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# Improving the Immune and Health Status and Blood Constituents of Egyptian Buffaloes and their Offspring in Response to Treating Dams with Selenomethionine and Levamisole

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## ABSTRACT

The present study was conducted to evaluate the effect of treating Egyptian buffaloes with some immunopotentiators as selenomethionine and levamisole HCl separately or in combination, during the late stage of pregnancy and early lactation period, on immune and health status and blood constituents of the dams and their offspring as well as growth performance of their calf. Total of 28 Egyptian buffaloes weighing 600 ± 50 kg in $2^{nd}$ – $4^{th}$ lactation season at late stage of pregnancy (60 days before parturition) were selected to carry out this study. All experimental animals were randomly allocated in randomized design to four similar experimental groups (7 buffaloes / each). Animals in the $1^{st}$ group were without treatment and served as a control group, while those in the $2^{nd}$ group were orally administrated with 0.9 mg/kg of DM twice weekly of selenomethionine (Se-Met). The $3^{rd}$ group were subcutaneously injected with 0.5 mg/kg of BW/week of levamisole HCl (LEV) and the $4^{th}$ group were orally administrated with 0.9 mg / kg of DM twice weekly of selenomethionine plus 0.5 mg/kg of BW/week, subcutaneously of levamisole HCl (Se-Met+LEV) respectively, during 60 days pre- and post-partum. The obtained results showed that blood plasma TP, AL, $T_3$ and $T_4$ hormones, selenium and glucose concentrations as well as activity of transferases enzymes (ALT and AST) and antioxidant enzymes (GSH and SOD) were improved (P<0.05) in dams that administrated with Se-Met, LEV and Se-Met+LEV and their calf compared with untreated dams and its calves. The best (P<0.05) values recorded with dams received Se-Met+LEV together and their newborn calves. Administration of Se-Met, LEV and their combination Se-Met+LEV to dams achieved significantly (P<0.01) enhanced levels of immunoglobulins IgA, IgM and IgG, and significantly (P<0.01) reduced levels of nitric oxide and cortisol in blood plasma of dams and their newborn calves as compared to the control dams and their offspring. The greatest (P<0.01) values of immunoglobulins IgA, IgM and IgG and least (P<0.01) values of nitric oxide and cortisol were detected in dams administrated with Se-Met+LEV together and their calf. Also, dams treated with a combination of Se-Met+LEV ($G_4$) recorded significantly (P<0.01) increased concentrations of all immunoglobulins fractions (IgA, IgM and IgG) in their colostrum as compared to the control dams or dams treated with Se-Met ($G_2$) or LEV ($G_3$) separately. Moreover, there were no recorded cases of newborn affection between calves delivered from all administrated dams compared with calves delivered from the control dams that shows more severe pneumonia and enteritis as a result of which two calves (28.57%) died in the first month of life. Average birth and weaning weights and daily gain of buffalo calves were significantly (P<0.05) higher by Se-Met, LEV and Se-Met+LEV administration to their dams, begin the highest (P<0.05) values of birth and weaning weights and daily gain of calf produced from buffalo dams received Se-Met+LEV together ($G_4$). In conclusion, administration of some immunopotentitors as selenomethionine and levamisole HCl single or in combination to Egyptian buffaloes during the late stage of pregnancy and early lactation period (the transition period) had a valuable impact on immune and health status and blood constituents of dams and their calves as well as growth performance of their calves. Therefore, it can be recommend to administer the Egyptian buffalo dams with a combination of selenomethionine at level of 0.9 mg/kg of DM/twice weekly, orally plus injection subcutaneously with levamisole at level of 0.5 mg/kg of BW/weekly during the transition period for improving immune and health status and blood constituents of dams and their newborn calves as well as growth performance of their offspring, which allow for raising good female calves for milk production or male calves for breeding.

**Keywords:** selenomethionine, levamisole, immunoglobulins, health status, blood constituents, buffaloes, calves

## INTRODUCTION

The late stage of pregnancy and early lactation times are periods of increased nutritional allowance and risk to metabolic and infections diseases in dairy cows due to a suppressed immune system (Huozha *et al.,* 2010). It is of important to notice that the proper nutrients intake during these periods helps in enhancing immune response and improving health status of cows and their offspring with good performance (Awadeh *et al.,* 1998 and Mamdouh *et al*., 2000). Also, in dairy cows, there are relationships between nutrition plan and levels of immunoglobulins and biochemical in calf blood as reported by Bayram *et al.* (2016).

Most regions in the world are naturally deficient in micro minerals in soil and crop plants, particularly in selenium (Se) as reported by Borowska (2002). Also, selenium level in the feedstuffs in Egypt may be lower than the adequate levels set at 0.3 ppm which was recommended by NRC (2001). Se-deficiency affects animals living in areas with low natural level of selenium causes a wide range of health problems such as suppressed immune function (Cortinhas *et al.,* 2012), and endocrine disorder, especially thyroid dysfunction, as selenium is vital for thyroid function and thyroid homeostasis (Kohrle *et al.,* 2007). Some minerals exert an immunomodulatory effects on the immune response, between them is selenium as reported by Pechova *et al.* (2012). Selenium is present in all cells and tissue of humans and animals and it is an essential component of at least 25 seleno-proteins with antioxidant, anti-inflammatory and chemo-protective properties (Pappas *et al.,* 2008). Pechova *et al.* (2012) reported that the most important of selenoproteins are glutathione peroxidases, selenophosphate synthetase, iodothyronine deiodinases, thioredoxin reductases and selenoproteins P and W, which plays an important structural and enzymatic function. Blood plasma selenium levels of cows treated with selenium were significantly increased as compared to the control cows, which resulted in increase in blood plasma selenium of their newborn calves (Pavlata *et al.,* 2003 and Abd El-Hady *et al.,* 2005). Ghany-Hefnawy *et al.* (2007) reported that in newborn animals and during sucking the saturation of kids with selenium depends on saturation of mother, as selenium permeates placental and mammary barriers. The level of selenium in milk is significantly influenced mainly by products containing selenium organically bounds in the form of selenomethionine (Pechova *et al.,* 2008). Organic form of selenium (like selenomethionine) is natural, stable, better absorbed and metabolized, and lesser side and residual effects as compared to inorganic from (like sodium selenite, Cao *et al.*, 2014), as well as, selenomethionine uses as a source of selenium and methionine, which is necessary for protein synthesis (Espinosa *et al.,* 2015). Serum immunoglobulin is an important part of humoral immune system of vertebrates as reported by Wafa (2017). Gilles *et al.* (2009) demonstrated that selenium and iodine administration to cows during pre-calving enhanced ($P<0.05$) the calf immune system defenses by improving the maternal mineral status. Plasma immunoglobulins IgA, IgG and IgM levels were significantly greater in buffalo-cows treated with Se+E and their offspring than the control cows and their newborn calves (Abd El-Hady *et al.,* 2005). Niwinska and Andrzejewski (2013) reported that cows administrated with selenomethionine achieved increase in thyroid hormones levels and cow health resulted from better immune system.

Levamisole hydrochloride is one imidazothiazoles group and its levo- isomer of tetramisole (2,3,5,6 tetrahydro-6-phenylimidazole). Levamisole is used as an anthelmintic drug against adult and larval stage of gastrointestinal nematodes and lung worms in a large number of hosts (horse, sheep, cattle, pig and chicken, Spence *et al.*, 1992) and immunodulatory agent (Ayman, 2013). Also, Nadeen *et al.* (2010) suggested that prophylactic application of levamisole helps in achieving efficient immunization in buffalo calves. Gurbulak and Klcarslan (2004) reported that levamisole can be used as an alternative and reliable drug in decreasing the rates of mastitis and fetal deaths and increasing immunoglobulins levels in Holstein Friesian cows and their calves. Blood plasma immunoglobulins concentrations were significantly increased in cow's immunestimulated with levamisole and their newborn calves as compared to non-stimulated cows and their newborn calves (Sattar *et al.,* 2003 and Ayman, 2013). Amer and Badr (2008) concluded that immunoglobulins IgA and IgG concentrations were significantly enhanced in both the colostrum of dams and blood serum of newborn calves after administration of dams with levamisole. Also, Krakowski *et al.* (1999) reported that the colostrum from mares supplemented with levamisole showed higher contents of IgG and IgM compared with the colostrum from un-supplemented mares, in addition to higher level of immunity in foals from dams supplemented with levamisole. Treatment of dairy cows with immunepotentiating doses of levamisole during late gestation resulted in improving activity of the mammary gland immune system by increasing the amount of immunoglobulins and proportion of B lymphocytes in milk, and minimizes the risk of infectious diseases and mortality rate in their newborn calves (Sattar *et al.,* 2003).

The purpose of this study was to clarify the impact of administration of some immunopotentiators as selenomethionine and levamisole HCl singly or in combination to Egyptian buffaloes during the late stage of pregnancy and early lactation periods (the transition period) on immune and health status and blood constituents of dams and their newborn calves as well as growth performance of their offspring.

## MATERIALS AND METHODS

The present experiment was performed at El-Gemmizah Experimental Station, El-Gharbiya Governorate, belonging to the Animal Production Research Institute , Agricultural Research Center, Ministry of Agriculture , Egypt .

**Animals and experimental groups:**

Total of 28 lactating Egyptian buffalo-cows in apparently good health conditions and clinically free of external and internal parasites at two months before the expected calving date, within an average live body weight (LBW) of 600 ± 50 kg, aged 4-6 years and 2-4 lactation seasons were randomly divided into four similar groups (7/each) based on body condition scores (BCS), live body weight, the expected calving date and age at the beginning of the experiment. Buffaloes in the 1$^{st}$ group were without treatment and served as a control group, while those in the 2$^{nd}$ group were orally administrated with 0.9 mg/kg of DM twice weekly of selenomethionine (Se-Met, produced by Dexiberica Co., Spain). The 3$^{rd}$ group were subcutaneously injected with 0.5 mg/kg of BW/week of levamisole HCl (LEV, produced by Memphis Co., Cairo, Egypt) and the 4$^{th}$ group were orally administrated with 0.9 mg / kg of DM twice weekly of selenomethionine plus 0.5 mg/kg of BW/week, subcutaneously of levamisole HCl (Se-Met+LEV) respectively, during 60 days pre- and post-partum. Animals were fed individually on a concentrate feed mixture (CFM), berseem hay (BH) and rice straw (RS), in order to meet the nutritional allowance of lactating Egyptian buffaloes during the late gestation and early lactation periods according to NRC (2001), while fresh and clean water and minerals blocks were available as free choice through the day time. Animals were maintained in a common exercise area with open housing and given individual feeds twice daily at 8.00 a.m. and 4.00 p.m. during the experiment. 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) |
| Concentrate* | 91.38 | 90.43 | 16.81 | 16.22 | 2.90 | 54.50 | 9.57 | 0.089 |
| Rice straw | 92.11 | 85.94 | 4.85 | 39.89 | 1.61 | 39.59 | 14.06 | 0.034 |
| Berseem hay | 91.27 | 89.37 | 11.69 | 33.48 | 1.31 | 42.89 | 10.63 | 0.064 |
| Calf starter** | 92.31 | 90.81 | 18.10 | 10.51 | 3.90 | 58.30 | 9.19 | 0.075 |

*** Composition: 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.**

**** Composition: Soybean meal (44%), yellow corn, wheat bran, lin seed cake, molasses, calcium carbonate and sodium chloride.**

Each calving was supervised as soon as possible to care the dam during parturition and to help the neonatal calve to suckle the colostrum from its dam soon after 20 minutes of birth. The calves were suckled individually from their dams' milk at the rate of 10% of its body weight twice daily for six weeks of age. After that, the milk allowance was reduced gradually by 1% of body weight weekly until weaning time at fifteenth week of age. Calf starter and berseem hay were available for *ad libitum* feeding from the third week of age until weaning time; also, drinking fresh and clean water was available as free choice during the day time. The calves were kept under continuous observation for detection of any case of pneumonia, diarrhea, enteritis and mortality.

**Growth performance of calves:**

Newborn calves were individually weighted immediately after calving and then biweekly until the weaning time at 105 days of age. Also, total gain and daily gain of buffalo calves were calculated.

**Blood samples and analysis:**

Blood samples were taken from the jugular vein from all buffaloes in each group in heparinized test tubes biweekly during two months before and after parturition. Also, blood samples were drawn from all newborn calves before receiving colostrum at first, second and third day post-calving, then at 4, 8, 12 and 15 weeks of age. Blood samples were centrifuged at 1000 *g* for 15 minutes to separate plasma and stored at -20°C until biochemically analysis.

**Protein fractions and enzymatic activity:**

Plasma concentrations of total proteins and albumin were determined calorimetrically according to the method of Peters (1968) and Doumas *et al.* (1971). In addition, activity of alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) enzymes were determined calorimetrically using the method described by Reitman and Frankle (1957).

**Antioxidant enzymes:**

Blood plasma levels of glutathione peroxidase (GSH) and superoxide dismutase (SOD) enzymes were estimated according to Goldberg and Spooner (1983) and Madesh and Balasubramanian (1998).

**Hormonal profile:**

Blood plasma concentrations of triiodothyronine ($T_3$), thyroxine ($T_4$) and cortisol hormones were determined 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.

**Immunoglobulins and other parameters:**

Blood plasma immunoglobulins and nitric oxide were estimated according to Killingsworth and Savory (1972) and Rajaraman *et al.* (1998). Also, glucose was determined using commercial kits (Sigma kit # 315, Sigma Diagnostics) according to Trinder (1969). In addition, plasma samples were diluted by distilled water at 1: 9 ratio and analyzed for selenium (Se) directly using Graphite Furnace Atomic Absorption Spectrometry (ATI Unicam Model 939, UK).

**Colostrum samples and analysis:**

Colostrum samples were taken at parturition twice daily for three days postpartum to estimate the immunoglobulin contents (IgA, IgM and IgG) according to Killingsworth and Savory (1972). Se level was evaluated in feed samples similar to that of blood plasma after wet ashing with nitric acid and $H_2O_2$ in microwave unit.

**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

**Blood constituents of dams:**

Results presented in Table 2 reflecting that blood plasma concentrations of total protein (TP) and albumin (AL) were significantly (P<0.05) greater and globulin (GL) level was insignificantly higher in all administrated dams, while activities of transferases (ALT and AST) enzymes were significantly (P<0.05) lower than in untreated dams. In general, dams treated with a combination of Se-Met+LEV had the greatest (P<0.05) values of TP and AL and least (P<0.05) values of ALT and AST as compared to other treated and control dams, begin the lowest (P<0.05) values of TP and AL and highest (P<0.05) values of ALT and AST with the control dams. These findings evidently suggested that administration of Se-Met or LEV separately or in combination to dams had a valuable effect on liver functions, particularly with a combination of Se-Met+LEV, which achieved the highest improvement of liver function without any harmful effect on hepatic cells. The detected increase of TP and AL levels in blood plasma in different treatments, particularly with Se-Met+LEV was mainly related to the high content of methionine in Se-Met, which essential for protein synthesis in accordance with Espinosa *et al.* (2015). The positive effect of organic selenium on blood protein and its fractions may be due to the anabolic action of insulin mediated through increasing the amino acids synthesis by liver enhancement the incorporation of several amino acids into protein (Cao *et al.,* 2014). Also, it may be due to enhance $T_3$ and $T_4$ concentrations in blood of treated dams (Table 2), which stimulates the protein synthesis by reducing the proteolytic action of glucocorticoids, or activity enhancements of hepatic immune system related to the reduced nitric oxide and cortisol concentrations in plasma of treated dams, especially with Se-Met+LEV (Table 3). Guyot *et al.* (2011) reported that thyroid hormones influence the function of most organs and stimulate the basic metabolic rate through regulation of the metabolism of proteins, carbohydrates and lipids. Biswal *et al.* (2014) found that cows treated with levamisole achieved increase of total proteins and decrease of ALT and AST in blood plasma.

Treatment with Se-Met and LEV separately or together (Se-Met+LEV) to dams induced significantly (P<0.05) increase in activities of superoxide dismutase (SOD) and glutathione peroxide (GSH) enzymes, concentrations of $T_3$ and $T_4$ hormones, as well as Se and glucose levels in blood plasma as compared to the control dams (Table 2). Among treatment groups, dams administrated with Se-Met and LEV separately showed lower (P<0.05) activities of SOD and GSH enzymes, levels of $T_3$ and $T_4$ hormones and GL in blood plasma than those administrated with a combination of Se-Met+LEV; however, the difference between Se-Met and LEV treatments was insignificant, whereas the highest was with Se-Met. In general, the greatest (P<0.05) values of activities of SOD and GSH enzymes, concentrations of $T_3$

and $T_4$ hormones as well as GL levels were recorded with Se-Met+LEV ($G_4$), while the least (P<0.05) values of activities of SOD and GSH, $T_3$, $T_4$, Se and GL levels were detected with the control ($G_1$). The observed enhance of activities of SOD and GSH enzymes in blood plasma of dams after administration of Se-Met, LEV or Se-Met+LEV may be attributed to increase of Se, which is a vital component of selenoproteins, which plays important structural and enzymatic functions according to Pappas *et al.* (2008) or an increase of methionine content in selenomethionine. Chung *et al.* (2007) and Espinosa *et al.* (2015) indicated that methionine acts as precursor of amino acids for glutathione in the protection of all cell membranes in the body from oxidative damage by free radicals; also the thiol-group of methionine was shown to chelate lead and remove it from all tissues in the body.

Concerning the thyroid hormones, the higher $T_4$ and $T_3$ concentrations in blood plasma of administrated groups were mainly related to direct impact of treatments on increasing thyroid stimulating hormones (TSH) and immune potentiating impact on thyroid metabolism, particularly with Se-Met+LEV together. These findings are in accordance with Gunter *et al*. (2003) and Atessahin *et al.* (2004). Beckett (1992) reported that about 80% of $T_3$ in blood plasma is produced in the kidney, liver and muscle and all these tissues contain the Se-depending enzyme type IDI. Niwinska and Anderzejewski (2013) found that cows treated with selenomethionine showed increase in $T_4$ and $T_3$ hormones and better health due to better immune system. Also, Awadeh *et al.* (1998) reported that cows supplemented with 60 ppm selenized yeast salt recorded the highest (P<0.01) $T_3$ and $T_4$ levels in blood plasma as compared to other supplemented groups. Moreover, Atessahin *et al.* (2004) reported that administration of levamisole may be cause significant changes in $T_3$ and $T_4$ levels, a significant increase in albumin and decrease in total cholesterol in ewes. These results also may be due to protected thyrocytes from free radicals by treatments. Kohrle *et al.* (2007) indicated that selenium is an essential component of selenoproteins enzymes, which plays a protective role of the thyrocytes from free radicals.

It is of interest to note that all treatments recorded higher levels of Se and glucose in blood plasma, especially with Se-Met+LEV together. These findings are in accordance with those of Cao *et al.* (2014). Abd El-Hady *et al.* (2005) found that Se and glucose levels of buffaloes supplemented with Se+E were significantly greater than unsupplemented buffaloes. Also, Calamari *et al.* (2010) found that Se level in whole blood and blood plasma was lower (P<0.05) in cows administrated with inorganic Se compared with cows administrated with organic Se. Moreover, ewes supplemented with selenomethionine recorded significantly increased blood plasma Se level.

**Table 2. Blood constituents of dams as affected by the experimental treatments**

| Item | Control (G1) | Treatments | | |
|---|---|---|---|---|
| | | Se-Met (G2) | LEV (G3) | Se-Met+LEV (G4) |
| Protein fractions: | | | | |
| Total protein (g/dl) | $5.50 \pm 0.22^c$ | $7.19 \pm 0.30^b$ | $6.53 \pm 0.25^b$ | $8.11 \pm 0.27^a$ |
| Albumin (g/dl) | $3.40 \pm 0.15^c$ | $4.59 \pm 0.22^b$ | $4.40 \pm 0.14^b$ | $5.41 \pm 0.25^a$ |
| Globulin (g/dl) | $2.10 \pm 0.23$ | $2.60 \pm 0.15$ | $2.13 \pm 0.20$ | $2.70 \pm 0.14$ |
| Enzymatic activity: | | | | |
| ALT (U/100ml) | $25.00 \pm 1.04^a$ | $15.33 \pm 0.63^c$ | $17.78 \pm 0.54^b$ | $11.00 \pm 0.38^d$ |
| AST (U/100ml) | $58.33 \pm 4.34^a$ | $40.61 \pm 2.30^b$ | $43.94 \pm 2.50^b$ | $30.94 \pm 1.84^c$ |
| Antioxidative status: | | | | |
| GSH (U/ml) | $10.61 \pm 0.80^c$ | $15.83 \pm 0.34^b$ | $15.00 \pm 0.32^b$ | $19.11 \pm 0.42^a$ |
| SOD (U/mg protein) | $14.50 \pm 1.18^c$ | $19.44 \pm 0.65^b$ | $18.56 \pm 0.73^b$ | $25.28 \pm 0.59^a$ |
| Thyroid hormones: | | | | |
| T3 (ng/ml) | $2.50 \pm 0.10^c$ | $3.75 \pm 0.14^b$ | $3.53 \pm 0.12^b$ | $4.32 \pm 0.18^a$ |
| T4 (ng/ml) | $89.10 \pm 3.05^c$ | $110.10 \pm 4.34^b$ | $101.60 \pm 4.07^b$ | $124.30 \pm 5.23^a$ |
| Other parameters: | | | | |
| Selenium (ng/ml) | $46.74 \pm 3.29^c$ | $72.90 \pm 3.33^{ab}$ | $64.39 \pm 2.31^b$ | $75.02 \pm 2.92^a$ |
| Glucose (mg/dl) | $55.70 \pm 3.67^c$ | $70.30 \pm 2.07^b$ | $68.90 \pm 2.18^b$ | $78.40 \pm 2.43^a$ |

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

**Humoral immune response of dams:**

Data in Table 3 revealed that administration of Se-Met and LEV separately or together (Se-Met+LEV) to dams induced significantly (P<0.01) increased concentrations of immunoglobulins Ig (Ig A, IgM and IgG) and significantly (P<0.01) decreased concentrations of nitric oxide and cortisol in blood plasma as compared to the control dams. In general, dams treated with a combination of Se-Met+LEV had the highest (P<0.01) values of IgA, IgM and IgG and the lowest (P<0.01) values of nitric oxide and cortisol in blood plasma compared with those administrated with Se-Met and LEV separately and the control dams, which recorded the lowest (P<0.01) values of IgA, IgM and IgG and the highest (P<0.01) values of nitric oxide and cortisol in blood plasma. These findings reflecting that treatment of Se-Met, LEV and their combination to dams during pre- and post-calving is able to enhance immune response of dams and improving their general health condition and body metabolism, particularly with Se-Met+LEV together. Similar results were reported by Gunter *et al.* (2003) and Rook *et al.* (2004), who demonstrated that selenium supplementation, could increase serum immunoglobulins IgM, IgG and the production of secondary antibodies to antigen. Abd El-Hady *et al.* (2005) found that buffalo-cows injected with Se+E showed significantly (P<0.01) increased blood plasma levels of IgA, IgM and IgG as comparted to the control cows. Gurbulak and Klcarslan (2004) and Ayman (2013) reported that concentrations of plasma immunoglobulins were greater in cows supplemented with levamisole than that un-supplemented cows.

The higher improvement of immunoglobulins levels of blood plasma in different treatments, particularly with Se-Met+LEV together may be attributed to absolute increased in number of immunoglobulins antibodies producing cells in the germinal center of the spleen and other lymphoid organs (Mamdouh, 2000). Also, it may be due to activity enhancement of immune response of lymphoid cells in response to the reduce in nitric oxide and cortisol levels in different treatments, especially with Se-Met+LEV (Table 3) in accordance with Huozha *et al.* (2010); Singh *et al.* (2011) and El-Desouky (2014). Selenomethionine can protect all cell membranes in the body including of lymphoid cells and subcellular organelles from oxidative damage by free radicals, which are produced through normal cellular metabolism (Bula and Ositis, 2008). Such finding may be due to increase in levels of total protein, albumin and globulin or an increase in thyroid hormones levels (Table 2) in dams received Se-Met or LEV separately or in combination which resulted in enhancement of the immune system, being more efficient of dams received Se-Met+LEV together. Guyot *et al.* (2011) demonstrated that $T_3$ and $T_4$ hormones play essential role in the control of several metabolic processes of protein, fat, carbohydrates, minerals and vitamins.

**Table 3. Blood plasma immunoglobulins, nitric oxide and cortisol hormone concentrations of dams as affected by the experimental treatments.**

| Item | Control (G1) | Treatments | | |
|---|---|---|---|---|
| | | Se-Met (G2) | LEV (G3) | Se-Met+LEV (G4) |
| IgA (g/dl) | $0.078 \pm 0.008^c$ | $0.141 \pm 0.01^b$ | $0.112 \pm 0.01^b$ | $0.178 \pm 0.02^a$ |
| IgM (g/dl) | $0.29 \pm 0.03^d$ | $0.46 \pm 0.01^b$ | $0.42 \pm 0.02^c$ | $0.59 \pm 0.02^a$ |
| IgG (g/dl) | $1.38 \pm 0.10^d$ | $2.23 \pm 0.06^b$ | $2.00 \pm 0.05^c$ | $2.73 \pm 0.11^a$ |
| Nitric oxide (μM/L) | $10.19 \pm 0.60^a$ | $6.92 \pm 0.40^c$ | $8.66 \pm 0.39^b$ | $5.51 \pm 0.27^d$ |
| Cortisol (ng/ml) | $6.74 \pm 0.43^a$ | $4.48 \pm 0.11^b$ | $5.01 \pm 0.14^b$ | $4.53 \pm 0.13^b$ |

**a-d: Means within the same row with different superscripts are significantly different at (P<0.01)**

**Colostral immunoglobulins (Ig):**

Data in Table 4 showed that colostrum immunoglobulins' concentrations including IgA, IgM and IgG were affected significantly (P<0.01) by Se-Met, LEV and Se-Met+LEV treatments of dams. Dams that were administrated with a combination of Se-Met+LEV ($G_4$) achieved the greatest (P<0.01) levels of immunoglobulins IgA, IgM and IgG in colostrum as compared to those that were administrated with Se-Met ($G_2$) and LEV ($G_3$) separately or the control one ($G_1$), although the differences between $G_2$ and $G_3$ were insignificant, being higher with $G_2$. Concerning colostral collection period, immunoglobulins IgA, IgM and IgG concentrations reduced (P<0.01) gradually at intervals with all experimental groups. In general, the greatest (P<0.01) values of immunoglobulins IgA, IgM and IgG were recorded in dams treated with a combination of Se-Met+LEV, particularly in the first day post-calving, while the least (P<0.01) values were detected in untreated dams, particularly in the third day post-calving. These findings go in hand with the findings of Rowntree *et al.* (2004) and Abd El-Hady *et al.* (2005). Awadeh *et al.* (1998) demonstrated that beef cows supplemented with 60 ppm of Se-yeast had the highest levels of IgA and IgM in colostrum as compared to other supplemented groups. A positive impact of selenium supplementation on the concentration of antibodies in the colostrum was observed by Pavlata *et al.* (2004). Treatment of Holstein-Friesian cows with immunopotentiating doses of levamisole during late gestation resulted in significantly enhance concentration of IgG in colostrum compared with the control cows as reported by Gurbulak and Klcarslan (2004). Also, Amer and Badr (2008) concluded that buffalo-cows immunostimulated with levamisole had the greatest levels of IgA and IgG in colostrum compared with non-stimulated buffalo-cows. Our findings were supported by Murphy *et al.* (2005) and Wafa (2017), who indicated that the amount of all immunoglobulin (Ig) fractions in colostrum of buffalo-cows depend mainly upon pre-partum administration of immunepotentiators to dams.

**Table 4. Colostrum immunoglobulins (intervals) of dams as affected by the experimental treatments.**

| Item | | Control (G1) | Treatments | | | Overall mean |
|---|---|---|---|---|---|---|
| | | | Se-Met (G2) | LEV (G3) | Se-Met+LEV (G4) | |
| IgA (g/dl) | 1st day | 0.218 ± 0.018 | 0.430 ± 0.021 | 0.367 ± 0.04 | 0.532 ± 0.024 | $0.387 \pm 0.025^a$ |
| | 2nd day | 0.142 ± 0.006 | 0.393 ± 0.023 | 0.342 ± 0.014 | 0.507 ± 0.021 | $0.346 \pm 0.029^b$ |
| | 3rd day | 0.133 ± 0.05 | 0.353 ± 0.025 | 0.277 ± 0.012 | 0.438 ± 0.015 | $0.300 \pm 0.024^c$ |
| Overall mean | | $0.164 \pm 0.02^d$ | $0.392 \pm 0.015^b$ | $0.328 \pm 0.011^c$ | $0.492 \pm 0.014^a$ | 0.344 ± 0.016 |
| IgM (g/dl) | 1st day | 0.628 ± 0.051 | 0.977 ± 0.039 | 0.800 ± 0.04 | 1.153 ± 0.045 | $0.890 \pm 0.057^a$ |
| | 2nd day | 0.458 ± 0.043 | 0.922 ± 0.032 | 0.750 ± 0.03 | 1.068 ± 0.037 | $0.799 \pm 0.051^b$ |
| | 3rd day | 0.363 ± 0.024 | 0.813 ± 0.045 | 0.670 ± 0.041 | 0.987 ± 0.032 | $0.708 \pm 0.04^c$ |
| Overall mean | | $0.483 \pm 0.035^d$ | $0.904 \pm 0.049^b$ | $0.740 \pm 0.025^c$ | $1.069 \pm 0.027^a$ | 0.799 ± 0.03 |
| IgG (g/dl) | 1st day | 3.10 ± 0.09 | 4.26 ± 0.13 | 4.01 ± 0.08 | 5.44 ± 0.38 | $4.21 \pm 0.19^a$ |
| | 2nd day | 2.73 ± 0.14 | 4.15 ± 0.11 | 3.88 ± 0.06 | 5.25 ± 0.40 | $4.00 \pm 0.20^a$ |
| | 3rd day | 2.65 ± 0.16 | 3.98 ± 0.08 | 3.56 ± 0.12 | 4.82 ± 0.35 | $3.75 \pm 0.17^b$ |
| Overall mean | | $2.83 \pm 0.08^d$ | $4.13 \pm 0.06^b$ | $3.82 \pm 0.07^c$ | $5.17 \pm 0.15^a$ | 3.99 ± 0.11 |

**a-d: Means within the same row or column with different superscripts are significantly different at (P<0.01).**

**Humoral immune response of calves:**

Data in Table 5 cleared that concentrations of immunoglobulins, including IgA, IgM and IgG in blood plasma were significantly (P<0.01) higher and levels of nitric oxide and cortisol were significantly (P<0.01) lower in calves delivered from dams received Se-Met ($G_2$) and LEV ($G_3$) separately or in combination Se-Met+LEV ($G_4$) as compared to calves delivered from untreated dams ($G_1$). In general, calves produced from dams treated with ($G_4$) achieved the highest (P<0.01) values of IgA, IgM and IgG and the lowest (P<0.01) values of nitric oxide and cortisol hormone compared with calves delivered from dams treated with ($G_2$ or $G_3$) and untreated dams ($G_1$). However, the difference between $G_2$ and $G_3$ was insignificant. The present results evidently reflecting that treatment of Se-Met and LEV separately or in combination of Se-Met+LEV to dams during pre-and post-partum lead to enhance calf immunity in terms of increase concentrations of IgA, IgM and IgG in plasma of their calves, particularly with a combination of Se-Met+LEV. Similar results were reported by Gurbulak and Klcarslan (2004) in Holstein-Friesian cows, Pavlata *et al.* (2004) in dairy cows, and Amer and Badr (2008) in buffaloes. Also, Abd El-Hady *et al.* (2005) found that concentrations of plasma IgA, IgM and IgG of calves from dams injected with Se+E at dry-off period were greater (P<0.01) than those from control dams. The observed increase in immunoglobulins, IgA, IgM and IgG concentrations in plasma of calves from dams with different treatments was mainly related to enhanced levels of immunoglobulins in colostrum of their dams, particularly those treated with Se-Met+LEV together (Table 4). Similar trend was reported by Murphy *et al.* (2005) and Wafa (2017). Jezek *et al.* (2012) found that quality of colostrum was positively correlated with the levels of IgM, IgA and IgG in the calves' serum in the first week and with the IgG in the fourth week of life. Also, Heriazon *et al.* (2011) indicated that the immune status of a cow during the transition period importantly influences passive immunity and health status of a newborn calf due to mechanisms that allow the transport of immunoglobulins and cells from blood plasma into mammary gland secretions. Moreover, Krakowski *et al.* (1999) reported that the colostrum from mares supplemented with levamisole showed higher contents of IgG and IgM compared with the colostrum from un-supplemented mares, in additions to higher concentration of immunity in foals from dams supplemented with levamisole. Also, it may be due to enhancement of the immune status of calves in response to the reduction of plasma nitric oxide and cortisol concentrations in calves from dams treated with $G_2$, $G_3$ and $G_4$, particularly with $G_4$ (Table 5).

**Table 5. Blood plasma immunoglobulins, nitric oxide and cortisol hormone of buffalo calves as affected by the experimental treatments of their dams.**

| Item | Control (G1) | Treatments | | |
|---|---|---|---|---|
| | | Se-Met (G2) | LEV (G3) | Se-Met+LEV (G4) |
| IgA (g/dl) | $0.064 \pm 0.003^c$ | $0.121 \pm 0.01^b$ | $0.101 \pm 0.004^b$ | $0.145 \pm 0.02^a$ |
| IgM (g/dl) | $0.20 \pm 0.03^c$ | $0.38 \pm 0.02^b$ | $0.32 \pm 0.01^b$ | $0.48 \pm 0.03^a$ |
| IgG (g/dl) | $1.15 \pm 0.09^c$ | $1.96 \pm 0.05^b$ | $1.91 \pm 0.04^b$ | $2.43 \pm 0.03^a$ |
| Nitric oxide (μM/L) | $8.63 \pm 0.44^a$ | $5.49 \pm 0.20^b$ | $6.28 \pm 0.26^b$ | $4.22 \pm 0.16^c$ |
| Cortisol (ng/ml) | $5.63 \pm 0.44^a$ | $4.12 \pm 0.19^b$ | $4.45 \pm 0.30^b$ | $3.27 \pm 0.18^c$ |

**a-c: Means within the same row with different superscripts are significantly different at (P<0.01)**

**Blood constituents of calves:**

Influence of administration of Se-Met, LEV and their combination, to dams, on blood metabolites of their calves are presented in Table 6. Calves delivers from dams supplemented with Se-Met, LEV and Se-Met+LEV had significantly (P<0.05) increased levels of TP, AL, $T_3$, $T_4$, Se and GL as well as activity of SOD and GSH enzymes and significantly (P<0.05) decreased activities of ALT and AST enzymes in blood plasma as compared to calves delivered from control dams. Also, there were significantly (P<0.05) differences among treated groups. In general, buffalo calves produced from $G_4$ recorded the highest (P<0.05) values of TP, AL, $T_3$, $T_4$, Se and GL as well as activities of GSH and SOD enzymes and the lowest (P<0.05) values of ALT and AST as compared to those of $G_1$ or $G_2$ and $G_3$, respectively. This finding came in agreement with previous reports by Pechova *et al.* (2012) and Zarczynska *et al.* (2013). The observed enhancement in TP and AL concentrations in plasma of calf delivered from dams treated with ($G_2$, $G_3$ and $G_4$) may be attributed to higher increase in TP and AL concentrations or an increase of Se and glucose levels in plasma of their dams, particularly in $G_4$ (Table 2). Abd El-Hady *et al.* (2005) reported that calves of the cows supplemented with Se+E have adequate plasma Se level, which resulted in prevention of nutritional muscular dystrophy (NMD). Selenomethionine supplementation of goats from six weeks before delivery significantly enhanced selenium and total protein levels in plasma of their kids (Pechova *et al.,* 2012). Also, Zarczynska *et al.* (2013) demonstrated that selenomethionine can be incorporated into GSH, which helps to protect all body cells from free radicals which are produced during normal metabolic activities and also be incorporated in other proteins in substitution for methionine. Moreover, Schrauzer and Surai (2009) indicated that selenomethionine is the only naturally occurring Se compound that is significantly incorporated into body proteins. Atessahin *et al.* (2004) reported that administration of levamisole significantly enhanced the serum albumin and reduced the total cholesterol in ewes.

The observed reduce of transferases (AST and ALT) enzymes activities and enhancement of anti-oxidant enzymes (GSH and SOD) activities in plasma of calves from treated dams was associated with normal liver functions (Table 6), as well as improve immune response (Table 5) and body metabolic activities of calves which may be related to effect of treating their dams with Se-Met ($G_2$) and LEV ($G_3$), particularly with Se-Met+ LEV ($G_4$). Similar results were reported by Gunter *et al.* (2003) and Rowntree *et al* (2004). The highest levels of Se and glutathione peroxidase in blood were recorded in the group of kids whose mothers supplemented with selenomethionine as compared to control and other supplemented groups (Pechova *et al.,* 2012). Also, Ebrahimi *et al.* (2009) reported that glutathione peroxidase activity was 110% higher in Sel-plex treated group than the control group. Moreover, Cao *et al.* (2014) demonstrated that diets supplemented with 0.30 or 0.70 mg/kg of DL-Se Met seemed to be more effective and had advantages in improving the oxidation resistance of weaning pigs. Mamdouh (2000) reported that administration of levamisole HCl and vitamin E-selenium to pregnant cows at dry-off period improve blood metabolites of their newborn calves compared with calves from control cows.

The marked improvement in thyroid hormones ($T_3$ and $T_4$) of calves delivered from dams treated with ($G_2$, $G_3$ and $G_4$) compared with calves delivered from untreated dams ($G_1$) is in agreement with the findings of Awadeh *et al.* (1998) in calves and Pechova *et al.* (2012) in newborn lambs. Also, Ebrahimi *et al.* (2009) reported that plasma levels of $T_3$ hormones were greater (P<0.01) in claves supplemented with Sel-plex than in the control calves. It was observed that administration of levamisole may cause significantly changes in serum levels of $T_3$ and $T_4$ hormones (Atessahin *et al.*, 2004).

The obviously enhanced glucose levels in calf produced from $G_2$, $G_3$ and $G_4$ groups (Table 6) may be due to the increase in plasma glucose concentrations of their dams (Table 2), which may be related to a reasonable improvement in gluconeogenesis and enhanced lactose absorption. In accordance with Abd El-Hady *et al.* (2005) who reported that dams injected with Se-E at dry-off-period had increased (P<0.05) levels of glucose in plasma and lactose in colostrum as compared to the control dams.

**Table 6. Blood constituents of buffalo calves as affected by the experimental treatments of their dams.**

| Item | Control (G1) | Treatments | | |
|---|---|---|---|---|
| | | Se-Met (G2) | LEV (G3) | Se-Met+LEV (G4) |
| Protein fractions: | | | | |
| Total protein (g/dl) | $4.00 \pm 0.56^{c}$ | $6.25 \pm 0.42^{b}$ | $5.90 \pm 0.33^{b}$ | $7.70 \pm 0.49^{a}$ |
| Albumin (g/dl) | $2.50 \pm 0.33^{c}$ | $4.55 \pm 0.29^{b}$ | $4.10 \pm 0.23^{b}$ | $5.70 \pm 0.81^{a}$ |
| Globulin (g/dl) | $1.50 \pm 0.34$ | $1.70 \pm 0.20$ | $1.80 \pm 0.30$ | $2.00 \pm 0.25$ |
| Enzymatic activity: | | | | |
| ALT (U/100ml) | $28.00 \pm 1.34^{a}$ | $18.70 \pm 0.90^{b}$ | $21.4 \pm 1.01^{b}$ | $15.20 \pm 0.83^{c}$ |
| AST (U/100ml) | $75.70 \pm 5.39^{a}$ | $58.90 \pm 2.17^{b}$ | $61.40 \pm 2.54^{b}$ | $47.90 \pm 1.71^{c}$ |
| Antioxidative status: | | | | |
| GSH (U/ml) | $8.90 \pm 1.12^{c}$ | $13.40 \pm 0.60^{b}$ | $11.80 \pm 0.67^{b}$ | $16.00 \pm 0.45^{a}$ |
| SOD (U/mg protein) | $13.8 \pm 1.04^{d}$ | $19.90 \pm 0.79^{b}$ | $17.20 \pm 0.84^{c}$ | $23.90 \pm 0.87^{a}$ |
| Thyroid hormones: | | | | |
| T3 (ng/ml) | $1.69 \pm 0.18^{d}$ | $2.97 \pm 0.13^{b}$ | $2.53 \pm 0.10^{c}$ | $3.57 \pm 0.11^{a}$ |
| T4 (ng/ml) | $60.10 \pm 3.80^{d}$ | $81.40 \pm 1.90^{b}$ | $71.20 \pm 2.71^{c}$ | $97.20 \pm 2.27^{a}$ |
| Other parameters: | | | | |
| Selenium (ng/ml) | $38.3 \pm 2.58^{c}$ | $52.00 \pm 1.81^{b}$ | $47.30 \pm 1.76^{b}$ | $61.70 \pm 1.01^{a}$ |
| Glucose (mg/dl) | $43.70 \pm 2.97^{d}$ | $59.10 \pm 2.01^{b}$ | $51.00 \pm 2.30^{c}$ | $65.70 \pm 1.20^{a}$ |

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

**Health status of delivered calves:**

Results regarding viability and health status of newborn calves presented in Table 7 reflecting that the newborn calves delivered from dams treated with Se-Met, LEV and their combination (Se-Met+LEV) have excellent healthy condition compared with newborn calves delivered from control dams. Moreover, there were no recorded cases of newborn affection among calves delivered from dams received Se-Met, LEV and Se-Met+LEV compared with calves delivered from control dams that show more severe pneumonia and enteritis as a result of which two (28.57%) calves died in the first month. These finding are in accordance with those of Sattar *et al.* (2003), Pavlata *et al.* (2004) and Amer and Badr (2008) who demonstrated that bovine dams supplemented with levamisole or Se+E at dry-off period gave rise to calves with excellent healthy condition and low morbidity and mortality rates. This explained that administration of levamisole or Se+E to cows during late gestation period improved immune status, blood constituents and colostral immunoglobulins levels of cows which resulted in enhancement of immune response and health status of their calves with good performance (Krakowski *et al.,* 1999). Gilles *et al.* (2009) reported that the transfer of adequate amounts of IgG from dams to claves minimize the risk of infectious diseases and mortality in claves. Ayman (2013) demonstrated that diarrhea and pneumonia which occur frequently in calf can be prevented by active immune response against pathogenic bacteria responsible for such diseases. Therefore, there is a need for an immunestimulator as an effective measure to enhance the calves' defense mechanism against these infections (Mamdouh, 2000). Nadeen *et al.* (2010) reported that prophylactic application of levamisole helps in achieving efficient immunization in buffalo calves. Sattar *et al.* (2003) found that survival rate up to weaning was the lowest in the calves from untreated dams and the highest in the calves from dams treated with levamisole and Se-E.

**Table 7. Viability and health status of newborn calves as affected by the experimental treatments to their dams.**

| Item | Control (G1) | Treatments | | |
|---|---|---|---|---|
| | | Se-Met (G2) | LEV (G3) | Se-Met+LEV (G4) |
| Viability of calves | Good | Excellent | Excellent | Excellent |
| Pneumonia | 1/7 (14.28%) | 0% | 0% | 0% |
| Enteritis | 2/7 (28.57%) | 0% | 0% | 0% |
| Mortality rate at 1st month | 2/7 (28.57%) | 0% | 0% | 0% |
| Survival rate | 5/7 (71.43%) | 7/7 (100%) | 7/7 (100%) | 7/7 (100%) |

**Birth weight and growth rate of calves:**

The administration of Se-Met and LEV separately or in combination (Se-Met+LEV) to buffalo-cows achieved significantly (P<0.05) greater values of birth weight (BW), weaning weight (WW), total gain (TG) and daily gain (DG) of calves than the control group (Table 8). In general, buffalo-cows received Se-Met+LEV achieved the greatest (P<0.05) values of BW, WW, TG and DG of calves as compared to those received Se-Met and LEV separately and the control group, reflecting the highest percentage of improvement of gain and survival rate of calves produced from dams received Se-Me+LEV. Such results reflecting that beneficial impacts of administrating dams with Se-Met and LEV separately or together Se-Met+LEV on improving blood constituents and immune response of dams (Tables 2 and 3) and their calves (Tables 5 and 6), respectively, increased concentrations of all immunoglobulin (Ig) fractions in colostrum (Table 4), which led to increase the calf birth and weaning weights and enhance growth performance of calf with good health status, especially in calves from dams received Se-Met+LEV ($G_4$). These results are in agreement with those of Amer and Badr (2008) and Gilles *et al.* (2009). Sattar *et al.* (2003) found that the highest and the lowest values of birth and weaning weights and growth rate were recorded in calf from dams treated with levamisole and the control group, respectively. The calves from dams injected with Se-E and Levamisole showed an increase in growth rate and better health conditions as compared to the control group (Mamdouh, 2000). Also, cows given Se+E and levamisole in the late stage of pregnancy produced high quality of colostrum and milk which is suitable to feed and growth of their newborn calves (Amer and Badr, 2008).

The improvement of growth rate of calf with good health status after administration of their dams with $G_2$ or $G_3$ and particularly with $G_4$ may be attributed to enhance the activities of antioxidants enzymes (GSH and SOD) and to levels of $T_3$ and Se in plasma of administrated dams (Table 2) and their calves (Table 6). In accordance, Pechova *et al.* (2012) indicated that Se deficiency can decrease pituitary level of growth hormone by impairing production of $T_3$ hormone by type of II5-deiodinase enzyme in the pituitary gland that reflected a vital role for selenium in growth. Also, selenomethionine supplementation to the diet significantly improved the growth performance of weaning pigs by enhancing its antioxidant ability and plasma selenium content. Moreover, Ebrahimi *et al.* (2009) reported positive effects of Sel-Plex supplementation on weight gain and/or average daily gain in Holstein suckling calves.

In conclusion, administration of some immunopotentitors as seleno-methionine and levamisole HCl separately or in combination to Egyptian buffaloes during the late stage of pregnancy and early lactation period (the transition period) had a valuable impact on immune and health status and blood constituents of dams and their calves as well as growth performance of their calves. Therefore, it can be recommend to administer the Egyptian buffalo dams with a combination of selenomethionine at level of 0.9 mg/kg of DM/twice weekly, orally plus injection subcutaneously with levamisole at level of 0.5 mg/kg of BW/weekly during the transition period for improving immune and health status and blood constituents of dams and their newborn calves as well as growth performance of their offspring, which allow for raising good female calves for milk production or male calves for breeding.

**Table 8. Birth weight and growth rate of buffalo calves as affected by the experimental treatments to their dams.**

| Item | Control (G1) | Treatments | | |
|---|---|---|---|---|
| | | Se-Met (G2) | LEV (G3) | Se-Met+LEV (G4) |
| Birth weight (kg) | $33.00 \pm 2.02^c$ | $38.71 \pm 1.14^b$ | $37.14 \pm 1.12^b$ | $45.86 \pm 0.80^a$ |
| Weaning weight (kg) | $80.57 \pm 3.10^c$ | $97.28 \pm 1.47^b$ | $95.14 \pm 1.40^b$ | $113.43 \pm 1.91^a$ |
| Total gain (kg) | $47.57 \pm 2.52^c$ | $58.57 \pm 0.61^b$ | $58.00 \pm 0.72^b$ | $67.57 \pm 2.03^a$ |
| Daily gain (kg) | $0.453 \pm 0.024^c$ | $0.558 \pm 0.005^b$ | $0.552 \pm 0.006^b$ | $0.644 \pm 0.017^a$ |

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

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## تحسين الحالة المناعية والصحية ومكونات الدم للجاموس المصرى ونسلها كاستجابة لمعاملة الأمهات بالسيلينوميثيونين والليفاميزول

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أجريت هذه الدراسة بمحطة بحوث الإنتاج الحيوانى بالجميزة بهدف دراسة تأثير معاملة أمهات الجاموس المصرى ببعض المنشطات المناعية مثل السيلينوميثيونين والليفاميزول هيدروكلوريد منفردين أو مجتمعين معاً (السيلينوميثيونين + الليفاميزول) قبل وبعد الولادة بشهرين (الفترة الإنتقالية) على الحالة المناعية والصحية ومكونات الدم للأمهات ونسلها وكذا أداء نسلها. تم اختيار 28 جاموسة بمتوسط وزن 600 كجم ± 50 كجم فى موسم الحليب الثانى إلى الرابع قبل الولادة بـ 60 يوماً، قُسمت عشوائياً إلى أربعة مجموعات متشابهة بكل منها 7 حيوانات، المجموعة الأولى لم يتم معاملتها وتُركت كمجموعة ضابطة، بينما المجموعة الثانية تم معاملتها بالسيلينوميثيونين (0.9 مجم / كجم من المادة الجافة، مرتان فى الأسبوع تجريعاً)، المجموعة الثالثة تم معاملتها بالليفاميزول هيدروكلوريد (0.5 مجم / كجم من وزن الجسم، مرة فى الأسبوع حقناً تحت الجلد)، والمجموعة الرابعة تم معاملتها بالسيلينوميثيونين (0.9 مجم / كجم من المادة الجافة، مرتان فى الأسبوع تجريعاً) بالإضافة إلى الليفاميزول هيدروكلوريد (0.5 مجم / كجم من وزن الجسم، مرة فى الأسبوع حقناً تحت الجلد)، وذلك خلال 60 يوما قبل وبعد الولادة. أظهرت الدراسة أن إعطاء كلاً من السيلينوميثيونين والليفاميزول والسيلينوميثيونين + الليفاميزول للأمهات أحدث تحسناً معنوياً ملحوظاً على مستوى 5% فى تركيزات كل من البروتين الكلى والألبيومين وهرمونات الغدة الدرقية وعنصر السيلينيوم والجلوكوز بالإضافة إلى نشاط الإنزيمات الناقلة لمجموعة الأمين (AST , ALT) والإنزيمات المضادة للإكسدة (GSH , SOD) فى دم الأمهات ونتاجها مقارنة بالمجموعة الضابطة ونتاجها، وكانت المفاضلة لصالح الأمهات المعاملة بالسيلينوميثيونين + الليفاميزول معاً وأيضا نتاجها. أيضا أدت معاملة الأمهات بالسيلينوميثيونين أو الليفاميزول منفردين أو مجتمعين معاً إلى إرتفاع معنوي ملحوظ على مستوى 1% فى تركيز الجلوبيولينات المناعة (IgA, IgM, IgG) وانخفاض معنوي ملحوظ على مستوى 1% فى تركيز أكسيد النيتريك وهرمون الكورتيزول فى بلازما دم الأمهات المعاملة ونسلها مقارنة بالمجموعة الضابطة ونسلها. سجلت مجموعة الأمهات المعاملة بالسيلينوميثيونين + الليفاميزول معاً أعلى تركيز معنوى على مستوى 1% للجلوبيولينات المناعية (IgA, IgM, IgG) وأقل تركيز معنوي على مستوى 1% لأكسيد النيتريك وهرمون الكورتيزول فى بلازما دم الأمهات المعاملة ونسلها. أوضحت النتائج أن مجموعة الأمهات المعاملة بالسيلينوميثيونين + الليفاميزول معاً سجلت أعلى ارتفاعاً معنوياً ملحوظاً على مستوى 1% فى تركيز الجلوبيولينات المناعية (IgA, IgM, IgG) فى السرسوب مقارنة بأمهات المجموعات المعاملة بالسيلينوميثيونين أو الليفاميزول منفردين أو المجموعة الضابطة. أيضا اتضح الأثر الإيجابى لمعاملة الأمهات بالمنشطات المناعية مثل السيلينوميثيونين والليفاميزول منفردين أو مجتمعين معاً على تحسين الحالة الصحية وحيوية ومقاومة نسلها للأمراض مقارنة بنسل المجموعة الضابطة. أوضحت الدراسة زيادة معنوية على مستوى 5 % فى متوسط وزن الميلاد و الفطام ومعدل الزيادة الوزنية اليومية للمواليد الناتجة من الأمهات المعاملة بالسيلينوميثيونين أو الليفاميزول منفردين أو مجتمعين معاً، وكانت الأفضلية لصالح مواليد المجموعة المعاملة بالسيلينوميثيوين + الليفاميزول معاً، وبناء عليه ومن الناحية الاقتصادية والتطبيقية، توصى الدراسة بمعاملة أمهات الجاموس المصرى خلال المرحلة الإنتقالية (قبل وبعد الولادة بـ 60 يوماً) ببعض المنشطات المناعية مثل السيلينوميثيونين بمعدل (0.9 مجم / كجم من المادة الجافة مرتان فى الأسبوع تجريعاً) بالإضافة إلى الليفاميزول هيدروكلوريد بمعدل (0.5 مجم/ كجم من وزن الجسم مرة فى الأسبوع حقناً تحت الجلد) لتحسين الحالة المناعية والصحية ومكونات الدم لكلا من الأمهات ونسلها وكذا أداء نسلها مع وضع صحى جيد.