2012) found in dairy ewes that rosemary (*Rosmarinus officinalis*) extract supplementation increased ($P < 0.05$) blood concentrations of creatinine.

Means of aspartate transaminase (AST) and alanine transaminase (ALT) or AST/ALT did not differ among treatments (Table 3). In contrast, Seleem *et al.* (2007) found that adding 3% marjoram (*Origanum majorana*) to growing NZW rabbit diets increased ($P \leq 0.05$) the concentrations of AST and ALT, but the increase was still within normal range.

Serum testosterone level was higher ($P \leq 0.05$) in rabbit bucks treated with ZR and MX compared to other treatment groups. Means of RS and MR groups did not differ from that of the CO group. (Table 3). Similarly, Amr and Hamza (2006) found testosterone level was elevated in ginger supplemented rabbits and suggested that the increase may be related to its contents which have potent androgenic activity in male rat. Moreover, Seleem *et al.* (2007) found that serum concentration of testosterone was significantly ($P < 0.05$) higher (4.7 ng/ml) in NZW rabbit bucks fed diets

containing 3% marjoram (*Origanum majorana*) compared with the control (3.6 ng/ml). Data on the effects of other medicinal plants on testosterone level are scarce.

Semen characteristics:

Semen color (Table 4) did not differ significantly ($P>.05$) due to feeding medicinal plants. Semen density means were higher ($P\leq 0.05$) in treatment groups ZR and MX than the control. Means of RS and MR did not differ significantly from the control. Bucks of the RS, ZR and MX groups had higher ($P\leq 0.05$) mass motility score compared with the control group but MR did not differ from the control (Table 4).

Table 4. Semen color, density and mass motility(score) of NZW rabbit bucks as affected by supplementation with medicinal plants.

Items	Treatment groups				
	CO	RS	MR	ZR	MX
Color (score 1-3)*	2.0 ±0.25	2.0 ±0.25	2.2 ±0.18	2.2 ±0.12	2.4 ±0.21
Density (score 0-3)**	1.6 ^b ±0.39	1.8 ^{ab} ±0.44	1.8 ^{ab} ±0.31	2.2 ^a ±0.18	2.0 ^a ±0.28
Mass motility (score 0-5)***	3.0 ^c ±0.25	3.4 ^b ±0.2	2.8 ^c ±0.28	3.8 ^a ±0.11	3.6 ^{ab} ±0.2

^{a, b and c}: Means within the same row with different superscripts differ ($P<0.05$) using Chi-Square.

SE= Standard error , CO= Control; RS=Rosemary; MR= Marjoram; ZR= Ginger; MX= mixture of RS, MR and ZR , *Color 1-2= milky, 2-3= creamy, **Density 1-2= milky, 2-3= creamy , ***Mass motility 2= moderate, 3= strong, 4= very strong.

The present results of semen color in NZW rabbit bucks are in agreement with those obtained by El-Sherbiny (1987) and Abd El-Ghaffar (1992). Also, Abd El-Azim and El-Kamash (2011) indicated that density and mass motility score of semen were 1.99 and 2.90 in NZW rabbits. In sheep, Mudhaffar and Saad (2012) reported that Awassi male lamb supplemented with 2.5% Ginger (*Zingiber officinale*) increased ($P<0.05$) mass motility of semen as compared with the control group.

Ejaculate volume of rabbit bucks in all treatment groups are presented in Table 5. Overall means of ejaculate volume were significantly higher in MR, ZR and MX groups compared with the control but RS group did not differ significantly from the control. Also, the effect of collection week on ejaculate volume was significant ($P<0.001$) . It was observed that ejaculate volume was significantly ($P<0.05$) lower in the 1st week compared to all other weeks. In contrast, Ogbuewu *et al.* (2013 ; 2014a & b) indicated that

dietary inclusion of ginger (*Zingiber officinale*) rhizome powder at 5, 10 and 15g per kg feed had deleterious effects on ejaculate volume of pubertal rabbit bucks.

Table 5. Means of semen characteristics of NZW rabbit bucks as affected by medicinal plants dietary treatments (LS-means \pm SE).

Items	Treatment groups					\pm SE
	CO	RS	MR	ZR	MX	
Ejaculate volume (ml)	0.81 ^c	0.86 ^c	1.07 ^{ab}	1.01 ^b	1.12 ^a	0.05
Advanced sperm motility (%)	63.6 ^c	71.3 ^b	61.3 ^d	73.8 ^a	72.7 ^{ab}	3.6
Live spermatozoa (%)	78.9 ^c	83.8 ^a	81.4 ^b	82.8 ^a	84.0 ^a	2.7
Sperm cell conc. ($\times 10^6$ /ml)	409.1 ^d	473.2 ^c	361.9 ^e	553.3 ^a	507.6 ^b	32.7
Total sperm output ($\times 10^6$ /ejac.)	330.4 ^c	409.5 ^b	396.7 ^b	558.8 ^a	569.4 ^a	42.7
Live normal sperm output ($\times 10^6$ /ejac.)	227.1 ^d	311.0 ^c	287.8 ^c	417.7 ^b	443.7 ^a	39

a, b ...e: Means within the same row with different superscripts differ ($P < 0.05$), SE= standard error.

CO= Control; RS=Rosemary; MR= Marjoram; ZR= Ginger; MX= mixture of RS, MR and Z

The present results might indicate the beneficial effects of marjoram and ginger and mixture on the accessory sex glands (seminal vesicles and prostate) to produce large volume of seminal plasma and/or might be attributed to an increase in testosterone concentration in the blood (Etchu and Egbunike, 2002). The secretory functions of the accessory sex glands are very sensitive to dietary changes and the slight changes in feed chemical composition goes a long way in influencing the semen volume (Ogbuewu *et al.*, 2013). The accessory sex glands are functionally controlled by testosterone (Hafez, 2000).

Sperm motility percentages in rabbit bucks semen of different treatment groups are presented in Table 5. Overall means of RS, ZR and MX treatments were higher ($P < 0.05$) than the CO treatment. However, MR group had the lowest mean of all ($P < 0.05$). The increase in sperm motility of ZR group in comparison to the control group could be due to the protective effect of ginger administration. Ginger rhizome contains a wide variety of both antioxidative (Sekiwa *et al.*, 2000) and androgenic activity (Kamtchouing *et al.*, 2002). The major active phenolic ingredients isolated from *Z. officinale* (Zingerone, Gingerdiol, Zingibrene, gingerols and shogaols) have antioxidant activity (Zancan *et al.*, 2002; Kamtchouing *et al.*, 2002; Jorsaraei *et al.*, 2008).

Table 6. Overall means of semen characteristics of NZW rabbit bucks as affected by medicinal plants dietary treatments at different weeks (LS- means \pm SE).

Collection week	Semen characteristics		
	Ejaculate volume (ml)	Advanced sperm motility (%)	Sperm cell concentration ($\times 10^6$ /ml)
1	0.80 ^b	65.0 ^b	323.9 ^c
2	0.99 ^a	66.4 ^b	356.0 ^d
3	0.99 ^a	67.0 ^b	427.2 ^c
4	0.98 ^a	66.6 ^b	474.8 ^b
5	0.96 ^a	70.6 ^a	490.9 ^b
6	0.99 ^a	70.8 ^a	508.3 ^a
7	0.97 ^a	70.4 ^a	518.0 ^a
8	1.04 ^a	69.8 ^a	524.2 ^a
9	1.03 ^a	70.2 ^a	526.3 ^a
\pm SE	± 0.03	± 0.89	± 6

^{a, b, c, d}: Means within the same column with different superscripts differ ($P < 0.05$), SE= standard error.

CO= Control; RS=Rosemary; MR= Marjoram; ZR= Ginger; MX= mixture of RS, MR and ZR.

In addition, Hamza (2006) reported that ginger have protective effects against cisplatin–induced testicular damage and oxidative stress in rats. Amrand Hamza (2006) showed that ginger has the ability to increase the activities of testicular antioxidant enzymes and restoring sperm motility of cisplatin–treated rats. While, the low sperm motility recorded in MR group could be attributed in part to the rapidity of sperm cell development in the seminiferous tubules, in the sense that spermatozoa may need a moderate but progressive development for them to have excellent motility (Ogbuewu *et al.*, 2013).

Live sperm percentages of rabbit bucks in all treatment groups are presented in Table 5. No interaction was found between dietary treatment and week. Overall means of all treatment groups were higher ($P < 0.05$) than that of the control. The MR group had the lowest mean compared to the other treated groups. The improvements in the percentage of live sperms may be attributed to the antioxidant effects of medicinal plants. Antioxidants actually brake or prevent the lytic activity within semen fluid (Rajeev *et al.*, 2006 and Yang *et al.*, 2006), thereby reducing lipid oxidation which lead to the deterioration of semen quality (Sekiwa *et al.*, 2000). The increase in the percentage of dead

spermatozoa could be attributed to acceleration of the release of immature spermatozoa in marjoram group (MR). Marjoram at this inclusion level might also inhibit the maturation of normal sperm cells by suppressing semen antioxidant systems that protect spermatozoa from oxidative stress caused by reactive oxygen species (Ogbuewu *et al.*, 2013).

Sperm cell concentration ($\times 10^6$) in semen of rabbit bucks in all treatment groups are presented in Table 5. The effect of dietary treatment on sperm cell concentration was significant. The ZR group had the highest mean followed by MX, RS, CO and MR groups. Sperm cell concentration increased significantly within weeks until wk. 6 then the increase was not significant (Table 6). These results indicated that sperm cell concentration was associated with the development of the testes and consequently spermatogenesis increased with the advancement of age. This increase could be due to increased serum testosterone level which stimulated the spermatogenic cells to undergo successful spermatogenesis, sperm maturation in the epididymes and increased the secretory activity of the accessory sex glands (Greenspan and Stawler, 1997; Gelain *et al.*, 2005).

The interaction between dietary treatment and week for total and live normal sperm outputs were significant ($P < 0.01$). Total and live normal sperm output in semen of rabbit bucks in all treatment groups at different collection weeks are presented in Tables 7 & 8. The ZR and MX treatments from wk. 1, 2, 4, 5, 6, 7, 8 and 9 had the highest mean ($P < 0.05$) than the CO.

In general, the observed improvement in all semen characteristics of rabbit bucks fed medicinal plants (Rosemary, Ginger, Marjoram and their mixture) may be attributed to their protective function against oxidative damage of spermatogonia and spermatocytes membranes through the reduction of endogenously formed hydroperoxides of unsaturated fatty acids (Fujii *et al.*, 1984).

Sperm abnormality percentages in semen of rabbit bucks of different treatment groups at different collection weeks are presented in Table 9. The interaction of experimental diet \times collection week on sperm abnormality was significant. The MX treatment from wk. 4, 7, 8 and 9 had the lowest mean ($P < 0.05$) than the CO treatment. No differences were found between the other medicinal plants treated groups and the control (Table 9). Seleem *et al.*, (2007) reported that feeding NZW rabbit bucks on diets containing 3% marjoram (*Origanum majorana*) decreased ($P \leq 0.05$) their abnormal sperm cells from 16% in the control diet to 12.7% in marjoram diet. In general, the range of abnormal sperms in this study during the collection period ranged from 3.85 to 12.95% for all groups, which are within the normal range of abnormal sperm (7.5-13.17) as reported by many authors with different breeds.

Table 7. Total sperm output ($\times 10^6$ /ejaculate) in semen of NZW rabbit bucks in treatment groups at different weeks (LS-means \pm SE).

Collection week	Treatment groups					\pm SE
	CO	RS	MR	ZR	MX	
1	178.9 ^b	255.1 ^{ab}	194.4 ^b	347.4 ^a	345.4 ^a	31.2
2	309.5 ^{bc}	350 ^{abc}	245.9 ^c	425.9 ^a	459.3 ^a	44.8
3	386.3	465.3	312.5	474.5	472.7	55.3
4	354.1 ^c	409.5 ^{bc}	376.6 ^c	616.8 ^a	603.9 ^{ab}	66.5
5	338.4 ^c	399.3 ^{bc}	461.7 ^{abc}	564.9 ^{ab}	593.2 ^a	58.3
6	321.4 ^c	428.7 ^{bc}	520.8 ^{ab}	625.1 ^a	625.9 ^a	53.9
7	342.9 ^c	432.7 ^{bc}	473.0 ^{abc}	579.7 ^{ab}	665.7 ^a	68.5
8	362.1 ^b	475.1 ^b	497.9 ^b	696.1 ^a	701.9 ^a	52.1
9	379.9 ^b	469.9 ^b	487.3 ^b	699.1 ^a	656.3 ^a	42.7

^{a, b and c}: Means within the same row with different superscripts differ ($P < 0.05$), SE= standard error.

CO= Control; RS=Rosemary; MR= Marjoram; ZR= Ginger; MX= mixture of RS, MR and ZR.

Table 8. Live normal sperm output ($\times 10^6$ /ejaculate) in semen of NZW rabbit bucks in treatment groups at different weeks (LS-means \pm SE).

Collection week	Treatment groups					\pm SE
	CO	RS	MR	ZR	MX	
1	118.1 ^b	189.8 ^{ab}	134.3 ^b	254.1 ^a	261.7 ^a	27.4
2	195.0 ^b	260.7 ^{ab}	172.2 ^b	318.3 ^a	335.5 ^a	34.6
3	261.4	344.6	224.3	332.8	352.2	46.5
4	232.7 ^b	328.5 ^{ab}	259.7 ^b	474.2 ^a	485.3 ^a	53.3
5	246.2 ^b	305.4 ^{ab}	330.3 ^{ab}	413.8 ^a	470.3 ^a	52.6
6	233.1 ^b	320.6 ^{ab}	387.2 ^{ab}	475.7 ^a	480.6 ^a	50.6
7	236.5 ^c	321.1 ^{bc}	338.9 ^{bc}	436.6 ^{ab}	523.3 ^a	56.4
8	240.0 ^c	369.3 ^{bc}	374.9 ^{bc}	524.9 ^{ab}	554.8 ^a	50.9
9	280.8 ^b	358.9 ^b	368.4 ^b	528.7 ^a	526.8 ^a	40.8
Overall	227.1^d	311.0^c	287.8^c	417.7^b	443.7^a	
Mean\pmSE	± 39					

^{a, b ...d}: Means within the same row or column with different superscripts differ ($P < 0.05$), SE= standard error.

CO= Control; RS=Rosemary; MR= Marjoram; ZR= Ginger; MX= mixture of RS, MR and ZR.

Table 9. Abnormal spermatozoa (%) in semen of NZW rabbit bucks in treatment groups at different weeks (LS-means \pm SE).

Collection week	Treatment groups					\pm SE
	CO	RS	MR	ZR	MX	
1	10.8	8.2	12.6	10.2	8.0	1.5
2	11.0	9.6	10.0	8.8	9.4	1.3
3	9.0	9.6	9.2	10.6	8.8	1.3
4	9.8 ^{ab}	6.4 ^{bc}	10.8 ^a	7.8 ^{abc}	4.2 ^c	1.3
5	11.0	8.2	9.8	8.4	7.0	1.8
6	9.2	10.0	9.0	8.0	6.4	1.1
7	10.0 ^a	7.2 ^{ab}	10.0 ^a	9.0 ^a	5.2 ^b	1.0
8	11.0 ^a	8.0 ^{ab}	8.2 ^{ab}	8.8 ^{ab}	6.0 ^b	1.4
9	9.0 ^a	7.0 ^{ab}	9.0 ^a	7.2 ^{ab}	5.0 ^b	1.0

^a, ^b and ^c: Means within the same row with different superscripts differ ($P < 0.05$), SE= standard error.

CO= Control; RS=Rosemary; MR= Marjoram; ZR= Ginger; MX= mixture of RS, MR and ZR.

Histological Examination:

In this work, the internal organs; testes, liver, and kidney were histologically examined to study the effect of using medicinal plants in the diet of NZW rabbit bucks on the histological structure of the studied organs. Testicular sections of control group (CO) revealed that convoluted seminiferous tubules were lined with sertoli cells and spermatogenic cells at different stages of development (Figure 1, Plate a & b). All the experimental groups were similar to the control group except that ZR group (Figure 1, Plate 4b) showed plentiful and more apparent elongated spermatids. In addition to in MX group (Figure 1, Plate 5a); leydig cells were more developed in comparison to other experimental groups. Bordbar *et al.*, (2013) reported that the increase of the testosterone level might have resulted from the Leydig cells which are found in the interstitial tissue and secret testosterone. It could be explained on the basis that enhancement of volume or number of Leydig cells resulted in elevation of the testosterone

level. It has been established that ginger has antioxidant properties which can be contributed to its protective components such as zingerone, gingerdiol, zingiberene, gingerols and shogaols (Kamtchoung *et al.*, 2002; Shalaby and Hamowieh, 2010; Memudu *et al.*, 2012). These compounds prevent DNA damages and destruction of genome induced by H₂O₂ (Zancan *et al.*, 2002 and Khaki *et al.*, 2009). It would be expected that ginger cause an increase in the spermatogenesis number and seminiferous tubules volume by preventing apoptosis in the spermatogenesis process.

Many studies showed that ginger was scavenging free radical by its potent antioxidant properties. Amin and Hamza (2005) demonstrated that *Zingiber officinal* increased the activities of testicular antioxidant enzymes, superoxide dismutase, glutathione and catalase and reduced level of malondialdehyde.

Histological examination of the liver sections of control group (CO) revealed normal histological structure (Figure 2, Plate 1a&b); anastomosing laminae of hepatocytes were separated by blood sinusoids. Kupffer cells were observed in the blood sinusoids (Fig.2, Plate 1b). The current finding revealed that hepatic blood sinusoids were dilated in the RS group, this was in accordance with Monira *et al.* (2012) who stated that animals fed rosemary extract exhibited a normal hepatic architecture with slight dilatation between hepatic cords. Although this finding was recorded in animals subjected to high doses (500mg/kg) of *Zingiber officinale* (Amabe, 2014).

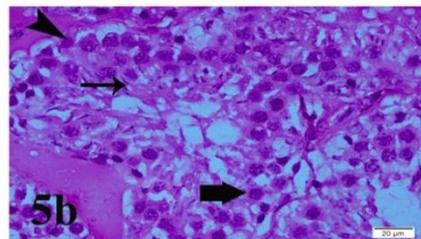
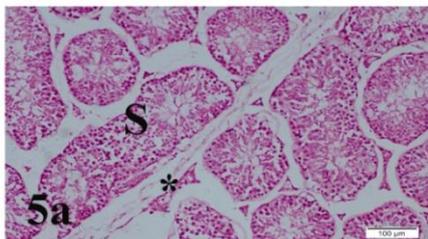
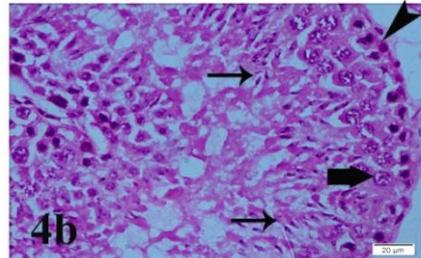
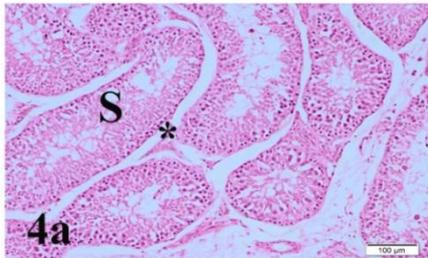
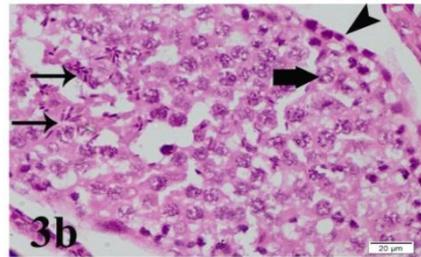
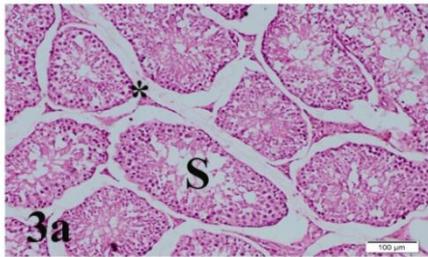
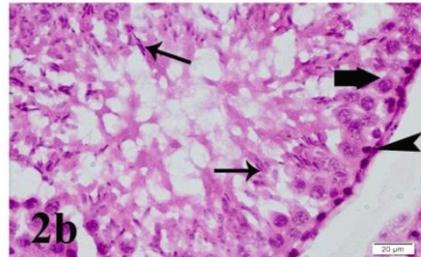
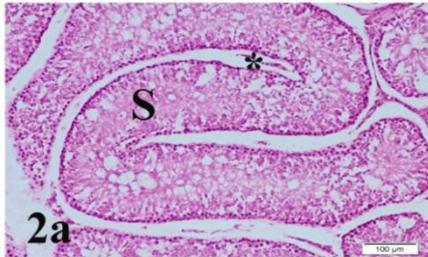
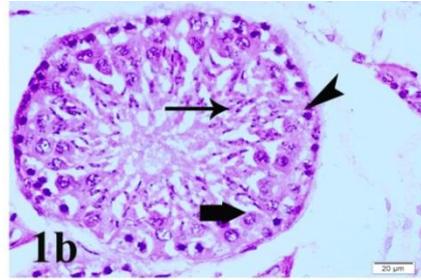
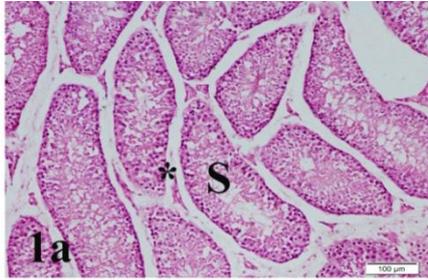
Our investigation emphasized that there was an increase in the number of mononuclear phagocytic cells in the MR group (Figure 2, Plate 3a) and ZR group (Figure 2, Plate 4a); especially in the portal tracts and among the hepatic lobules. In addition to, ZR group (Figure 2, Plate 4b) showed enlarged Kupffer cells. Dilated blood sinusoids were distinguished in the RS group (Figure 2, Plate 2b) and MR group (Figure 2, Plate). This was in agreement with Nusaibah *et al.* (2013) who reported that aggregation of mononuclear cells mainly macrophage and lymphocyte in liver parenchyma was observed in liver section of mice treated with Ginger (*Zingiber officinale*). Du *et al.* (2010) recorded that *Zingiber officinale* can maintain the normal immune function of immune system because it increases the higher titer of antibody such as IgG, IgA and IgM.

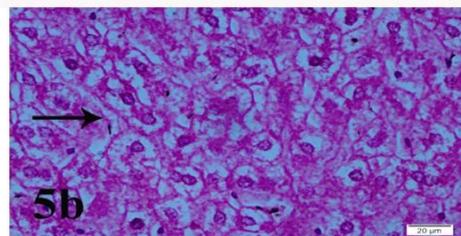
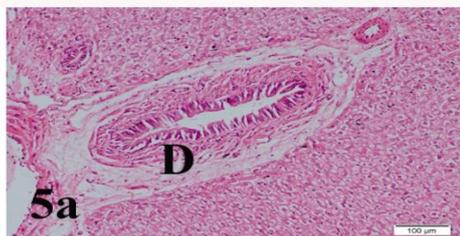
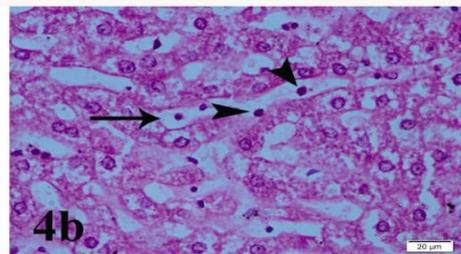
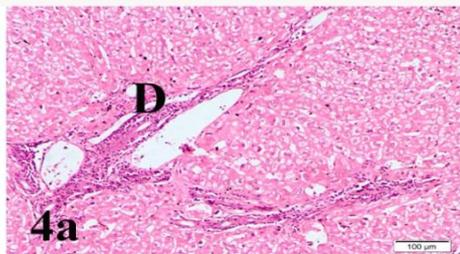
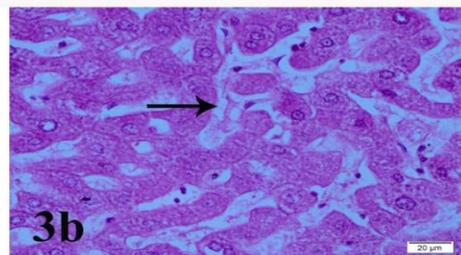
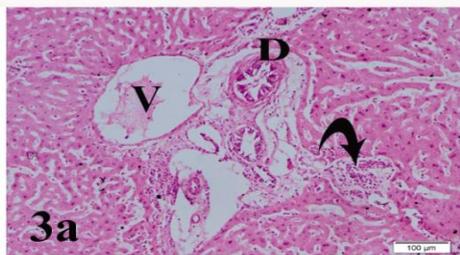
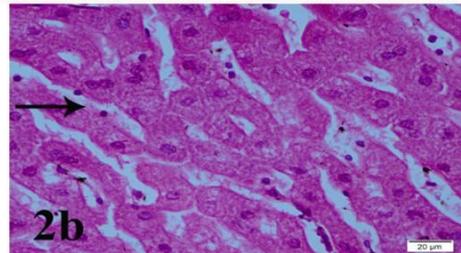
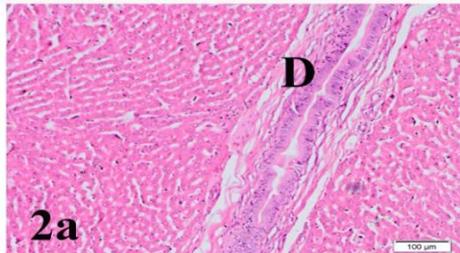
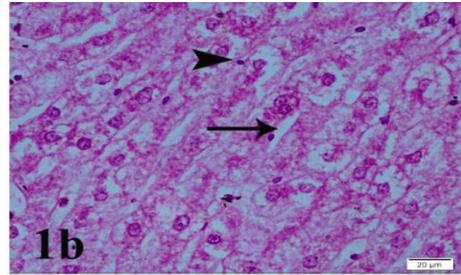
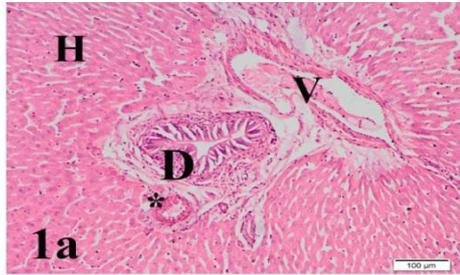
The present study had revealed an increase in the size and number of bile ducts of the experimental groups; the significant changes were apparent in the RS group (Figure 2, Plate 2 a & b) and to a lesser extent in the MR group (Figure 2, Plate 3a) and MX group (Figure 2, Plate 5a). This was in agreement with Fernando *et al.* (2008) who attributed the bile duct

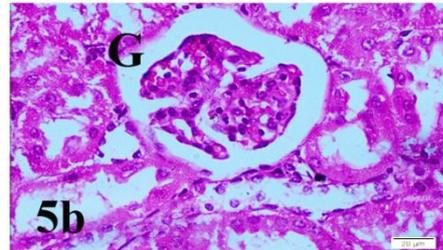
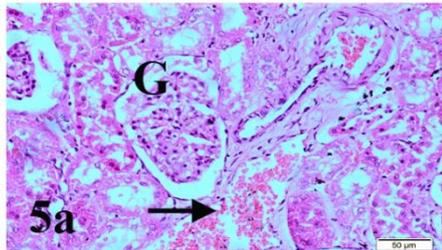
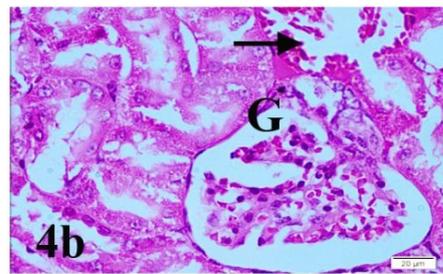
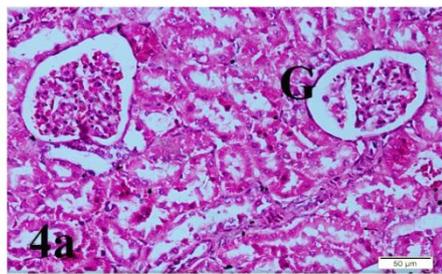
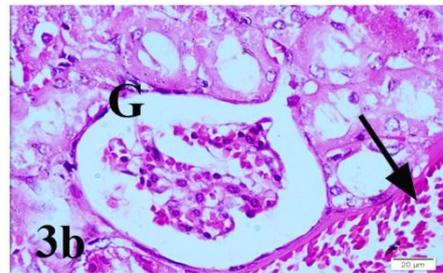
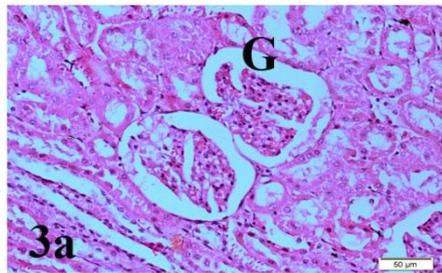
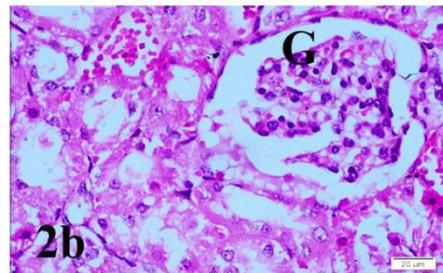
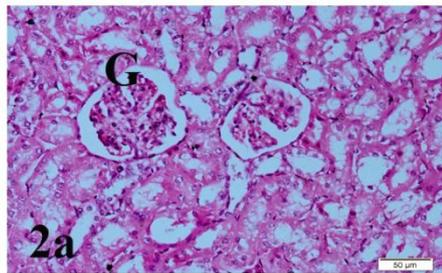
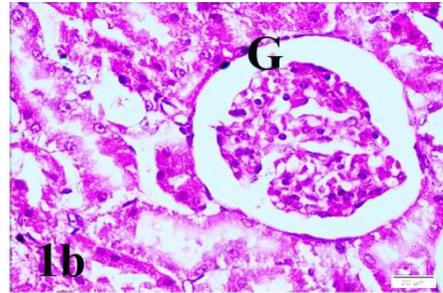
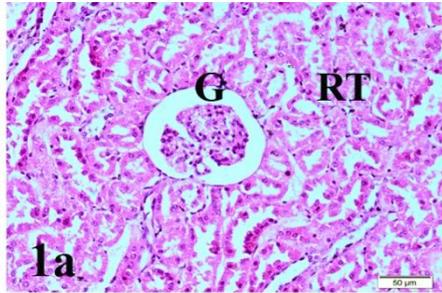
hyperplasia of animals subjected to RS, probably due to the extremely high level of RES uptake relative to body weight. Liver sections of animals fed rosemary leaves showed dilated and congested portal vein (Yasamin *et al.*, 2013); who suggested that the physiological activity of the rosemary (*Rosmarinus officinalis*) can be attributed to the volatile oil. Alpha-pinene, camphene, verbenone, camphor, cineole, bronyl-acetate and borneol are all active compounds of the rosemary (*Rosmarinus officinalis*) and determine the biological Properties of the essential oil and can act in synergic manner or regulate one another.

Examined sections of kidney show normal renal corpuscles surrounded by renal tubules (Figure 3, Plate 1a&b). There was no magnificent changes in the kidney sections of experimental groups (Figure 3, Plates 2, 3, 4 &5) except that few congested blood vessels were observed in the MR group (Figure 3, Plate 3b) and ZR group (Figure 3, Plate 4b) and Mix group (Figure 3, Plate 5a). Monira *et al.* (2012), Histological study of the kidneys of the control rats revealed normal glomerulus surrounded by the Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes. Rosemary has no histopathological effects in renal tissues of rats. Rosemary is one of the plants rich in different phytochemical derivatives such as triterpenes, flavonoids or polyphenols. Its extracts are able to donate electrons to reactive radicals, converting them to more stable and non-reactive species, therefore preventing them from reaching biomolecules, such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars, in susceptible biological systems. It was concluded that RS extracts have a high scavenging capacity of different types of reactive oxygen species which is one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals Mohammad *et al.*, (2013) stated that long term treatment of diabetic rats with *Origanum majorana* can partially protect the renal tissue via attenuation of oxidative stress and glomerular expansion. Ibrahim *et al.* (2005) concluded that *Origanum majorana* plays an important role in ameliorating liver and kidney functions and genotoxicity induced by lead toxicity.

Conclusively, the medicinal plants, Rosemary (*Rosmarinus officinalis*), Marjoram (*Origanum majorana*), Ginger root (*Zingiber officinale*) and their mixture could be added to NZW rabbit diets within the limits recorded in this study to improve semen characteristics and reproductive performance of NZW rabbit bucks without any detrimental effects on their health.







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تأثير اضافة بعض النباتات الطبية على صفات السائل المنوي و خصائص الدم والقطاعات الهستولوجية لذكور ارانب النيوزلندي الابيض فى شمال سيناء

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ذكور الأرانب النيوزيلندي الأبيض (عمر 9 شهور وبمتوسط وزن 3,280.1± كجم) تم تقسيمها عشوائيا إلى خمس مجاميع متماثلة (5 لكل مجموعة) غذيت على خمسة علائق تجريبية متوازنة حتى الشبع. العليقة الأولى كمنترول بدون إضافة اى نباتات طبية، والعليقة الثانية محتوية على 3 % حصالبان، الثالثة 3 % بردقوش، الرابعة 1,5 % زنجبيل والخامسة خليط من هذه النباتات بنسب (1% حصالبان + 1% بردقوش + 0,5% زنجبيل)

وقد أوضحت النتائج المتحصل عليها مايلى: جلوكوز الدم انخفض معنويا نتيجة تعريض الأرانب للتغذية على عليقة الزنجبيل مقارنة ، بينما لم توجد فروق معنوية بين باقى المجاميع. تركيز البروتين الكلى فى سيرم الدم ازداد معنويا فى معاملات الحصالبان والبردقوش والخليط مقارنة بالكنترول، فى حين معاملة الزنجبيل لم تختلف معنويا عن الكمنترول. أظهرت النتائج عدم وجود فروق معنوية فى تركيز كل من الالبيومين ونسبة الالبومين جلوبيولين بين جميع المعاملات. فى حين اتجه تركيز الجلوبيولين الى الزيادة عند التغذية على النباتات الطبية. التغذية على علائق الحصالبان، الزنجبيل، البردقوش وخليطهم ادت الى انخفاض معنوفى تركيز الجليسيريدات الثلاثية بسيرم الدم عن الكمنترول. الارانب المغذاة على علائق الزنجبيل والخليط اعطت اقل متوسط معنوى لتركيز الكوليسترول مقارنة بالكنترول،

الحصالبان والبردقوش. ارتفع مستوى هرمون التستوستيرون معنويا بصورة مرتفعة في الارانب المغذاة على عليقة الزنجبيل والخليط مقارنة بباقي المجاميع. كثافة السائل المنوي كانت مرتفعة معنويا في مجاميع الزنجبيل والخليط عن الكنترول، في حين ان مجاميع الحصالبان والبردقوش لم تختلف معنويا عن الكنترول. كذلك الحركة الجماعية كانت مرتفعة معنويا في مجاميع الحصالبان والزنجبيل والخليط عن الكنترول، في حين ان مجموعة البردقوش لم تختلف معنويا عن الكنترول. حجم الفذفة ازداد معنويا في معاملات البردقوش والزنجبيل والخليط مقارنة بالكنترول في حين ان مجموعة الحصالبان لم تختلف معنويا عن الكنترول. ارتفعت النسبة المئوية للحركة الفردية التقدمية معنويا في معاملات الحصالبان والزنجبيل والخليط عن الكنترول. في حين ان مجموعة البردقوش كانت اقل متوسط. وجد ان اعلى قيم تركيز الحيوانات المنوية سجلت لمجموعة الزنجبيل ثم مجموعة الخليط ثم مجموعة الحصالبان ثم مجموعة الكنترول وأخيرا مجموعة البردقوش. أظهر الفحص الهستولوجي للخصية أن مجموعة الزنجبيل كانت أكثر تطورا حيث لوحظ اتساع وتطور الأنبيبات المنوية وظهرت الاسبرماتيدات على امتداد الأنبيبات المنوية بوضوح، في حين كانت مجموعة الخليط أكثر تطورا ووضوحا في النسيج البيني المحتوى على خلايا ليدج المفرزة لهرمون التستوستيرون. أظهر الفحص الهستولوجي لقطاعات الكبد زيادة ملحوظة في حجم وعدد القنوات الصفراوية والعديد من خلايا الكبد ثنائية الأنوية في مجموعة الحصالبان في حين ظهر بوضوح زيادة في عدد القنوات الصفراوية فقط في مجموعة البردقوش، وفي مجموعة الزنجبيل ظهرت الخلايا الليمفاوية المحيطة بالقناة الصفراوية وزيادة في عدد خلايا كوبفر، أما مجموعة الخليط لم يلاحظ أي تغييرات تذكر سوى اتساع في حجم القناة الصفراوية. أظهر الفحص الهستولوجي لقطاعات الكلى في المجموعات التجريبية المختلفة اذحام بعض الأوعية الدموية في مجموعة البردقوش والزنجبيل ZR والخليط.

التوصية: من النتائج السابقة يمكن التوصية بإضافة النباتات الطبية الحصالبان، البردقوش والزنجبيل وخليطهم الى علائق ذكور الأرانب النيوزيلندا الأبيض بالنسب المذكورة لتحسين خصائص السائل المنوي والأداء التناسل بدون حدوث اي اثار جانبية قد تضر بصحة الأرانب او تؤثر على مكونات الدم او على التركيب الهستولوجي لبعض اعضاء الجسم.