

The effect of dietary doum supplementation on- productive and reproductive performance of male rabbits in Upper Egypt

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Abstract: The objective of this work was conducted to study the effect of Doum (*Hyphaenethebaica*) supplementation on productive and reproductive performance of rabbit bucks in Upper Egypt during spring season. Thirty- two males 16 California (CAL) and 16 Newzland (NEZ) rabbits 8 months old with average initial body weight 3.409 ± 0.05 kg were randomly divided into four groups of 4 bucks each/breed (CAL and NEZ). Group 1 served as control fed a basal diet. Groups 2, 3 and 4 fed basal diets supplemented with 0.3, 0.6 and 0.9 g doum/kg diets, respectively for 12 weeks. The results revealed that doum supplementation significantly ($p \leq 0.01$) increased bucks live body weight compared with control group. Ejaculate volume, mass motility, sperm concentration, total sperm output, total motile sperms, live normal sperm, total functional sperm fraction and initial fructose were significantly increased, while reaction time significantly decreased in rabbit bucks received doum compared to control group. Administration of doum increased ($P < 0.05$) seminal plasma total proteins, globulins, alkaline phosphatase, acid phosphatase and lactate dehydrogenase. Conversely, seminal plasma aspartate aminotransferase and alanine aminotransferase were significantly decreased compared to the control group. Seminal plasma lipid peroxidation as indicated by thiobarbituric acid-reactive substances was significantly decreased while, seminal plasma antioxidant enzymes were significantly increased due to doum supplementation Also, doum supplementation significant increased serum testosterone level. In conclusion, doum supplementation improved semen quality, and seminal plasma antioxidant status of rabbit bucks. Thus, the doum could help to improve productive and reproductive efficiency of rabbit bucks under Upper Egypt condition.

Key words: Doum - semen quality- antioxidant status- male rabbits

INTRODUCTION

Exposure of rabbits to high ambient temperature (30°C) negatively affected the fertility (Nagwa et al., 2005), growth and reproductive traits (Marai *et al.*, 2002). High environmental temperature tends to have a detrimental effect on semen-

-production and fertility of men (Sinclair, 2000), bulls and goats (Murugaiyah, 1992). Exposure to hyperthermia is harmful for spermatogenesis by decreases testosterone levels (Murray, 1997). A high ambient temperature causes also, an increase in oxidative stress due to the increase in production of reactive oxygen species (ROS), which determines semen characteristics and sperm-oocyte fusion (Akiyama, 1999).

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Formation of ROS can lead to DNA damage in the form of mutations, base deletions, degradation, and single-strand scission (Imlay, 2003). Surplus ROS caused lipid peroxidation that disrupts cell membrane fluidity and can lead to apoptosis (Green and Reed, 1998). In long hot period exposure, rabbits have difficulty to eliminate body heat due to the unfunctional sweat glands (Marai *et al.*, 2002). Alleviation of heat stress effect can be carried out by either chemical (Ayyatet *al.*, 1997) or physical or nutritional techniques (Marai *et al.*, 1994).

The World Health Organization has encouraged research on medicinal plants as ant diabetics (Shehuet.,*al.*, 2014) and (Abdel-Rahimet.,*al.*,2011). Doum (*Hyphaene thebaica*) is an African palm tree, common in Upper Egypt (Aremu2011), originally native to the Nile valley, bearing an edible fruit which is glubose-quadrangular , about 6 x 5 cm with a shiny orange-brown to deep chestnut skin (epicarp). The rind (mesocarp) in some palm is inedible but of other it is very palatable , highly aromatic and sweet with a taste like ginger bread hence the English name. When eaten it serves as vermifuges and parasite expellant (Burkill, 1997). The chloroform extract of the fruits improve spermatic count of male rats at low concentration (Hetta and Yassin 2006) but decrease it at high concentration (Hetta *et al.*, 2005). It was considered sacred by the Ancient Egyptians and its seeds were found in many pharaoh's tombs e.g.Tutankhamun's tomb (Hetta *et al.*, 2005). Mervat Ghazal (2016) concluded that supplementing doum to Hy-Plus rabbit diets caused significant improvement in semen quality and fertilizing ability of

bucks. Improving fertility traits of rabbit does treated with doum can be attributed mainly to improve of semen quality, as recorded previously by (Ali and Mervat Ghazal, 2013). (Lavaraa *et al.* 2005) observed significant correlations between fertility rate of does and semen quality. Also, it can be due to doum contain Zn, Mn and copper, which have been reported in improving reproduction in males and females. The objective of this work was to study the effects of doum (*Hyphaenethebaica*) supplementation on reproductive performance of rabbit bucks in Upper Egypt during spring condition.

Materials and methods

The present experimental work was carried out at the Experimental Farm of Poultry Production Department, Faculty of Agriculture South Valley University, Qena. This study was undertaken during the spring season of Qena City in the period from April to June 2017. The investigation was carried out under warm conditions having average ambient temperature ranging from 25.8⁰C (min) to 41.1⁰C (max), relative humidity 16.3% minimum to 41.5% maximum (Table 1) recorded maximum, minimum and estimation of average air temperature (C⁰) and relative humidity during the experimental period.

Temperature-humidity index (THI) was calculated according to Marai *et al.* (2001):

$$THI = db \text{ } ^\circ C - [(0.31 - 0.31 \times RH) \times (db \text{ } C - 14.4)]$$

Where, db ⁰C = dry bulb temperature and RH = relative humidity %. The THI values were classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8),

severe heat stress (28.9-29.9) and very severe heat stress (>30.0) (Marai *et al.*, 2002).

Animals, management and experimental design:

Thirty-two males (16 CAL and 16 NEZ) rabbits 8 months old (average initial body weight 3400g) were used. Rabbits were individually housed in galvanized wire cages provided with feeders and automatic stainless- steel nipple drinkers where basal diet and water were offered *ad libitum*. Rabbits were randomly divided into four equal groups. Group 1 served as control (fed basal diet). Groups 2, 3 and 4 fed the basal diet supplemented with 0.3, 0.6 and 0.9g doum /kg diets, respectively, for 12 weeks formulation and chemical composition of the diets was showed in (Table 2)

Data collection:

Live body weight (LBW) and feed intake (FI) of rabbit bucks were weekly recorded through 12 weeks. Semen collection weekly occurred through 12 weeks (experimental period). Ejaculates were collected using an artificial vagina maintained at 45-46°C and a teaser doe. Reaction time (RT) was the time interval from the introduction of the teaser doe into the male's cage to ejaculation and it was measured by seconds with a stopwatch and considered as an indication of libido. Immediately after collection, semen was kept at 35 °C in water bath for evaluation. Ejaculate volume (EV) was recorded after removal of the gel mass. Percentage of sperm motility (SM) from at least three fields was examined at 37°C under a phase

microscope at 400× with scale from 0 to 100%. Initial hydrogen ion concentration (pH) of semen samples was determined immediately after collection using a pH paper (Universalindikator pH 0–14 Merck, Merck KgaA, 64271 Darmstadt, Germany). A weak eosin solution was used at a dilution of 1:99 before counting the cells for evaluation of sperm concentration (SC), ×10⁶/ml according to (Smith and Mayer 1955) using hemocytometer slide. Total sperm output (TSO) was calculated through multiplying semen ejaculate volume by semen concentration. Total number of motile sperm (TMS) was calculated by multiplying percentage of motile sperm and total sperm output. Assessment of live and abnormal spermatozoa was performed using an eosin-nigrosine staining (Kamel 2012).

Seminal plasma samples

At the end of experiment Seminal plasma samples were analyzed for total proteins (TP), albumin (Alb), globulins (Glob), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), acid phosphatase (AcP) and lactate dehydrogenase (LDH) were measured using commercial kits of bio-diagnostic company (Recycling Crusher-SBM®). Seminal plasma samples were analyzed for thiobarbituric acid-reactive substances (TBARS) using the method of (Tappel and Zalkin 1959). Seminal plasma glutathione content (GSH) was determined using commercial glutathione reduced kits.

Table1. Least square means and standard errors of air temperature (AT, °C) and relative Humidity (RH, %) in the area of south valley experimental animal farm during the period of study.

MONTH	AIR TEMPERATURE, °C			RELATIVE HUMIDITY, %			THI
	Minimum	Maximum	Mean	Afternoon	Morning	Mean	
APRIL	15±0.22	32±0.19	23.5±0.37	48.6±0.27	80±1.05	64.3±0.36	22.49
MAY	25.8±0.27	41.1±0.17	33.4± 0.27	16.3±0.61	41.5±0.36	28.9±1.10	29.20
JUNE	26±0.29	42±0.18	34± 0.29	17.8±0.63	42.5±0.39	30.15±0.51	29.68

Table 2. Formulation and chemical composition of the diets (g/kg)

Ingredients %	Control	tested one ration	tested two rations	tested three rations
Alfalfa hay	342	342	342	342
Soybean meal (44% CP)	125	125	125	125
Corn meal	225	225	225	225
Whole sunflower meal	70	69.7	69.4	69.1
Doum (Hyphaene thebaica)	-	0.300	0.600	0.900
Barley meal	140	140	140	140
Wheat bran	50	50	50	50
Beet molasses	12	12	12	12
Calcium carbonate	13.72	13.72	13.72	13.72
Calcium diphosphate	6.71	6.71	6.71	6.71
Sodium chloride	5	5	5	5
DL-methionine	0.570	0.570	0.570	0.570
Vitamin-mineral premix *	10	10	10	10
Total	1000	1000	1000	1000
Calculated chemical composition of the diets%				
Dry matter	89.2	89.8	89.5	89.5
Crude protein	17.3	17.2	17.00	17.00
Ether extract	5.3	5.2	5.3	5.3
Crude fibre	14.9	14.8	14.8	14.8
Ash	8.8	8.6	8.7	8.7
Digestible energy kcal/kg	2610	2600	2600	2600

* per kg diet: Vit. A 11,000 UI; Vit. D3 2,000 UI; Vit. B1 2.5 g; Vit. B2 4 g; Vit. B6 1.25 g; Vit. B12 0.01 g; Alpha-tocopheryl acetate 50 g; Biotine 0.06 g; Vit. K 2.5 g; Niacine 15 g; Folic acid 0.30 g; Dpanthotenic acid 10 g; Choline 600 g; Mn 60 g; Fe 50 g; Zn 15 g; I 0.5 g; Co 0.5 g.

Glutathione peroxidase (GPx) activity was assayed using the method of (Chiu *et al.* 1976). Superoxide dismutase (SOD) activity was assayed according to (Misra and Fridovich 1972). Glutathione S-transferase (GST) activity was determined according to (Habig *et al.* 1974) using P-nitrobenzylchloride as a substrate.

Blood samples were weekly up to 4 weeks collected from the ear vein from each buck and placed immediately on ice serum were separated from blood by centrifugation for 20 min and stored at -18 °C. Testosterone concentration and estradiol 172 α and progesterone levels in blood serum were measured using immunoassay (Biosource-Europe S.A. 8, rue de L'Industrie.B-1400 Nivelles. Belgium).

Bucks reproductive performance:

At the same period for fertility criteria evaluation bucks of each group were mated to 15 receptive nulliparous female rabbits. Kindling rate and litter size at birth (total and alive), and at weaning were recorded, litter weight at birth and 28 days were recorded. Kindling rate was estimated as the number of kindled does divided by the number of mated does \times 100 .

Statistical Analysis:

The data were analyzed using General linear method of statistical analysis system SAS (2004) Duncan Multiple range Test was used to compare the differences among means (Duncan, 1955), As all two-way interactions of the study were

significant. Data presented as percentages were transformed to the corresponding arcsine values (Warren and Gregory, 2005) before being statistically analyzed

$$Y_{ijk} = \mu + D_i + B_j + DB_{ij} + E_{ijk}$$

Where: Y_{ijk} = any observation of the rabbit in the treatment; μ = Overall mean, common element to all observations; D_i = Effect of the treatments (dour levels) ($i = 1, 2, 3$ and 4); B_j = Effect of the breeds ($j = 1$ and 2 NZW and CAL); DB_{ij} = The interaction between two factor; E_{ijk} = Random error component assumed to be normally distributed.

Results and Discussion

Body weight (BW) and feed intake (FI)

Bucks supplemented with dour significantly increased LBW and FI (Table 3) compared with control group. Our result are agreement with (Huda AL-amer, and Nabila, Rashwan 2012) they found, there was a significant increase in final weight, weight gain and feed intake consumed dour powder ($P < 0.05, 0.01$ & 0.001), extract, dour powder with selenium and dour extract with selenium ($P < 0.01$ & 0.001) rat groups compared with control. On the other hand (Shehu 2015) which recorded that dour significant ($p > 0.05$) difference on feed intake, this may be due to the absence of tannin in both the stem and bark extracts. No different between the breeds on feed intake and body weight.

Table 3. Live body weight and feed intake of CAL and NZW male rabbits as affected by supplementation with doum through 12 weeks

Items	Doum levels (g/kg)				<i>P</i> <i>value</i>	Species		<i>P</i> <i>value</i>	Interaction	
	T0 (0g)	T1 (0.3g)	T2 (0.6g)	T3 (0.9g)		NZE	CAL		SEM	<i>P</i> <i>value</i>
LBW (g)	3400 ^c	3450 ^b	3500 ^b	3620 ^a	0.0001	3450 ^b	3500 ^a	0.016	0.014	0.024
Feed intake (g/kg/day)	50.5 ^c	57.6 ^b	57.1 ^b	59.4 ^a	0.0001	57.6	57.1	0.540	0.440	0.740

^{abc} Means with different superscript letters within rows are significantly different ($P < 0.05$)

Semen characteristics:

Results indicated that semen volume, sperm concentration, total sperm output TFSF and fructose increased significantly ($p < 0.05$) in all treated groups compared with control (Table 4) and increased significantly with CAL compared with NZE. Sperm motility was increased significantly ($p < 0.05$) in treated groups compared with control but Not significant change ($p > 0.05$) with breed CAL or NZE. The highest sperm concentration was found in treatment 3. Supplemented rabbit bucks with doum induced a significant increase in normal sperm compared with control group. Mervat Ghazal (2016) concluded that supplemented doum to Hy-Plus rabbit diets caused significant improvement in semen quality and fertilizing ability of bucks. Improving fertility traits of Rabbit Does treated with doum can be attributed mainly to improve of semen quality, as recorded previously by (Ali and Mervat Ghazal, 2013).

Reaction time was decreased with doum and the lowest Reaction time found in group 3.

Biochemical constituents of seminal Plasma:

Biochemical evaluation of seminal plasma constituents of rabbit bucks is presented in

Table (5). Seminal plasma TP, Glob, ALP, AcP and LDH were significantly increased due to doum supplementation compared with control group. Bucks supplemented with the 0.9g doum/ kg diet was significantly better for all previous traits than for group 1 and group 2 separately but difference between the later groups was not significant except in AcP parameter. Seminal plasma albumin was not significantly differed among different groups. Bucks supplemented with doum showed a significant decrease in both seminal plasma ALT and AST activities compared with control group. No significant differences among 0.3g or 0.6g doum/kg diet in ALT activity were shown but 0.9 gDoum /kg diet (group3) significantly decreased AST activity compared to 1 or 2 groups (Table 5).

Seminal Plasma Antioxidant Constituents:

Data presented in Table (6) showed that seminal plasma antioxidant GPx, SOD and GST activities of rabbit bucks were significantly increased due to doum supplementations, while seminal plasma TBARS concentration was significantly ($P < 0.05$) decreased as compared with the control group. These improvements in antioxidant constituents were significantly

maximized of group 3. Meanwhile, TBARS concentration was significantly minimized of group3 only compared with control group. Bucks supplemented with 0.9gdoum /kg diet had significantly more GSH concentration than the control and the group fed on 0.3gdoum /kg diet. (Huda AL-amer, and Nabila, Rashwan 2012) reported that values of serum GPX and SOD were significantly increase in all treated groups ($P < 0.05$, 0.01 & 0.001), but the value of NO was decreased in doum powder rat groups ($P < 0.05$ & 0.01) compared with control. with a non-significant differences in GPX among doum powder, doum extract and doum powder rat groups.

Reproductive performance of rabbit bucks:

Fertility of rabbit bucks supplemented with doum as indicated of kindling rate was significantly improved compared with control group (Table 7). Litter size at birth and weaning were significantly increased compared with the control group. Bucks supplemented with group 3 showed significantly higher values of pervious parameters than those of group1 and control. Body weight at birth and at 28 days of age was significantly increased compared with control (Table 7). The rabbit bucks supplemented with the group 3 recorded the highest weight at birth and at 28 days of birth followed by 1 , 2 groups, respectively. California bucks recorded significantly increased compared with the NZW bucks.

Sexual hormones

Regarding, sexual hormones of male was represented by concentration of testosterone; estradiol 172α and progesterone (Table 8). High level of

doum recorded a significant ($p \leq 0.01$) increase in descending order, due to diet supplemented with 0.9, 0.6 and 0.3 g doum/kg diet, respectively (Table 8). These results agree with (Mervat et al., 2016). California bucks were recorded significantly increased compared with the NZW bucks. The interaction between feed additive and breeds was appositive effect that California rabbits which fed on 0.3 g doum /kg diet were recorded the highest value.

Conclusion

The results observed that doum treatments improved semen quality, antioxidant status and reproductive traits of rabbit bucks. Thus, the doum seems to be benefit for productive and reproductive efficiency of rabbit bucks in Upper Egypt.

Table 3. Live body weight and feed intake of CAL and NZW male rabbits as affect by supplementation with doum through 12 weeks

Items	Doum levels (g/kg)				<i>P value</i>	Species		<i>P value</i>	Interaction	
	T0	T1	T2	T3		NZE	CAL		SEM	<i>P value</i>
	(0g)	(0.3g)	(0.6g)	(0.9g)						
Live body weight (g)	3400 ^c	3450 ^b	3500 ^b	3620 ^a	0.0001	3450 ^b	3500 ^a	0.016	0.014	0.024
Feed intake (g/kg/day)	50.5 ^c	57.6 ^b	57.1 ^b	59.4 ^a	0.0001	57.6	57.1	0.540	0.440	0.740

^{abc} Means with different superscript letters within rows are significantly different (P<0.05).

Table 4. Effect of doum supplementation in diet of rabbit bucks on semen characteristics at 12 week

Items	Doum levels (g/kg)				<i>P value</i>	Species		<i>P value</i>	Interaction	
	T0	T1	T2	T3		NZE	CAL		SEM	<i>P value</i>
	(0g)	(0.3g)	(0.6g)	(0.9g)						
Ejaculate volume (ml)	0.65 ^d	0.83 ^c	0.90 ^b	1.00 ^a	0.0001	0.80 ^b	0.89 ^a	0.0001	0.025	0.293
Reaction time (sec.)	14.80 ^a	10.90 ^b	10.30 ^b	6.70 ^c	0.0001	10.90 ^a	10.50 ^a	0.195	0.531	0.216
Ph	7.72 ^a	7.00 ^b	6.84 ^b	6.88 ^b	0.0001	7.13 ^a	7.00 ^a	0.626	0.080	0.233
Sperm motility (%)	58.68 ^c	78.13 ^b	87.27 ^a	87.3 ^a	0.0001	77.85 ^a	77.84 ^a	0.997	2.142	0.053
Sperm concentration (10 ⁶ /ml)	285.18 ^d	334.32 ^c	367.33 ^b	416.66 ^a	0.0001	342.45 ^b	359.29 ^a	0.0001	9.02	0.0001
Total sperm output (10 ⁶)	186.92 ^d	273.14 ^c	333.65 ^b	421.45 ^a	0.0001	284.32 ^b	323.26 ^a	0.0001	16.13	0.0183
Total motile sperm (10 ⁶)	109.31 ^d	212.9 ^c	292.06 ^b	364.22 ^a	0.0001	231.84 ^b	257.413 ^a	0.0002	17.37	0.4229
Normal sperm (%)	78.25 ^c	83.41 ^b	86 ^a	86.01 ^a	0.0001	82.80 ^b	84.00 ^a	0.0174	0.619	0.1682
Live sperm (%)	66.99 ^c	81.17 ^b	81.87 ^b	84.71 ^a	0.0001	78.11 ^b	79.26 ^a	0.028	1.26	0.1375
TFSF (10 ⁶)	113 ^d .00	154.4 ^c	218.9 ^b	276.56 ^a	0.0001	167.76 ^b	213.75 ^a	0.0001	12.20	0.0001
fructose (g/100 ml)	167.22 ^d	223.16 ^c	238.8 ^b	248.4 ^a	0.0001	218.69 ^b	220.10 ^a	0.0038	5.65	0.5476

^{abcd} Means with different superscript letters within rows are significantly different (P<0.05).

pH=initial hydrogen ion concentration; TFSF= total functional sperm fraction.

Table 5 . Effect of doum Supplementation in Diet of Rabbit bucks on seminal plasma TP, Alb, Glob, ALT, AST, AIP, AcP and LDH

Items	Doum levels (g/kg)				<i>P value</i>	Species		<i>P value</i>	Interaction	
	T0	T1	T2	T3		NZE	CAL		SEM	<i>P value</i>
	(0g)	(0.3g)	(0.6g)	(0.9g)						
TP (g/dl)	5.97 ^c	6.70 ^b	6.65 ^b	6.92 ^a	0.0001	6.71	6.62	0.32	0.05	0.21
Alb (g/dl)	3.09	3.09	2.92	3.02	0.868	3.07	2.93	0.23	0.03	0.86
Glob(g/dl)	2.88 ^c	3.61 ^b	3.73 ^b	3.90 ^a	0.0001	3.64	3.69	0.54	0.06	0.29
ALT (IU)	33.30 ^a	25.50 ^b	26.20 ^b	24.90 ^b	0.0001	24.30	24.90	0.45	0.14	0.25
AST (IU)	55.60 ^a	49.40 ^b	49.10 ^b	46.30 ^c	0.0001	49.40	49.00	0.75	0.61	0.79
AIP (U/L)	53.20 ^c	59.43 ^b	60.00 ^b	63.70 ^a	0.0001	59.12	59.00	0.25	0.99	0.32
AcP (U/L)	48.10 ^d	55.50 ^b	53.20 ^c	60.40 ^a	0.0001	55.50	55.70	0.79	0.56	0.89
LDH (IU)	1120 ^c	1294 ^b	1306 ^b	1358 ^a	0.0001	1284	1288	0.21	17.00	0.41

^{abcd} Means with different superscript letters within rows are significantly different ($P < 0.05$). TP= total proteins; Alb=albumin; Glob=globulins; ALT=alanine aminotransferase; AST=aspartate aminotransferase; AIP=alkaline phosphatase; AcP=acid phosphatase; LDH=lactate dehydrogenase.

Table 6. Seminal plasma TBARS, GSH, GPx, SOD and GST of NZE and CAL rabbits as affect by supplementation with doum through 12 weeks.

Items	Doum levels (g/kg)				<i>P value</i>	Species		<i>P value</i>	Interaction	
	T0	T1	T2	T3		NZE	CAL		SEM	<i>P value</i>
	(0g)	(0.3g)	(0.6g)	(0.9g)						
TBARS (nmol/ml)	1.41 ^a	1.06 ^b	1.03 ^c	0.98 ^d	0.0001	1.12 ^b	1.14 ^a	0.88 ^{NS}	0.03	0.008
GSH (g/dl)	12.16 ^d	12.34 ^c	15.42 ^b	18.20 ^a	0.0001	15.03	14.57	0.88	0.51	0.89
GPx (g/L)	5.14 ^d	6.15 ^c	7.47 ^b	7.68 ^a	0.0001	6.61 ^a	6.46 ^b	0.75 ^{NS}	0.19	0.64
GST (IU)	1.07 ^c	1.24 ^b	1.31 ^a	1.32 ^a	0.0001	1.237	1.234	0.27	0.19	0.25
SOD (IU)	6.70 ^d	8.51 ^c	9.07 ^b	9.33 ^a	0.0001	8.41 ^a	8.20 ^b	0.37 ^{NS}	0.01	0.07

^{abc} Means with different superscript letters within rows are significantly different ($P < 0.05$). TBARS= Thiobarbituric acid-reactive substances; GSH=glutathione content; GPx=Glutathione peroxidase; SOD= Superoxide dismutase; GST=Glutathione S-transferase

Table 7. Reproductive indices of NZE and CAL rabbits as affect by supplementation with doum through 12 weeks.

Items	Doum levels (g/kg)				<i>P value</i>	Species		<i>P value</i>	Interaction	
	T0	T1	T2	T3		NZE	CAL		SEM	<i>P value</i>
	(0g)	(0.3g)	(0.6g)	(0.9g)						
Kindling rate %	54.65 ^d	73.4 ^c	89.05 ^b	90.60 ^a	0.0001	73.42 ^b	80.42 ^a	0.0001	0.90	0.0001
Litter size at birth	7.12 ^c	8.06 ^b	9.37 ^a	9.57 ^a	0.0001	8.18 ^b	8.78 ^a	0.0001	0.13	0.8186
Litter size at weaning	5.93 ^c	7.12 ^b	8.87 ^a	9.16 ^a	0.0001	8.01 ^b	8.28 ^a	0.0001	0.19	0.4596
Bunny weight at birth (g):	56.0 ^c	60.9 ^b	68.62 ^a	69.20 ^a	0.0001	62.12 ^b	65.17 ^a	0.0001	0.73	0.0080
Bunny weight at 28 days (g):	629.87 ^c	706.75 ^b	725.31 ^a	730.93 ^a	0.0001	666.09 ^b	730.34 ^a	0.0001	7.02	0.9477

Table 8. Sexual hormones concentration of NZE and CAL rabbit males fed diets with different levels of Doum

Sexual hormone	Doum levels (g/kg)				<i>P value</i>	Species		<i>P value</i>	Interaction	
	T0	T1	T2	T3		NZE	CAL		SEM	<i>P value</i>
	(0g)	(0.3g)	(0.6g)	(0.9g)						
Testosterone concentration (ng/ ml)	5.63 ^d	5.81 ^c	6.00 ^b	6.525 ^a	0.0001	5.90 ^b	6.09 ^a	0.0001	0.0681	0.0001
Estradiol 172 α (pg/ ml)	27.13 ^d	27.88 ^c	29.31 ^b	30.85 ^a	0.0001	28.75 ^b	28.84 ^a	0.0001	0.25564	0.0001
Progesterone (pg/ ml)	0.715 ^d	0.730 ^c	0.804 ^b	0.840 ^a	0.0001	0.768 ^b	0.777 ^a	0.0015	0.0093	0.1699

^{abcd} Means with different superscript letters within rows are significantly different (P<0.05).

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