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EFFECTS OF INJECTABLE TRACE MINERALS ON THE IMMUNE RESPONSES AND CYTOKINES IN DAIRY COWS

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ABSTRACT

The health and performance of cattle have been related to trace minerals that are necessary for the immune system. So, our goal was to assess the effects of an injectable trace mineral (ITM) supplement; the effects of copper, zinc, manganese, and selenium on the immune responses of dairy cows vaccinated with multivalent viral vaccine (MLV) against foot and mouth disease (FMD), infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), bovine ephemeral fever (BEF), and lumpy skin disease (LSD) vaccines. Two groups of dairy cows consisted of twenty cows per group and ranged in age from 3.5 to 4 years. The first group was vaccinated with multiviral vaccines (FMDV, IBRV, BVDV, BEFV, and LSDV vaccines only). The second group's cows received ITM injections with MLV vaccinations following farm policy. On days 0, 7, and 14, blood samples were taken to determine total leucocytic count, antibody titer, neutrophil cells, monocytes, lymphocytes, interleukins 1β and 6, and milk's somatic cell count (SCC). The simultaneous administration of MLV and ITM On the seventh week following vaccination, the vaccines in the second group produced greater antibody titers than the initial group. ITM-treated cows displayed an early increase in mononuclear cells and lymphocyte proliferation to MLV after immunization in contrast to the first group. Additionally, cows after the seventh week of immunization, the second group's interleukins 1β and 6 productions increased, compared to day 0 ($P < 0.01$). In conclusion, dairy cows' antibody titer against BVDV, IBRV, FMDV, BEFV, and LSDV increased higher than in the first group when ITM was given to them concurrently with the MLV vaccine. This suggests that ITM may be a viable way to enhance the immune responses of dairy cows to MLV vaccinations.

Keywords: Trace minerals, multivalent viral vaccine, interleukins, immune response, cytokines

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INTRODUCTION

The cattle sector is under more strain due to the world's expanding population (7.8 billion people). Among other things. The expansion and intensification of the production of milk and meat invariably result in an elevated risk of the spread and worsening of infectious diseases. This suggests that more knowledge of how cattle's immune systems work is required to develop the best means of preventing the enhancement of food security and combating current and emerging illnesses (Vlasova and Saif, 2021). Immunization against LSDV, BEFV, FMDV, IBRV, and BVDV is regarded as a crucial management measure and method to reduce dairy cow mortality and financial losses. But vaccinations aren't 100% successful. Numerous elements influence the defence and immunological response triggered by the vaccination of dairy cows, taking into account factors including diet, stress, weather, and the existence of maternal antibodies, route of immunization and management of vaccines (Palomares *et al.*, 2016).

Adult dairy cow vaccination aims to provide a sufficient degree of protection and elevated antibody levels. Pregnant cows can safely get vaccinations; heifers should receive at least two MLV vaccinations prior to breeding. Vaccination ought to be as extensive as possible. During the crucial first four months of pregnancy, protection is obtained to optimize and improve defence against infection in the fetus. MLV vaccinations are frequently administered to dairy heifers at 30–60 days before breeding and at 6 months of age to optimize protection against fetal infection. Every year, four weeks before breeding, cows should have a revaccination (Bonaventura *et al.*, 2015). Cattle performance and health have been linked to mineral levels and nutritional conditions (Enjalbert *et al.*, 2006). Trace metals such as copper (Cu), manganese (Mn), zinc (Zn), and selenium (Se) are necessary for healthy growth and immunological response in cattle, particularly in highly stressed cows (Spears and Kegley, 2002; Duff and Galyean, 2007).

Zinc helps more than 2,500 enzyme systems in their structure, function, and metabolism (Andreini *et al.*, 2009). Zinc activates the enzyme superoxide dismutase, which is necessary to shield cell membranes from reactive oxygen species (Bonaventura *et al.*, 2015). Zinc aids in DNA replication and is necessary for lymphocyte proliferation and differentiation through the action of ribonucleotide reductase. Zinc's primary functions in the immune response include neutrophil and macrophage adhesion, monocyte generation of proinflammatory cytokines, control of IL-1 release, T-cell activation signal transmission, clonal growth, differentiation, B-cell activity, antibody synthesis, and polarization (Tomlinson *et al.*, 2008; Haase and Rink, 2014).

When it comes to the mitochondrial metabolic cascades that produce energy for various immunological systems, among other organs (Failla, 2003). Additionally, copper contributes to the activity of superoxide dismutase and reactive oxygen species (ROS) neutralization (Maggini *et al.*, 2007). B-lymphocyte and neutrophil activity were significantly reduced in cattle fed a diet low in copper. Selenium plays a critical role in neutrophil migration into tissues and ensuing inflammation (Cerone *et al.*, 1998). Glutathione peroxidase is an enzyme that contains selenium which inhibits the generation of ROS and stops released ROS from damaging cells (Maddox *et al.*, 1999). Reduced neutrophil migration and its killing capacity, as well as decreased antibody synthesis and B-cell responsiveness have been linked to Se deficiency. Additionally, Se suppl-ementation improved immunological

responses, humoral responses, and cell-mediated responses (Maggini *et al.,* 2007). Total IgM levels and specific antibody titers were influenced by the amount of Se present in tissues and blood (Reffett *et al.*, 1988). The impact of an ITM supplement was assessed in a study including copper, zinc, manganese, and selenium for the cytokines and immunological responses in MLV vaccinations for dairy cows that contain BVDV, IBRV, FMDV, BEFV, and LSDV.

MATERIAL AND METHODS

This study was carried out at Nubaria Dairy Farm in compliance with the Care and Utilizing Animals for Research and Education in Agriculture requirements. The dairy cows were taken and handled by them. The Institutional Animal Care and Use Committee at the University of Cairo gave its approval to the research protocol (cu II F 424/2024), which was assigned the number 0000588. Experiment design the 14-week trial had 40 dairy cows with an average weight of 350–400 kg, and each group comprised 20 youngsters aged 3–5 years. Every cow had no antibodies to the FMD, BVD, IBR, BEF, or LSD viruses, and they were all in good clinical health (Table 1). Two groups of cows were formed. Twenty Holstein cows were vaccinated against the FMDV, BVDV, IBRV, BEFV, and LSDV in the first group. In addition to several viral vaccinations, the second group got 7 ml of a subcutaneous injectable trace mineral supplement (MultiMin 90, USA) including Cu, Zn, Mn, and Se (Table 2). Blood samples were obtained on days 0, 7, and 14 following vaccination, and each cow's weight and milk production were measured.

Table 1: The vaccination regimen in the dairy farm**.**

Type of vaccines	Group No.	Route of vaccination	Time of vaccination
BEFV (Zoetis pharm)	1 2	2 ml S/C	1st week
BVDV (Bovilis pharm)	1 2	2 ml I/M	3rd week
FMDV (Biolabs pharm)	1 2	2 ml S/C	5th week
LSDV (Biolabs pharm)	1 2	1 ml S/C	7th week Booster dose 13th week
IBRV (Bovilis pharm)	1 2	5 ml S/C	11th week

S/C: Subcutaneous I/M: Intra muscular

Multiviral vaccines:

Ultravac BEF vaccine (ZOETIS for Vet):

An attenuated live virus vaccination in a solution that has been freeze-dried. The BEF strain 919 underwent modifications through cell culture and growth in heterologous tissue. Cattle in the early stages of pregnancy. It is safe to subcutaneously inject a 2 ml dose of the ephemeral fever vaccination during pregnancy vaccination. The reduction in antibody titers takes a minimum of one year.

BOVILIS BVD vaccine:

With a TCID50 of 7.7 log10 per dosage, it contains the cytopathogenic strain of the BVD virus C86. Beta-propiolactone is employed to make the virus inactive once it has grown in cell culture. An adjuvant made of aluminum salts facilitates the

absorption of the antigen. The vaccine includes methyl para preservative: hydroxybenzoate, as well as traces of calf serum and antibiotics, which are byproducts of the antigen's synthesis.

FMD biolab vaccine:

The viral capsids of the O and SAT2 serotypes of the FMD virus are relatively more thermolabile. Readily divide into non-immunogenic pentameric subunits, which may lessen FMD's effectiveness. Vaccines designed to increase the size of neutralizing-antibody titers produced by SAT2 antigenicity. Serotype O field samples were gathered from 2010 to 2021 in East, South, Central, and Southeast Asia.

LSD vaccine (biolabs):

The majority of live attenuated vaccines used to protect cattle from LSD are created from cell-cultured attenuated wild isolate strains. Dermatosis in cows the Gorgan goat pox (GTP) vaccine, the Kenyan sheep and goat pox (KSGP) O-180 strain vaccine, and the LSDV Neethling vaccine are the three approved vaccines that can prevent LSD.

Bovilis IBR Marker vaccine:

The GK/D virus's TCID50 in each dose of the live attenuated marker vaccine is at least 5.7 log10 strain of BHV-1. The synthesis of antigens may leave behind cell debris and antibiotic residues. In order to decrease nasal field viral excretion and the severity and duration of clinical respiratory symptoms brought on by a BHV-1 infection, a current vaccine is required for livestock.

ITM:

Group (1) got a subcutaneous injection of sterile saline (1 ml/45 kg) as part of the ITM protocol at the time of the vaccine, while injectable trace minerals (MultiMin 90, MultiMin USA) (1 ml/45 kg of BW) Inc., Fort Collins, CO) were injected under the skin alongside the immunization in (2) Group (Table 2). Commercial cattle feed (about 2.7 kg/d per cow), hay, and water that are high in the grazing cows were given energy, protein, minerals, and vitamins twice a day.

Table 2: The chemical constituents of the MultiMin 90.

Ingredients	Amount per 50 g
Copper sulfate (mg)	139.3
Zinc sulfate (mg)	264.3
Selenium (mg)	1.35
Magnesium sulfate (mg)	288.1
Calcium pantothenate (mg)	3.2
Choline chloride (mg)	4.75
Cobalt sulfate (mg)	2.4
Manganese (mg)	238.7
Iron sulfate (mg)	369.05
Iodine (mg)	1.45
Sodium chloride (g)	7.05
Potassium iodate (ppm)	11.9

Blood and milk samples:

Jugular venipuncture was used to obtain about 20 ml of blood samples, which were then placed in vacuum tubes (Vacutainer®, BD). Obtaining whole blood and serum, both with and without an anticoagulant (acid citrate dextrose), in turn. Blood was drawn on days 0, 7, and 14 in relation to the priming vaccination and ITM injection in order to measure antibody titers, produce interferon gamma (IFN), interleukins 1 and 6, total leucocytic count, and mononuclear cell (MC). Two hours after blood samples were collected and brought to the lab on ice. The blood specimens in the anticoagulant-free tubes were centrifuged for 15 minutes at 800 g. After that, the serum was eliminated and kept in aliquots at -80°C until the antibodies against BVDV, IBRV, FMDV, BEFV and LSDV were examined. Fresh milk samples of about 50 ml were gathered and delivered to the lab for SCC analysis.

Body weight and milk yield evaluation:

Using a Coburn bodyweight tape, the average weight was measured before

immunization, seven weeks into the trial, and fourteen weeks after the challenge (kg). Additionally, the quantity of milk. Reproductive performance and yield were measured from day 0 to the completion of the experiment.

Antibody titer:

For the first and second groups, serum samples were obtained from dairy cows on days 0, 7, and 14 and analyzed using the ELISA test for antibody titers by kits against BVDV (IDEXX BVDV Ag/Serum Plus Test, USA), FMDV (PrioCHECK™ FMDV NS Antibody ELISA Kit, Ireland), IBRV (antibody ELISA kit Ring Biotechnology Co, US**)**, LSDV (ID Screen® Capri pox Double Antigen Multi-species, France), and BEFV (Bovine ephemeral fever virus antibody Elisa Kit, AFG Scientific, USA).

Sample processing for cellular immune:

1. Mononuclear cell (MCs): Blood was diluted 1:1 using HYCLONE RPMI-1640 (GE, USA), as well as inverted mixing. In a 50 ml conical tube, the mixture was carefully placed over 7–10 ml of Histopaque ®-1077 (Sigma-Aldrich, USA). Differential centrifugation was used to separate the MCs for 30 minutes at room temperature at 400×g. They cleaned the MCs. Centrifuged twice with RPMI-1640 at 300× g for 10 minutes at 4°C. The pellet was suspended in one milliliter of lysis buffer (1:10 dilution of 0.17 M Tris-HCl to 0.16 M NH_4Cl) for two to five minutes in order to lyse any leftover erythrocytes. RPMI-1640 was added as a final wash, and 2 ml of 10% L-glutamine in enriched medium RPMI-1640 was used to reconstitute the final pellet. Heat-inactivated fetal bovine serum (FBS), 100x Thermo Fisher Scientific, USA; 1× non-essential amino acids (Gibco MEM NEAA); streptomycin (100 µg/ml); and penicillin (100 IU/ml). After being divided into 1×10^6 cells per well, the PBMCs were either stimulated with 10 µg/ml Johnin PPD or left unstimulated for 72 hours. The supernatants were frozen at -80°C after being removed from the MCs. According to Phanse *et al.* (2020).

2. IFN-γ production: A monoclonal antibody was used to assess it in culture supernatants. Based on sandwich ELISA with samples diluted 1:10 (Bovigam®, Thermo Fisher Scientific, USA). According to Palomares *et al.* (2016), ELISA plates were examined at OD 450 nm.

3. Total leucocyte count: Trypan blue was used to measure blood neutrophils; the deceased while live cells seem colorless, cells that absorb the dye appear blue. Under a microscope, TLC was determined by counting on a hemocytometer. A smear of blood was made on a spotless glass. For a precise cell count, the slide was stained with Giemsa stain and viewed in an oil immersion. Everybody to ascertain the percentage of distinct leucocytes, leucocytes were categorized.

4. Somatic cell count in milk samples: Two groups' milk samples' SCC was determined using a somatic cell counter and also examined using a milk smear on a glass slide at ×40 magnification. To ascertain whether various cell types were present, differential cell counting was also done. Similar to milk's macrophages, neutrophils, and lymphocytes. 40°C was reached by heating milk in water. Soak for 15 minutes, then stir-cool to 20°C. After spreading 0.01 ml of milk on a slide and drying it horizontally, it was fixed with 96% ethyl alcohol for three minutes, allowed to air dry, and then defatted with xylol for eight minutes, then well cleaned with 60% ethyl alcohol, allowed to air dry, and then dyed with pure May-Giemsa solution (20 min), Grünwald (2 minutes), at 50% ethyl alcohol (2 min), air dried, and dehydrated in xylols and alcohols (Dang *et al.,* 2008).

5. Cytokines:

The ELISA test was used to measure the variations in the blood levels of interleukin 1 beta (IL-1β) and interleukin 6 (IL-6) by bovines during the experiment. Bovine IL-6 ELISA kit for measuring bovine IL-6 in cow serum samples (abcam) and interleukin 1 beta ELISA kit (Catalog Number: MBS703996, My BioSource, Inc. USA). The levels of circulating cytokines in serum samples were measured using ab205080, Italy from two groups of cows in the experiment at weeks seven and fourteen. Amounts of cytokines in a standard curve created using a set of known cytokine values were used to calculate the samples.

Statistical analysis:

All of the variables in the data were analyzed using independent sample t-tests using the SPSS software (version 23.027). The obtained result was displayed as mean ± standard deviation. error (SE), where $P < 0.05$ is regarded as a sign of statistical significance.

RESULTS

Body weight:

There were no discernible variations in the two groups' changes in body weight over the duration of the experiment ($P = 0.138$).

Milk yield:

On average, group 2 produced less variance and more milk than group 1 during the seventh week implying more stability. Although there was some fluctuation, group 2 continued to produce milk at a little greater rate in the fourteenth week than ITM injections. This may indicate that the first group's standard deviation was higher, indicating a positive effect on body weight and milk production (Table 3 & Fig. 1).

Health and reproductive performance:

The goal of the experimental investigation was to improve stressed materials' ITM status. ITM has been found to benefit dairy cows by increasing feed efficiency lowering morbidity and enhancing the ability to reproduce (Table 4 & Fig. 2).

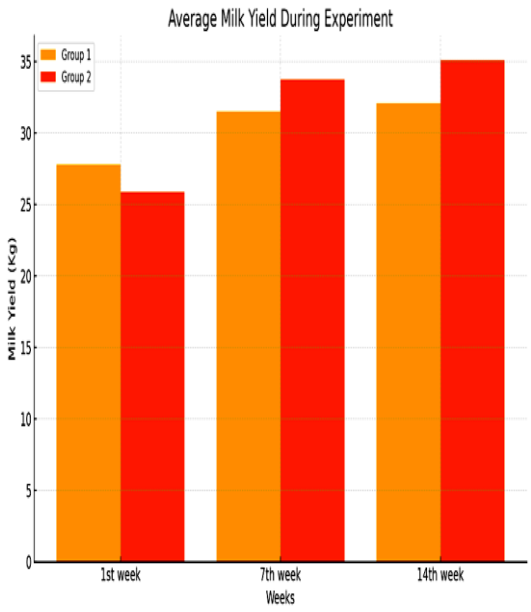

Fig. 1: The average of milk yield/Kg during 14 weeks in two Groups

Table 3: Average of milk yield during experiment in two groups.

Weeks	Milk yield (Kg)	
	Group 1(n+20)	Group 2 (n+20)
1st week	27.8 kg	25.9 Kg
7th week	31.5 Kg	33.75 Kg
14th week	32.1 Kg	35.1 Kg

Table 4: Reproductive performance during the experiment in two groups.

Weeks	Reproductive performance	
	Group 1(n+20)	Group 2 (n+20)
1st week	Pregnant cows 3	Pregnant cows 3
7th week	Pregnant cows 4	Pregnant cows 5 Birth cow (1/20)
14th week	Pregnant cows 3 Birth cows (2/20)	Pregnant cows 5 Birth cows (1/20)

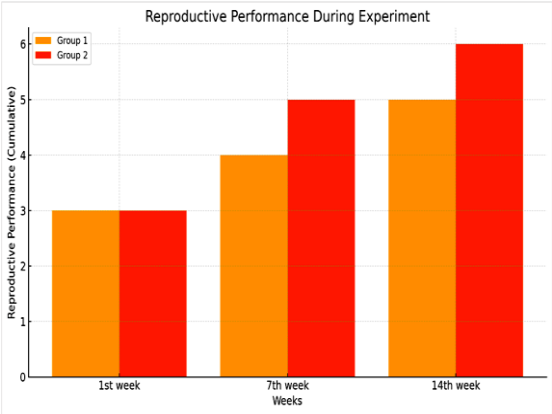

Fig. 2: Reproductive performance during the experiment in two groups

Antibody titer for Multiviral Vaccines:
The antibody titers to MLV were above cut-off values in group 2 than in group 1; after the first immunization in the FMD vaccine, group 2 was higher than in group 1. However, no statistically significant differences ($p > 0.05$) were observed between groups. Moreover, the MLV showed that group 2 was significantly higher than group 1 at the 7th and 14th weeks ($p < 0.05$) (Table 5 & Fig.3).

Table 5: Antibody titers of dairy cow serum in two groups vaccinated by viral vaccines.

Viral Vaccines	7th week		14th week		P value*
	Group1	Group2	Group1	Group2	
FMDV	1.5	1.9	3.7	4.2	0.375± 0.452
BEFV	4.6	5.7	3.9	4.4	1.179±1.295
BVDV	5.6	5.9	5.2	5.6	1.076±1.053
LSDV	0.1	0.65	1.75	1.95	0.057±0.033
IBRV	0.2	0.25	4.0	5.2	0.05±0.48

Mean ± SD (n=20): $P<0.05$.

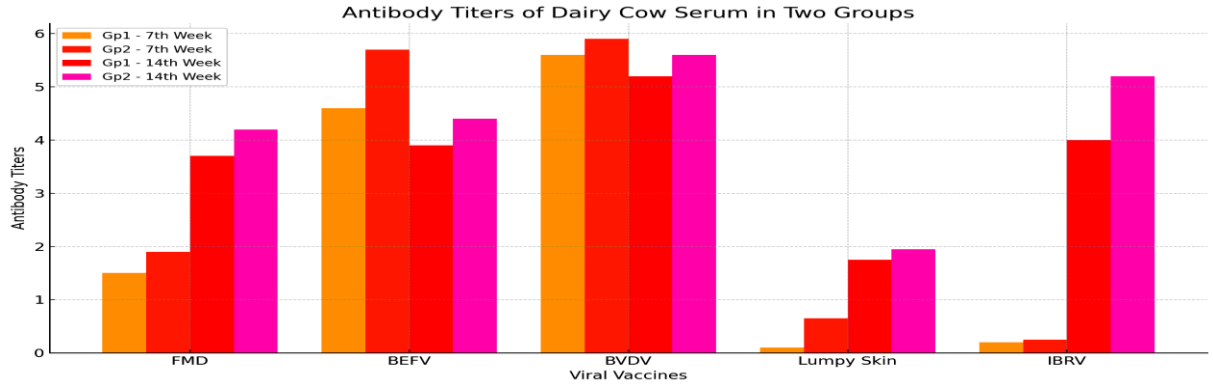

Fig. 3: Antibody titers of dairy cow serum in two groups vaccinated by viral vaccines.

Total leucocytic, lymphocyte, and neutrophil count:

The blood neutrophil proportion of dairy cows significantly increased; almost 93% of the neutrophils displayed segmented nuclei, whereas 2-3% were group 1's immature nuclei. 97% of the dairy cows in group 2 had viable blood neutrophils. Compared to blood, the viability of the lymphocyte cells increased greater in group 2. Significantly ($p < 0.05$) in group 1 (Table 6 & Fig. 4).

Monocyte cells (MCs):

Both groups showed a notable increase in MCs proliferation following stimulation on distinct post-vaccination days compared to day 0 ($P<0.05$). ITM-treated cows consistently displayed increased MC proliferation after receiving MLV immunization (on the 7^{th} and 14^{th} week compared to day 0). It was noteworthy that group 2 showed a notable rise in MC stimulation earlier than group 1 (Table 6 & Fig. 4).

IFN-γ production:

In the seventh week, there was a notable rise in IFN-γ production following in vitro stimulation with MLV in both experimental groups compared to the priming vaccination day (day 0) ($P<0.01$); however, there was a decline in the 14^{th} week. When ITM was administered alongside the MLV vaccine, it caused an increased IFN-γ synthesis following priming immunization compared to day 0 ($P<0.01$) (Table 6 & Fig. 4)

Interleukins 1 β & 6:

When tissue infection or antigen stimulation occurs, macrophages release the cytokine IL-1 β, engaged in controlling inflammation and the immunological response, boosting defensive mechanisms. No discernible variation in the alterations of the IL-1 β level was found in this investigation between the two groups, indicating that ITM supplementation did not affect this cytokine. Additionally, IL-6, another immunological signal, contributes to protective actions through certain receptors and immunological mediators, were considerably greater in group 2 than in group 1, suggesting that dairy cows' immune systems may be strengthened by trace elements (Table 6 & Fig. 4).

Milk somatic cells (SCs):

They are a blend of immunological and milk-producing cells. Milk secretes these cells throughout the typical milking process and serves as a gauge for assessing the health of the mammary glands and quality of dairy cows' milk. Regarding SCC, the variance in milk composition has been shown in Table (6) and Fig. (4). In group 2, which received ITM and MLV, milk SCC was low compared to group 1, which only received multiviral vaccinations.

Table 6: Hematological parameters; leucocytic count, neutrophil, lymphocyte, cytokines and somatic cell count in milk

Hematological parameters	7^{th} week		14^{th} week	
	Group1	Group2	Group1	Group2
WBC	11.2	10.0	9.2	10.1
Lym	4.8	5.2	4.1	4.6
Neu	5.3	4.3	4.5	4.9
Mon	5.7	5.9	5.3	6.1
IFN-γ	0.60	0.70	0.62	0.63
IL-1β	0.70	0.80	0.90	0.95
IL-6	0.50	0.57	0.48	0.53
SCC in milk (10^5cells/ml)	>2.2	>1.5	>1.6	>1.2

WBC: white blood cell count, Lym: lymphocyte count, Neu: neutrophil count, Mon: monocyte cell, IFN-γ; interferon gamma, IL-1β: interlukin-1 beta, IL-6: interlukin-6, SCC: somatic cell count, count (cells/ml).

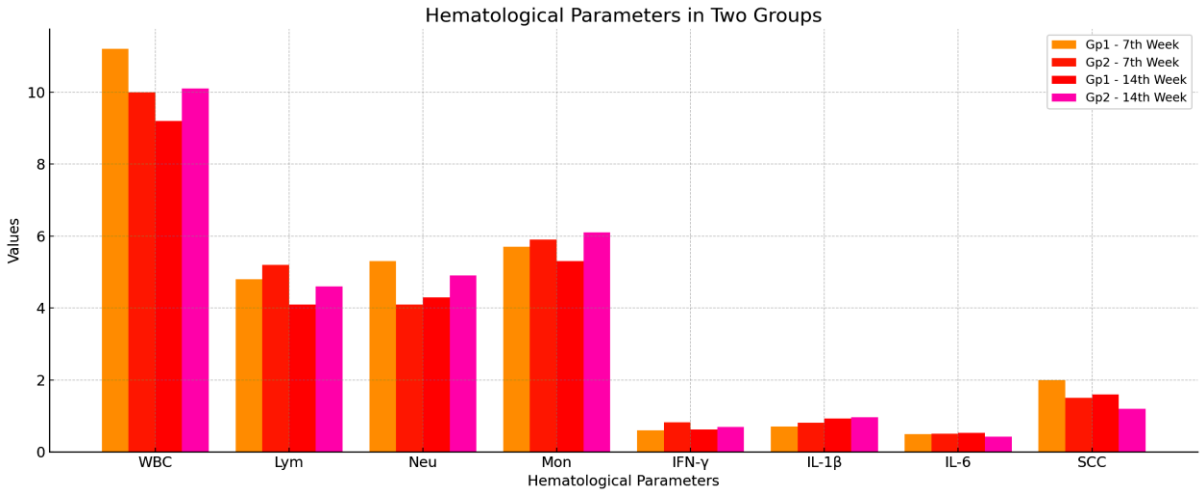

Fig. 4: Hematological parameters; leucocytic count, neutrophil, lymphocyte, monocyte, cytokines and somatic cell count in milk

DISCUSSION

The titers of antibodies were altered in both groups in different ways during the experiment. After the first vaccination, group 1 antibody titers declined; however, they increased on days seven and fourteen. However, titers of group 2 antibodies rose much earlier and more steadily (from the 7^{th} week to the 14^{th} week) than titers of group 1 antibodies ($P < 0.01$). The first dosage of the vaccination appears to have primed the immune system; thus, Srinand *et al.* (1996) state that animals were able to react to immunization more successfully.

In this study, group 2's FMDV antibody titers were considerably raised by ITM injection. Likewise, it has been demonstrated that trace minerals raise cows' antibody titers (Nemec *et al.*, 2012). Further evidence that ITM may boost the immunological response in dairy cows comes from Shinde *et al.* (2006); Cao *et al.* (2015), who also observed that zinc could improve the immune response. This study found that the main immunization for LSDV had a weak antibody response. Vaccination on the ninth week, followed by a booster shot four weeks later to achieve a notable level of humoral immunity, as previously documented by Larson and Step (2012). According to Fulton *et al.* (2002), who reported that antiviral antibody neutralization is thought to be an essential defense against viral infections and is favorably associated with improved dairy performance. For all viral strains, group 2 antibody levels are often higher than group 1 antibody levels, according to estimates of the averages for each group. To ascertain whether these temporal fluctuations were statistically significant, one could employ analysis of variance, or ANOVA. The present investigation indicates that, at the $p < 0.05$ threshold of significance, the antibody levels of the two groups (1 and 2) differ statistically significantly. These variations imply that the immunological reactions. There are notable differences between the two groups, and after receiving viral vaccines, group 2 was probably going to have a higher immune response (Palomares *et al.*, 2016).

This study showed that when ITM was given with the MLV vaccine following vaccination, compared to group 1, group 2's mononuclear leukocyte proliferation was higher. It was improved by ITM. Protecting against viral infection by the MLV vaccine, assisting in the maintenance of appropriate health and cows' performance. Moreover, lymphocyte activation contributes to cellular growth for defense. preventing infection by viruses

(Failla, 2003). The improvement of these fundamental biological processes helped explain the second group's greater responsiveness to leukocyte proliferation. In this study, two groups showed peaks in neutrophil cell growth after being stimulated with MLV. In group 2 receiving ITM, the initial peak of neutrophil proliferation happened earlier and was noticeably stronger than in group 1. This discovery has been previously shown by Malmgaard *et al.* (2000); Vollstedt *et al.* (2004).

Both groups showed a markedly elevated IFN-γ production in response to MLV and showed that the nonspecific reaction to the viral infection included the production of IFN-γ. When ITM and MLV immunization were administered together, there was an increase in production. On the seventh week following immunization, IFN-γ by immune response with recall activation was compared to day zero. It appears that stimulation with MLV at week 14 reduced IFN-γ production. Prior research has demonstrated that both in vitro and in vivo viral infections downregulate IFN-γ (Rhodes *et al.,* 1999; Ellermann-Eriksen, 2005). Cytokines produced by the immune response have the power to eradicate infected cells when infected by a virus. Cell signaling and cytokine production have been found to depend on an adequate availability of trace minerals (Puertollano *et al.,* 2011). In this investigation, there was no noteworthy difference between the two groups' IL-1β level fluctuations, indicating that this ITM had no effect on cytokine (Table 6 & Fig. 4). These findings were originally noted by Akira *et al.* (1990), who noted that IL-6 participates in the acute phase and affects immunological modulation answer. It promotes the synthesis of proteins and aids in B cell development into cells that produce antibodies (LeBlanc *et al.,* 1999). Interleukin-6 is important for the immune response, and inflammation happens in a variety of cell types, including fibroblasts and macrophages (Beatrice *et al.,* 2001). Following MLV vaccination, the experiment revealed a significantly higher level of IL-6 in group 2 compared to group 1 at weeks 7 and 14 (Fig. 4).

White blood cells and epithelial cells that shed from the lining are known as somatic cells in milk during milking of the mammary gland (Swain *et al.,* 2014). They act as a common indicator to determine the quality of the milk and the mummery's health (Al-Hussien *et al.,* 2016). The breed, health, parity, lactation stage, and production of the cow all affect milk SCC. Any changes to stressful circumstances, poor management practices, and environmental factors significantly increase the amount of SCC in milk (Varshney *et al.,* 2016). Better feeding practices and hygiene help reduce milk SCC. Milk with a low SCC yields better milk products with a longer shelf life according to Al-Hussien and Dang (2018). The current study outlined the function of milk's immune cells for SCC. ITM combined with MLV may help lower milk consumption, SCC, and create standards for differentiating SCC.

CONCLUSION

For cattle to build an immunological response, trace minerals are essential, particularly in animals under stress. Dairy farms have seen improvements in their trace mineral condition, feed efficiency, lowering treatment expenses, morbidity, enhancing performance, and reproduction. The advantages of trace mineral supplementation on cattle's immunological response to MLV vaccinations indicated that including ITM in management procedures could be a promising instrument to enhance the health of dairy farm animals.

CONFLICT OF INTEREST

The authors of this paper claim that there are no conflicts of interest.

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تأثير حقن المعادن النادرة على الاستجابات المناعية والسيتوكينات في الأبقار الحلوب

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لقد ارتبطت صحة وأداء الماشية بالمعادن النادرة الضرورية للجهاز المناعي. لذلك كان هدفنا هو تقييم آثار المكملات المعدنية االنادرة للحقن (ITM) مثل النحاس والزنك والمنغنيز والسيلينيوم مع اللقاحات الفيروسية المتعددة وتأثيرها على الاستجابات المناعية للأبقار الحلوب المحصنة ضد مرض الحمى القلاعية (FMD)، والتهاب القصبة الهوائية (IBR)، والإسهال الفيروسي البقري (BVD)، الحمى البقرية الزائلة (BEF) ولقاحات مرض الجلد العقدي (LSD). تم اختيار مجموعتين من الابقار الحلابة عدد كل مجموعة ٢٠ بقرة تتراوح اعمارها بين ٣,٥ الى ٤ سنوات. تلقت أبقار المجموعة الاولى اللقاحات الفيروسية الخمسة بدون حقن المكملات المعدنية النادرة وتلقت المجموعة الثانية حقن العناصر المعدنية النادرة مع اللقاحات الفيروسية المتعددة وفقًا لسياسة المزرعة. منذ اليوم الاول والاسبوع السابع والاسبوع ١٤ ، تم أخذ عينات الدم من أجل تحديد العدد الإجمالي للكرات الدم البيضاء، و الأجسام المضادة، والخلايا المتعادلة، والخلايا الليمفاوية، والإنترلوكينات βI، و٦ وعدد الخلايا في الحليب (SCC). لـ لابقار المحصنة باللقاحات الفيروسية المتعددة وحقن العناصر المعدنية النادرة في الأسبوع السابع مع التطعيم. أنتجت اللقاحات في المجموعة الثانية عيارًا أكبر من الأجسام المضادة مقارنة بالمجموعة الأولى. أظهرت الأبقار فى المجموعة الثانية المعالجة بالعناصر المعدنية النادرة زيادة في الخلايا وحيدة النواة وانتشار الخلايا الليمفاوية بعد التحصين على عكس المجموعة الأولى. بالإضافة إلى ذلك، بعد الأسبوع السابع من التحصين، زاد إنتاج الأبقار في المجموعة الثانية من الإنترلوكينات β١ و ٦. مقارنة باليوم صفر (P <٠,٠١). الخلاصة : ارتفع الأجسام المضادة لأبقار الألبان ضد BVDV وIBRV وFMDV وBEFV وLSDV أعلى مما كان عليه في المجموعة الأولى عندما تم إعطاء محلول الاملاح المعدنية النادرة بالتزامن مع لقاحات الفيروسية المتعددة للابقار الحلابة. يشير هذا إلى أن حقن الاملاح المعدنية النادرة مع اللقاحات الفيروسية المتعددة قد يكون وسيلة فعالة لتعزيز الاستجابات المناعية للأبقار الحلوب.