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IMPACT OF ROYAL JELLY TO IMPROVE REPRODUCTIVE PERFORMANCE OF MALE RABBITS UNDER HOT SUMMER CONDITIONS

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Abstract: To alleviate the deleterious effect of heat stress during summer conditions on male rabbits' reproduction, 40 V Line adult rabbit bucks (on av. 8 mo old) were divided into 4 experimental groups and exposed to temperatures ranging from 23 to 36°C. Bucks in the 1st, 2nd, 3rd, and 4th group were supplemented with 0, 50, 100 or 150 mg of Chinese royal jelly (RJ)/kg twice per week, respectively, over a 20-wk period. Semen quality and blood biochemical constituents were evaluated. RJ at any dose exhibited a significant increase (P<0.05) in rabbits' sperm concentration, total sperm output, sperm motility, live sperm and normal sperm compared to the untreated controls. Plasma total protein, albumin, globulin, glucose and high density lipids (HDL) concentrations were significantly (P<0.05) boosted in the RJ groups compared to the controls. In contrast, RJ treatment resulted in a significant (P<0.05) reduction in plasma total lipids, triglycerides, cholesterol and low density lipids (LDL) concentrations. Treatment with RJ significantly boosted (P<0.05) testosterone concentration in the RJ groups to reach 110, 120 and 128%, respectively, of the control group. Improved kidney and liver functions were observed in the RJ bucks groups where plasma creatinine, urea concentrations, aspartate aminotransferase and alanine aminotransferase enzyme activities were significantly (P<0.05) decreased by RJ treatments. Treating bucks subjected to heat stress by different RJ doses increased (P<0.05) total antioxidant capacity to 106, 111 and 115% of basal, but significantly reduced (P<0.05) malondialdehyde and thiobarbituric acid reactive substances compared to the untreated. It was concluded that Chinese royal jelly supplementation for heat-stressed male rabbits can counteract summer infertility and improve their physiological status.

Key Words: rabbit, heat stress, royal jelly, litter size, blood constituents.

INTRODUCTION

Royal jelly (RJ) is a secretion produced in the cephalic glands of nurse bees and serves as the most important part of honeybee larvae diet, while playing a major role in caste differentiation (Moritz and Southwick, 1992). RJ is widely used in both folk and official medicine and is a controversial effectual and beneficial dietary supplement. It has a complex composition (water, proteins, lipids, carbohydrates, amino acids, mineral salts, vitamins, enzymes, hormones, oligo-elements and natural antibiotics) comprising 67% water, 12.5% crude protein (including small amounts of many different amino acids), 11% simple sugars (monosaccharides) and 5% fatty acids. It also contains many trace minerals, some enzymes, antibacterial, antibiotic components and trace amounts of vitamin C (Graham, 1992). RJ has been associated with antioxidant, blood pressure regulatory, antitumor, antibiotic, anti-inflammatory, immunomodulatory, anti-allergic and general tonic pharmacological activities (Mărghitaş, 2008). According to Pizzorno *et al.* (2007), RJ is effective for reducing and controlling triglycerides and cholesterol in humans. RJ has been found to protect DNA against oxidative damage.

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In elderly people, eating RJ (10 g/daily for 14 d) showed an increase in serum high density lipids (HDL) level and a decrease in low density lipids (LDL) level, without affecting serum triglycerides (Münstedt *et al.,* 2009). In rabbits, adding 50-100 mg RJ/d reduced serum total cholesterol level by 14% and total lipids by 10% (Vittek, 1995).The same results were found by Guo *et al.* (2007) and Abou-Hozaifa *et al.* (1995) in rats fed a cholesterol-enriched diet. RJ also had a dose-dependent improvement on serum renal and hepatic parameters (El-Nekeety *et al.,* 2007) and increased oxygen flow to the liver (Vittek, 1995). In addition, adding RJ to mice for 16 wk, the levels of 8-hydroxy-2-deoxyguanosine (an oxidative stress marker) were significantly reduced in kidney and serum (Inoue *et al.,* 2003). It has also been shown to inhibit lipid peroxidation both *in vitro* and *in vivo* (Hang *et al.*, 2008).

RJ has been associated with positive effects on human fertility, where it is effective on perimenopausal symptoms, osteoporosis, improving hormonal equilibrium and fertility in women and men by increasing ovules and sperm quality (Lewis, 2005). It also could help in cases of low libido and impotence, especially in the elderly (Destrem, 1956). In a recent study, RJ was reported to contain testosterone and to have steroid hormone-type activities (Hidaka *et al.*, 2006). Moreover, El-Banby (1987) reported that when royal jelly was administered as a 10% solution either intraperitoneally or through a stomach tube to male rats, it stimulated the production of luteinising hormone, testosterone and progesterone. RJ can counter "summer infertility" (significant improvement of a series of spermography parameters) and improve physiological status in male rabbits (Elnagar, 2010).

Therefore, the aim of this research was to investigate the use of RJ to improve fertility traits and semen quality of V Line male rabbits and enhance their physiological status under harsh summer conditions.

MATERIALS AND METHODS

Forty male *V Line* rabbits (on av. 8 mo old) were randomly distributed into 4 homogeneous groups (10 bucks each). The groups were fed *ad libitum* the same commercial pelleted diet, containing 17.27% crude protein and 2640 kcal dry energy/kg. Three groups received RJ (Anhui Tianxin Bee Products Co., Ltd) in a water solution at 50, 100 and 150 mg/kg body weight (BW). The RJ was given orally, using an insulin syringe directly in the oral cavity, twice per week over a 20-wk treatment period. The 4th group did not receive RJ and served as a control group, similarly treated except that the solution was water only. The experiment was carried out in summer from May to September, 2012, for 20 wk.

Animal husbandry

Bucks were housed in a naturally ventilated building and kept in individual Italian galvanised wire cages (60×55×40 cm). Batteries were equipped with feeders for pelleted rations and automatic drinkers. Animals were kept under similar management and hygienic conditions. The lighting schedule provided was 14L:10D a day. The average temperature and relative humidity during this period were on av. 30°C (23 to 36°C) and 75% relative humidity (68 to 81%), respectively. Feed and fresh water were given *ad libitum*.

Data collection

Semen Quality

Semen was collected every 2 weeks over the 16 wk, after a 4-wk adaption period. During this training period, bucks were trained for semen collection and adaptation to the experimental conditions, so 80 ejaculates were obtained per treatment. Ejaculates were collected using an artificial vagina maintained at 45-46°C and a teaser doe. The volume of each ejaculate was recorded after removal of the gel mass. Reaction time, the time between introducing the teaser doe to the buck and ejaculation (RT/s), was estimated. Sperm motility was estimated using phase contrast optics at 40× and assessed from 0 to 100%. A weak eosin-formalin (10% formalin) solution was used to evaluate the sperm concentration (Elkomy, 2003), using the improved Neubauer haemocytometer slide (Smith and Mayer, 1955). Assessment of live and abnormal spermatozoa was performed using an eosin-nigrosine blue staining mixture (Blom, 1950). The percentage of live, dead and abnormal spermatozoa was determined using stains that penetrate cells with damaged membranes. Normal live sperm were not stained by the eosin stain and appeared white in colour, whereas dead sperm were stained by eosin and appeared pinkish in colour due to loss of membrane integrity. Normal sperm

have an oval head with a long tail. Abnormal sperm have head, mid piece or tail defects, such as a large or misshapen head or a crooked or double tail.

Reproductive traits

For each group, 30 *V line* nulliparous rabbit does (at the same age) were mated by 10 bucks. For each treatment, the fertility was estimated by dividing the number of pregnant does by the number of mated does ×100). Does that failed to become pregnant after 3 successive natural mating attempts were discarded from the experiment.

Blood biochemical constituents, antioxidants enzymes and lipid peroxidation biomarkers

Heparinised tubes were used to collect the blood samples obtained from the ear vein of each buck at the end of the experiment. Blood samples were centrifuged at 3500 rpm for 20 min to obtain plasma and stored at –20°C for later analysis. Total protein, albumin, globulin, glucose, total lipid, urea, creatinine, the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and also blood lipids such as triglycerides, total cholesterol, high density lipoproteins (HDL) cholesterol and low density lipoproteins (LDL) cholesterol were determined.

Plasma lipid peroxidation biomarkers such as thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) were assayed in the blood plasma according to Conti *et al.* (1991), respectively. Total antioxidant capacity was according to Erel (2004). Glutathione S-transferase (GST), glutathione (GSH), glutathione peroxidase (GPx) and superoxide dismutase activity (SOD) activities were estimated according to the method of Habig *et al.* (1974), Beutler *et al.* (1963), Chiu *et al.* (1976) and Misra and Fridovich (1972), respectively. Blood testosterone concentration in plasma was measured using enzyme immunoassay method. Humoral immune response was evaluated using haemagglutination (HA) test following the method described by Prescott *et al.*, (1982). Titres were measured as $\log_2$ values. Five bucks from each group received 0.5 mL of 50% sheep red blood cells (SRBCs) via intramuscular injection as T-dependent antigen. Haemagglutination antibodies were assessed 7 d later by HA test. Lysozyme activity was according to Schultz (1987). Serum immunoglobulin types (IgG, IgM and IgA) were determined using ELISA technique.

Statistical analysis

Statistical analysis was performed by Anova (SAS, 2002). Before analyses, all percentages were subjected to logarithmic transformation (log10 $x + 1$) to normalise data distribution. Mean difference at $P<0.05$ was tested using Student-Newman-Keuls-test.

RESULTS AND DISCUSSION

Semen characteristics

The libido of bucks (measured by the reaction time, seconds) was significantly reduced when they received RJ at any dose and this effect was clearly apparent with 100 and 150 mg/kg (17.5 and 16.9 *vs.* 31.7 s for 100, 150 mg/kg and the control group, respectively). The previous effect may be due to the increased testosterone level in the treated rabbits, which had higher levels of testosterone hormone than the control animals. This result was in agreement with that found by Kamel *et al.* (2009), who reported a good relation between increased testosterone hormone concentration and increase libido in male rabbits.

Chinese royal jelly significantly ($P<0.05$) improved most semen characteristics except the volume and pH (Table 1). Sperm motility was increased significantly ($P<0.05$) in groups treated with RJ by 9.7, 23.6 and 25.9% compared to the control group, and this effect was dose-dependent. The enhancement observed in sperm motility is consistent with the findings of Karacal and Aral (2008), who reported higher sperm motility when male mice were treated with RJ. Ejaculate volume of bucks that received 100 and 150 mg of RJ was non-significantly increased compared to the control group or the group that received 50 mg, but the lower dose showed a slightly decreased ejaculate volume than the control group. Increased ejaculate volume in 100 and 150 RJ groups may be due to increased secretion seminal fluid from the sex accessory glands due to increased testosterone concentration compared to the control. Previous

Table 1: Effect of Chinese royal jelly on libido, semen characteristics, sperm output, pH and fertility after natural mating.

	Chinese royal jelly (mg/kg BW)				
Parameters	0	50	100	150	RMSE
Reaction time (s)	31.7[a]	25.3[b]	17.5[c]	16.9[c]	0.5
Ejaculate volume (mL)	0.711	0.704	0.720	0.718	0.034
Sperm motility (%)	53.7[c]	58.9[b]	66.4[a]	67.5[a]	3.7
Sperm concentration (10^6/mL)	443[c]	487[b]	527[a]	531[a]	16
Total sperm output (10^6)	314.9[c]	342.8[b]	379.4[a]	381.3[a]	23.8
Total motile sperm (10^6)	169.1[c]	201.9[b]	251.9[a]	257.4[a]	21.7
Total motile normal sperm (10^6)	144.6[c]	174.8[b]	223.4[a]	231.9[a]	20.3
Alive sperm (%)	85.5[b]	86.6c[b]	89.8[a]	90.1[a]	1.2
Dead sperm (%)	14.5[a]	12.3[b]	10.2[c]	9.9[c]	0.9
Abnormal sperm (AbNS %)	15.9[a]	13.4[b]	11.3[c]	11.1[c]	1.2
pH	7.4	7.2	7.3	7.1	0.3
Fertility after natural mating (%)	85.4[d]	89.6[c]	92.7[b]	94.3[a]	3.6

[abc]means in the same row having different superscripts are significantly different ($P<0.05$).

RMSE: Root mean square error.

results of Fujihara *et al.* (1983) found that exogenous testosterone caused ductus deferens fluid secretion in juvenile, ducks and castrated chickens. RJ at any doses led to a significant and gradually increased ($P<0.05$) live sperm and normal sperm count compared to the control group. The opposite trend was shown in the abnormal sperm and dead sperm, which showed a significant decrease, and this effect was dose-dependent.

Treated bucks had a significantly and gradually increased ($P<0.05$) sperm concentration, total sperm output (TSO), total motile sperm (TMS) and total motile normal sperm (TMNS), live sperm and normal sperm compared to the control group. Sperm concentration was increased in RJ groups to reach 9.9, 18.7 and 19.9% over the control group. Kohguchi *et al.*, (2004) demonstrated that golden hamsters treated with RJ showed more intensive spermatogenesis than the control group. This could explain the significant increase in sperm concentration and therefore total sperm output.

Increased sperm concentration and sperm motility in the RJ treated groups were influenced by seminal plasma hydrogen ion concentration. The seminal plasma pH was non-significantly decreased in treated groups compared to the control group and these differences may be due to increased sperm activities which might have affected the seminal plasma hydrogen ion concentration.

These results suggest that RJ had beneficial effects on male reproductive functions, as RJ resulted in improved sexual system functions reflected in increased semen production from seminiferous tubules to produce complete sperm with high motility. Our results were in agreement with the findings by Elnagar (2010), who reported that oral administration of RJ can counteract summer infertility (significant improvement of a series of spermography parameters) and improve physiological status in male rabbits. RJ intragastric administration can promote the development of male reproductive organs, such as hypothalamus and testicular, improving the motility and density of sperm in rabbits (Xu *et al.*, 2011). In human, Lewis (2005) indicated that RJ is effective in improving hormonal equilibrium and fertility in women and men by increasing ovules and sperm quality.

Some blood biochemical constituents

Results presented in Table 2 indicated that RJ treatment significantly increased plasma total protein, albumin and globulin concentration and this effect was dose-dependent. Our results are comparable to the results of Elnagar *et al.* (2010) who reported that in stressed male rabbits, RJ led to a significantly increase in serum total protein, albumin and globulin. In contrast, RJ did not influence serum total protein, albumin and globulin in growing rabbits (Elnagar *et al.*, 2010). In chicken, Kurkure *et al.* (2000) reported an increase in plasma albumin concentration in White Leghorn cockerels, orally treated with RJ at 10 mL/bird d.

Table 2: Effects of Chinese royal jelly on some blood biochemical constituents and testosterone concentration (means±standard error) in heat stressed male rabbits.

	Chinese royal jelly (mg/kg BW)				
Parameters	0	50	100	150	RMSE
Testosterone (ng/mL)	0.528^{d}	0.583^{c}	0.634^{b}	0.671^{a}	0.045
Total protein (g/dL)	5.67^{c}	5.98^{b}	6.34^{a}	6.41^{a}	0.04
Albumin (g/dL)	2.95^{c}	3.12^{b}	3.31^{a}	3.37^{a}	0.36
Globulin (g/dL)	2.72^{c}	2.86^{b}	3.03^{a}	3.04^{a}	0.31
Glucose (mg/dL)	115.2^{d}	118.5^{c}	123.6^{b}	129.1^{a}	3.6
Total lipids (g/dL)	6.18^{a}	5.88^{b}	5.38^{c}	5.07^{d}	0.51
Triglycerides (mg/dL)	124.23^{a}	113.17^{b}	85.47^{c}	87.61^{c}	3.55
Total cholesterol (mg/dL)	167.36^{a}	158.46^{b}	146.28^{c}	145.74^{c}	3.23
high density lipoproteins cholesterol (mg/dL)	50.16^{c}	52.06^{b}	54.37^{a}	55.02^{a}	1.76
low density lipoproteins cholesterol (mg/dL)	107.41^{a}	93.73^{b}	81.45^{c}	80.87^{c}	3.16
Urea (mg/dL)	24.6^{a}	20.1^{b}	16.3^{c}	18.7^{bc}	1.7
Creatinine (mg/dL)	1.25^{a}	1.06^{b}	0.85^{c}	0.94^{bc}	0.82
AST (IU/L)	29.7^{a}	25.1^{b}	22.8^{c}	22.6^{c}	2.6
ALT (IU/L)	26.3^{a}	24.9^{b}	23.6^{c}	23.1^{c}	2.6

^{abc}means in the same row having different superscripts are significantly different (P<0.05).

AST: aspartate aminotransferase. ALT: alanine aminotransferase. RMSE: Root mean square error.

Glucose level increased significantly (P<0.05) with RJ treatments to reach 2.86, 6.79 and 12.06%, over heat stressed control level, with the three doses of RJ, respectively. The same trend of RJ effect on blood glucose level was found by Elnagar, *et al.* (2010) in rabbits kept under heat stress conditions.

Treatment of heat-stressed male rabbits with RJ resulted in a significant (P<0.05) reduction in plasma total lipids concentration by 4.9, 12.9 and 18.0% and plasma triglycerides concentration by 8.9, 31.2 and 29.5%, less than heat stressed control value, with the 3 doses, respectively. A similar trend was observed with plasma cholesterol concentration, which significant reduced (P<0.05) by 5.31, 12.59 and 12.94%, less than the control value, with the 3 doses of RJ, respectively. Serum LDL concentration was gradually and significantly reduced in treated rabbits with RCJ at any doses and the values were 12.73, 24.17 and 24.71% below the control group value, while serum HDL concentration was increased by 3.78, 8.39 and 9.68% above the control level and this effect was significant (P<0.05) at any RJ dose. These results support the findings of Münstedt *et al.,* (2009), as they showed that in elderly people eating RJ 10 g/d for 14 d showed an increase in serum HDL and a decrease in LDL levels. Similarly, Elnagar (2010) reported that serum total lipids, cholesterol and triglycerides were decreased in heat-stressed male rabbits treated with 200, 400 or 800 mg RJ/kg BW. Rabbits treated with 50-100 mg RJ/d experienced reduced serum total cholesterol level by 14% and total lipids by 10% (Vittek, 1995). Similar results were obtained by Guo *et al.* (2007) in rats fed a cholesterol-enriched diet. According to Shinoda *et al.* (1978) the mode of action of RJ on decreasing cholesterol level is by attaching the phytosterol-like biosterol in the intestinal tract.

Creatinine and urea concentrations in blood were significantly (P<0.05) affected by RJ treatments. Creatinine concentration was reduced by 18.3, 33.7 and 24.0% and urea was reduced by 15.2, 32 and 24.8%, less than the heat stressed control value, with the 3 doses of RJ, respectively. The medium dose (150 mg RJ/kg BW) had the highest beneficial effect compared to the lower and higher RJ doses. This indicates an improved kidney function The present results conform to those reported by Elnagar *et al.*, (2010) in growing rabbits and Elnagar, (2010) in heat stressed male rabbits, which were treated with 200, 400 and 800 mg RJ/kg BW.

During heat stress, liver enzyme activities (AST and ALT) tend to rise, suggesting some liver damage in mammals and birds (Faisal *et al.*, 2008). RJ treatments caused a gradually and significant (P<0.05) reduction in both AST and ALT enzymes activities, and this effect reveals an improvement in liver function. From the literature, RJ had a dose-dependent improvement on serum renal and hepatic parameters (El-Nekeety *et al.,* 2007) and increased the oxygen flow to the liver (Vittek, 1995). Likewise, Elnagar *et al.* (2010) observed a non-significant reduction in both AST and ALT enzyme activities.

Treated heat-stressed male rabbits with 50, 100 and 150 mg RJ/kg BW boosted blood testosterone concentration to reach 10, 20 and 28%, respectively above the untreated group values. The effect of RJ on testosterone is in agreement

with the findings of Kohguchi *et al.* (2004), who demonstrated that feeding golden hamsters on diet containing RJ increased testosterone level in a dose dependent manner. In addition, Elnagar (2010) showed a significant increase in testosterone level when heat stressed male rabbits were orally administered 200, 400 and 800 mg RJ/kg BW.

Immunoglobulins, sheep red blood cells (Antibody Titre) and lysozyme activity

RJ treatments significantly ($P<0.05$) increased IgG, IgM and IgA levels in heat-stressed buck rabbits and this effect was dose-dependent (Table 3). Similarly, Elnagar *et al.* (2010) found that RJ treatments increased IgG and IgM levels in heat-stressed growing rabbits. Yamada *et al.* (1990) stated that when substances known to have Ig production stimulating factor activity, such as RJ, were tested on lymph node lymphocytes from breast cancer patients, IgM concentration was increased by 2.25 folds.

In comparison with control animals, RJ treatments significantly ($P<0.05$) increased sheep red blood cells (SRBCs) in heat stressed buck rabbits, and this increase was dose-dependent. Crenguta *et al.* (2011) mentioned that RJ contains amino and gamma globulin, unsaturated fatty acids, hormones, enzymes, proteins, vitamin E and A that help the immune system fight infection. It has been recently shown that fatty acids isolated from RJ (10HDA and 3-10- dihydroxydecanoic acid) modulate the immune response in rat dendritic cell and T-cell cultures in different ways depending on concentration (they stimulate the proliferation of T cells, Vucevic *et al.*, 2007). Moreover, AL-Mufarrej *et al.* (1997) reported that chickens challenged with sheep erythrocytes (SRBC) significantly increased antibodies production when RJ was administered.

Lysozyme activity showed a gradual and significant decrease ($P<0.05$) by 3.6, 9.5 and 11.6% for groups treated with 50, 100 and 150 mg RJ/kg BW compared to the control group.

Blood antioxidant content and lipid peroxidation biomarkers

Data for plasma antioxidant enzymes activities and lipid peroxidation biomarkers MAD and TBARS are shown in Table 4. There was a significant effect of RJ on Total Antioxidant Capacity (TAC), glutathione S-transferase (GST), glutathione content (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and malondialdehyde (MDA). Treated bucks subjected to heat stress by different RJ doses (50, 100 and 150 mg RJ/kg BW) significantly increased ($P<0.05$) TAC by 5.6, 10.5 and 15.1% above the control value, and this effect was dose-dependent. In a similar way, there were significant increases in antioxidant enzyme activities (GST, GSH, GPx and SOD when heat stressed bucks were treated with RJ at any doses compared to control. On the other hand, the influence of dietary antioxidant supplementation on the antioxidant defence system of heat-stressed rabbits can be evaluated by measuring biomarkers of oxidative stress. The present results showed that treated heat-stressed bucks with RJ at any tested doses had significant reduction ($P<0.05$) of malondialdehyde (MDA) by 23.2, 37.4 and 45.6% and thiobarbituric acid reactive substances (TBARS) by 19.3, 34.0 and 43.0% compared to the control group. RJ has pharmacological antioxidant activity, so it can protect against cellular damage, and this effect may be associated with reduced oxidative damage to lipids proteins and DNA. According to Pizzorno *et al.* (2007), RJ is efficient to protect DNA against oxidative damage. It has also been shown to inhibit lipid peroxidation both *in vitro* and *in vivo* (Hang *et al.*, 2008). When mice are fed RJ for 16 wk, the levels of 8-hydroxy-2-deoxyguanosine (an oxidative stress marker) were significantly reduced in kidney,

Table 3: Effects of Chinese royal jelly on immunoglobulins (Gamma inmmunoglobulin IgG, Mu immunoglobulin IgM, Alpha immunoglobulin IgA), against sheep red blood cells antibodies titre (SRBCs) and lysozyme activity (means±standard error) in heat stressed male rabbits.

	Chinese royal jelly (mg/kg BW)				
Parameters	0	50	100	150	RMSE
IgG (mg/mL)	3.67^{d}	4.13^{c}	4.41^{b}	4.63^{a}	0.14
IgM (mg/mL)	1.09^{c}	1.25^{b}	1.44^{a}	1.47^{a}	0.11
IgA (mg/mL)	0.486^{d}	0.507^{c}	0.536^{b}	0.564^{a}	0.075
Antibody titre (SRBCs)	4.48^{c}	5.11^{b}	6.28^{a}	6.37^{a}	0.14
Lysozyme (mmol/mL)	80.13^{a}	77.24^{b}	72.55^{c}	70.81^{d}	7.94

^{abc}means in the same row having different superscripts are significantly different ($P<0.05$).

RMSE: Root mean square error.

Table 4: Effects of Chinese royal jelly on plasma total antioxidant capacity (TAC), antioxidant enzyme activities (Glutathione S-transferase (GST), Glutathione content (GSH), Glutathione peroxidase (GPx), Superoxide dismutase (SOD)), malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) (means±standard error) in heat stressed male rabbits.

	Chinese royal jelly (mg/kg BW)				
Parameters	0	50	100	150	RMSE
TAC (mm/L)	146.5^{d}	154.8^{c}	161.9^{b}	168.7^{a}	2.3
GST (IU/L)	1.588^{d}	1.659^{c}	1.746^{b}	1.885^{a}	0.004
GSH (mg/dl)	19.86^{d}	22.35^{c}	25.42^{b}	27.19^{a}	0.48
GPx (mg/L)	4.36^{c}	4.81^{b}	5.28^{a}	5.31^{a}	0.17
SOD (IU/L)	7.23^{c}	7.69^{b}	8.17^{a}	8.22^{a}	0.16
MDA (mg/dl)	1.317^{a}	1.012^{b}	0.825^{c}	0.716^{d}	0.084
TBARS (nmol/ml)	1.225^{a}	0.989^{b}	0.809^{c}	0.698^{d}	0.006

^{abc}means in the same row having different superscripts are significantly different (P<0.05).

RMSE: Root mean square error.

DNA and serum. Moreover, the average life expectancy of C3H/HeJ mice was increased through the mechanism of reduced oxidative damage (Inoue *et al.,* 2003).

From Table 1 it can be observed that the fertility rate in RJ treated heat-stressed bucks was gradually and significantly restored compared to the control group and this improvement was 4.9, 8.5 and 10.4% above the control average.

CONCLUSION

From the results, it can be concluded that Chinese royal jelly administered in a water solution at 150 mg/kg body weight to adult male rabbits subjected to summer heat conditions has a positive effect on libido, semen quality, sperm output, blood concentration of testosterone, total proteins, glucose and fertility. Moreover, abnormal and dead sperm concentrations were reduced. This treatment seems to improve rabbits' physiological status, reflected in better liver and kidney functions with reduced oxidative stress biomarkers such as malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS).

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