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Physiological Response, Semen Quality and Blood Metabolites of Friesian Bulls Treated with Zinc-Selenomethionine under Hot Summer Conditions in Egypt

El-Hawary, A. F. A.; A. A. Gabr and M. A. M. El-Kishk
Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt
*** Corresponding author's e-mail: dr.ashraf016@gmail.com**

ABSTRACT

The present study aimed to evaluate the impact of administration of zincmethionine and selnomethionine to Friesian bulls during hot summer conditions in Egypt on physiological thermoregulatory response, sexual desire, some physical and chemical semen characteristics and blood metabolites. Twenty sexually mature Friesian bulls were divided into five similar groups (4 bulls/ each). Bulls in the 1st group were unsupplemented and served as a control, while those in the 2nd,3rd,4th and 5th groups were orally supplemented with zincmethionine (Z-Met) at a levels of 2 and 4 mg/kg of BW/day and selenomethionine (Se-Met) at a levels of 0.3 and 0.6 mg/kg of BW/day, respectively, for two months as preliminary period and other three months as a main collection period. Rectal and skin temperatures, pulse rate (PR) and respiration rate (RR) were measured once each week through the experimental period. During the main collection period, semen was collected twice weekly and evaluated for semen-ejaculate volume (SEV), percentage of progressive sperm motility (PSM), live sperm (LS) and sperm abnormalities (SAB), sperm-cell concentration (SCC/ml) and total-sperm output (TSO/ejaculate). Blood samples were collected pre-treatment and monthly during the main collection period to determine concentration of total proteins (TP), albumin (AL) and globulin (GL); thyroxin (T_4), triiodothyronine (T_3), cortisol and testosterone hormones and zinc (Zn) and selenium (Se) levels in blood plasma. Also, activity of ALT, AST, ALP, ACP, SOD and GSH enzymes, as well as concentration of fructose, zinc and selenium were estimated in seminal plasma.The obtained results revealed that reaction time (RT) was shorter ($P<0.05$) and blood plasma testosterone concentration was higher ($P<0.05$) in all treated groups than the control group, begin the shortest ($P<0.05$) and highest ($P<0.05$) values of RT and testosterone concentration were recorded for bulls treated with Z-Met, especially in G3 group. Physical semen characteristics (SEV, PSM, LS, SCC/ml, TSO/ejaculate and SAB) were improved ($P<0.05$) in all treated groups as compared with the control one, being the best ($P<0.05$) values for bulls treated with Z-Met, especially in G3 group. Also, bulls treated with Z-Met in G3 group showed the lowest ($P<0.05$) activity of ALT, AST, ALP and ACP enzymes, highest ($P<0.05$) activity of SOD and GSH enzymes, and fructose, Zn and Se concentrations in seminal plasma as compare to other treated groups and control one. Bulls treated with Z-Met and Se-Met showed increased ($P<0.05$) levels of AL, T_4, T_3, Zn and Se, while GL and cortisol levels decreased ($P<0.05$) in blood plasma as compared to control one. In conclusion, orally administration of zincmethionine and selenomethionine to Friesian bulls had a valuable impact on sexual desire, semen quality and blood metabolites. Therefore, it can be recommended to treating the Frisian bulls with zincmethionine at a level of 4 mg/kg of BW/day during two preliminary months and three main semen collection months for improving the sexual desire, semen quality and blood metabolites under hot summer conditions in Egypt.

Keywords: Bulls, zincmethionine, selenomethionine, blood, semen, physiological parameters.

INTRODUCTION

Summer in Egypt is characterized by high ambient temprature, intense solar raditation and high relative humidity Habeeb *et al.* (2015). Farm animals have been exposed to heat stress through summer months, especially during June, July and August as reported by Abdel-Khalek (2000) on Friesian bulls and Wafa *et al.* (2017) on buffalo bulls. Heat stress caused significant increase in cortisole level and significant decrease in T_4 and T_3 levels in blood plasma in Friesian bulls Abdel-Khalek (2000). Blood and seminal plasma antioxidant levels were dropped by heat as reported by Nichi *et al.* (2006) in bulls and El-Tohamy *et al.* (2012) in male rabbits.

Most regions in the world are naturally deficient in micro minerals in soil and crop plants, especially in selenium (Se) and zinc (Zn) Borowska (2002) and Rashid and Ryan (2008). In Egypt, Se concentration in the feedstuffs may be lower than the adequate levels set at 0.3 pp which was recommended by NRC (2001). Also, marginal deficiency of zinc in ruminants has been reported in many parts of the tropical countries, especially in Egypt and thus needs supplementation in their diet Shams (2008) and Khalifa *et al.* (2011) . On the other hand, Se and Zn have to be supplied via the feed as they can not be produced by the animal itself Ullrey (1980).

Se is a vital component of selenoproteins (including iodothyronine deiodinase, gluatathione peroxidase and selenoprotein P and W and thioredoxine reductase), which plays an important structural and enzymatic functions Kohrle *et al.*(2007). Se is involved in protein synthesis and the immune system of animal Cortinhas *et al.* (2012) and thyroid hormones metabolism McDowell (2003). A deficiency of Se affects animals living in areas with low natural levels of selenium causes a wide range of health problems including disrupt spermatogenesis, changes of mid-piece architecture leading to breakage of head and tail of spermatozoa and impaired of sperm motility Suttle (2010). Se is incorporated into the mitochondrial capsule and is associated with Cys-rich protein of the mitochondrial sheath thus, affecting the structure development of spermatozoa and other functional aspects Marin-Guzman *et al.* (1997). Se supplementation has been found to increase semen quality by increasing antioxidative defense of seminal plasma in ram Kendall *et al.* (2000) and in buck Shi *et al.* (2010).

Zinc plays significant role in different enzyme system needed in nucleic acid metabolism, protein synthesis and carbohydrate metabolism Khalifa *et al.* (2011). Zinc is vital for a multitude of body functions, including thyroid metabolism and many of other physiological processes Baltaci *et al.* (2004). Also, zinc is involved intimately in many aspects of sperm morphology, physiology and biochemistry, although a considerable controversy exists over mechanism of action of zinc in the male reproductive system Lord and Averill (2002). Smith and Akinbamizo (2000) indicated that more than 200 zinc dependent enzymes and hormones have been identified in all the main biochemical pathways. Dismulation of superoxide radial is done by superoxide dismutase, which contains zinc Keen and Groham (1989). Hartoma *et al.* (1977) reported that zinc is a structural part of protein involved in synthesis and secretion of testosterone hormone. Spermatogenesis requires the amino acids, especially arginine, methionine and cysteine Young *et al.* (2008) and minerals (Zn and Se) Cheah and Yang (2011). Mandal *et al.* (2008) found that supplementation of organic chelated zinc has been found more bioavailable as compared to inorganic zinc. Imam *et al.* (2009) reported that the highest values of serum testosterone and zinc levels were recorded for bulls supplemented with zinc propionate, while the lowest values were observed with the control bulls. Kumar *et al.* (2013) found that Zn and Se supplementation can improve the antioxidative status and hormone levels by increasing the Zn and Se level in blood serum and seminal plasma of goat.

Spears (2003) and Imam *et al.* (2009) reported that the use of trace element from organic sources in animals nutrition (i.e. complexed and chelated amino acids), which is more bioavailable as compared to those from inorganic sources. Organic forms of zinc and selenium (like zincmethionine and selenomethionine) are natural, stable, better absorbed and metabolized, lesser side and residual effects as compared to inorganic form (like zinc oxide and sodium selenite, respectively) El-Tohamy *et al.* (2012) and Cao *et al.* (2014).

Therefore, the current study aimed to define the effect of zincmethionine and selenomethionine administration on physiological response, sexual desire, semen quality and blood metabolites of Friesian bulls under hot summer conditions in Egypt.

MATERIALS AND METHODS

The present study was carried out at El-Gemmizah Experimental Station, El-Gharbiya Governorate, belonging to the Animal Production Research Institute, Agricultural Research Center during the period from the first of April to the end of August 2016.

Animals and experimental groups:

Twenty sexually mature Friesian bulls aged 3.5 – 4 years old were used in the present study. All bulls were in healthy condition and clinically free of external and internal parasites with a sound history in the herd. Palpation of the external genetalia tract showed that they were typically normal. Copulatory patterns for all tested bulls at the beginning of the experiment were judged to be normal. Bulls were randomly divided into five similar groups (4 each). Bulls in the 1st group were untreated acting as control group. While, those in the 2nd,3rd,4th and 5th groups were orally supplemented with zinc-methionine (Z-Met) at a levels of 2 and 4 mg/kg of BW/day and selenomethionine (Se-Met) at a levels of 0.3 and 0.6 mg/kg of BW/day, respectively. All treatments were orally supplemented once/daily for five months as an experimental period, two months as preliminary period and other three months as a main collection period. Bulls were fed individually on a concentrate feed mixture (CFM), berseem hay (BH) and rice straw (RS). The CFM was composed of 65% un-corticated cotton seed meal, 9% wheat bran, 20% rice polish, 3% molasses, 2% limestone and 1% sodium chloride. Bulls were given individual feeds twice daily at 8.00 a.m. and 3.00 p.m., in order to meet the nutritional allowances according to NRC (2001), while fresh water was available as free choice. Bulls were housed individual under semi-open sheds.

Environmental conditions:

Average of highest and lowest ambient air temperature (AT, °C) and relative humidity (RH%) were recorded once each week in the same time of carrying out the physiological measurements for five months as an experimental period. Temperature-humidity index (THI) was calculated (Table 1) according to Thom (1959) using the following equation: THI= (0.8 X AT°C) + [(RH / 100) X (AT°C – 14. 4)] + 46.4. The obtained values of THI were classified as follows: < 72 = absence of heat stress, 72 to <74 = moderate heat stress, 74 to <78 = severe heat stress and more than 78 = very severe heat stress.

Table 1. Ambient temperature (AT, °C), relative humidity (RH, %) and temperature-humidity index (THI) during the experimental period.

Experimental period		Environmental conditions			Status of heat stress (HS)
		AT (°C)	RH (%)	THI	
Preliminary period	April	25.45 ± 0.64	35.15 ± 1.85	70.64 ± 0.75	Absence HS
	May	27.10 ± 0.41	45.19 ± 2.65	73.82 ± 0.64	Moderate HS
Main collection period	June	31.90 ± 1.20	51.05 ± 1.93	80.85 ± 1.53	Very severe HS
	July	33.30 ± 0.85	49.95 ± 2.75	82.48 ± 1.35	Very severe HS
	August	35.20 ± 0.58	50.40 ± 1.86	85.04 ± 0.78	Very severe HS

Thermoregulatory parameters:

Individual skin temperature, rectal temperature, pulse rate (PR) and respiration rate (RP) as a physiological measurements of bulls were recorded once each week through the experimental period. Skin temperature and rectal temperature were measured by digital thermometer. Counts of pulse rate (number of pulses/min) and respiration rate (number of breaths/min) were estimated using stop watch.

Sexual desire:

Sexual desire was determined in term of reaction time (RT) as described by Chenoweth (1981). The RT as a time elapsed between the exposure of a bull to a suitable stimulus and the first copulation was recorded using stop-watch. Also, testosterone concentration was determined in blood plasma and was taken as sexual desire indicator.

Semen collection and evaluation:

Semen samples were collected from all bulls of experimental groups twice weekly by means of an artificial vagina for three months (main collection period). One false mount had been always allowed before collection of the first ejaculates. Two successive ejaculates were obtained from each bull at each day of collection during main collection period of 12 weeks. Semen was collected in a graduated collecting tube. Semen-ejaculate volume (ml), percentage of progressive sperm motility, live sperm and sperm abnormalities, sperm-cell concentration ($\times 10^9$/ml) and total-sperm output ($\times 10^9$/ml) were estimated for each ejaculate according to Salisbury *et al.*(1978). Semen samples were centrifuged at 1000 *g* for 20 minutes. The supernatant was collected and kept at -20° C till analysis.

Enzymatic activities:

Acid phosphatase (ACP) was estimated as described by Kind and King (1954) by using colorimetric method. Alkaline phosphatase (ALP) was measured according to Guder (1996) using Roche kits. Alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) were estimated as described by Tietz (1995) using Roche Biological Kits. All enzymatic activities in the seminal plasma (AC,ALP,ALT and AST) were adjusted according to sperm-cell concentration (U/10^9 spermatozoa) according to Reitman and Frankle (1957).

Antioxidant enzymes:

Superoxide dismutase (SOD) and glutathione peroxidase (GSH) were determined according to Madesh and Balasubramanian (1998) and Goldberg and Spooner (1983) respectively.

Micro minerals:

Zinc (Zn) and selenium (Se) concentrations were determined using Atomic Absorption Spectophotometer (Pye Unicam) according to the method of Kolmer *et al.* (1951). In addition, fructose concentration was estimated according to Barakat and El-Sawaf (1964).

Blood samples:

Blood samples were collected from the jugular vein from bulls in each group in heparinized test tubes pre-treatment and monthly during main collection period of 12 weeks and centrifuged at 1000 *g* for 20 minutes. The blood plasma samples were separated and stored at -20°C till biochemically analysis. Total proteins and albumin concentrations were estimated colourimetrically according to Weichselbaum (1946) and Doumas *et al.* (1971), respectively. In addition, testosterone concentration was estimated by radio-immunoassay (RIA) according to Ekins (1984). Also, thyroxine (T_4), triiodothyronine (T_3) and cortisol hormones were evaluated by RIA procedure using the coated tubes kits purchased from (Diagnostic Products Corporation, Los Angeles, CA, USA) according to the procedure outlined by manufacturer. Zn and Se concentrations were evaluated similar to that of seminal plasma.

Statistical analysis:

Statistical analysis of data was carried out according to Snedecor and Cochran (1982) using General Linear Models procedure (GLM) of SAS (2004). Percentage values were transformed to arcsines before being analyzed. Means were compared using Duncan's multiple rang test Duncan (1955).

RESULTS AND DISCUSSION

Physiological response:

Data presented in Table 2 showed that skin and rectal temperatures, pulse rate (PR) and respiration rate (RR) of Friesian bulls were significantly ($P<0.05$) lowest for bulls supplemented with Z-Met in G_3 group as compared to other supplemented groups and control one, reflecting the highest physiological response of bulls supplemented with Z-Met at level of 4 mg/kg of BW/day under hot summer conditions in Egypt. Farm animals have been exposed to heat stress through summer months, in especially during June, July and August as reported by Abdel-Khalek (2000) on Friesian bulls and Wafa *et al.* (2017) on buffalo bulls. Habeeb *et al.* (2015) reported that summer in Egypt is characterized by high ambient temperature, intense solar radiation and high relative humidity, particularly in June, July and August months. In accordance with the present results, Khalifa *et al.* (2011) reported similar physiological response of buffalo cows treated with zincmethionine under hot environmental conditions during June, July and August months in Egypt.

Table 2. Physiological response of Friesian bulls supplemented with zinc-selenomethionine under hot summer conditions.

Item	Control	Supplemented groups			
		Z-Met		Se-Met	
	G1	(2mg/kg/day) G2	(4mg/kg/day) G3	(0.3mg/kg/day) G3	(0.6mg/kg/day) G4
Skin temperature (°C)	38.52 ± 0.2^a	36.58 ± 0.22^c	35.67 ± 0.18^d	36.75 ± 0.18^{bc}	37.33 ± 0.14^b
Rectal temperature (°C)	39.7 ± 0.41^a	37.91 ± 0.19^c	36.67 ± 0.14^d	38.25 ± 0.14^{bc}	38.83 ± 0.20^b
Pulse rate (pulse/min)	51.08 ± 1.25^a	44.33 ± 1.12^c	41.17 ± 0.80^c	47.33 ± 1.53^b	48.83 ± 1.61^b
Respiration rate (Br./min)	69.25 ± 2.52^a	61.67 ± 1.09^c	58.90 ± 1.10^d	64.25 ± 1.10^b	65.75 ± 1.08^b

a-e, Means within the same row with different superscripts are significantly different at ($P<0.05$)

Sexual desire:

Data presented in Table 3 showed that reaction time (RT) was significantly ($P<0.05$) shorter and testosterone concentration in blood plasma was significantly ($P<0.05$) higher in all supplemented groups than in the control group. Generally, the shortest ($P<0.05$) and highest ($P<0.05$) values of RT (51.25 second) and testosterone concentration (1.60 ng/ml) were recorded for bulls supplemented with Z-Met (G_3), while the longest ($P<0.05$) and lowest ($P<0.05$) values of RT (155.28 second) and testosterone concentration (0.69 ng/ml) were observed for un-supplemented bulls (G_1), respectively. Such results indicated that administration of Z-Met and Se-Met to Friesian bulls had a valuable impact on decreasing RT and increasing testosterone concentration, especially with Z-Met at a level of 4mg of BW, which recorded the best and optimal values under hot summer conditions in Egypt.

Sexual desire (libido) is an important factor in evaluation of male reproductive capacity, especially in insemination programs and depends on testosterone level in blood plasma El-Gohary *et al.* (2008) and Habeeb et al. (2015). Abd El-Latif (2001) reported that buffalo bulls supplemented with Se, E or Se+E recorded significantly decreased in RT and increased testosterone level in blood serum as compared to the control one. Abdel-Khalek *et al.* (2010) reported that the highest testosterone concentration and shortest RT were observed for bulls treated with Vit. E+Z, reflecting the highest sexual desire as compared to other treated groups and the control group.

Supplementation of organic zinc increased the testosterone concentration in crossbreed bulls Kumar *et al.* (2006) and in Murrah buffalo bulls Imam *et al.* (2009). Furthermore, El-Tohamy *et al.* (2012) reported that zincmethionine supplemented bucks recorded significantly increased in serum testosterone level and significantly decreased in reaction time as compared to other supplemented groups and the control one, especially during summer season in Egypt. In contrary, Mandal *et al.* (2008) reported that Zn supplementation did not improve serum testosterone concentration. This difference may be due to species variation, the frequency of the treatment given and duration of Zn supplementation.

The increase in testosterone concentration in different treatments, especially with Z-Met in G_3 may be attributed to the stimulatory effect of Se and Zn on pituitary gland and testicular steroidogenesis. Roy *et al.* (2013) reported that the synthesis of testosterone from lydig cells in the testes depends on the adequacy of Zn in the diet. Moreover, Hartoma *et al.* (1977) demonstrated that Zn is a structural part of protein involved in synthesis of testosterone. El-Masry *et al.* (1994) reported beneficial effect of Zn on testicular function by activating adenly cyclase system, which stimulates the synthesis of testosterone from leydig cells. Bedwal and Bahuguna (1994) reported that Zn plays a vital role in production and secretion of prolactin, LH and FSH and these in term, regulate spermatogenesis and testosterone production.

Table 3. Reaction time and testosterone concentration of Friesian bulls supplemented with zinc-selenomethionine under hot summer conditions.

Item	Control G1	Supplemented groups: Z-Me (2mg/kg/day) G2	Z-Me (4mg/kg/day) G3	Se-Me (0.3mg/kg/day) G3	Se-Me (0.6mg/kg/day) G4
Reaction time (second)	155.28 ± 3.67^{a}	63.97 ± 2.94^{d}	51.25 ± 1.90^{e}	84.95 ± 4.38^{b}	74.28 ± 3.15^{c}
Testosterone (ng/ml)	0.69 ± 0.11^{d}	1.44 ± 0.11^{ab}	1.60 ±0.12^{a}	1.07 ±0.08^{c}	1.17 ±0.09^{c}

a-e, Means within the same row with different superscripts are significantly different at (P<0.05)

Physical semen characteristics:

Results regarding physical semen characteristics of Friesian bulls presented in Table 4 reflecting that semen-ejaculate volume (SEV), percentage of progressive sperm motility (PSM) and live spermatozoa (LS), sperm-cell concentration (SCC/ml) and total-sperm output (TSO/ejaculate) were significantly (P<0.05) increased for bulls supplemented with G_2, G_3, G_4 and G_5 as compared to unsupplemented bulls G_1. On the other hand, percentage of sperm abnormalities (SAB) was significantly (P<0.05) decreased in all supplemented groups as compared to the control one. Generally, the highest (P<0.05) values of SEV, PSM, LS, SCC/ml and TSO/ejaculate and lowest (P<0.05) value of SAB were recorded for bulls supplemented with G_3 as compared to other supplemented bulls. Such results indicated the beneficial effects of treating Friesian bulls with Z-Met and Se-Met on sperm function to have ability of sperm movement within the female reproductive tract beside high fertilizing ability, especially with Z-Met at a level of 4 mg of BW under hot summer conditions in Egypt. In this respect, Bertelsmann *et al.* (2007) and Alavi-Shoushtari *et al.* (2009) reported that Zn and Se levels in both blood serum and seminal plasma are positively correlated with improved semen quality. These findings are in accordance with Abdel-Khalek *et al.* (1999), Hafez and Hafez (2000) and Habeeb *et al.* (2015), who reported that accessory glands; seminal vesicle and prostate gland, responsible for seminal plasma production are functionally controlled by testosterone and may be affected by dietary protein source. Furthermore, secretion of testosterone which activates the accessory sex gland plays the main role for secretion of large amount of seminal plasma from the accessory glands Mahmoud *et al.* (2005).

The increase in PSM, LS, SCC/ml and TSO/ejaculate and decrease in SAB in the present results is in accordance with the reports of improvement of semen quality in buffalo El-Hawary (2010), bulls, Kumar *et al.* (2006), buck, Shi *et al.* (2010) and in goats, Kumar *et al.* (2014).

The significant improvement in physical semen characteristics after administration of selenomethionine or zinc methionine may be due to the pronounced impacts of Se-Met and Z-Met, especially with Z-Met in G_3 as antioxidants on improving most sperm characteristics and may be attributed to the prevention of excessive generation of free radicals produced by sperm metabolism by means of antioxidant properties of Se-Met and Z-Met. Selenomethionine can protect the cell from free radicals which are produced during the phagocytosis process and normal cellular metabolism Spears (2003). Nizza *et al.* (2000) and Singh *et al.* (2000) who reported that dietary supplementation with methionine had a beneficial impact on semen quality by enhancing intracellular glutathione. Spermatogenesis requires the amino acid, especially arginine, methionine and cysteine Young *et al.* (2008) and minerals (Zn and Se) Cheah and Yang (2011). Patra *et al.* (2001) indicated that methionine acts as a precursor of amino acid for glutathione in the protection of cell from oxidative damage, also the thiol group of methionine was shown to chelate lead and remove it from tissues. Also, these results may be attributed to increasing testosterone, T_3 and T_4 levels in blood plasma of bulls treated with Se-Met and Z-Met which resulted in improvement of semen quality, being more efficient in bulls treated with Z-Met in G_3. These findings were supported by Kumar *et al.* (2013) who reported that Se and Zn supplementation increased T_3, T_4 and testosterone concentrations in blood serum of goat, which may play a vital role in production of good quality semen.

Table 4. Physical semen characteristics of Friesian bulls supplemented with zinc-selenomethionine under hot summer conditions.

Item	Control	Supplemented groups			
		Z-Me		Se-Me	
		(2mg/kg/day)	(4mg/kg/day)	(0.3mg/kg/day)	(0.6mg/kg/day)
	G1	G2	G3	G3	G4
Semen-ejaculate volume (ml)	2.05 ±0.25[c]	2.87 ±0.20[ab]	3.09 ±0.19[a]	2.37 ±0.17[bc]	2.63 ±0.18[ab]
Sperm motility (%)	72.67 ±1.18[d]	81.92 ±0.73[b]	86.0 ±1.21[a]	77.00 ±0.79[c]	80.25 ±0.80[b]
Live spermatozoa (%)	70.33 ±2.06[d]	83.42 ±1.70[b]	88.50 ±1.88[a]	78.08 ±1.44[c]	80.67 ±1.55[bc]
Sperm abnormalities (%)	19.08 ±1.51[a]	13.92 ±0.81[b]	10.67 ±0.82[c]	15.33 ±0.91[b]	14.67 ±0.79[b]
Sperm cells concentration ($\times 10^9$/ml)	1.19 ±0.08[d]	1.63 ±0.04[ab]	1.73 ±0.05[a]	1.46 ±0.03[c]	1.53 ±0.04[bc]
Total-sperm output ($\times 10^9$/ejaculate)	2.44 ±0.24[d]	4.68 ±0.39[b]	5.35 ±0.41[a]	3.46 ±0.24[c]	4.02 ±0.28[bc]

a-c, Means within the same row with different superscripts are significantly different at (P<0.05)

Bio-chemical semen characteristics:

Enzymatic activity:

Data in Table 5 revealed that the administration of bulls with Z-Met and Se-Met recorded significantly (P<0.05) decreased in the activities of transferases (ALT and AST) and phosphatases (ALP and ACP) enzymes in seminal plasma as compared to the control bulls, being the lowest (P<0.05) for bulls treated with Z-Met, especially in G_3 under hot summer conditions in Egypt. With regard to transferases activities (ALT and AST) in semen they are a good indicator of semen quality because it measures sperm membrane stability Zedda *et al.* (1996). Yousef *et al.* (2003) found that there was a negative correlation among increase of AST and ALT activities and decrease of semen ejaculate volume, sperm-cell concentration, total sperm output and sperm motility index. Therefore, the increase of semen quality was associated with the decrease in the activities of ALT and AST enzymes. Dhami *et al.*(1994) and Tabl *et al.* (2012) reported that the phosphatases (ALP and ACP) enzymes in semen plays a vital role in phosphorylation and transamination processes in sperm metabolism. The observed decrease of activities of ALT, AST, ALP and ACP enzymes in semen of bulls after administration of Se-Met and Z-Met may be attributed to increase the activities of antioxidant enzymes (GSH and SOD) in seminal plasma, which may provide better protection to spermatozoa from oxidative damage by free radicals or an increase of Se and Zn concentration in blood and seminal plasma in different treatments, especially with Z-Met in G_3. Similar results were reported by Khalifa (1997) and El-Hawary (2010). Zn supplementation may induce the membrane stabilizing action of zinc that prevent leakage of proteins, enzymes and other essential components of spermatozoa in order to extend the functional life of spermatozoa Kumar *et al.* (2006).

Antioxidative status:

Concerning the antioxidative status, the activity of superoxide dismutase (SOD), and glutathione peroxides (GSH) in seminal plasma were significantly (P<0.05) higher with all supplemented groups than the control one. Generally bulls supplemented with Z-Met in G_3 and Se-Met in G_5 showed the highest (P<0.05) activity of SOD and GSH in seminal plasma, respectively. However, differences between G_3 and G_5 groups on activity of GSH were insignificant (Table 5). These findings indicated that Z-Met and Se-Met supplementation had marked effect on increasing antioxidant defense system in seminal plasma of bulls under hot summer conditions in Egypt. In accordance with Kendall *et al.* (2000) in ram, and Shi *et al.* (2010) in buck, who reported that Se supplementation increased GSH and SOD concentration in seminal plasma, which are the main defense against free radicals. The percent findings are supported by El-Tohamy *et al.* (2012), who found that the administration of zinc methionine to male rabbits had a valuable impact on antioxidative status of seminal plasma which may provide better protection to spermatozoa from oxidative damage during heat stress.

Fructose and micro minerals:

Data presented in Table 5 cleared that bulls treated with Z-Met and Se-Met recorded significant (P<0.05) increase in fructose, zinc and selenium concentrations in seminal plasma as compared to untreated bulls. Generally, the highest (P<0.05) values of fructose, zinc and selenium in seminal plasma were recorded with G_3 group, while the lowest values were observed with the control G_1. Such results indicated the beneficial effect of treating Friesian bulls with Z-Met and Se-Met on increasing fructose, zinc and selenium concentrations in seminal plasma, especially with Z-Met at a level of 4mg of BW under hot summer conditions in Egypt. Fructose is secreted mainly from the seminal vesicles Hafez and Hafez (2000), so the observed increase in fructose concentration in seminal plasma of treated groups may be attributed to direct effect of Z-Met and Se-Met, especially with Z-Met in G_3 on functional of seminal vesicles and/or indirectly by increasing testosterone concentration, which had vital role on accessory sex gland function Abdel-Khalek *et al.* (1999) and Habeeb *et al.* (2016). The present findings are supported by El-Tohamy *et al.* (2012) who reported that male rabbits treated with zincmethionine showed the highest fructose level in seminal plasma as compared to other treated groups and the control during summer months. Concerning of Se and Zn level in seminal plasma, similar findings were reported by Massanyi *et al.* (2003) in bulls, and Shi *et al.* (2010) in goat, who reported that Se supplementation significantly increased the Se level in seminal plasma. Kumar *et al.* (2013) found that goat treated with Se and Zn recorded the highest value of Se and Zn in seminal plasma, while the lowest value of Se and Zn was observed in the control one.

Table 5. Bio-chemical semen characteristics of Friesian bulls supplemented with zinc-selenomethionine under hot summer conditions.

Item	Control	Supplemented groups			
		Z-Me		Se-Me	
	G1	(2mg/kg/day) G2	(4mg/kg/day) G3	(0.3mg/kg/day) G3	(0.6mg/kg/day) G4
Enzymatic activity (U/10^9 spermatozoa):					
ALT	23.25 ±1.43^{a}	15.25 ±1.07^{bc}	13.33 ±1.03^{c}	17.83 ±1.04^{b}	16.83 ±1.12^{b}
AST	56.67 ±2.41^{a}	35.33 ±1.72^{b}	32.91 ±1.64^{b}	37.42 ±1.90^{c}	35.58 ±1.31^{b}
ALP	179.08 ±5.43^{a}	109.42 ±3.97^{c}	96.83 ±3.45^{d}	125.17 ±3.21^{b}	117.42 ±3.31^{bc}
ACP	105.33 ±9.37^{a}	71.08 ±3.12^{bc}	64.92 ±3.35^{c}	85.58 ±3.27^{b}	76.25 ±2.91^{b}
Antioxidative status :					
Superoxide dismutase (U/ mg protein)	32.19 ±2.53^{d}	50.86 ±0.94^{b}	64.89 ±1.40^{a}	41.09 ±1.49^{c}	44.95 ±1.12^{c}
Glutathione peroxidase (U/ml)	5.70 ±0.44^{c}	11.46 ±0.54^{b}	13.37 ±0.62^{a}	10.09 ±0.42^{b}	14.33 ±0.67^{a}
Fructose (mg/100 ml)	326.17 ±14.43^{c}	394.67 ±7.11^{b}	424.17 ±9.12^{a}	370.67 ±7.46^{b}	387.92 ±6.71^{b}
Micro minerals:					
Zinc (ppm)	61.27 ±3.05^{d}	98.71 ±3.29^{b}	118.53 ±2.21^{a}	83.70 ±2.80^{c}	90.49 ±3.01^{c}
Selenium (ppb)	57.81 ±2.11^{c}	83.79 ±2.13^{b}	96.33 ±2.78^{a}	99.29 ±2.82^{a}	102.89 ±3.60^{a}

a-d, Means within the same row with different superscripts are significantly different at (P<0.05)

Blood metabolites:

Protein fractions:

Results presented in Table 6 reported that concentration of total proteins (TP) in blood plasma tended to be insignificantly higher with all treated groups than the control one. Concentration of albumin (AL) in blood plasma of bulls treated with Z-Met and Se-Met was significantly (P<0.05) increased, while globulin (GL) concentration was significantly (P<0.05) decreased as compared to the control bulls. Bulls treated with Z-Met in G_3 recorded the highest values of TP and AL and lowest (P<0.05) value of GL in blood plasma. This results may be indicated that increasing TP concentration was mainly related to increasing AL rather than GL concentration as affected by Z-Met and Se-Met treatment, especially with Z-Met at a level of 4mg of BW under hot summer condition in Egypt. Similar results were reported by Gunter *et al.* (2003), Chung *et al.* (2007) and El-Hawary (2010). The increase of total proteins and albumin level in blood plasma in different treatments, especially with Z-Met in G_3 was mainly attributed to the increase in methionine content in Z-Met, which essential for protein synthesis. Also, Singh *et al.* (2000) in buffalo bulls, and Patra *et al.* (2001) in rats, reported that dietary supplementation with methionine had a beneficial impact on the increase of blood total proteins and its fractions.

Hormonal profile:

Concerning the hormonal profile of Friesian bulls presented in Table 6, T_4 and T_3 concentrations in blood plasma were significantly (P<0.05) increased in treated groups, while cortisole concentrations were significantly (P<0.05) decreased as compared to the control group. Among treated groups, the difference between treated groups in T_4 concentration was insignificantly, being the highest value with G_3 group.

Generally, the highest (P<0.05) and lowest (P<0.05) values of T_3 and cortisole were recorded with G_3 group (1.41 and 3.71 ng/ml), respectively. While, the lowest (P<0.05) and highest (P<0.05) values of T_3 and cortisole were observed with G_1 (0.77and 5.54 ng/ml), respectively. The higher T_4 and T_3 levels in blood plasma of treated groups were mainly related to direct effects of Z-Met and Se-Met on increasing the production of thyroid stimulating hormone (TSH), which stimulates thyroid gland to produce thyroid hormones (T_4 and T_3), or immune-potentiating effects of treatments of thyroid gland. These finding are in accordance with McDowell (2003), Chung *et al.* (2007) and Ebrahimi *et al.* (2009). These results also may attribute to protect the thyrocytes from free radicals by treatments. Kohrle *et al.* (2007) reported that Se is a vital component of deiodinase enzyme and other selenoproteins, which play an essential role for the thyrocytes from free radicals which are produced during thyroxine synthesis. In Barbari bucks Kumar *et al.* (2013) showed that animals supplemented with Se and Zn recorded significantly increased T_4 and T_3 levels in blood serum as compared to the control one, which may play a vital role in production of high quality semen. Lowering cortisol levels in bulls treated with Z-Met and Se-Met, especially with Z-Met in G3 may be due to the decrease in adrenocorticotrophic hormone (ACTH). Our findings are supported by El-Hawary *et al.* (2017), who reported that T_4 and T_3 levels were significantly increased in buffalo cows supplemented with selenomethionine, while cortisol levels were significantly decreased as compared to the control one.

Micro minerals:

Regarding to blood plasma zinc and selenium concentration, bulls in treated groups showed significant (P<0.05) increase in zinc and selenium concentration in blood plasma as compared to the control one, being the highest (P<0.05) values of zinc in G_3 and selenium in G_5 and G_3. However, the differences between G_5 and G_3 group were insignificant (Table 6). This means that dietary Z-Met and Se-Met supplementation had marked effect on increasing zinc and selenium concentration in blood plasma of Friesian bulls, especially with Z-Met at a level of 4mg of BW under hot summer conditions in Egypt. In accordance with the present results, Massaryi *et al.* (2003) in bulls, reported that Se supplementation was significantly responsible for increased Se concentration in blood plasma. Also, Kumar *et al.* (2013) demonstrated that Zn and Se concentrations in blood serum significantly increased in animals supplemented with Se and Zn as compared to the control one. Also, Espinosa *et al.* (2015) in ewes and El-Hawary *et al.* (2017) in buffalo cows, reported that dietary supplementation with selenomethionine had a beneficial impact on increasing Se level in blood plasma as compared to the control.

In conclusion, orally administration of zinc-methionine and seleno - methionine to Friesian bulls had a valuable impact on sexual desire, semen quality and blood metabolites. Therefore, it can be recommended to treating the Frisian bulls with zincmethionine at a level of 4 mg/kg of BW/day during two preliminary months and three main semen collection months for improving the sexual desire, semen quality and blood metabolites under hot summer conditions in Egypt.

Table 6. Blood metabolites of Friesian bulls supplemented with zinc-seleno-methionine under hot summer conditions.

Item	Control	Supplemented groups			
		Z-Me		Se-Me	
	G1	(2mg/kg/day) G2	(4mg/kg/day) G3	(0.3mg/kg/day) G3	(0.6mg/kg/day) G4
Protein fractions:					
Total protein (g/dl)	6.45 ±0.19	6.76 ±0.12	6.89 ±0.11	6.50 ±0.20	6.83 ±0.10
Albumin (g/dl)	2.90 ±0.14[b]	3.90 ±0.07[a]	4.04 ±0.08[a]	3.83 ±0.07[a]	3.88 ±0.06[a]
Globulin (g/dl)	3.55 ±0.24[a]	2.86 ±0.14[b]	2.85 ±0.15[b]	2.67 ±0.27[b]	2.95 ±0.13[b]
Hormonal profile :					
Cortisole (ng/ml)	5.54 ±0.21[a]	4.38 ±0.26[b]	3.71 ±0.07[c]	4.54 ±0.18[b]	4.47 ±0.19[b]
T_4 (ng/ml)	107.33 ±8.68[b]	143.87 ±8.22[a]	159.09 ±4.43[a]	139.17 ±5.71[a]	152.20 ±6.30[a]
T_3 (ng/ml)	0.77 ±0.04[c]	1.29 ±0.06[ab]	1.41 ±0.07[a]	1.16 ±0.06[b]	1.28 ±0.07[ab]
Micro minerals:					
Zinc (ppm)	2.74 ±0.11[c]	3.82 ±0.12[b]	4.33 ±0.16[a]	3.53 ±0.13[b]	3.78 ±0.09[b]
Selenium (ppb)	139.25 ±4.11[d]	179.50 ±2.10[bc]	184.50 ±2.90[ab]	173.75 ±3.50[c]	1914.25 ±3.20[a]

a-d, Means within the same row with different superscripts are significantly different at (P<0.05)

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الإستجابة الفسيولوجية وجودة السائل المنوى ومكونات الدم لطلائق الفريزيان المعاملة بالزنك- سيلينوميثيونين تحت ظروف الصيف الحار فى مصر

أشرف فرج السعيد الهوارى ، عبد الفضيل عبد الحفيظ جبر و محمد عبد الحكيم الكشك
معهد بحوث الإنتاج الحيوانى – مركز البحوث الزراعية – الدقى – الجيزة - مصر

أجريت هذه الدراسة بمحطة بحوث الإنتاج الحيوانى بالجميزة بهدف دراسة تأثير معاملة طلائق الفريزيان بالزنك ميثيونين و السيلينوميثيونين على الإستجابة الفسيولوجية والرغبة الجنسية و بعض الصفات الطبيعية و الكيميائية للسائل المنوى ومكونات الدم. استخدم فى هذه الدراسة عشرين طلوقة فريزيان تم تقسيمهم إلى خمسة مجاميع متشابهة بكل منها أربعة طلائق ، المجموعة الأولى لم يتم معاملتها و تركت كمجموعة ضابطة ، بينما المجموعات الثانية و الثالثة و الرابعة و الخامسة تم معاملتها بالزنك ميثيونين بمستويات (٢ ، ٤ ملجم / كجم من وزن الجسم /يوم ، تجريع) ، بالسيلينوميثيونين بمستويات (٠.٣ ، ٠.٦ ملجم / كجم من وزن الجسم/ يوم ، تجريع) على التوالى لمدة شهرين كفترة تمهيدية ثم ثلاثة أشهر كفترة رئيسية لتجميع السائل المنوى ، و التى أخذت أثناءها القياسات التالية : الرغبة الجنسية (زمن التجاوب الجنسى ، تركيز هرمون التستوستيرون) ، الصفات الطبيعية للسائل المنوى (حجم القذفة ، النسبة المئوية لحركة الحيوانات المنوية ، النسبة المئوية للحيوانات المنوية الحية و الشاذة ، تركيز الحيوانات المنوية / مل ، تركيز الحيوانات المنوية / قذفة). تم تجميع عينات الدم قبل المعاملة ثم شهرياً خلال الفترة الرئيسية لتجميع السائل المنوى وذلك لتقدير تركيز البروتين الكلى و الألبيومين و الجلوبيولين وهرمونات الغدة الدرقية و الكورتيزول و التستوستيرون وكذلك عنصرى الزنك و السيلينيوم فى بلازما الدم. أيضا تم تقدير النشاط الإنزيمى لكل من الإنزيمات الناقلة لمجموعة الأمين AST وALT و الفوسفاتيز القاعدى (ALP) و الحامضى (ACP) و الإنزيمات المضادة للأكسدة (GSH و SOD) بالإضافة إلى الفركتوز و الزنك و السيلينيوم فى بلازما السائل المنوى . أظهرت الدراسة وجود نقصاً معنوياً على مستوى ٥% فى زمن التجاوب الجنسى و إرتفاعاً معنوياً على مستوى ٥% لتركيز هرمون التستوستيرون فى بلازما الدم فى كل المجموعات المعاملة مقارنة بالمجموعة الضابطة ، حيث سجلت مجموعة الطلائق المعاملة بالزنك ميثيونين وبخاصة المجموعة الثالثة أقل زمن للتجاوب الجنسى و أعلى تركيز لهرمون التستوستيرون . أوضحت النتائج وجود تحسناً معنوياً ملحوظاً على مستوى ٥% فى الصفات الطبيعية للسائل المنوى من حيث حجم القذفة و حركة الحيوانات المنوية و الحيوانات المنوية الحية و الشاذة وتركيز الحيوانات المنوية / مل و تركيز الحيوانات المنوية /قذفة وذلك فى كل المجموعات المعاملة مقارنة بالمجموعة الضابطة ، وكانت أفضل النتائج لمجموعة الطلائق المعاملة بالزنك ميثونين ، و بخاصة المجموعة الثالثة. أظهرت الدراسة أن المجموعة الثالثة المعاملة بالزنك ميثيونين أحدثت إنخفاضاً معنوياً على مستوى ٥% فى الإنزيمات الناقلة لمجموعة الأمين و الفوسفاتيز القاعدى و الحامضى وإرتفاعاً معنوياً على مستوى ٥% فى الإنزيمات المضادة للأكسدة بالإضافة إلى الفركتوز و الزنك و السيلينيوم فى بلازما السائل المنوى مقارنة بالمجموعات المعاملة الأخرى أو المجموعة الضابطة. أيضا وجد أن المجموعات المعاملة بالزنك ميثيونين و السيلينوميثيونين أظهرت تحسنا معنوياً على مستوى ٥% فى مكونات الدم ، حيث إرتفع تركيز كلا من الألبيومين و هرمونات الغدة الدرقية وعنصرى الزنك و السيلينيوم ، بينما انخفض تركيز كل من الجلوبيولين و هرمون الكورتيزول مقارنة بالمجموعة الضابطة. وبناء عليه ومن الناحية التطبيقية توصى الدراسة بمعاملة طلائق التلقيح الإصطناعى بالزنك ميثيونين (٤ ملجم / كجم من وزن الجسم /يوم ، تجريع) لمدة خمسة أشهر (شهرين كفترة تمهيدية ثم ثلاثة أشهر كفترة رئيسية لتجميع السائل المنوى) لتحسين الرغبة الجنسية وجودة السائل المنوى و مكونات الدم تحت ظروف الصيف الحار فى مصر.