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FOCUS ON EFFECT OF PUMPKIN OIL ON ENDOCRINE AND ANTIOXIDANT LEVEL IN RABBIT

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ABSTRACT

The present study was designed to investigate the effect of pumpkin oil on semen characteristics, testosterone and estradiol hormones of rabbit buck, oxidative and antioxidative status of rabbit both sex. Progesterone and estradiol of doe were determined as a result of pumpkin oil supplementation. Eight bucks and twenty - four doe of New Zealand white rabbits of about 4 months age and an average body weight (2000 g) were experimentally used. The animals were divided randomly to two groups each of 4 bucks and 12 does. One group was considered as control group and fed on basal diet (C) while the second group supplemented with 5 g pumpkin seed oil/kg diet (P) each sub group was divided to two subgroups for making cross mating as follows, control does were mated with control bucks (CC), pumpkin does were mated with control bucks (CP), control does were mated with pumpkin bucks (PC) and the fourth one was pumpkin does which were mated with pumpkin bucks (PP). Results revealed a significant improvement in sperm count, mass and individual motility, sperm concentration, live and dead ratio and testosterone hormones and anti-oxidative parameters of bucks. Moreover, significant increase in estradiol hormone and antioxidant parameters of does supplemented with pumpkin oil. On conclusion, the benefits shown in this study support further research into the use of pumpkin oil in view of increasing reproductive hormone and anti-oxidative parameters of rabbit**.**

Keywords: Rabbit bucks, rabbit doe, pumpkin oil, reproduction, semen characteristics.

INTRODUCTION

Pumpkin seeds have been used to improve sexual stimulation and improvement of sexual performance in terms of intromissions and ejaculatory latency which also improved sexual sensation and copulatory efficiency (Gundidza *et al*., 2009). Pumpkin considered as natural source of zinc, zinc is an essential mineral, important in prostate gland function and the growth of the reproductive organs, also zinc is found more in male reproductive fluid than anywhere else in the body, moreover; It is required for protein synthesis and collagen formation, promotes a healthy immune system, the healing of wounds and synthesis of DNA and RNA, also zinc is an important fertility nutrient for both sexes and zinc deficiency can cause sperm counts to drop below the point of technical sterility (Feng *et al*., 2002).

Pumpkin are a rich source of unsaturated fatty acids, antioxidants and fibers, known to have anti-atherogenic and hepatoprotective activities (Makni *et al*., 2008). The intake of a whole extract of pumpkin seeds is correlated to reduced benign prostate hyperplasia-associated symptoms (Stevenson *et al*., 2007). Pumpkin improve growth performance, milk yield and composition and reproductive performance of does as well as the best results regarding litter size and weight, mortality rate and growth performance of their offspring and economic efficiency (Gaafar *et al*.,2014).

The objective of this study was to investigate the effect of feed additive of pumpkin seeds oils on antioxidant, reproductive hormone of male and female rabbit.

MATERIALS AND METHODS

Animals and experimental design

Eight bucks and twenty - four doe of New Zealand white rabbits of about 4 months age and an average body weight (2000 g) were experimentally used. The animals were divided randomly to two groups each of 4 bucks and 12 does. One group was considered as control group and fed on basal diet (C) while the second group supplemented with 5 g pumpkin seed oil/kg diet (P). Each subgroup was divided to two subgroups for making cross mating as follows: control does were mated with control bucks (CC), pumpkin does were mated with control bucks (CP), control does were mated with pumpkin bucks (PC) and the fourth one was pumpkin does which were mated with pumpkin bucks (PP). Pumpkin seed oil was obtained from Arab Company for Pharmaceutical and Medicinal Plants, MEPACO", Egypt. The experiment lasted for 2 month. Rabbit were housed individually in commercial cages (55x60x34), equipped with automatic drinkers and j-feeders. Clean and fresh water was available all time. The whole rabbitry was well ventilated through both natural windows and electric fans and illuminated to 14:10 light dark cycle through natural and fluorescent lighting. The rabbitry average ambient temperature and relative humidity ranged from 20 to 30ºC and 70-80%, respectively. Basal and experimental diets were formulated to cover the nutrient requirements of and offered for all animals ad libitum. Samples collection and analysis. Semen collection was done by using a teaser female and artificial vagina (containing water at 50ºc) according to (Moce *et al*., 2000).semen collection six times all over experiment from control and pumpkin groups

Chemical Analyses

The blood samples of all animals were collected from the ear vein into tubes, and immediately centrifuged at 1000g for 10 min at 4 ºC. Timing of the blood samples were a beginning of experiment for male and female, just 5 -10 min after mating, day 16th (mid pregnancy) and 26th (late pregnancy) day and after parturition for treated and control does and bucks. Serum was stored at –20 ºC until assayed for serum Superoxide dismutase activity (SOD) according to (Jewett and Rocklin, 1993), total antioxidant capacity (TAC) by koracevic *et al*. (2001) and lipid peroxidation expressed in Malondialdehyde (Yoshioka *et al*. 1979) were performed using kits purchased from Biodiagnostic Company, Dokki, Egypt. Buck serum testosterone , doe estradiol 17 ^{ß} and progesterone hormones were accomplished according to the method of Tietz *et al.* (1995) using enzyme immunoassay kits purchased from "DRG" Germany. The sensitivity of testosterone is <0.084 ng/ml, and estradiol hormones is <9.7pg/ml, and progesterone hormones is <0.045 ng/ml .

Statistical Analysis

Statistical Analysis: Data are presented as means ±S.E. and analyzed by one way ANOVA according to the method of Snedecor and Cochran (1980). Differences of P value<0.05 were considered significant among the experimental groups.

RESULTS AND DISCUSSION

Effect of pumpkin oil supplementation on semen characteristics of bucks (means ± SE).

The present results revealed significant increase in the sperm characteristics while the semen PH and the percentage of abnormal sperm remain as the control. Pumpkin seeds are an excellent source of magnesium, phosphorus, manganese, zinc, copper and iron, which are vital for male reproductive system (Bombardelli & Morazzoni, 1997). Pumpkin is rich in antioxidants such as vitamin C, vitamin E and vitamin A (Christian, 2007). According to Fukuchi *et al*. (2004), vitamin A protects the testis against lipid peroxidation, hence promotes spermatogenesis and improves structural differentiation of epithelial cells of the epididymis.

Table 1: Semen characteristics of bucks

Items	Control	Pumpkin
Nº ejaculates (6 ejaculates)		
Semen Volume(ml)	0.71±0.07[b]	1.10± 0.06[a]
Semen PH	7.35±0.20	7.32± 0.11
Mass motility	3.55±0.12[b]	4.18± 0.07[a]
Individual motility%	72.77±0.40[b]	88.34± 1.57[a]
Sp. conc x 10^6 (ml)	305.20±5.61[b]	313.90± 5.56[a]
Live& dead ratio%	75.50±2.87[b]	91.30± 1.54[a]
Abnormal sperm%	9.53±0.46	8.31± 0.25

Means with different letters on the same raw differ significantly at P≤ 0.05.

Effect of pumpkin oil supplementation on serum testosterone and estradio17$^{\beta}$ hormones level of bucks (means ± SE).

From table (2) results revealed significant increase of testosterone of rabbits supplemented with pumpkin oil as compared with control value. The results are agree with those of Nagata *et al*. (2000) who showed that Pumpkin seeds are rich in unsaturated fatty acids such as omega 3, 6 and 9 as well as its high protein therefore the rabbit buck which fed diets rich in monounsaturated fats had greater –dehydrogenase activity which is a key enzyme in the testosterone synthesis pathway in the male.

Table 2: Effect of pumpkin oil supplementation on testosterone hormone and estradiol-17$^{\beta}$ level of bucks (means ± SE).

Parameters	*Control*	*Pumpkin*
Nº samples(6 samples)		
Testosterone(ng/mg)	4.40± 0.71[b]	4.85± 0.04 [a]
Estradiol-17$^{\beta}$(pg/mg)	13.04± 0.81	13.11± 0.21

Means with different letters on the same raw differ significantly at P≤ 0.05.

Impact of pumpkin oil supplementation on antioxidant parameters of buck and doe:

Rabbit male and female supplemented with pumpkin oil showed significant increase of antioxidant parameters and decrease of lipid peroxide. The reported results proud with those of Xu (2000) who found that pumpkin polysaccharide could increase the SOD and GSH-Px activity and reduce the MDA content in tumor mice serum. pumpkin are a rich source of unsaturated fatty acids, antioxidants and fibers (Makni *et al.*, 2008).

Table 3: Impact of pumpkin oil supplementation on antioxidant parameters of buck and doe (means ± SE).

Parameters		Control	Pumpkin
Buck	SOD(U/g)	613.51± 7.27[b]	661.10± 8.35[a]
	TAC (µm/L)	2.66± 0.08[b]	2.97± 0.15[a]
	MDA('nmol/g)	15.10± 0.62[a]	14.46 ± 0.65[b]
Doe	SOD(U/g)	635.10± 8.60[b]	669.55±0 .51[a]
	TAC (µm/L)	2.70± 0.07[b]	3.03± 0.07[a]
	MDA('nmol/g)	15.12±0 .57[a]	14.45 ± 0.05[b]

Means with different letters on the same raw differ significantly at P≤ 0.05.

SOD (superoxide Dismutase), TAC (Total antioxidant capacity), MDA (Malondialdehyde).

Effect of pumpkin oil supplementation on serum estradiol-17$^{\beta}$ and progesterone level.

The results confirm the former finding of (Bataineh *et a*l., 2002) who reported that increase in doe estradiol levels may be due to the zinc content of pumpkin oil which considered as a main requirement for all the steroid hormone receptors to maintain their secondary structure and function. The increased estradiol 17^{ß} level and receptivity of (CP and PP) groups may be attributed to the increased amount of

energy available for the doe which favor the re-establishment of the hypothalamus – pituitary – ovary cycle (Rodriguez de Lara *et al*., 2000 and Theau Clement *et al.*, 2005).

Table 4: Impact of pumpkin oil supplementation on estradiol-17$^{\beta}$ level of doe (means ± SE).

Groups	CC	CP	PC	PP
Nº samples(6 samples)				
At mating	13.44± 0.27^{b}	18.10± 1.15^{a}	13.52± 1.10^{b}	19.12± 1.35^{a}
Mid pregnancy	9.76± 0.38^{b}	11.87± 0.33^{a}	9.77± 0.30^{b}	11.97± 0.39^{a}
late pregnancy	11.26± 0.48^{b}	13.37± 0.23^{a}	11.47± 0.25^{b}	14.47± 0.30^{a}
After parturition	15.52± 0.62^{b}	17.44 ± 1.15^{b}	15.47 ± 1.25^{b}	18.46 ± 1.05^{a}

Means with different letters on the same raw differ significantly at P≤ 0.05.

Table 5: Impact of pumpkin oil supplementation on progesterone level of doe (means ± SE).

Groups	CC	CP	PC	PP
Nº of samples(6 samples) At mating	1.55± 0.36	1.91± 2.20	1.33± 0.55	1.93± 0.53
Mid pregnancy	12.41± 0.24	12.93±0.22	12.43± 0.15	13.03± 0.1
late pregnancy	10.71± 0.22	10.82± 0.23	10.80± 0.20	10.92± 0.2
After parturition	1.12± 0.07	1.11 ± 0.05	1.25 ± 0.10	1.35 ± 0.1

Means with different letters on the same raw differ significantly at P≤ 0.05.

CC (control buck x control doe), CP(control buck x pumpkin doe), PC (pumpkin buck x control doe), PP (pumpkin buck x pumpkin doe).

CONCLUSION

Supplementation of male rabbit with pumpkin seed oil was associated with elevates the antioxidative parameters of buck and doe and decrease oxidative MDA. Additionally, pumpkin supplementation improved level of testosterone hormone, sperm count, mass and individual motility, sperm concentration, live and dead ratio in male rabbit and increased in estradiol hormone in rabbit doe.

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