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Effect of dietary supplementation of wheat germ on some reproductive performances and oxidative status of rabbit bucks under heat stress

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The benefit from cereals is pivotal feed especially in human and animal life cycle including their reproductive performance. Wheat is the dowel in this category of aliments. Hence, the aim of this study was to evaluate the effect of dietary supplementation of wheat germ on semen quality, testosterone level and antioxidant status in heat stressed rabbit bucks. 24 apparently healthy mature New Zealand white bucks were allocated randomly into three equal groups (n=8). Group 1 served as control fed on commercial equilibrated maintenance ration, group 2 and 3 were fed on 2 and 4 g wheat germ/day respectively early in the morning for 5 days/week, in addition to the maintenance ration. Semen parameters, testosterone level and serum antioxidant status were assessed. Reaction time, ejaculate volume, PH, mass motility%, individual motility%, live sperm% were significantly increased in group 3 compared to 2 compared to the control. Testosterone level increased significantly (P<0.0366) in group 3 (4 g wheat germ / buck) compared to the control and group 2. Also, the abnormal sperm % was increased significantly (P<0.0001) from group 3 to group 2 to the control one. Nitric oxide and ascorbic acid were significantly (<0.0305) decreased in group 2 than the control and group 3. While, the glutathione reduced showed a significant (P<0.0006) decrease in both group 2 and 3 compared to the control group. Thus, it was concluded that the incorporation of 2 – 4 g wheat germ daily to the bucks during hot season enhanced the reproductive performance of bucks and can alleviate the heat stress load.

Keywords: wheat germ; rabbit bucks, semen parameters; antioxidants.

INTRODUCTION

Cereals, fruits, and vegetables are considered ubiquitous natural antioxidants which have been extensively studied as effective free radical scavengers. Among cereals, wheat is one of the main cereals consumed over the world for human and animal nutrition, which contains a wide range of antioxidant such as flavonoids (Zhou et al., 2004; Liyana-Pathirana and Shahidi, 2007and Vaher et al.,2010). Wheat germ constitutes about 2-3% of the kernel weight and it is produced as a by-product in the wheat milling process. It contains many nutritional ingredients, such as proteins, carbohydrates, oils, vitamins B, pigments, and minerals and some functional micro-components (Ge et al., 2000; Zhu et al., 2006a, Zhu et al., 2011). Additionally, wheat germ provides nutrients of superior biological value and known to have prebiotic effects (Matteuzzia et al., 2004).Rabbits are considered as the most vulnerable animal to heat stress due to lack of efficient sweat glands and insufficient heat evaporation during hot seasons from May to September in Egypt (Attia et al., 2011). Heat

stress has a great impact on the productive and reproductive performances which in turn stops continuance of the breeding season for about 5 months (García-Tomás et al., 2008). Various physiological and reproductive disorders such as disturbances in blood metabolites, antioxidant enzymes, hormonal secretions, semen quality and fertility were altered by heat stress (Alvariño, 2000; Marai et al., 2002; Altan et al., 2003 and Sahin et al., 2001). Heat stress resulted in reduction in ejaculate volume, sperm cell concentration, and total sperm number per ejaculate (Marai et al., 2003) in rabbit and the increases in both temperature and humidity was correlated with poor sperm production (Ghasemi et al., 2009; Roca et al., 2005; Schwalm et al., 2007). Heat stress in rabbit can be ameliorated via supplementation of cool water and mineral mixture (Habeeb et al., 1994), vitamins (Al-Shanty, 2003), and antioxidants (Türk et al., 2016) or enzyme mixture preparations (Tawfeek, 1996).Many researches are focused on the powerful antioxidative potential of various natural resources such as wheat germ instead of the probable health risks of various synthetic antioxidants. Thus, the present study aimed to evaluate the effect of dietary supplementation of wheat germ on semen quality, testosterone level and antioxidant status in heat stressed rabbit bucks.

MATERIALS AND METHODS

Animals and diets

The present study was conducted between April and September 2016 at national research centre experimental animal house, Dokki, Giza, Egypt. Twenty seven, 6 month old male, New Zealand White rabbits with average body weight of 1.964 – 2.311 kg were used in the current study. Bucks were individually kept in well ventilated tagged wire cages and subjected to a photopheriod of 16 h light/day. Clean fresh water was supplied *ad libitum.*Bucks were randomly allocated and kept individually into three equal groups (8 bucks / group): group 1 (used as control) fed on 200g/ d commercial diet (berseem hay, yellow corn, wheat bran, soybean meal, molasses, minerals and vitamins) which is formulated according (NRC, 1994) to contain 17%, 14% and 2.5% crude protein, fiber and fat, respectively. Group 2 was fed on 200g/ d commercial diet with a daily free access supplementation of 2 gram whole wheat germ/ animal in a separate feeder, and group 3 was fed on 200g/ d commercial diet with a daily free access supplementation of 4 g of whole wheat germ / animal for 10 weeks. The whole wheat germ (WWG) was obtained from Miller Company at 6 October city, Egypt. Samples of wheat germ were taken for chemical analysis. The contents of crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen free extract (NFE) and ash were tabulated in table (1).

Semen collection and evaluation

Males were trained for semen collection on an artificial vagina adjusted at 42-45°C for 2 wks. Two ejaculates / week/ male were collected with an interval of 3 days between them. The reaction time (in seconds) was the interval calculated from the introduction of the “teaser” doe into the male’s cage till the first ejaculation and was considered as an indicator for libido. Ejaculates containing urine and calcium carbonate deposits were discarded, and gel plugs were removed. All ejaculates were stored in a water bath at 37 °C until evaluation for 15 min maximum after collection. Directly following semen collection, ejaculate volume, pH, mass motility and individual motility were conducted as described by (Boussit 1989). The volume of each ejaculate was recorded after removal of the gel mass using graduated collecting tube. Initial hydrogen ion concentration (pH) was determined immediately after collection using pH cooperative paper (Universal indicator pH 0–14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Mass motility (MM) was assessed according to a subjective scale ranging from 0-9, individual progressive motility % (IM) of the ejaculate was measured in aliquots under a microscope with a phase-contrast optic (Nikon) at ×400. Aliquots (10 µl) of raw semen were mixed with equal volume of vital nigrosin–eosin staining (Bamba ,1988) and spread a thin film to allow the measurements of sperm quality traits (percentage of viable spermatozoa, percentage of total sperm spermatozoa abnormalities) by examining 200 spermatozoa under a light microscope at ×1000 (oil immersion).

Blood collection and biochemical analyses

Blood samples were collected into clean sterilized plain tubes from the ear vein of each buck on days at biweekly intervals from the start of experiment (day 0). Serum was separated from blood by centrifugation at 700 ×g for 20 min and stored at -80°C until chemical analysis.Blood serum testosterone was determined by using solid-phase enzyme immunoassay (ELISA) total

testosterone commercial kit (Biosource, Testo ELISA, Belgium). The sensitivity of the assay was 0.05 µg/L and intra-assay coefficients of variation were 6.3 and 8.3. Serum glutathione (GSH) reduced; nitric oxide (NO), lipid peroxide indicator (Malondialdehyde, MDA) and ascorbic acid were measured using commercial kits (Bio Diagnostic, Egypt).

Statistical analysis

All data were analyzed using the SAS statistical software (SAS 2009). The data were expressed as mean ± standard error of means (SEM). Simple one way ANOVA test were used. Duncan Multiple Range test was used to differentiate between significant means at $P < 0.05$.

RESULTS AND DISCUSSION

Data output in table 2 showed the reaction time was significantly ($P<0.0182$) shorter on feeding a supplement of 2 and 4 gram of WWG (16 and 12.86 seconds, respectively) to mature bucks compared to the control group (23.86 sec). Also, the volume of the ejaculate was significantly ($P<0.0001$) increased on feeding the supplement of 4 gram WWG to 0.97 ml compared to the control and those supplemented with 2 gram WWG (0.57 and 0.50, respectively). A significant ($P<0.0006$) increase of the potential of hydrogen ion in the semen of group 2 and 3 (8.23 and 8.24, respectively) compared to the control group (PH=7.59). The mass motility, individual motility % and life sperm % were significantly ($P<0.0001$~0.0002) increased in group 3 (8.64 score, 88.33%, 94.87%, respectively) than in group 2 (8.29 score, 82.86%, 93.35%, respectively) than in the control group (6.29 score, 65.00%, 89.62%, respectively). On the contrary, the abnormal morphological sperm trait was significantly ($P<0.0001$) decreased from the control group (8.23%) to 6.65% in group 2 and at last 5.27% in group 3.

Although, the testosterone was significantly ($P<0.0366$) increased when a 4 g / buck was added compared to the 2 g / buck while, it was not significantly increased than the control.

Naturally occurring antioxidants, which are ever-present in wheat germ, fruits, vegetables, cereals, and herbal plants, have accepted a great research attention due to their efficient free radical scavenging capacity and are indicated to hove lower toxicity as compared to synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxyltoluene (Ratnam et al., 2006). Wheat germ is broadly noticed as a nutritious material for integration into food product. In Egypt, it was reported that the amount of wheat germ production was about 120,000 tons wheat germ in 2012 as a by-product of wheat milling (Mahmoud et al., 2015) which is mostly utilized in animal feeding **(**Megahad and El Kinawy, 2002**)**. It is advisable therefore that this by-product would be rather utilized for oil production. During the hot season in Egypt, rabbit bucks undergo severe heat stress as revealed by the elevated values of temperature humidity index and impaired reproductive performances (Hashem et al., 2013). The results of current study noticeably demonstrate the efficacy of wheat germ dietary supplementation on libido and semen quality in rabbit. Results showed that wheat germ supplemented bucks had better libido ($P>0.05$) which is manifested by shorter reaction time in both treated groups compared to the control group. These libido improvements in wheat germ supplemented bucks were in concomitant with their higher testosterone concentrations as compared to control. These results were in accordance with (Hashem et al., 2013) who noticed association between higher concentration of testosterone and libido in rabbit bucks and stated that libido of bucks are testosterone-dependent processes. Data revealed obvious improvements semen characteristics and oxidative status of bucks as a result of dietary supplementation with wheat germ. Wheat germ is rich in protein (about 30%) and contains many other nutritional as carbohydrates (about 46%), lipid (about 9%), pigments, minerals, and B vitamins (Mahmoud et al.,2015 and Zhu et al., 2006)is comparable to those reported in our study (table, 1). Therefore, wheat germ, with its ubiquitous nutritional value, could be used as a high-quality substitute to imported vegetable oils in rabbit rations to improve reproductive performance and semen quality. The nutritive and antioxidant effects exhibited by wheat germ could be related to the effect of the phenolic compounds, vitamins B, vitamin E. pigments, and minerals and some functional micro-components (Nichelatti and Hidvegi , 2000). Beside, Wheat germ is one of the valuable supplies of low cost proteins which is rich in 17 amino acids, particularly lysine, threonine, and methionine (Yiqiang et al., 2001 and Muhammad et al., 2007).

Table 1: Chemical analysis of wheat germ.

Item	%						Food energy value(kcal/100g)
	Moisture	Ash	Crude fat	Crude protein	Crude fiber	Carbohydrate	
Wet germ	12.80	3.12	8.80	28.11	1.43	45. 74	375.95
Dry weight	0.0	3.53	10.29	31.6	1.76	52.82	430.3

Table 2: The effect of wheat germ dietary supplementation on semen quality of mature male New Zealand rabbit.

Parameter	Control	Whole wheat germ (WWG)		F-cal	P> F
		2 g/kg b.wt.	4 g/kg b.wt.		
Reaction time (sec)	23.86 ± 1.87^{a}	16.00 ± 3.13^{b}	12.86 ± 2.40^{b}	5.05	0.0182
Volume (cc)	0.57 ± 0.04^{b}	0.50 ± 0.04^{b}	0.97 ± 0.03^{a}	48.93	< 0.0001
PH	7.59 ± 0.15^{b}	8.23 ± 0.06^{a}	8.24 ± 0.11^{a}	11.38	0.0006
Mass motility (score 0-9)	6.29 ± 0.42^{b}	8.29 ± 0.36^{a}	8.64 ± 0.18^{a}	14.31	0.0002
Individual motility (%)	65.00 ± 1.54^{c}	82.86 ± 2.86^{b}	88.33 ± 0.89^{a}	39.39	<0.0001
Life sperm (%)	89.62 ± 0.70^{c}	93.35 ± 0.45^{b}	94.87 ± 0.45^{a}	23.26	<0.0001
Abnormal sperm (%)	8.23 ± 0.44^{a}	6.65 ± 0.29^{b}	5.27 ± 0.40^{c}	14.37	<0.0001
Testosterone (ng/ml)	3.65 ± 0.19^{ab}	3.32 ± 0.60^{b}	5.03 ± 0.50^{a}	3.81	0.0366

Data represented in Mean ± Std. Error.
Different superscript (a, b…etc) are significantly different using Duncan multiple range test at P<0.05**.**

Table 3. The effect of whole wheat germ dietary supplementation on oxidative status and of male rabbit.

Parameter	Control	Whole wheat germ (WWG)		F-cal	P> F
		2 g/kg b.wt.	4 g/kg b.wt.		
Nitric oxide (nmol/ mL)	22.88 ± 0.92^{a}	15.79 ± 0.33^{b}	19.43 ± 1.01^{a}	4.05	0.0305
ReducedGlutathione(mg/dL)	5.41 ± 1.11^{a}	1.17 ± 0.09^{b}	2.75 ±0.32^{b}	10.34	0.0006
Ascorbic acid ((mg/L)	3.77 ± 0.04^{a}	2.87 ± 0.01^{b}	3.69 ± 0.21^{a}	16.93	0.0001
Malondialdehyde (nmol/ mL)	0.93 ± 0.0^{4a}	0.88 ± 0.05^{a}	0.83 ± 0.05^{a}	1.13	0.3408

Data represented in Mean ± Std. Error.
Different superscript (a, b…etc) significant difference between means using Duncan multiple range test at P<0.05.

Wheat germ is customarily integrated in healthy foods as it utilized as a richest vitamin E, thiamine, riboflavin and niacin sources from plant origin (Boros et al., 2005).Dietary supplemental of Vitamin E has been indicated to increase sperm production in boars (Brzezinska-Slebodzinska et al., 1995).

(Yousef et al., 2003) showed that inclusion of vitamin E in rabbit buck rations improved sexual desire, ejaculate volume, sperm number, fructose level and reduced lipid peroxidation. In addition, a combination of vitamin E and selenium dietary supplementation able to elevate testosterone levels and increase sperm number in heat stressed rabbit bucks (El-Maasry et al., 1994). (Hashem et al., 2013) indicated that heat stress can be counteracted by vitamin E dietary supplementations which enhance the sexual activity, semen quality and oxidative status of bucks .Data in table 3 showed that nitric oxide and ascorbic acid that were significantly (P<0.0305, P<0.0001) decreased on using 2 g / buck when compared to the control and the addition of 4 g / buck. The reduced glutathione was significantly (P<0.0006) decreased in both wheat germ additions (2 and 4 g / buck) compared to the control. The lipid peroxidation was not significantly (P<0.34) different between the three treatments.The main antioxidants included in wheat germ are carotenoids, tocopherols, flavonoids and phenolic acids (Vaher et al.,2010 and Zhu et al., 2011)**.** Vitamin E is considered to

be the chief constituents of the antioxidant defense system of the sperm cells (Surai et al., 1998), and is one of the key membrane protectants from lipid peroxidation (Akiyama et al., 1999). Dietary supplementation with wheat germ in the present study reduced oxidative stress which is revealed by lowered activity of the malondialdehyde without any significant differences ascorbic acid levels. These results were in agreement with (Castellini et al., 2003 and Hashem et al., 2013) who indicated that vitamin E can counteract oxidative stress during the hot season by enhancing total antioxidant capacity and lowering activity of the malondialdehyde. (Yousef et al., 2003) showed that vitamin E supplementation resulted in caused a significant decline in the production of reactive oxygen species and enhanced the capacity of glutathione S-transferase. (Zhu et al., (2011) and Mahmoud et al., (2015) stated that wheat germ posses a powerful free radical inhibitor capacity, which may hinders the deleterious effects of of free radical in animal body. Moreover, the wheat germ shows a good natural antibacterial activity against some bacteria such as *Staphylococcus aureus* and *Escherichia coli* (Huang et al., 2006 and Mahmoud et al., 2015)

CONCLUSION

The data obtained in the current study advocated that incorporation of rabbit bucks diets with 2 and 4 gram wheat germ is an efficient option to enhance and maintain their reproductive performance during hot season. The enhancement in reproductive performance could be interrelated to the nutritive and antioxidant effects exhibited by wheat germ and the biological value of its constituents such phenolic compounds, vitamins B, vitamin E. pigments, and minerals and other functional micro-components.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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AUTHOR CONTRIBUTIONS

El-Sisy, Khalifa and El-Nattat designed the experiment and animal managements, perform semen collection and evaluation, hormone and antioxidant analysis, statistical analysis and also wrote the manuscript. Abusinaa and Maghraby share in blood sampling and antioxidant analysis and writing the article. All authors read and approved the final version.

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