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Original research article

Beneficial roles of boron supplementation in the ration of rams on productive activity and semen quality

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ABSTRACT

The aim of this study is to determine the effects of boron supplementation in ration of rams on their productive activity and semen quality. For that purpose, twelve rams were divided into two groups (6 rams/ each). Rams of the first control group were fed the basal ration. Concerning the second group (boron group), the rams were fed the basal ration plus 400 mg boric acid (BA) / kg diet as a source of boron. The rams in both groups fed their corresponding ration for 4 months *ad libitum*. Blood samples were collected monthly throughout the experimental period. Semen samples were collected at the 12th and 16th weeks of the experiment. The results clarified that the serum levels of tetraiodotyrosine (T4) were observed to be elevated significantly ($P<0.05$) with growing of age in the rams fed B, but they didn't change in the control group. In addition, the semen quality was maintained normal with boronsupplementation as the individual sperm motilities and live/dead sperm cells percentages were similar to control. Also, boronsupplementation didn't induce any adverse effects on the serum levels of Mg when compared to control group. Thus, boronis considered a potential and useful supplement in ration of rams to improve the thyroid activities and didn't induce any adverse effects on either semen quality or serum magnesium level in rams.

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1- Introduction:

Boron is a trace element playing an important role in mineral and hormonal metabolism, cell membrane functions, and enzyme reactions. Boron also affects osteoporosis, heart trouble, paralysis, diabetes and senility. Experiments with boronsupplementation or deprivation show that its effects are more marked when cholecalciferol and magnesium are deficient. Boron may be involved in cerebral function due to its effects on the transport across membranes. Boron is involved in the synthesis of extracellular matrix and is helpful in wound healing. Compounds of boron have been shown to be potent anti-osteoporotic, anti-inflammatory, hypolipemic, anti-coagulant, and anti-neoplastic agents both in vitro and in vivo in animals **(Hunt, 2007 and Korkmazet al., 2011)**.

Popova et al. (2017) hypothesize that in terms of overexposure boron may serve as a potential goiterogen. It is proposed that boronoverload may impair thyroid physiology ultimately leading to goiter formation. It has been demonstrated that serum and urinary boronlevels were characterized by a negative association with thyroid hormone levels in exposed subjects. Single indications on the potential efficiency of boronin hypothyroidism also exist. Moreover, the levels of boronwere found to be interrelated with thyroid volume in children environmentally exposed to boron. Experimental studies also demonstrated a significant impact of boronon thyroid structure and hormone levels. Finally, the high rate of boronaccumulation in thyroid may also indicate that thyroid was the target for boronactivity. Chemical properties of iodine and boronalso provide a background for certain competition. If such association will be confirmed and the potential mechanisms elucidated, it will help to regulate the incidence of hypothyroidism and goiter in endemic regions with high boron levels in soil and water.

Boron is essential for magnesium and calcium metabolism, and is probably involved in estrogen and testosterone metabolism. There are a lot of reasons to suspect that a borondeficiency is involved in hyperthyroidism **(Kenney and McCoy, 2000)**. They also recorded that boron appeared to lessen effects of a low-Mg diet on body growth, serum cholesterol, and ash concentration in bone, but exacerbated deficiency symptoms, without affecting the concentration of Mg in serum.

Because the shortageof information about the benefits of boronsupplementation in rations of sheep, the current study was designed to investigate the influence of boronsupplementation in ration of rams on thyroid gland activity and semen quality of rams.

2- Material and methods

The practical course of the present study was implemented in the farm of Faculty of Agriculture, Minia University, Minia Governorate starting from mid-February to mid-June.

2.1. Animals:

The present study was conducted on twelve healthy Osemi rams, 4 months old, located at the farm of Faculty of Agriculture, Minia University. The body weight of rams was (17.6 ± 1.26 kg).

2.2. Ration:

Basal diet was formed from commercial normal diet, containing corn 42%, soybean 16% and bran 42% with adding 0.03% minerals, 0.5 % sodium chloride, 0.03% premix and probiotic. This mixture is represents16% crude proteins and 2446 kcal kgG1.

2.3. Boron:

Boric acid (BA) "H_3PO_3" (Nasr company for chemicals, Egypt) as a source of boron was used.

2.4. Experimental design:

Rams were classified randomly into two equal groups of 6 rams each. Rams of the first control group fed basal diet*adlibitum*.The second group (boron group),the rams fed *adlibitum* the same basal diet supplemented with400 mgBA/kg dietwhich represent 70 mg B/kg diet.The rams in both groups fed their corresponding ration for 4 months till the rams reach 8 months old (2 months after age of maturity).

2.5. Sampling:

2.5.1. Blood Samples:

Blood samples were collected monthly throughout the experimental period.Blood samples were collected via jugular veinpuncture under clean and sterile conditions into sterile dry vacationer tubes. Blood samples were left for 30 minutes then centrifuged at 3000 g for 10 minutesat room temperature. Sera were carefully harvestedand kept at -20°C until analysis.

2.5.2. Semen sample collection:

Semen samples were collected using the electro-ejaculator (EE) method according (**Jiménez-Rabadán et al., 2012**).

2.6. Biochemical analysis:

2.6.1. Determination ofserum T4:

Serum level of total T4 was measured using The Micro enzyme immune linked assay (EILA) test according to **Felig et al. (1987)**.

2.6.2. Determination of of serum calcium magnesium (Mg) levels:

Serum Magnesium (Mg) was determined by anatomic absorption spectrophotometer (Perkin-Elmer. Corporation, USA) usingdirect sample dilution according **Suzuki et al. (1965)**.

2.7. Evaluation of the ejaculated semen samples:

2.7.1. Individual sperm motility:

For evaluation of progressive forward sperm motility, one drop of collected semen on a clean dry warm (37°C) slide then few drops of warm sodium citrate (2.9 % in distilled water) buffer solution were added and gently mixed with the semen. The slide was covered and examined microscopically according to method described by **World Health Organization, (2010).**Two hundred sperm in four different fields were examined. Sperm motility was expressed as percentages.

2.7.2. Live / dead ratio:

Live / dead percentage of sperms was determined by using eosin nigrosine stain as performed by **Jequier (2010)**. Sperm Live / dead ratio were expressed as percentages.

2.9. Statistical analysis:

Collected data for the two groups were statistically analyzed in comparison for the mean and standard error of mean using statistical software program (SPSS for windows, version 16, USA) according to **(Coakes et al., 2009).**

3. Results:

3.1. Effect of boron on serum levels of thyroid hormones:

The data in table 1pointed that serum levels of T4increased significantly ($P<0.05$) with growing of ageduringthe experimental period in B-treated rams but they didn't in control group.

3.2. Effect of boron on serum level of calcium and magnesium in rams:

The results in table 2 clarified that there were no significant differences between the boron and control groups on the serum levels of Mgalong in the experiment.

3.3. Effect of boron on semen parameters:

Table 3 displayed that there were no significant differences between the boron and control groups on the sperm individual motilities and live/dead percentages.

1-Effect of boron supplementation on serum Levels of T4 hormone (ng/ml) in rams (Mean ± SE):

Parameter	Period	control group	B-Group
T4 (ng/dl)	**0 days**	15.65±0.68 ^{aA}	11.97±0.79 $^{a\,A}$
	8th week	16.23±2.01 ^{aA}	14.58±0.93 $^{a\,AB}$
	12th week	18.10±0.23 ^{aA}	15.58±0.64 $^{a\,B}$
	16th week	18.08±0.55 ^{aA}	15.90±0.9 $^{a\,B}$

SE: Standard error
The values in the same raw have different small superscript letters are significantly different from each other at $P< 0.05$.

The values in the same column have different capital superscript letters are significantly different from each other at $P<0.05$.

2-Effect of boron supplementation on serum levels of magnesium ions in rams(Mean ± SE):

Parameter	Period	Control group	B-Group
Magnesium (mg/dl)	**0 days**	1.95±0.06 [aA]	1.90±0.02 [aA]
	8^{th} week	1.93±0.06 [aA]	2.00±0.04 [aA]
	12^{th} week	1.95±0.01 [aA]	2.01±0.08 [aA]
	16^{th} week	1.98±0.05 [aA]	2.01±0.07 [aA]

- **SE:** Standard error.
- The values in the same raw have similar small superscript letters didn't differ significantly from each other at P>0.05.
- The values in the same column have similar capital superscript letters didn't differ significantly from each other at P>0.05.

3- Effect of boron on semen parameters (Mean ± SE):

Treatments	Individual motility %		Live /dead ratio %	
	12^{th} week	16^{th} week	12^{th} week	16^{th} week
Control	84.00 ± 1.34 [a]	84.33 ±1.05 [a]	89.40 ±0.87 [a]	91.33 ± 0.89 [a]
Boron	84.16 ± 1.44 [a]	86.00 ±1.23 [a]	90.00 ± 0.70 [a]	90.83 ± 1.17 [a]

- SE: Standard error
- The values in the same column have different small superscript letters are significantly different from each other at P
- <0.05.

4-Discusion:

The present study was implemented to inspect the impact of supplementation ofram's ration with boronon productive activities and semen quality.

Data in table 1pointed that serum levels of T4increased significantly ($P<0.05$) with growing of age during the experimental period in boron-treated rams but they didn't in control group. In this regard, **Skalny et al. (2016)**hypothesize that in terms of overexposure, boron may serve as a potential goiterogen. It is proposed that boron overload may impair thyroid physiology ultimately leading to goiter formation. It has been demonstrated that serum and urinary boronlevels were characterized by a negative association with thyroid hormone levels in exposed subjects. Single indications on the potential efficiency of boronin hypothyroidism also exist. Moreover, the levels of boronwere found to be interrelated with thyroid volume in children environmentally exposed to boron. Experimental studies also demonstrated a significant impact of boron on thyroid structure and hormone levels. Finally, the high rate of boronaccumulation in thyroid may also indicate

that thyroid was the target for boronactivity. Chemical properties of iodine and boron also provide a background for certain competition. If such association will be confirmed and the potential mechanisms elucidated, it will help to regulate the incidence of hypothyroidism and goiter in endemic regions with high boron levels in soil and water.

The results in table 2 clarified that there were no significant differences between the boronand control groups on the serum levels of Mgalong the experiment. In this regard, **Kabu et al. (2015)** found that borax administration did not have any negative effects on the health of Austrian Simmental (Fleckvieh) cows during early lactation. **Abnosi (2015)** found that the low dose of boric acid (6 ng/ml) is not toxic and might be a necessary factor for osteogenic differentiation and induction of early matrix deposition in rat bone marrow mesenchymal stem cells (BMSCs).

Table 3displayed that there were no significant differences between the boronand control groups on the sperm individual motilities and live/dead percentages.Studies on human population exposed to boron at dose of 6.7 mg/day did not find significant alterations in human fertility **(Bolt, 2015;Duydu et al., 2016 and Duydu and Üstündağ, 2017)**. Similar observations dealing with boron and semen parameters have been reported no correlation between boron in urine, blood and semen parameters, although there was a no significant increase in the percentage of normal sperm morphology in workers exposed to boron versus a control population **(Robbins et al., 2010 and Duydu et al., 2011)**.

5- Conclusions:

Therefore, it is highly recommended to add boron to the ration of rams to improve thyroid activitiesand consequently the productive performance.

Conflict of interest statement

The authors declare they have no conflict of interest.

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