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Effect of Selenomethionine and Levamisole Administration on Productive and Reproductive Efficiency and Blood Metabolites of the Egyptian Dairy Buffaloes

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ABSTRACT

The present study aimed to investigate the effect of administration of selenomethionine, levamisole HCl and selenomethionine plus levamisole HCl to Egyptian buffalo-cows during the late gestation and early lactation period on productive traits (milk yield and composition), reproductive performance, blood metabolites and economical efficiency. Twenty-eight of Egyptian buffalo-cows (2-4 lactation seasons) in the late gestation (two months before calving) were divided into four similar groups (7 buffaloes/each). Buffaloes in the 1st group were untreated and served as a control, while those in the 2nd, 3rd and 4th were orally treated with 0.9 mg/kg DM twice weekly of selenomethionine (Se-Met), 0.5 mg/kg BW/week, subcutaneously of levamisole HCl (LEV), and 0.9 mg/kg DM orally twice weekly of selenomethionine plus 0.5 mg/kg BW/week, subcutaneously of levamisole HCl (Se-Met + LEV) respectively, during the late gestation and early lactation period (two months before and after calving). The obtained results revealed that the interval from calving to complete fetal membranes drop (FMD) and uterine involution (UI) were significantly ($P<0.05$) shorter with all treated groups than untreated grou p, being the shortest ($P<0.05$) and longest ($P<0.05$) values of interval from calving to FMD and UI were recorded for buffaloes treated with Se-Met+LEV(G4) and untreated buffaloes (G1), respectively. Buffaloes treated with Se-Met+LEV (G4) showed significantly ($P<0.05$) decrease in the interval from calving to ovarian structure, first estrus postpartum, service period and days open as compared to the control buffaloes (G1) or buffaloes treated with Se-Met (G2) or LEV (G3). The highest ($P<0.05$) and lowest ($P<0.05$) values of number of service /conception were observed with the control buffaloes and Se-Met+LEV (G4), respectively. The significantly ($P<0.05$) reduction of calving interval in all treated groups, especially with Se-Met+LEV. Also, based on cumulative values of conception rate, all buffaloes in Se-Met+LEV and Se-Met groups were conceived with 103 days postpartum, followed by 85.71% in LEV group, while non-buffaloes in control group. Buffaloes treated with Se-Met, LEV and Se-Met+LEV recorded significantly ($P<0.05$) increase in the actual milk yield as compared to the control buffaloes (9.41, 8.89 and 10.33 vs. 7 kg/day), respectively. Moreover, values of RBC's, WBC's , Hb, Ht, Gl, T_4 and T_3 were significantly ($P<0.05$) increased in all treated groups, while cortisol level was significantly ($P<0.05$) decreased as compared to the control group. In conclusion, the administration of selenomethionine, levamisole HCl and their combination to Egyptian buffalo-cows during the late gestation and early lactation period exerted a beneficial effect on reproductive performance, milk yield and composition, blood metabolites and economical efficiency. Therefore, it can be recommended to treatment of buffalo-cows with selenomethionine at a level of 0.9 mg/kg of DM/twice weekly, orally and injected subcutaneously with levamisole at a level of 0.5 mg/kg BW/week for improve of the reproductive efficiency, milk yield and economical efficiency.

Keywords: Buffalo-cows, selenomethionine, levamisole, milk yield, reproductive efficiency.

INTRODUCTION

The transition period is a time of increased nutritional demands and risk to metabolic and infectious diseases in dairy cows due to a suppressed immune system (Huozha *et al.,* 2010). The occurrence of health problems during the transition period is clearly a major complexed factor for subsequent reproductive performance (Ferguson, 2001 and El-Desouky, 2014). Selenium (Se) is physiologically important, because it acting as an integral component of selenoproteins (including glutathione peroxidase, iodothyronine deiodinase, and selenoprotein P and W, and thioredoxine reductase), which plays an important structural and enzymatic functions (Flohe *et al.,* 2000). Selenium is an inactivator of toxic heavy metals, causes apoptosis in tumor cells (Behne *et al.,* 1996), prevents or delays the cell aging by protecting the mitochondrial membranes (Wesolowski and Ulewicz, 2000), and protects the cell lipids from damaging effects of reactive oxygen species (Musik *et al.*, 2003). Selenium is involved in vitamin metabolism, protein synthesis, the immune system of animal (Cortinhas *et al.*, 2010), and metabolism of thyroid hormones (McDowell, 2003). Selenium deficiency affects animals living in areas with low natural concentrations of Se causes a wide range of health problems including retained placenta, metritis, mastitis, milk fever, ketosis, postpartum anestrus, and severely suppressed immune function (Sattar *et al.*, 2007 and Cortinhas *et al.*, 2010 and 2012). In Egypt, selenium level in the feedstuffs may be lower than the adequate levels set at 0.3 ppm which was recommended by NRC (2001). Various methods of supplementation have been developed to increase intake of selenium. Recently, close attention has been paid to the development of new preparations containing organically bound selenium, from the organic compounds; selenomethionine has been described most thoroughly. Selenomethionine fulfils the criteria of essential amino acids for human and monogastric animals. The same can be said for ruminants, even though these carry bacteria in their rumen that produce Se-met (Schrauzer and Surai, 2009). Thus, use of trace element from organic sources in animals nutrition (i.e, complexed and chelated amino acids), which is more bioavailable (Spears, 2003) compared with those from inorganic sources, can be an important tool in maximizing milk production and maintaining health.

Levamisole hydrochloride is used as an antinematodal (Spence *et al.*, 1992) and immunomodulatory agent (Mamdouh, 2000; Pancarci *et al*., 2007 and Ayman, 2013). The reproductive performance of cattle was believed to be improved following treatment with levamisole. Levamisole therapy during the dry period was significantly decreased the incidences of mastitis, fetal death, and endometritis due to its immunopotentiating activity in cows (Flesh *et al.*, 1982) and in bubaline and bovine (Arfan, 2009). Kacar *et al.* (2007) concluded also that the administration of levamisole of pregnant cows significantly decreased the incidence of abortion, fetal death, retention of placental, mastitis, metritis and uterine and vaginal prolapse. The important role of levamisole as immunostimulant agent appeared not only in improving the reproductive performance, but also in controlling mastitis and udder heath. Injection of cattle by levamisole during late gestation period was observed to reduce rate of incidence and severity of subclinical and clinical mastitis comparing to untreated cattle (Anderson, 1984). In addition to controlling the under health, levamisole was noticed to increase the rate of milk production up to 4.8% per cow per lactation (Spence *et al.*, 1992).

Therefore, the present study aimed to define the effect of administration of selenomethionine, levamisole HCl and selenomethionine plus levamisole HCl to Egyptian buffalo-cows during the late gestation and early lactation period on productive traits (milk yield and composition), reproductive performance, blood metabolites and economic efficiency

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.

Animals and experimental groups

Twenty-eight Egyptian buffalo-cows healthy and clinically free of external and internal parasites at 60 day prepartum with an average live body weight of 600 ± 50 kg, 2-4 parities and aged 4-6 years were used in the present study.

Animals were randomly divided into four similar groups (7 each) based on live body weight (LBW), body condition score (BCS) according to Veerkamp *et al.* (2001), expected calving date and age at the beginning of the experiment. Animals in the 1st group were untreated and served as a control, while those in groups 2nd, 3rd and 4th were orally treated with selenomethionine (Se-Met) at level of 0.9 mg/kg of DM twice weekly, levamisol HCl (LEV) at level of 0.5 mg/kg of BW/week, subcutaneously and Se-Met at level of 0.9 mg/kg of DM twice weekly, orally plus LEV at level of 0.5 mg/kg of BW/week, subcutaneously, respectively, during two months before and after calving. Buffalo-cows maintained in a common exercise area with open housing and fed the same dry cow ration. Also, animals were fed individually on a concentrate feed mixture (CFM), which composed of (28% undecorticated cotton seed meal, 30% wheat bran, 25% yellow corn, 10% rice bran, 3.5% molasses, 2% limestone, 1% sodium chloride and 0.5% minerals mixture), berseem hay (BH) and rice straw (RS), in order to meet the nutritional allowance of buffalo-cows during the late gestation and early lactation periods according to NRC (2001). While fresh water and mineral blocks were available as free choice. Chemical composition of feedstuffs (Table 1) was analyzed according to A.O.A.C. (1995).

Table 1. Chemical composition of different feedstuffs used in the experimental rations

Item	Chemical composition (% on DM basis)							
	DM	OM	CP	CF	EE	NFE	Ash	Se (ppm)
CFM	91.38	90.43	16.81	16.22	2.90	54.50	9.57	0.089
BH	91.27	89.37	11.69	33.48	1.31	42.89	10.63	0.064
RS	92.11	85.94	4.85	39.89	1.61	39.59	14.06	0.034

Reproductive measurements

All buffalo-cows were put under continual observation immediately after calving to record normal dropping and retention of the fetal membranes. Uterine involution was estimated by rectal examination at regular interval (twice weekly) till complete involution of internal genital organs according to Ibrahim (2004). Ovarian structures were calculated as the first appearance of corpus luteum (CL) on the ovary after calving. Animals in all groups were visually observed for detection of estrus behavior, using teaser bull introduced for 3 times/day at 6 am, 12 pm and 6 pm. The teaser bull was allowed to run with females for 30 minutes on each occasion. Natural mating was adapted by proven buffalo bulls, within the second half of day of estrus. The interval from calving to first estrus and conception (days open) were recorded. Then, service period (SP) length, number of services per conception (NS/C), gestation period (GP) length, calving interval (CI) and conception rates (CR) were calculated.

Milk samples

After calving, all buffalo-cows were allowed to nurse their calves for only one week postpartum (period of colostrum intake); thereafter, the dams were transferred to the milking unit and milked twice daily at 6 am and 5 pm. Daily milk yield of each animal was recorded. Composite and representative milk samples (2 milking/ day) were well mixed and analyzed biweekly using Milkoscan apparatus.

Blood samples

Blood samples were collected biweekly from the jugular vein in heparinized test tubes during two months before and after calving from all buffalo-cows. Blood samples were divided into two portions. The first portion was immediately centrifuged at 600 for 15 minutes and plasma were the separated and stored at -25°C until analysis. Plasma concentrations of thyroxine (T4), triiodothyronine (T3) and cortisol hormones were evaluated by the radioimmunoassay (RIA) procedure using the coated tubes kits purchased from (Diagnostic products corporation, Los Angeles, CA, USA) according to the procedure outlined by manufacturer. Glucose (Gl) and beta-hydroxy butyrate (BHB) concentrations were estimated in the plasma using commercial chemical regent kits (Sigma kit #315 and 310-A, Sigma Diagnostics) according to Trinder (1969) and Williason *et al.* (1962). While, plasma samples were diluted by distilled water at 1:9 ratio and analyzed for Se directly using Graphite Furnace Atomic Absorption Spectrometry (ATI Unicam Model 939, UK). Also, Se concentration was estimated in feed samples using the same procedure after wet ashing with nitric acid and H2O2 in microwave unit. The second portion was taken to determined hemoglobin (g/dl), hematocrit (%), red blood cells (RBC's × 106/mm3) and white blood cells (WBC's × 106/mm3)

Statistical analysis

Data were analyzed using the General Linear Models procedure (GLM) of SAS (2004). Means were compared using Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Fetal membranes drop and uterine involution

Data in Table 2 revealed that the time elapsed from calving to complete fetal membranes drop (FMD) was significantly ($P<0.05$) shorter with all treated groups than the control group. Among treated groups, the time of FMD was significantly (P<0.05) shorter with G4 as compared to G2 and G3 groups. However, the difference between G2 and G3 on time of FMD was insignificant.

Results presented in Table 2 showed also that the interval from calving to complete cervical closure (CC), normal position of the uterus (PU) and uterine horns symmetricity (UHS) occurred significantly (P<0.05) earlier with all treated groups than untreated group. Among treated groups, buffalo-cows treated with Se-Met (G2) and LEV (G3) showed significant (P<0.05) increase the interval from calving to CC, PU and UHS as compared to those treated with a combination of Se-Met+LEV (G4); however, the differences between Se-Met and LEV treatments were insignificant. Generally, the shortest (P<0.05) and longest (P<0.05) values of interval from calving to FMD, CC, UP and UHS were recorded with G4 and G1 groups, respectively. Such results indicated the beneficial effects of treating the Egyptian buffalo-cows with Se-Met, LEV and Se-Met+LEV on shorting the interval from calving to complete fetal membranes drop and uterine involution, especially with Se-Met+LEV (G4).

In accordance, Ayman (2013) reported that time required from calving to complete fetal membranes drop and uterine involution was significantly decreased in buffaloes treated with levamisole as compared to untreated buffaloes. Placental expulsion period and uterine involution period was significantly shorter in cows treated with vitamin E-selenium than in the control cows (Sattar *et al*., 2003a and 2007). Also, Niwinska and Andrzejewski (2013) reported that dairy cows supplemented with selenomethionine had significantly reduced incidence of retained fetal membranes and metritis and improvement of the immune system as compared to control cows. Moreover, Bula and Ositis (2008) demonstrated that placental expulsion period was significantly shorter in beef cattle treated with organic trace minerals than in control cows. The injection of vitamin E-selenium and levamisole resulted in faster fetal membranes drop and uterine involution than of control cows (Mamdouh, 2000). In contrary, Gwazdaushas *et al*. (1979) and Hassan (1994) reported that injection of vitamin E-selenium did not improve the reproductive performance. This difference may be attributed to differences in the pretreatment selenium status of the animals, the frequency of the treatment given or the dose rate (Awad *et al.,* 1985).

The rapid and earlier FMD and UI after administration of levamisole and selenomethionine may be due to immune-potentiating effect of levamisole (El-Desouky, 1997 and Ayman, 2013). Selenomethionine can protect the cell from free radicals which are produced during the phagocytosis process and normal cellular metabolism (Spears, 2003; Bula and Ositis, 2008 and Cortinhas *et al*., 2010). These results also probably attributed to the higher T3 and T4 hormones and glucose concentrations in buffaloes treated with Se-Met, LEV and Se-Met+ LEV which resulted in improvement of uterine muscular contraction and function, being more efficient of buffaloes treated with a combination of Se-Met+ LEV (G4).

Table 2. Fetal membranes drop and uterine involution of Egyptian buffalo-cows as affected by the experimental treatments

Item	Control (G1)	Treatments		
		Se-Met (G2)	LEV (G3)	Se-Met+LEV (G4)
Fetal membranes drop (hours)	11.57 ± 1.70[a]	6.50± 0.59[b]	6.07±0.77[b]	4.43 ± 0.40[c]
Uterine involution (day):				
Closure of the cervix/day	28.86 ± 3.25[a]	24.14 ± 1.96[b]	26.85 ± 1.84[b]	19.00 ± 0.90[c]
Position of the uterus/day	46.57 ± 4.52[a]	36.00 ± 3.09[b]	33.86 ± 1.44[b]	26.57 ± 1.77[c]
Uterine horns symmetricity / day	65.14 ± 8.86[a]	42.71 ± 3.29[b]	41.43 ± 2.33[b]	33.00 ± 2.77[c]

a-c, Means denoted within the same row with different superscripts are significantly different at (P<0.05)
control: untreated buffalo-cows; Se-Met: selenomethionine and LEV: levamisole

Reproductive parameters

Data presented in Table 3 revealed that buffaloes treated with Se-Met, LEV and Se-Met+LEV showed significantly (P<0.05) reduction in the interval from calving to ovarian structure (OS), first estrus postpartum (FE), days open (DO) and service period (SP) as compared to untreated buffaloes. Among treated groups, all values, except gestation period (GP) were significantly (P<0.05) shorter with Se-Met+LEV than other treated group. However, the difference between Se-Met and LEV was insignificant. Generally, the shortest (P<0.05) and longest (P<0.05) values of OS, FE, DO and SP were recorded for buffaloes treated with Se-Met+LEV and untreated buffaloes, respectively. Such results indicated that administration of Se-Met, LEV and their combination to buffalo-cows during late gestation and early lactation period had a valuable impact on reproductive performance, especially with Se-Met+LEV. The findings are in accordance with Qureshi *et al.* (1997) and Mamdouh (2000), who showed that cows treated with levamisole recorded significant decrease in the first estrus postpartum, days open and service period length as comported to the control cows.

Table 3. Some reproductive parameters of Egyptian buffalo-cows as affected by the experimental treatments

Item	Control (G1)	Treatments		
		Se-Met (G2)	LEV (G3)	Se-Met+LEV (G4)
Ovarian structure/day	87.14 ±14.13[a]	51.71 ± 5.50[b]	52.00 ± 3.80[b]	43.85 ± 4.31[c]
First estrus postpartum /day	110.71 ± 18.7[a]	68.57 ± 5.80[b]	65.57 ± 5.36[b]	54.00 ± 4.34[cb]
Days open/day	172.57 ± 14.20[a]	75.71 ± 6.31[b]	77.28 ± 8.40[b]	54.00 ± 4.34[c]
Service period/day	61.86 ± 16.52[a]	7.14 ± 7.14[b]	11.71 ± 8.63[b]	0.00 ± 0.00[c]
Gestation period/day	314.71 ± 3.21[a]	312.71 ± 2.94[a]	314.57 ± 2.84[a]	316.14 ± 2.50[a]

a-c, Means denoted within the same row with different superscripts are significantly different at (P<0.05)
Control: untreated buffalo-cows; Se-Met: selenomethionine and LEV: levamisole

The shorter days open after levamisole injection was expected as a result of its immune-potentiating effect and improve the general health condition and body metabolism of the animal (Ayman, 2013). First estrus postpartum and service period were significantly reduced in cows treated prepartum with vitamin E-selenium as compared to untreated cows (Sattar *et al.*, 2007). It has been reported that cattle supplemented with either the organic trace minerals (Bula and Ositis, 2008) or selenomethionine (Niwinska and Andrzejewski, 2013) had significant improvement in reproductive efficiency in cattle. Our findings were supported by Kotowski (1991) and Mamdouh (2000), who reported that there was significant reduction in the time from calving till the first estrus postpartum and days open in cows treated with levamisole and vitamin E-Selenium as compared to control cows. In contrary, Hassan (1994) did not found any significant effect of levamisole and vitamin E-selenium injection on reproductive performance of buffaloes.

Number of service/conception and calving interval

Results of number of service/conception (NS/C) shown in Table 4 cleared that values of NS/C were significantly ($P<0.05$) lower in buffaloes treated (G2, G3 and G4) than untreated buffaloes (G1), being significantly ($P<0.05$) lower in buffaloes treated in G4 than other treated and untreated buffaloes. It is interested to note that the marked variation among those groups were related to that about 85.71, 71.43 and 100% of buffaloes in G2, G3 and G4 groups, respectively, were conceived from the 1st service verses 14.29% in G1. Also, 14.29, 28.57 and 28.57% were conceived from the 2nd service in G2, G3 and G1, respectively. However, about 57.14% in G1 required 3rd service to be conceived (Table 4). In accordance with Qureshi *et al*. (1997) and Mamdouh (2000), the administration of levamisole was significantly decreased the number of service/conception and increase the conception rate in cows. Our findings were supported by those obtained by Ayman (2013), who showed that number of service/conception was significantly ($P<0.05$) lesser in buffalo-cows treated with levamisole than that in the control (1.17 vs. 1.83 services, respectively). Also, about 83.33% of animals in levamisole group were conceived from the 1st service compared with 33.3% in control animals. Also, reproductive performance of cattle was significantly improved by supplementation with organic trace minerals (Bula and Ositis, 2008), or selenomethionine (Niwinska and Andrzejewski, 2013). Moreover, Huang *et al*. (2002) reported that dairy cows treated with selenomethionine showed significantly decreased in number of service/conception and the incidence of obstetrical diseases.

Results presented in Table 4 showed that calving interval (CI) was significantly ($P<0.005$) shorter for buffaloes treated (G2, G3 and G4) than untreated buffaloes in G1 (388.42, 391.85 and 370.14 vs. 487.28 days, respectively). Among treated groups, CI was significantly ($P<0.05$) shorter in G4 than G2 and G3 groups. However, the difference between G2 and G3 was insignificant. The marked shortness of CI for the treated buffaloes (G2, G3 and G4) groups was associated with about 100, 85.71 and 100% of buffaloes in these groups, respectively, showed CI from 360 to 420 days as comparted to 0.00% for untreated buffaloes (G1). However about 71.43% of buffaloes in G1 showed CI from 481 to 540 days (Table 4).

Table 4. Number of service/conception, calving interval and frequency distribution (%) of Egyptian buffalo-cows as affected by the experimental treatments

Item		Control (G1)	Treatments		
			Se-Met (G2)	LEV (G3)	Se-Met+LEV(G4)
Number of service/ conception		2.43 ± 0.3^{a}	1.14 ± 0.14^{b}	1.29 ± 0.18^{b}	1.00 ± 0.00^{c}
1st service	Frequency	14.29	85.71	71.43	100
2nd service	distribution	28.57	14.29	28.57	-
3rd service	(%)	54.14	-	-	-
Overall		100 %	100 %	100 %	100 %
Calving interval (day)		487.28 ± 14.31^{a}	388.42 ± 4.98^{b}	391.85 ± 8.35^{b}	370.14 ± 3.4^{c}
360 - 420	Frequency	-	100	85.71	100
421 - 480	distribution	28.75	-	14.29	-
481 - 540	(%)	71.43	-	-	-
Overall		100 %	100 %	100 %	100 %

a-c, Means denoted within the same row with different superscripts are significantly different at ($P<0.05$)
Control: untreated buffalo-cows; Se-Met: selenomethionine and LEV: levamisole

The observed reduction in CI of treated groups was mainly related to decreasing first estrus postpartum, service period and days open length as compared to untreated group. Similarly, Kotowski (1989) and Ayman (2013) demonstrated that prepartum injection of cows by levamisole in the dry period resulted in shorten calving interval as compared to the control.

Generally, Se-Met, LEV and Se-Met+LEV treatments reduced NS/C and CI of Egyptian buffalo cows, especially with a combination of Se-Met+LEV, which had a valuable impact on NS/C and CI.

Conception rate (CR %)

Data in Table 5 showed that within 60 days postpartum, buffaloes in G4 showed the highest conception rate (85.71%), followed by G2 (28.58%) and G3 (14.29%), while 0.00% in the control group G1 were conceived. Within 61 – 103 days, the corresponding rates were 14.29, 71.42, 71.42 and 0.00%, respectively. Also, within 104 – 146 days postpartum, about 14.29 and 28.58% of buffaloes treated in G3 and untreated buffaloes G1 were conceived, meanwhile, about 71.42% of buffaloes in G1 required more than 147 days postpartum to be conceived. Based on cumulative values of conception rate, all buffaloes in G4 and G2 were conceived within 103 days postpartum, followed by 85.71% in G3, while 0.00% in G1. Within 146 days postpartum, all buffaloes in G3 were conceived. However, all buffaloes in G1 required more than 146 days postpartum to be conceived (Table 5). The concurrent results come in the same line with finding with Ayman (2013), who reported that within 90 days postpartum, conception rate was higher in buffaloes treated with levamisole than untreated buffaloes (100 vs. 50%, respectively). Cows supplemented with organic trace minerals showed significantly increase in conception rate as compared to untreated cows (Bula and Ositis, 2008). The injection of levamisole and vitamin E-selenium to cows caused significant (P<0.01) increase in conception rate (Mamdouh, 2000). The increase of CR% in different treatments, especially with Se-Met+LEV may be due to the increase in T4 and T3 and glucose levels, which are necessary for secretion of GnRH and LH, which stimulates development of ovarian luteal cells. Also, it may increase of progesterone concentration from steroidogenic cells that is associated with inhibit production of prostaglandin F2α (PGF2α) from the uterine tissue and estradiol-17β in order to increase the lifespan of the corpus luteum (CL), potentially improving survival of the embryo LHRH-LH releasing hormone. These findings are in accordance with those of Thatcher and Staples (2000), who showed that glucose has direct effect on the central nervous system and gonadal tissues, and on hypothalamus-pituitary-ovary axis. Glucose is also necessary for progesterone production by steroidogenic cells (Zakar and Hertelendy, 1980).

Table 5. Actual and cumulative conception rate (CR) of Egyptian buffalo-cows at successive postpartum days as affected by the experimental treatments

Postpartum day	Control (G1)	Treatments Se-Met (G2)	LEV (G3)	Se-Met+LEV (G4)
Actual conception rate (CR %):				
Within 60 days	-	28.58	14.29	85.71
Within 61-103 days	-	71.42	71.42	14.29
Within 104 - 146 days	28.58	-	14.29	-
< 147 days	71.42	-	-	-
Cumulative conception rate (CR %):				
Within 60 days	-	28.58	14.29	85.71
Within 103 days	-	100	85.71	100
Within 146 days	28.58	-	100	-
After 147 days	100	-	-	-

a-c, Means denoted within the same row with different superscripts are significantly different at (P<0.05)
Control: untreated buffalo-cows; Se-Met: selenomethionine and LEV: levamisole

The administration of immunomodulatory or anthelmintic doses of levamisole HCl to ewes do not interfere with CI development. Also, the increase in progesterone concentration following estrus indicated that all ewes ovulated without the disruption of ovulation by levamisole (Pancarci *et al*., 2007).

Milk yield and composition

Data presented in Table 6 showed that buffalo-cows in G2, G3 and G4 groups were significant (P<0.05) increase in the actual milk yield (AMY) as compared to untreated group in G1 (9.41, 8.89 and 10.33 vs. 7.00 kg/day), respectively. The AMY was significantly (P<0.05) higher with G4 than G3 groups. However, the differences among G2 and G3 or G4 groups on AMY were insignificantly. Generally, the highest (P<0.05) and lowest (P<0.05) value of AMY were observed with G4 and G1 groups, respectively.

Percentages and milk yield constituents (fat, protein, lactose, SNF and TS) were significantly (P<0.05) higher in all treated groups than the control group, being the highest (P<0.05) and lowest (P<0.05) values in buffalo-cows in G4 and G1 groups, respectively. However, the differences among G3 and G2 or G1 groups on the percentage of fat was insignificant (Table 6). Fisher (1980) reported that cows treated with levamisole phosphate at calving and 8 weeks post-partum showed increase in milk yield and fat content compared with the control cows. Also, Sattar *et al*. (2003b) showed that the highest and lowest values of milk yield and lactation length were recorded in levamisole and control groups, respectively.

Table 6. Milk yield and composition of Egyptian buffalo-cows as affected by the experimental treatments

Item	Control (G1)	Treatments Se-Met (G2)	LEV (G3)	Se-Met+LEV(G4)
Milk yield:				
Actual milk yield (kg/day)	7.00 ± 0.46^{c}	9.41 ± 0.41^{ab}	8.89 ± 0.29^{b}	10.33 ± 0.23^{a}
Milk composition:				
Fat (%)	6.78 ± 0.23^{c}	7.36 ± 0.04^{ab}	6.97 ± 0.16^{bc}	7.72 ± 0.13^{a}
Fat yield (kg/day)	0.48 ± 0.03^{c}	0.69 ± 0.04^{b}	0.62 ± 0.03^{b}	0.80 ± 0.02^{a}
Protein (%)	4.05 ± 0.15^{c}	4.91 ± 0.16^{b}	4.61 ± 0.11^{b}	5.89 ± 0.12^{a}
Protein yield (kg/day)	0.28 ± 0.02^{c}	0.46 ± 0.03^{b}	0.41 ± 0.01^{b}	0.61 ± 0.01^{a}
Lactose (%)	3.81 ± 0.16^{d}	4.74 ± 0.08^{b}	4.21 ± 0.1^{c}	5.15 ± 0.07^{a}
Lactose yield (kg/day)	0.26 ± 0.02^{d}	0.45 ± 0.01^{b}	0.37 ± 0.01^{c}	0.53 ± 0.01^{a}
Solid non fat (SNF, %)	8.45 ± 0.14^{c}	9.39 ± 0.12^{b}	9.18 ± 0.10^{b}	10.51 ± 0.13^{a}
Solid non fat yield (kg/day)	0.59 ± 0.04^{c}	0.88 ± 0.04^{b}	0.81 ± 0.02^{b}	1.08 ± 0.02^{a}
Total solid (TS, %)	15.05 ± 0.37^{b}	17.58 ± 0.32^{a}	17.36 ± 0.28^{a}	18.35 ± 0.43^{a}
Total solid yield (Kg/day)	1.05 ± 0.08^{c}	1.65 ± 0.07^{b}	1.54 ± 0.06^{b}	1.89 ± 0.06^{a}
Ash (%)	0.86 ± 0.04^{a}	0.67 ± 0.02^{bc}	0.72 ± 0.02^{b}	0.62 ± 0.01^{c}

a-d, Means denoted within the same row with different superscripts are significantly different at (P<0.05)
Control: untreated buffalo-cows; Se-Met: selenomethionine and LEV: levamisole

Selenomethionine supplementation protects bovine mammary epithelial cells (BMEC) from induced apoptosis and increased proliferation and cell viability under conditions of oxidative stress (Miranda *et al*., 2011). Also, Cortinhas *et al*. (2010) showed that in fewer cases of subclinical mastitis occurred, an increase on milk yield was expected by cows fed organic Zn, Cu and Se. Moreover, Nocek *et al*. (2006) observed effects of organic mineral sources on both milk yield and composition. In contrary, Sattar *et al*. (2003a and 2007) reported that the differences effects between vitamin E- selenium and control groups on productive performance in Sahiwal and Exotic cows were insignificant. The increase in milk yield and constituents in different treatments, especially with Se-Met+LEV (G4) may be due to the increase in glucose and T_4 and T_3 levels, which stimulates the protein synthesis by decrease of the proteolytic action of glucocorticoids or an increase of glucose transport to provide energy required for peptide synthesis and milk production by mammary gland. Also, may be due to activity enhancement of mammary immune system related to the decrease in cortisol levels in different treatments, especially with Se-Met+LEV. Thyroid hormones play a major role in the control of several metabolic processes including carbohydrate, fat, protein vitamin and mineral metabolism (Guyot *et al*., 2011).

Blood metabolites

Data presented in Table 7 showed that values of RBC, WBC, Hb and Ht were significantly ($P<0.05$) increased in all treated groups as compared to untreated group. Among treated groups, values of RBC and Ht were significantly ($P<0.05$) greater with G2 and G4 groups than G3 group. Generally, the highest ($P<0.05$) and lowest values of RBC's, WBC's, Hb and Ht were observed with G4 and G1 groups, respectively. These findings are in accordance with that of Shaha *et al*. (2015). Moreover, Hadden (1986) reported that the injection of levamisole HCl stimulates the reticuloendothelial system and increase the proliferative rate of leukocytes, consequently increase total leukocytic count. The administration of levamisole and vitamin E-selenium to pregnant cows induced a significant increase in total leukocytic count, Mamdouh (2000).

As shown in Table 7, T_4 and T_3 levels were significantly ($P<0.05$) increased in treated groups. While cortisol levels were significantly ($P<0.05$) decreased as compared to control group. Among treated group, T_4 and T_3 levels were significantly ($P<0.05$) greater with G4 than G2 and G3 groups. Also, the difference between treated groups in cortisol level was insignificant, begin the lowest value with G4. Generally, the highest ($P<0.05$) value of T_4 and T_3 and the lowest ($P<0.05$) value of cortisol were recorded with G4 group (124.30, 4.30 and 4.53 ng/ml), respectively. While, the lowest ($P<0.05$) value of T_4 and T_3 and the highest ($P<0.05$) value of cortisol was observed with G1 (89.10, 2.50 and 6.74 ng/ml), respectively.

The increase in thyroid hormones may be due to that Se-Met, LEV and Se-Met+ LEV treatments stimulates the production of thyroid stimulating hormone (TSH) which induce thyroid gland to produce thyroid hormones (T_4 and T_3), or immune-potentiating effect of Se-Met and LEV on thyroid gland. Se is a significant part of deiodinase enzyme and other selenoporteins play a protective role from the thyrocytes against free radicals which is produced during thyroxine synthesis (Kohrie *et al*., 2007). Also, Beckett *et al*. (1992) found that about 80% of T_3 in plasma is produced in the liver, kidney and muscle, and all these tissues contain the selenium-depending enzyme type IDI. Dietary Se in pregnant ewes increased T_3 and T_4 levels in blood plasma compared with control ewes (Rock *et al*., 2001). Cows supplemented with seleno-methionine recorded increase in thyroid hormones and cow health resulted from better immune system (Niwinska and Andrzejewski, 2013). It was shown that the administration of levamisole may cause significant changes in thyroid hormones concentrations, a significant increase in serum albumin and decrease in total cholesterol in ewes (Atessahin *et al*., 2004).

Lowering cortisol levels in buffaloes treated with Se-Met, LEV and especially with Se-Met+ LEV may be due to the decrease in adrenocorticotrophic hormone (ACTH). The administration of ACTH to cows showed increase in cortisol level and decreased the immune functions and total leukocyte counts (Ferguson and Beach, 1990).

Regarding to blood plasma glucose and beta-hydroxy butyrate (BHB) levels, the buffaloes in G2, G3 and G4 groups showed significant ($P<0.05$) increase in blood plasma glucose and significantly ($P<0.05$) decrease in BHB concentrations as compared to untreated buffaloes (control). Generally, the highest ($P<0.05$) value of glucose and lowest ($P<0.05$) value of BHB were recorded with G4 group, while the lowest ($P<0.05$) value of glucose and highest ($P<0.05$) value of BHB were observed with the control group (Table 7). These results are in accordance with Spear (2003) and Cortinhas *et al.* (2010). Also, Abd El-Hady *et al.* (2005) found that buffalo-cows treated with Se+E recorded significant ($P<0.01$) increase in plasma glucose concentration as a compared to untreated buffaloes.

Table 7. Blood picture of Egyptian buffalo-cows as affected by the experimental treatment

Item	Control (G1)	Treatments		
		Se-Met (G2)	LEV (G3)	Se-Met+LEV (G4)
Blood picture:				
RBC's (x 10^6/mm^3)	6.11 ± 0.27^c	7.24 ± 0.13^{ab}	6.94 ± 0.16^b	7.83 ± 0.37^a
WBC's (x 10^6/mm^3)	6.27 ± 0.42^b	7.51 ± 0.35^a	7.46 ± 0.28^a	8.18 ± 0.34^a
Hemoglobin (Hb, g/dl)	7.41 ± 0.30^c	8.91 ± 0.43^b	8.63 ± 0.46^b	10.44 ± 0.36^a
Hematocrit (Ht, %)	30.01 ± 1.38^c	38.3 ± 0.97^a	35.36 ± 0.99^b	$39,80 \pm 1.04^a$
Blood hormones:				
T4 (ng/ml)	89.10 ± 3.05^c	110.10 ± 4.34^b	101.60 ± 4.07^b	124.30 ± 5.23^a
T3 (ng/ml)	2.50 ± 0.10^c	3.75 ± 0.14^b	3.53 ± 0.12^b	4.32 ± 0.18^a
Cortisole (ng/ml)	6.74 ± 0.43^a	4.48 ± 0.11^b	5.01 ± 0.14^b	4.53 ± 0.13^b
Other parameters:				
Glucose (Gl, mg/dl)	55.70 ± 3.67^a	70.30 ± 2.07^b	68.90 ± 2.18^b	78.40 ± 2.43^a
BHB (mg/dl)	23.30 ± 0.63^a	20.80 ± 0.62^b	21.30 ± 0.56^b	20.40 ± 0.72^b
Selenium (ng/ml)	46.74 ± 3.29^c	72.90 ± 3.33^{ab}	64.39 ± 2.31^b	75.02 ± 2.92^a

a-c, Means denoted within the same row with different superscripts are significantly different at ($P<0.05$)
Control: untreated buffalo-cows; Se-Met: selenomethionine and LEV: levamisole

Data presented in Table 7 revealed that blood plasma Se concentrations were significantly ($P<0.05$) increased in all treated groups as compared to control group, begin the highest ($P<0.05$) value with G4 group and lowest ($P<0.05$) value with G1 group. Similar findings were reported by Abd El-Hady *et al*. (2005), who indicated that plasma Se concentration of cows treated with Se or Se+ E was significantly increased compared with untreated cows. Also, Calamari *et al*. (2010) reported that Se concentration in whole blood and plasma was increased ($P<0.01$) in cows supplemented with organic Se as compared cows supplemented with inorganic Se. Moreover, Espinosa *et al.* (2015) demonstrated that ewes treated with selenomethionine had significantly increased plasma Se-concentration. In contrary, Cortinhas *et al*. (2012) reported that supplying organic or inorganic sources of Zn, Cu and Se did not effect on plasma concentration of selenium in cows from 60 days before calving to 80 days of lactation.

Economical efficiency

Data illustrated in Table 8 showed that buffaloes treated with Se-Met. LEV and Se-Met+LEV increased milk yield and economical efficiency as compared to untreated one. Also, the highest values of milk yield and economical efficiency were recorded for buffaloes treated with Se-Met+LEV. While, the lowest values of milk yield and economical efficiency were observed with untreated buffaloes. These results are in agreement with those obtained by Ibrahim (2004).

In conclusion, the administration of selenomethionine, levamisole HCl and their combination to Egyptian buffalo-cows during the late gestation and early lactation period exerted a beneficial effect on reproductive performance, milk yield and composition, blood metabolites and economical efficiency. Therefore, it can be recommended to treatment of buffalo-cows with selenomethionine at a level of 0.9 mg/kg of DM/twice weekly, orally and injected subcutaneously with levamisole at a level of 0.5 mg/kg BW/week for improve of the reproductive efficiency, milk yield and economical efficiency.

Table 8. Economic efficiency of milk production of lactating Egyptian buffalo-cows as affected by the experimental treatments

Item	Control (G1)	Treatments		
		Se-Met (G2)	LEV (G3)	Se-Met+LEV (G4)
	Average daily feed cost (LE):			
CFM	22.5	22.50	22.50	22.50
BH	5.10	5.10	5.10	5.10
RS	0.60	0.60	0.60	0.60
Se-Met, LEV or Se-Met+LEV	-	1.43	0.71	2.14
Total daily feed cost (LE)	28.2	29.63	28.91	30.34
Actual milk yield (kg/day)	7.00	9.41	8.89	10.33
Price of actual milk yield (LE)	42	56.46	53.34	61.98
Economic feed efficiency (%)	148.94	190.55	184.50	204.28

*** Average daily feed intake of CFM, BH and RS were 7.50, 3 and 3.5 (kg/h/day)**
*** price of feedstuffs (LE/ton) for 2016: CFM 3000, BH 1700 and RS 170.**
*** price of kg of milk of buffaloes was 6 LE.**
*** Economic Efficiency = (price of actual milk (LE)/Total feed cost) X 100**

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تأثير المعاملة بالسيلينوميثيونين والليفاميزول على الكفاءة الإنتاجية والتناسلية ومكونات الدم فى الجاموس المصرى الحلاب

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أجريت هذه الدراسة بمحطة بحوث الإنتاج الحيوانى بالجميزة بهدف دراسة تأثير معاملة الجاموس المصرى الحلاب بالسيلينوميثيونين و الليفاميزول و السيلينوميثيونين + الليفاميزول قبل وبعد الولادة بشهرين على الكفاءة الإنتاجية و التناسلية ومكونات الدم وكذا الكفاءة الاقتصادية. استخدمت فى هذه الدراسة 28 جاموسة فى موسم الحليب الثانى إلى الرابع خلال المرحلة الأخيرة من الحمل (قبل الولادة بشهرين) حيث تم تقسيمها إلى أربعة مجموعات متماثلة بكل منها 7 حيوانات. المجموعة الأولى لم يتم معاملتها وتركت كمجموعة ضابطة، بينما المجموعة الثانية تم معاملتها بالسيلينوميثيونين (0.9 ملجم/كجم من المادة الجافة / مرتين فى الأسبوع، تجريع)، المجموعة الثالثة تم معاملتها بالليفاميزول هيدروكلوريد (0.5 ملجم / كجم من وزن الجسم / مرة فى الأسبوع ، تم حقنها تحت الجلد) والمجموعة الرابعة تم معاملتها بالسيلينوميثيونين (0.9 ملجم/كجم من المادة الجافة / مرتين فى الأسبوع، تجريع) بالإضافة إلى الليفاميزول هيدروكلوريد (0.5 ملجم / كجم من وزن الجسم / مرة فى الأسبوع، تم حقنها تحت الجلد). أظهرت الدراسة وجود نقص معنوى ملحوظ على مستوى 0.05 فى الوقت اللازم لنزول الأغشية الجنينية بعد الولادة وعودة الرحم إلى وضعه الطبيعى فى كل المجموعات المعاملة مقارنة بالمجموعة الضابطة، وكان لتأثير السيلينوميثيونين + الليفاميزول مفاضلة عن باقى المعاملات. أظهرت الدراسة أن المجموعة المعاملة بالسيلينوميثيونين + الليفاميزول أحدثت انخفاضاً معنوياً ملحوظا على مستوى 0.05 فى الفترة من الولادة حتى ظهور تراكيب مبيضية، حتى أول شياع وحتى التلقيحة المخصبة (الفترة المفتوحة) وكذلك الفترة من أول شياع حتى التلقيحة المخصبة مقارنة بالمجموعات المعاملة الآخرى أو المجموعة الضابطة. أيضا أوضحت النتائج أن المعاملة بالسيلينوميثيونين + الليفاميزول أحدثت انخفاضاً معنوياً على مستوى 0.05 فى عدد التلقيحات اللازمة للإخصاب، بينما ارتفع عدد التلقيحات اللازمة للأخصاب معنوياً للمجموعة الضابطة. أشارت النتائج إلى وجود قِصر معنوى على مستوى 0.05 فى الفترة بين ولادتين فى كل المجموعات المعاملة و بخاصة المجموعة المعاملة بالسيلينوميثيونين + الليفاميزول. أظهرت الدراسة أنه خلال 103 يوما بعد الولادة ارتفع معدل الخصوبة فى كل من المجموعة المعاملة بالسيلينوميثيونين + الليفاميزول والمعاملة بالسيلينوميثيونين (100%) عن المجموعة المعاملة بالليفاميزول (85.71%) بينما المجموعة الضابطة (صفر %). أظهرت الدراسة وجود تحسن معنوى على مستوى 0.05 فى إنتاج اللبن ومكوناته فى المجموعات المعاملة بالسيلينوميثيونين، الليفاميزول والسيلينوميثيونين + الليفاميزول مقارنة بالمجموعة الضابطة (9.41 ، 8.89 ، 10.33 مقابل 7 كجم / يوم) على التوالى. أيضا وُجد تحسنا معنويا على مستوى 0.05 فى صورة الدم و كذلك مكوناته وهرمونات الغدة الدرقية، بينما حدث إنخفاضا فى مستوى هرمون الكورتيزول فى كل المجموعات المعاملة مقارنة بالمجموعة الضابطة. وبناء عليه ومن الناحية التطبيقية والاقتصادية ، تُوصى الدراسة بمعاملة الجاموس المصرى الحلاب خلال المرحلة الإنتقالية (قبل وبعد الولادة بشهرين) بالسيلينوميثيونين (0.9 ملجم / كجم من المادة مرتين فى الاسبوع ، تجريع) بالإضافة إلى الليفاميزول (0.5 ملجم /كجم من الجسم مرة فى الاسبوع ، حقناً تحت الجلد) لما لهما من تأثير إيجابى على الكفاءة الإنتاجية و التناسلية و صورة ومكونات الدم و كذا تحسين الكفاءة الاقتصادية.