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IN VIVO AND *IN VITRO* STUDIES ON THE EFFECT OF *Ganoderma* ON RABBIT REPRODUCTIVITY, SEMEN PRESERVATION AND ARTIFICIAL INSEMINATION

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ABSTRACT

This study was designed to evaluate mature New-Zealand White (NZW) rabbit reproductivety as affected by different levels of Ganoderma® in diets. Animals were divided into three comparable experimental groups (16 sexual mature bucks and 52 mono-parous does in each). The first group was fed a commercial diet and kept untreated (control group), while the 2nd and 3rd groups (treated groups) were fed the same diets but supplemented with 200 and 400 g Ganoderma®/ ton diet, respectively. Results obtained showed that, feeding diet supplemented with different levels of Ganoderma® improved significantly (P≤0.05) libido and physical semen quality of bucks, while values of semen enzymatic activity were decreased significantly (P≤0.05) and sperm penetration into estrus cow cervical mucus significantly (P≤0.05) increased due to treatments. Diluted semen ejaculated by NZW rabbit bucks fed diet supplemented with different levels of Ganoderma® or/ and added with 1 ml Ganoderma® aquas extract/100 ml diluted semen recorded significant (P≤0.05) increase in viability, quality and storagability, during the storage at refrigeration and incubation temperatures. Diluted semen ejaculated by rabbit bucks fed diet contained Ganoderma® and supplemented with Ganoderma® showed quality significantly (P≤0.05) better than that ejaculated by treated bucks and free Ganoderma®, followed by diluted semen of untreated bucks and free Ganoderma®, respectively. Physical semen quality and sperm penetration into estrus cow cervical mucus values significantly (P≤0.05) decreased and increased, respectively, with progressive of conservation time. Fertility traits of does represented in (rates of each of abortion, conception and kindling and values of each of litter size and weight at birth and at weaning, as well as, bunny weight at birth and at weaning were significantly (P≤0.05) better by adding different levels of Ganoderma® in diets. The improvement in parameters indicated fertilizing ability of bucks and fertility traits of does were arranged (P≤0.05) in descending order as recorded by 400, 200 then zero g Ganoderma®/ ton diet, respectively. While 400 g Ganoderma®/ton recorded insignificantly improvements in most studied parameters than 200 g Ganoderma®/ton.

In conclusion, NZW rabbit bucks or does feeding diets supplemented with different levels of Ganoderma® recorded significant (P≤0.05) improvement in all parameters studied, during mature period. This means that 200 g Ganoderma®/ ton diet is recommended for mature rabbits.

Keywords*:* Rabbit, semen, *Ganoderma*®, reproduction, fertility.

INTRODUCTION

After the appearance of avian influenza disease in several countries and the decrease of white meat production from poultry, rabbit industry can play an essential role in solving a part of this meat shortage and overcoming the gap between demand and supply of the animal protein.

Ganoderma is red algae sea used in human and animal feeding in order to improve body performance through amelioration of food properties, promotion of production performance, and improving the quality of animal origin food (Windisch *et al.*, 2008; Steiner, 2009 and El-Kholy *et al.*, 2012). This class of feed additives has recently gained interest, In addition, efficacy, application of *Ganoderma* feed additives to livestock has to be also safe to the animal, the user, the consumer of the animal product, and the environment (Mariey *et al.* 2012). *Ganoderma* has strong antibacterial properties, anticandidial, antiulcer, analgesic, antioxidant and hypocholesterolaemic activities (Mariey *et al.* 2012). The essential oil from red algae has acts as antimicrobial effects against animal and plant pathogens. Ali (2006) added that *Ganoderma* antioxidant capabilities are 20 times more powerful than vitamin C and 50 times more potent than vitamin E. *Ganoderma* was used as digestive stimulants, antiseptic, and appetite stimulants in human beings as well (Ali, 2006 and Mariey *et al.* 2012).

Wide range of products is available to help promote gut health of poultry and animals for improving performance under field conditions and stressors. The most commonly used compounds are antibiotics and anti-coccidials. But several other types of products now available especially after the ban on using antibiotic growth promoter 'AGP' (Marzo, 2001 and Seleem *et al.* 2007).

There are concerns that, the large-scale application of antibiotics may contribute to the evolution of antibiotics-resistant microbes. As a result, several countries have placed restrictions on its usage (Quirk, 2001 and Ali, 2006).

Many attempts have continuously been conducted to improve animal productive and reproductive performances under uncomfortable conditions by adding some minerals; vitamins; prebiotics; probiotics; synbiotics and natural herbs in drinking water and/ or diet to avoid several problems resulted from over using of chemical and drugs as a growth promoter or fertility stimulators.

Nowadays, a great attention has been focused to apply artificial insemination (AI) in most Rabbitries where intensive rabbit raising is practiced (Seleem and Rowida, 2007). Although AI maximizes the economical profit of rabbit industry by reducing the bucks and cages numbers (Khalifa *et al.*, 2002), it is still practiced on a very limited scale in Egyptian Rabbitries. One of the main constraints is that the disability of buck semen to be stored for a long period without the loss of its fertilization ability (Seleem *et al.*, 2008). The reason behind this disability may be due to the kind of the extender and additives used.

Recently, the artificial insemination (AI) technique is favorable and most suitable for both small and large commercial rabbitries (Lavara *et al.*, 2000 and Seleem and Rowida, 2007).

There are no available information in the literature on using *Ganoderma* as additive natural source in rabbit production. From this view, great attention must be focused on using *Ganoderma* in rabbit industry.

The present study was designed to throw some light on the effect of dietary supplementation of *Ganoderma* on reproductive performance of rabbit

bucks. Also, semen preservation and artificial insemination of rabbits as affected by different levels of *Ganoderma*® in diets were evaluated.

MATERIALS AND METHODS

Animals and general management:

The present work was carried out in an Industrial Rabbitry, El-Azeziah Vilage, near Sakarah City, Giza Province, Egypt. The laboratory work was conducted in Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. Rabbits were raised in semi-closed rabbitry with wire-netted windows on their sides for providing natural ventilation. The windows were oriented with an elevation of 2 meters from the floor. The floor was made from ceramic plates and has moderately slope (from the middle to both sides) to facilitate water drainage towards a large longitudinal gutter outside the Rabbitry.

The ingredients and chemical composition of the pelleted ration fed to rabbits, during the experimental period was as shown in Table (1)**.** Composition and chemical analysis of Ganoderma® used in the experiment are shown in Table (2).

Table (1): Ingredients and chemical composition of the pelleted ration fed to rabbits, during the experimental period (manufactured by IBEX International Co. L.®)

Ingredients	**(%)**	**Vitamins & Minerals premix per Kilogram.**	
Clover hay	40.50	Vit.A (IU)	10000
Wheat bran	25.00	Vit.D3 (IU)	9000
Yellow corn	14.00	Vit.E (IU)	10000
Soybean meal (44%)	11.00	Vit.K (IU)	3
Molasses	3.00	Vit.B1 (IU)	2
Vinass	3.00	Vit.B2 (IU)	6
Bone meal	1.75	Vit.B6 (IU)	2
Lime stone	0.70	Biotin (mg)	0.2
Sodium chloride	0.55	Choline (mg)	1200
Vitamins & Mineral Premix	0.35	Niacine (mg)	40
DL-Methionine	0.15	Zn. (mg)	60
Total	**100**	Cu. (mg)	0.1
Calculated chemical composition **		Mn. (mg)	85
Crude protein (CP)%	18.00	Fe. (mg)	75
Ether extract (EE)%	3.00	Folic acid (mg)	5
Crude fiber (CF)%	14.00	Pantothenic acid (mg)	20
Digestible energy (Kcal/Kg)	2720.00		

**** Calculated according to NRC (1994) for rabbits.**

Table (2): Composition and chemical analysis of *Ganoderma* used in the experiment.

Item	(%)
Glucose (mg/ dl)	21.90
Total protein (g/ dl)	03.70
Albumen (g/ dl)	00.60
Globulin (g/ dl)	03.13
Phosphorus (mg/ dl)	01.64
Calcium (mg/ dl)	48.59
Sodium (g/ dl)	01.45
Potassium (g/ dl)	02.04
Magnesium (g/ dl)	07.48
Iron (mg/ dl)	08.40
Zinc (mg/ dl)	00.54
Copper (mg/ dl)	00.53
pH	03.60

The experimental work:

A total number of 204 sexually mature New-Zealand White (NZW) rabbits (48 bucks aged 9 months, and 156 mono-parous does) was used in the present study. This study included two experiments. All animals in each experiment were divided into three equal comparable experimental groups (16 sexual mature bucks and 52 mono-parous does in each). The first group was kept untreated (control group) and fed a commercial diet covering the nutritional requirements of different physiological status of rabbits according to NRC (1994) recommendations, while the 2nd and 3rd groups (treated groups) were fed the same diet but supplemented with 200 and 400 g *Ganoderma*®/ ton, respectively. The experiments were as follow:

Aqueous extract of *Ganoderma*® was prepared by transfer of 5 grams of *Ganoderma*® powder to sterile wide-mouthed screw-capped bottles; 125 ml of sterile de-ionized distilled water was added to the powder samples and allowed to be soaked for 3 hours. The mixture was then centrifuged at 1000 rpm for half an hour. The supernatants were filtered through a 0.45 µm membrane (Kim *et al.,* 2002; Seleem and Rowida, 2005; Seleem *et al.,* 2007 and Rowida *et al.,* 2009).

Data collection:

Libido (sexual desire) was assessed in term of reaction time in seconds that was estimated just from the time of introducing doe to the buck until the buck start to mount (Daader *et al.,* 1999 a & b and Seleem, 2003). Semen was collected artificially twice a week for up to five weeks by means of an artificial vagina as described by Seleem (1996 and 2003). Semen samples ejaculated from each rabbit buck were evaluated individually microscopically and then semen ejaculate volume (ml); advanced sperm motility (%); dead spermatozoa (%); sperm abnormalities (%); sperm-cell concentration (N x 10^6/ ml) and total-sperm output (N x 10^6/ ejaculate) were estimated according to Salisbury *et al.* (1978). Acrosomal damages were determined by using a Giemsa stain procedure as described by Watson (1975).

The ejaculated semen of each buck within treated groups was evaluated microscopically and only ejaculates that showed advanced motility more than 70% were pooled and diluted with sodium chloride diluent (0.9 gm sodium chloride + 5.0 ml egg yolk + 50000 IU sodium penicillin + 5000 µg streptomycin sulfate and completed up to 100 ml with distilled water) at 1: 5 dilution rate. The diluted semen was divided into two equal parts; the first was kept untreated (free additives), while the second part was supplemented with 1 ml *Ganoderma*® aquas extract/ 100 ml diluted semen. Thereafter, each part of diluted rabbit semen was subdivided into two equal portions, the first was chilled at refrigeration temperature (4 - 6 ^{o}C) for up to 3 days, and the second was incubated at 37 ^{o}C for up to 4 hours. Percentages of advanced sperm motility, spermatozoa storagability, abnormal and dead spermatozoa and acrosomal damages were recorded at different stages of preservation according to Boiti *et al.* (2005); Castellini *et al.* (2006) and Fadila *et al.* (2013).

$$\text{Storageability} = \frac{\text{Final advanced sperm motility (after 4 hours)}}{\text{Initial advanced sperm motility (at zero time)}} \times 100$$

Diluted semen samples of treated rabbit bucks were centrifuged at 6000 r.p.m. for 20 minutes before removal of the supernatant and used for enzymatic assay. The activities of aspartate aminotransferase (AST) and alanine aminotranferase (ALT) were estimated according to Reitman and Frankel (1957) and Gella *et al*. (1985). Acid phosphatase (ACP) and alkaline phosphatase (ALP) enzymes were determined calorimetrically according to Graham and Pace (1967). Sperm penetration into estrus cow cervical mucus of extended semen was assessed during incubation condition at 37^{0}C for 4 hours as was studied by Daader *et al.* (1997); Seleem *et al.* (2009) and Rowida *et al.* (2009).

In the fertility traits, 156 mono-parous NZW rabbit does in three comparable experimental groups (52 rabbit does in each) and received the same treatments of rabbit bucks were artificially inseminated using diluted semen ejaculated by corresponding experimental groups of rabbit bucks. The artificial insemination was carried out as described by Boiti *et al.* (2005). Palpation of all rabbit does was carried out 12 days post inseminating to determine pregnancy and conception rate. At kindling, kindling and abortion rates and litter size and weight at birth and at weaning values were recorded. Bunny weight at birth and at weaning was also recorded.

Statistical analyses:

Data were subjected to analysis of variance according to Snedecor and Cochran (1982) using the General Linear Model Program of SAS (2001). Duncan's new multiple range tests were used to test the significance of the differences among means (Duncan, 1955). Data presented as percentages were transformed to the corresponding arcsine values (Warren and Gregory, 2005) before being statistically analyzed.

RESULTS AND DISCUSSION

Libido and physical semen characteristics:

Data presented in Table (3) showed that feeding diet supplemented with different levels of red algae (*Ganoderma*®), 200 or 400 gm/ton significantly (P<0.05) improved libido and physical semen characteristics in terms of ejaculate semen volume; percentages of mass and advanced motility; dead and abnormal and acrosomal damages of spermatozoa, and sperm cell concentration per ml and per ejaculate) in semen of NZW rabbit bucks.

The improvement in libido and physical semen characteristics of treated rabbit bucks may be brought through improving the efficiency of feed utilization**.** Dietary inclusion of red algae can partially offset the adverse effects of toxins on animal performance (Mariey *et al.*, 2012). Moreover, adding red algae (*Ganoderma*®) to the diet may enhance immune function (Qureshi *et al.*, 1994 and 1995).

These results emphasize the hypothesis that rabbit buck fertility could be improved by stimulation of testicular androgen secretion induced by feeding diet containing red algae (*Ganoderma*®).

Table 3. Libido and physical semen characteristics of NZW rabbit bucks fed diets supplemented with different levels of *Ganoderma*® (Mean ± SE).

Items	*Ganoderma* levels (g/ton)		
	(0.0) Control	(200) T_1	(400) T_2
Libido (Sexual desire) (Sec.)	45.71±3.45[a]	27.14±2.91 [b]	25.32±1.99 [b]
Semen ejaculate volume (ml)	0.396±0.034[b]	0.641±0.037[a]	0.673±0.044[b]
Mass sperm motility (Score)	2.87±0.32 [b]	3.93±0.43 [a]	3.98±0.36 [a]
Advanced sperm motility (%)	52.67±3.86 [b]	69.63±4.51 [a]	72.94±4.96 [a]
Dead spermatozoa (%)	29.34±2.75[a]	21.48±1.83 [b]	20.97±1.61[b]
Sperm abnormalities (%)	24.71±2.26 [a]	17.42±2.10 [b]	15.93±1.82 [b]
Acrosomal damages (%)	16.49±1.27 [a]	13.07±1.01 [b]	12.81±1.06 [b]
Sperm-cell concentration ($N \times 10^6$/ ml)	471.83±31.62[b]	632.94±37.82[a]	676.31±41.03[a]
Total-sperm output ($N \times 10^6$/ ejaculate)	186.84±11.73 [c]	405.71±23.64[b]	455.16±22.41[a]

Means bearing different letter superscripts (a, b, c) within the same row, are significantly (P ≤ 0.05) different.

Diluted semen preservation:

Tables (4-11) clearly showed that diluted semen ejaculated by NZW rabbit bucks fed diet supplemented with 200 or 400 gm/ton of red algae (*Ganoderma*®) with or without 1 ml of red algae (*Ganoderma*®) aqua's extract/100 ml diluted semen significantly (P≤0.05) recorded an increase in sperm motility and storagability, and a decrease in percentages of each of dead and abnormal spermatozoa and acrosomal damages, during the storage at refrigeration temperature (4-6 °C) up to 3 days and incubation at 37 °C for 6 hours.

Table (4): Effect of *Ganoderma*® supplementation to diet and/or semen on percentages of advanced sperm motility and storagability, during chilled storage up to 3 days of NZW rabbit bucks (Mean ± SE).

Chilled period (Day)	*Ganoderma*/ 100 ml diluted semen	*Ganoderma* levels (g/ton diet)			Mean ± SE
		(0.0) Control	(200) T_1	(400) T_2	
0.0	0.0	51.87±3.24	68.74±4.07	71.65±3.91	64.09±2.47
	1.0	51.95±3.11	68.81±3.96	71.79±3.88	64.18±2.54
Means ± SE		51.91±2.86 ^{b}	68.78±3.92 ^{a}	71.72±3.74 ^{a}	64.14±2.31 ^{A}
1.0	0.0	43.24±2.43	62.31±2.84	67.37±2.93	57.64±2.14
	1.0	46.87±2.71	64.99±3.12	68.98±3.03	60.28±2.41
Means ± SE		45.06±2.33 ^{b}	63.65±2.71^{a}	68.18±2.74 ^{a}	58.96±2.07 ^{B}
3.0	0.0	34.15±2.11	55.73±2.51	61.07±2.52	50.32±1.84 ^{B}
	1.0	39.72±2.27	59.82±2.84	64.19±2.93	54.58±2.27 ^{A}
Means ± SE		36.94±2.01 ^{c}	57.78±2.31 ^{b}	62.63±2.44 ^{a}	52.45±1.71 ^{C}
Overall means± SE		44.64±2.19 ^{b}	63.40±2.36 ^{a}	67.51±2.41 ^{a}	58.52±1.37
Spermatozoa storagability (%)	0.0	65.84±3.59	81.07±4.85	85.23±4.91	77.38±3.62
	1.0	76.46±3.71	86.94±4.81	89.41±4.96	84.27±3.94
Means ± SE		71.15±3.72 ^{b}	84.01±4.61 ^{a}	87.32±4.56 ^{a}	80.83±3.18

Means bearing different letter superscripts (a,b,c) within the same row, or (A,B,C,*A*,*B*) within the same column are significantly (P ≤ 0.05) different.

Table (5): Effect of *Ganoderma*® supplementation to diet and/or semen of NZW rabbit bucks on dead spermatozoa percentages, during chilled storage up to 3 days (Mean ± SE).

Chilled period (Day)	*Ganoderma*/ 100 ml diluted semen	*Ganoderma* levels (g/ton diet)			Mean ± SE
		(0.0) Control	(200) T_1	(400) T_2	
0.0	0.0	30.21±2.81	21.87±1.94	21.17±1.82	24.42±1.76
	1.0	29.92±2.57	21.81±1.96	21.12±1.67	24.28±1.53
Means ± SE		30.07±2.18 ^{a}	21.84±1.72 ^{b}	21.15±1.55 ^{b}	24.35±1.39^{C}
1.0	0.0	37.43±2.97	27.89±2.11	26.34±1.89	30.55±1.99
	1.0	34.99±2.69	25.92±1.99	24.16±2.01	28.36±1.83
Means ± SE		36.21±2.44 ^{a}	26.91±1.84 ^{b}	25.25±1.70 ^{b}	29.45±1.74^{B}
3.0	0.0	48.01±3.14	36.07±2.73	33.51±2.56	39.20±2.65
	1.0	43.14±2.80	32.11±2.47	30.21±2.37	35.15±2.31
Means ± SE		45.58±2.74 ^{a}	34.09±2.17 ^{b}	31.86±2.18 ^{b}	37.18±2.01^{A}
Overall means± SE		37.29±2.11 ^{a}	27.61±1.69 ^{b}	26.09±1.58 ^{b}	30.33±1.46

Means bearing different letter superscripts (a,b) within the same row, or (A,B,C) within the same column are significantly (P ≤ 0.05) different.

Table (6): Effect of *Ganoderma*® supplementation to diet and/or semen of NZW rabbit bucks on sperm abnormalities percentages, during chilled storage up to 3 days (Mean ± SE).

Chilled period (Day)	*Ganodermal* 100 ml diluted semen	*Ganoderma* levels (gm/ Ton diet)			Means ± SE
		(0.0) Control	(200) T_1	(400) T_2	
0.0	0.0	25.17±2.57	17.86±2.33	16.42±1.94	19.82±1.83
	1.0	25.12±2.43	17.82±2.25	16.40±1.88	19.78±1.69
Means ± SE		25.15±2.24[a]	17.84±2.11[b]	16.41±1.81[b]	19.80±1.54[B]
1.0	0.0	30.21±2.61	20.93±2.27	18.49±1.84	23.21±1.96
	1.0	29.11±2.48	19.81±2.14	17.50±1.68	22.14±1.89
Means ± SE		29.66±2.39[a]	20.37±2.01[b]	18.00±1.57[b]	22.68±1.61[B]
3.0	0.0	37.34±2.73	26.01±2.54	21.57±1.91	28.31±2.11
	1.0	35.10±2.68	21.94±2.21	19.64±1.96	25.56±2.03
Means ± SE		36.22±2.49[a]	23.98±2.17[b]	20.61±1.86[b]	26.94±1.97[A]
Overall means± SE		30.34±2.13[a]	20.73±1.86[b]	18.34±1.83[b]	23.14±1.74

Means bearing different letter superscripts (a,b) within the same row, or (A,B) within the same column are significantly (P ≤ 0.05) different.

Table (7). Effect of *Ganoderma*® supplementation to diet and/or semen of NZW rabbit bucks on acrosomal damages percentages, during chilled storage up to 3 days (Mean ± SE).

Chilled period (Day)	*Ganodermal* 100 ml diluted semen	*Ganoderma* levels (g/ton diet)			Means ± SE
		(0.0) Control	(200) T_1	(400) T_2	
0.0	0.0	16.89±1.41	13.46±1.22	12.97±1.14	14.44±1.11
	1.0	16.24±1.30	12.81±1.14	12.33±1.01	13.79±0.99
Means ± SE		16.57±1.26[a]	13.14±1.11[b]	12.65±0.97[b]	14.12±0.92[C]
1.0	0.0	22.91±1.52	18.49±1.27	16.99±1.09	19.46±1.02
	1.0	21.14±1.39	16.73±1.11	15.27±1.12	17.71±0.94
Means ± SE		22.03±1.38[a]	17.61±1.02[b]	16.13±0.86[b]	18.59±0.88[B]
3.0	0.0	30.81±1.53	24.54±1.16	21.13±1.03	25.49±1.02[*A*]
	1.0	28.09±1.44	21.69±1.14	18.07±1.07	22.62±0.96[*B*]
Means ± SE		29.45±1.39[a]	23.12±1.11[b]	19.60±0.94[c]	24.05±0.84[A]
Overall means± SE		22.68±1.12[a]	17.96±0.86[b]	16.13±0.87[c]	18.92±0.85

eans bearing different letter superscripts (a,b,c) within the same row, or (A,B,*A*,*B*) within the same column are significantly (P ≤ 0.05) different.

Table (8): Effect of *Ganoderma*® supplementation to diet and/or semen of NZW rabbit bucks on percentages of advanced sperm motility and storagability, during incubation condition up to 4 hours (Mean ± SE).

Incubation period (h)	*Ganoderma*/ 100 ml diluted semen	*Ganoderma* levels (g/ton diet)			Means ± SE
		(0.0) Control	(200) T_1	(400) T_2	
0.0	0.0	51.92±3.17	68.91±4.11	71.87±3.68	64.23±3.54
	1.0	52.11±3.14	68.99±3.84	71.94±3.91	64.35±3.61
Means ± SE		52.02±3.01 ^{b}	68.95±3.86 ^{a}	71.91±3.87 ^{a}	64.29±3.35 ^{A}
2.0	0.0	44.15±2.51	62.97±2.69	67.91±2.83	58.34±2.47
	1.0	47.12±2.69	67.53±3.18	69.41±3.37	61.35±2.75
Means ± SE		45.64±2.36 ^{b}	65.25±2.74 ^{a}	68.66±2.89 ^{a}	59.85±2.29 ^{A}
4.0	0.0	35.27±2.19	56.92±2.39	62.11±2.31	51.43±2.06
	1.0	40.07±2.34	60.82±2.80	65.00±2.69	55.30±2.12
Means ± SE		37.67±2.04 ^{c}	58.87±2.18 ^{b}	63.56±2.25 ^{a}	53.36±1.97 ^{B}
Overall means± SE		45.11±1.99 ^{b}	64.36±2.13 ^{a}	68.04±2.21 ^{a}	59.17±1.74
Spermatozoa storagability (%)	0.0	67.93±3.42	82.60±4.88	86.42±4.82	78.98±3.31
	1.0	76.90±3.59	88.16±4.71	90.35±4.93	85.14±3.86
Means ± SE		72.42±3.36 ^{b}	85.38±4.13 ^{a}	88.36±4.42 ^{a}	82.06±3.14

Means bearing different letter superscripts (a,b,c) within the same row, or (A,B) within the same column are significantly (P ≤ 0.05) different.

Table (9): Effect of *Ganoderma*® supplementation to diet and/or semen of NZW rabbit bucks on dead spermatozoa percentages, during incubation condition up to 4 hours (Mean ± SE).

Incubation period (h)	*Ganoderma*/ 100 ml diluted semen	*Ganoderma* levels (gm/ Ton diet)			Means ± SE
		(0.0) Control	(200) T_1	(400) T_2	
0.0	0.0	30.12±2.69	21.51±1.87	21.01±1.92	24.21±1.91
	1.0	29.84±2.64	21.65±1.85	20.92±1.59	24.14±1.83
Means ± SE		29.98±2.37 ^{a}	21.58±1.69 ^{b}	20.97±1.44 ^{b}	24.18±1.52^{C}
2.0	0.0	36.87±2.81	27.27±2.36	26.04±1.72	30.06±2.02
	1.0	34.51±2.74	25.59±1.83	23.91±2.19	28.00±1.99
Means ± SE		35.69±2.69 ^{a}	26.43±1.94 ^{b}	24.98±1.85 ^{b}	29.03±1.93^{B}
4.0	0.0	47.14±3.31	35.73±2.52	33.12±2.61	38.66±2.19
	1.0	42.61±2.69	31.83±2.53	29.63±2.30	34.69±2.22
Means ± SE		44.88±2.54 ^{a}	33.78±2.14 ^{b}	31.38±2.21 ^{b}	36.68±2.01^{A}
Overall means± SE		36.85±2.11 ^{a}	27.26±1.74 ^{b}	25.78±1.57 ^{b}	29.96±1.62

Means bearing different letter superscripts (a,b) within the same row, or (A,B,C) within the same column are significantly (P ≤ 0.05) different.

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Table (10). Effect of *Ganoderma*® supplementation to diet and/or semen of NZW rabbit bucks on sperm abnormalities percentages, during incubation condition up to 4 hours (Mean ± SE).

Incubation period (h)	*Ganodermal* 100 ml diluted semen	*Ganoderma* levels (g/ton diet)			Means ± SE
		(0.0) Control	(200) T_1	(400) T_2	
0.0	0.0	25.07±2.64	17.61±2.05	16.19±1.86	19.62±1.89
	1.0	25.03±2.31	17.54±2.14	16.13±1.80	19.57±1.95
Means ± SE		25.05±2.16 [a]	17.58±1.95 [b]	16.16±1.78 [b]	19.60±1.77 [B]
2.0	0.0	29.83±2.57	20.54±2.13	17.89±1.72	22.75±2.07
	1.0	28.71±2.51	19.34±2.02	17.06±1.43	21.70±2.00
Means ± SE		29.27±2.41 [a]	19.94±1.99 [b]	17.48±1.89 [b]	22.23±1.93 [B]
4.0	0.0	36.91±2.58	25.42±2.19	21.12±1.99	27.82±2.14
	1.0	34.87±2.71	21.63±2.11	19.31±1.82	25.27±2.11
Means ± SE		35.89±2.53 [a]	23.53±2.06 [b]	20.22±2.03 [b]	26.55±1.94 [A]
Overall means± SE		30.07±2.04 [a]	20.35±1.88 [b]	17.95±1.74 [b]	22.79±1.69

Means bearing different letter superscripts (a,b) within the same row, or (A,B) within the same column are significantly (P ≤ 0.05 or 0.01) different.

Table (11). Effect of *Ganoderma*® supplementation to diet and/or semen of NZW rabbit bucks on acrosomal damages percentages, during incubation condition up to 4 hours (Mean ± SE).

Incubation period (h)	*Ganodermal* 100 ml diluted semen	*Ganoderma* levels (g/ton diet)			Means ± SE
		(0.0) Control	(200) T_1	(400) T_2	
0.0	0.0	16.78±1.33	13.37±1.14	12.91±1.15	14.35±1.04
	1.0	16.11±1.27	12.63±1.03	12.28±1.12	13.67±0.99
Means ± SE		16.45±1.21 [a]	13.00±0.96 [b]	12.60±1.01 [b]	14.02±0.91 [C]
2.0	0.0	22.26±1.27	17.84±1.32	16.34±1.01	18.81±1.09
	1.0	20.49±1.29	16.14±1.19	14.55±1.01	17.06±1.16
Means ± SE		21.38±1.24 [a]	16.99±1.07 [b]	15.45±0.84 [b]	17.94±0.86 [B]
4.0	0.0	30.04±1.37	23.79±1.09	20.39±1.12	24.74±1.13
	1.0	27.43±1.26	20.97±1.03	17.42±1.11	21.94±1.11
Means ± SE		28.74±1.28 [a]	22.38±1.04 [b]	18.91±0.94 [c]	23.34±0.91 [A]
Overall means± SE		22.19±1.13 [a]	17.46±0.93 [b]	15.65±0.82 [c]	18.43±0.79

Means bearing different letter superscripts (a,b,c) within the same row, or (A,B,C) within the same column are significantly (P ≤ 0.05) different.

Diluted semen ejaculated by NZW rabbit bucks fed diet contained red algae (*Ganoderma*®) and supplemented with red algae (*Ganoderma*®) showed significantly (P≤0.05) better quality than that of those ejaculated by treated bucks without red algae (*Ganoderma*®), followed by diluted semen of untreated bucks and free from red algae (*Ganoderma*®).

Advanced sperm motility percentages significantly (P≤0.05) decreased, while percentages of each of dead and abnormal spermatozoa and acrosomal damages significantly (P≤0.05) increased with progressive storage time, either at refrigeration temperature (4-6 °C) up to 3 days or incubation at 37 °C up to 6 hours.

The decreasing recorded in the percentages of sperm motility may be due to the decrease in content of adenosine tri-phosphate (ATP) resulting in inactivation of spermatozoa (Zeidan *et al.,* 2008). This trend might attributed to an increase in lactic acid accumulated by time caused by the sperm anaerobic metabolism leading to changes in both osmotic pressure and pH value in the media exerting deleterious effect on the sperm cells (Seleem *et al.,* 2007).

Spermatozoa enzymatic activity:

Data shown in Table (12) showed that enzymatic activity in spermatozoa represented in leakage of AST; ALT; ACP and ALP into intracellular media of NZW rabbit bucks were decreased significantly (P≤0.05) due to feeding diet contained 0.02 or 0.04% red algae (*Ganoderma*®).

Table (12): Spermatozoa enzymatic activity in semen of NZW rabbit bucks fed diets supplemented with different levels of *Ganoderma*® (Mean ± SE).

Enzymatic activity	*Ganoderma* levels (g/ton diet)		
	(0.0) Control	(200) T_1	(400) T_2
AST (U/ L)	30.64±1.42 [a]	26.84±1.29 [b]	26.02±1.31 [b]
ALT (U/ L)	22.19±0.99[a]	18.06±0.83 [b]	17.63±0.81 [b]
Acid phosphatase ACP (U/ L)	28.94±1.17 [a]	26.41±1.12 [b]	25.17±1.12[b]
Alkaline phosphatase ALP (U/ L)	37.94±1.89 [a]	33.23±1.58 [b]	31.99±1.36 [b]

Means bearing different letter superscripts (a,b) within the same row are significantly (P ≤ 0.05) different.

The increase in leakage of the intracellular AST; ALT; ACP and ALP enzymes into the extra cellular medium may reflect the breakdown of the sperm cellular membrane (Seleem and Rowida, 2005; Zeidan *et al.,* 2008). However, leakage of these enzymes significantly (P≤0.05) decreased due to supplementation of red algae (*Ganoderma*®) to the diet. These results may emphasize that red algae (*Ganoderma*®) play a vital role in membrane integrity of spermatozoa in semen of of treated rabbit bucks, consequently decreasing activity of AST; ALT; ACP and ALP in extracellular medium. This is in parallel with level of red algae (*Ganoderma*®).

Sperm penetration into estrus cow cervical mucus:

Ability of spermatozoa in semen of NZW rabbit bucks to penetrate into estrus cow cervical mucus significantly (P≤0.05) increased due to feeding diet supplemented with 200 or 400 g red algae (*Ganoderma*®)/ton (Table 13). Sperm penetration into estrus cow cervical mucus values significantly (P≤0.05) increased with advancing incubation time at 37 °C up to 6 hours. .

These results may be attributed to the beneficial effect of red algae (*Ganoderma*®) on increasing advanced sperm motility in the reactivated media (Table 3) and consequently increasing sperm penetration into cervical mucus.

Table (13): Penetration of spermatozoa, of NZW bucks fed diets supplemented with different levels of *Ganoderma*®, into estrus cow cervical mucus (mm/hour) during incubation for 6 hours (Mean ± SE).

Incubation period (h)	*Ganoderma* levels (g/ton diet)			Means ± SE
	(0.0) Control	(200) T_1	(400) T_2	
1.5	17.52±1.21 ^{b}	21.43±1.42 ^{a}	23.22±1.37 ^{a}	20.72±0.91^{D}
3.0	27.18±1.87 ^{b}	35.24±1.99 ^{a}	38.46±2.13 ^{a}	33.63±1.84^{C}
4.5	38.51±2.63 ^{b}	51.06±3.82 ^{a}	55.63±3.91^{a}	48.40±2.41^{B}
6.0	51.92±2.97 ^{b}	66.99±3.91^{a}	73.71±4.11^{a}	64.21±3.22^{A}
Means ± SE	33.78±1.52 ^{c}	43.68±1.74 ^{b}	47.76±2.24 ^{a}	41.74±2.01

Means bearing different letter superscripts (a,b,c) within the same row, or (A,B,C,D) within the same column are significantly (P ≤ 0.05) different.

Fertility traits:

Fertility rate of NZW rabbit does represented in terms of rates of abortion, conception and kindling, values of litter size and litter weight at birth and at weaning, and bunny weight at birth and at weaning were significantly (P≤0.05 or 0.01) improved by adding different levels of red algae (*Ganoderma*®) in diets of rabbit bucks (200 or 400 gm/ ton).

The increase in kindling rate in rabbit does inseminated artificially by diluted semen ejaculated by red algae (*Ganoderma*®) treated bucks, can be attributed mainly to improve of semen quality (Table 14). In this respect, Lavaraa *et al.* (2005) showed significant correlations were observed between kindling rate and the percentage of total motile cells, and the percentage of abnormal sperm in the sample.

Table (14): Fertility traits of NZW rabbit does fed diets supplemented with different levels of *Ganoderma*® using artificial insemination (Mean ± SE).

Item	*Ganoderma* levels (gm/ Ton diet)		
	(0.0) Control	(200) T_1	(400) T_2
No. of inseminated does	52	52	52
No. of pregnant does	38	44	46
No. of kindled does	36	43	46
Abortion rate (%)	5.26 ^{a}	2.27 ^{b}	0.00 ^{c}
Conception rate (%)	73.08 ^{c}	84.62 ^{b}	88.46 ^{a}
Kindling rate (%)	69.23 ^{c}	82.69 ^{b}	88.46 ^{a}
Litter size at birth (n)	6.41±0.54 ^{b}	7.79±0.63 ^{a}	8.76±0.65 ^{a}
Litter weight at birth (g)	269.9±22.6 ^{b}	327.5±26.4 ^{a}	354.1±31.5 ^{a}
Bunny weight at birth (g)	42.12±2.04	42.05±1.84	40.43±1.99
Litter size at weaning (n)	5.38±0.31 ^{c}	7.04±0.36 ^{b}	8.43±0.38 ^{a}
Litter weight at weaning (g)	3721.7±87.3 ^{c}	5613.1±92.1 ^{b}	6849.2±103.3 ^{a}
Bunny weight at weaning (g)	691.6±27.4 ^{b}	797.3±34.6 ^{a}	812.4±37.1 ^{a}

Means bearing different letter superscripts (a,b,c) within the same row are significantly (P ≤ 0.05) different.

It is interested to note that the improvement in all parameters studied indicated fertilizing ability of rabbit bucks and fertility traits of rabbit does were

arranged (P≤0.05) in descending order as recorded by 400, 200 then zero g red algae (*Ganoderma*®)/ton diet, respectively. While, 400 g red algae (*Ganoderma*®)/ton recorded insignificantly improvements in most studied parameters than 200 g/ton.

Conclusion:

In conclusion, supplementing 200 or 400 g red algae (*Ganoderma*®)/ton diet of NZW rabbits, improved semen quality and storagability of buck semen and fertility traits of does. Based on the price of red algae, , 200 g red algae (*Ganoderma*®)/ ton diet is recommended for mature rabbit bucks.

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دراسات معملية وحقلية عن تأثير الطحالب الحمراء على التناسل فى الأرانب وحفظ السائل المنوى (الحيامن) والتلقيح الإصطناعى

وائل على حسن على و مرفت نبيل ابراهيم

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صممت هذه الدراسة لتقييم تأثير إضافة مستويات مختلفة من الطحالب الحمراء فى العلائق على معدلات الأداء التناسلى فى ذكور وأناث الأرانب النيوزيلندى الأبيض الناضجه قسمت الأرانب إلى ثلاثة مجموعات تجريبية متماثله، (١٦ ذكر ناضج جنسياً، بالإضافة إلى ٥٢ أم ولدت مره من قبل فى كل مجموعة). غذيت المجموعة الأولى على عليقة تجارية وبقيت بدون معامله (مجموعة المقارنة)، بينما غذيت المجموعتين التجريبتين الثانية والثالثة على نفس العليقة، ولكن أضيف إليها ٢٠٠ و ٤٠٠ جم من مستخلص الطحالب الحمراء لكل طن، على الترتيب.

أوضحت النتائج أن إضافة مستويات مختلفة من الطحالب الحمراء إلى علائق الأرانب حسن معنوياً (عند مستوى ٥%) من الرغبة الجنسية والخصائص الطبيعية للسائل المنوى للذكور، بينما القيم الداله على النشاط الإنزيمى للحيوانات المنوية تناقصت معنوياً (عند مستوى ٥%)، وتزايدت مقدرة الحيوانات المنوية على النفاذ فى مخاط عنق الرحم للبقرة الشائعة (عند مستوى ٥%) نتيجة لإضافة الطحالب الحمراء على العلائق.

السائل المنوى المخفف المقذوف من ذكور أرانب النيوزيلندى البيضاء والمغذاه على علائق مضاف إليها مستويات مختلفة من الطحالب الحمراء أو/ و مضاف إليه ١ مل مستخلص مائى من الطحالب الحمراء لكل ١٠٠ مل سائل منوى مخفف سجل زيادة معنوية (عند مستوى ٥%) فى كل من جودة وحيوية السائل المنوى وكذلك مقدرته التخزينية، خلال فترات الحفظ على درجة حرارة الثلاجة ٤-٦°م لمدة ثلاثة أيام، أو التحضين على ٣٧°م لمدة أربعة ساعات. السائل المنوى المخفف المقذوف من ذكور مغذاه على علائق محتوية على طحالب حمراء، ومضاف إلية مستخلص طحالب حمراء، كان أفضل فى مقاييس جودته معنوياً (عند مستوى ٥%) عن تلك المقذوف من ذكور معامله فقط بالطحالب وغير مضاف إليه مستخلص الطحالب، ثم سائل منوى الذكور الغير معامله ولكن المضاف إليه مستخلص الطحالب الحمراء، يليه فى المرتبة الأخيرة السائل المنوى المقذوف من ذكور أرانب غير معامله وغير مضاف إلية مستخلص الطحالب الحمراء، على الترتيب.

تناقصت القيم الداله على الخصائص الطبيعية للسائل المنوى، بينما تزايدت مقدرة الحيوانات المنوية على النفاذ فى المخاط الرحمى للبقرة الشائعة (عند مستوى ٥%)، بتقدم زمن حفظ السائل المنوى سواء على درجة حرارة التحضين، أو درجة حرارة الثلاجه.

معدلات خصوبة إناث الأرانب متمثلة فى (معدلات كل من الحمل، والإجهاضات، والولادات، ومعدلات كل من عدد ووزن خلفات البطن عند الميلاد وعند الفطام، وكذلك وزن الخلفة الواحدة عند الميلاد وعند الفطام، سجلت قيم أفضل معنوياً (عند مستوى ٥%) كنتيجة لإضافة مستويات مختلفة من الطحالب الحمراء على العليقة.

أوضحت الدراسة أن قيم التحسن فى المقاييس الداله على المقدرة الإخصابية للذكور، ومعدلات خصوبة الإناث كانت مرتبة معنوياً (عند مستوى ٥%) وفى ترتيب تنازلى كنتيجة لإضافة

٤٠٠ جرام، يليه ٢٠٠ جرام، مستخلص الطحالب الحمراء لكل طن عليقة، ثم مجموعة المقارنة والتى تغذت على عليقة خالية من الطحالب الحمراء، على الترتيب، فى حين أن المجموعة التى غذيت على عليقة مضاف إليها ٤٠٠ جرام مستخلص الطحالب الحمراء كانت أفضل غير معنوياً عن تلك التى غذيت على عليقة مضاف إليها ٢٠٠ جرام مستخلص طحالب، بالنسبة لكل الصفات المدروسة فى هذه الدراسة.

أوضحت النتائج فى مجملها أن ذكور أو إناث أرانب النيوزيلندى البيضاء المغذاه على عليقة مضاف إليها مستويات مختلفة من مستخلص الطخالب الحمراء، سجلت تحسن معنوى (عند مستوى ٥%) فى كل الصفات المدروسة، خلال مرحلة النضج الجنسى،فإنه يوصى بإضافة ٢٠٠ جرام مستخلص الطحالب الحمراء لكل طن عليقة أرانب ناضجة.

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