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Effect Of Injectable Trace Minerals on The Humoral Immune Response to Multivalent Viral Vaccine in Dairy Cows

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Abstract:
Background: *This study was done to investigate the effects of injectable trace minerals on the humeral immune response of dairy cows receiving multivalent viral vaccine.*
Materials and methods: *Forty cows divided into 2 groups; average BW = 350 - 420 kg. All were seronegative for FMD virus, lumpy skin, BFV, BVD and IBRV. First group (Gp1) n= 20 Holstein cows vaccinated with FMD virus, lumpy skin, BFV, BVD and IBRV, and the 2nd group (Gp2) vaccinated and treated with injectable trace mineral supplement 7 mL subcutaneous (MultiMin 90, USA) containing Cu, Zn, Mn (all as disodium EDTA salts), and Se. Humoral immunity was detected by neutralizing antibody titers measured on day one, 7th week and 14th weeks experiment relative to vaccines administration. All cows were seronegative for each of the 5 viruses on day zero, serum mineral concentrations were evaluated on day one, 7 w and 14 w.*
Results: *The higher average of milk production in 7th and 14th week in the Gp2 compared to Gp1, there are clear increases in the concentration of minerals (Cu, Zn, Se, Mo, Co, Mn, Fe) in Gp2 compared to Gp1, which indicates better absorption of these minerals when they are injection. The mean antibodies titers were gradually increased from 7th weeks post-vaccines till reached higher level at 14th weeks post- vaccines for all viral vaccines as measured by SNT and ELISA test. The antibodies peak titer was observed higher at 14th w post-vaccination in Gp2 significantly greater than Gp1.*
Conclusion: *Viral vaccination concurrent with ITM, induced a rapid protection against viral infection. In addition, administration of ITM at the time of vaccination was associated with increased humoral immune response, enhanced health status.*
Keywords: *Humoral immunity, FMD virus, Lumpy skin disease, IBR, BFV, Trace mineral*

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I. Introduction

The health of dairy cows may be significantly impacted by the protective immunity against infections that is brought about by vaccination1. Biosecurity, which prevents the introduction and transmission of viruses within the herd, the identification and removal of diseased animals, and vaccination are the cornerstones of the prevention and control of viral diseases2.

It is effective to prevent viral infections by immunizing against attenuated or inactivated vaccines3. The ability of a vaccine to trigger humoral and cell-mediated immune responses that can regulate viral replication, viremia, and shedding4 is a useful indicator of vaccination efficacy in preventing viral infection. The speed at which vaccinations trigger a sufficient immune response to shield cattle from acute infections and illness is another factor to consider when evaluating their effectiveness5.

Various stressors, including harsh weather, irregular vaccination schedules, prolonged transit, and dietary changes, can impair the immune system and the animal's reaction to vaccinations by causing oxidative stress, which can negatively impact the health and performance of dairy cows6.

The bovine ephemeral fever virus (BEFV) belongs to the Rhabdovirus family and is transmitted by arthropods. The virus affects cattle all across the world and has a big economic impact, mostly because it makes dairy cows produce less milk7. Regular immunization is the primary means of controlling BEF8. To prevent bovine ephemeral fever, three vaccination types are now employed in the field: subunit G protein-based, inactivated, and live attenuated (LA) vaccines9.

In Egypt, foot and mouth disease (FMD) is endemic; the latest available data (10) indicates that the incidence of FMD is "high," with O (O/EA-3) and, to a lesser extent, SAT 2 being the main FMD viral serotypes currently circulating. In endemic locations, controlling FMD in animals was thought to be crucial, as vaccinations can effectively stop the disease's spread11. The local commercial FMD vaccine is (trivalent O Panasia-2/A Iran-05/SAT2/EGYA-2012). Currently current FMD vaccines are mostly based on inactivated viral antigens combined with various adjuvants to boost vaccine efficacy in order to protection against infections.

A Capri pox virus is the cause of the economically significant cow disease lumpy skin disease (LSD), which has spread to an unprecedented level. In endemic and recently impacted areas, vaccination is the only method of stopping the virus from spreading. Choosing the most effective vaccine during an outbreak presents a significant obstacle for farmers and veterinary authorities. The live attenuated vaccines that are now on the market12.

The most significant cattle disease in terms of economic impact is bovine respiratory disease, or BRD. Bovine viral diarrhea virus is one of the infectious agents that is most frequently found in cases of BRD (BVDV). An acute BVDV infection suppresses the immune system. Biosecurity is the foundation for BVDV prevention and management since it prevents the virus from entering the herd and spreading there. Vaccination against persistently infected animals is therefore a key component of this strategy13.

Bovine Herpesvirus-1 (BHV-1) is the infectious respiratory illness that causes highly contagious Infectious Bovine Rhinotracheitis (IBR). Both young and aged animals may be impacted. This virus can also cause conjunctivitis, abortions, encephalitis, and generalized systemic infections in addition to respiratory diseases. Acute upper respiratory tract inflammation is a hallmark of IBR. Never completely eradicated is the virus after the initial infection. It remains a latent (hidden) infection in brain nerve cells for the duration of one's life. An animal that has contracted the virus cannot be deemed safe since it might reactivate at stressful times and cause expansion, usually from the eyes and nose. The primary source of new infections is the purchase of sick animals14.

Numerous studies have demonstrated the impact of trace minerals on cow immunity and health, including manganese (Mn), zinc (Zn), copper (Cu), and selenium (Se)15. The action of antioxidant enzymes, transcription and replication of nucleic acids, mitochondrial metabolism, which improves neutrophil migration and phagocytic function, lymphocyte proliferation, and antibody formation have all been linked to trace minerals16–17. In highly dairy cows, the use of trace mineral supplements may become very important18. In dairy calves, injectable trace mineral (ITM) administration has been demonstrated to have positive effects on the generation of antibodies and the cell-mediated immunological response to viral vaccines19.

A recent study showed that the use of ITM in conjunction with viral vaccines produced stronger and faster mononuclear cell proliferation upon stimulation in dairy cows as well as earlier and higher serum neutralizing antibody titers to the viral pathogen20. If a quick defense is needed following immunization, whether the use of ITM could accelerate the induction of a better and faster immune response and protection21. This study set out to investigate if administering the MLV vaccination to dairy cows that had received viral vaccines concurrently with ITM supplementation (which contains Se, Zn, Cu, and Mn) could improve their humoral immune response and speed up the commencement of protection.

II. Materials And Methods

This research was conducted in the field at the Nubaria dairy farm, where we looked at how ITM affected the humeral immunological response to multiviral vaccination21. According to the guidelines in the Care and Use of Agricultural Animals in Agricultural Research and Teaching22, the dairy cows were taken care of. The University of Cairo Institutional Animal Care and Use Committee, permission number 0000588, authorized the research protocol (cu II F 424/2024).

Experimental Design

For the duration of the 14-week trial, 40 dairy cows with an average body weight of 350–420 kg and ages between 4 and 5 were included in both groups. All of the cows were clinically healthy and free of antibodies against the FMD virus, lumpy skin, BFV, BVD, and IBRV (Table, 1). Cows were split into two groups. First group: 20 Holstein cows who received vaccinations against the FMD virus, BVD, IBRV, BFV, and bumpy skin. The second group received viral vaccines and 7 mL of injectable trace mineral subcutaneous (MultiMin 90, USA) treatment, which included Cu, Zn, Mn, and Se (all as disodium EDTA salts) (Table, 2). Every cow was weighed, the amount of milk they were breastfeeding was measured, and blood samples were taken one week prior to immunization seven weeks after vaccination, and fourteen weeks afterward.

Table no1: Vaccinal Program in the dairy farm

Type of vaccines	Group No.	Route of vaccination	Weeks of vaccination
BEFV (Zoetis pharm)	1 2	2 ml S/C	1st w
BVDV (Bovilis pharm)	1 2	2 ml I/M	3rd w
FMD (Biolabs pharm)	1 2	2ml S/C	5th w
Lumpy Skin V (Biolabs pharm)	1 2	1 ml S/C	9th w Booster dose 13th w
IBRV (Bovilis pharm)	1 2	5 ml S/C	11th w

Ultravac BEF vaccine (ZOETIS for Vet): A freeze-dried suspension of a live, attenuated virus vaccination. By passage through cell culture and growth in heterologous tissue, the BEF strain 919 was altered. Cattle in the early stages of pregnancy can safely receive a subcutaneous injection of a 2 mL dose of the ephemeral fever vaccine. Antibody titers do not drop for a minimum of a year.

BOVILIS BVD vaccine: It has the cytopathogenic BVD virus strain C86 corresponding to 7.7 log10 TCID50 per dosage. Beta-propiolactone is used to inactivate the virus after it has developed in cell culture. An adjuvant made of aluminum salts absorbs the antigen. The vaccine has traces of antibiotics and calf serum as leftovers from the manufacture of the antigen, as well as methyl Para hydroxybenzoate as a preservative.

FMD biolab vaccine: Because the FMD virus's O and SAT serotypes are comparatively more thermolabile and their viral capsids easily split into non-immunogenic pentameric subunits, this could reduce the efficacy of FMD vaccinations intended to produce larger neutralizing-antibody titers generated by SAT2 antigenicity. Field samples of serotype O collected between 2010 and 2021 throughout Southeast, Central, East, and South Asia.

Lumpy skin diseases vaccine (biolabs): The majority of live attenuated vaccines used to protect cattle against LSD are derived from attenuated strains of wild isolates that have been passage through cell culture. Bovine dermatosis (LSD) can be prevented by three licensed vaccines: the Gorgan goat pox (GTP) vaccine, the Kenyan sheep and goat pox (KSGP) O-180 strain vaccine, and the lumpy skin disease virus (LSDV) Neethling vaccine.

Bovilis IBR Marker vaccine: Each dose of the live, attenuated marker vaccine contains at least 5.7 log10 TCID50 of the GK/D strain of BHV-1. Antibiotic traces as well as cell debris may remain as leftovers from the generation of antigens. In order to decrease the severity and duration of clinical respiratory symptoms brought on by a BHV-1 infection and to lessen field viral excretion from the nose, cattle must receive an active vaccination.

ITM, Group (1) received an injection of sterile saline (1 mL/45 kg) subcutaneously at the time of vaccination, whereas injectable trace minerals (1 mL/45 kg of BW; MultiMin 90, MultiMin USA Inc., Fort Collins, CO) were administered subcutaneously concurrently with vaccination in Group (2) (Table, 2). Hay, water, and commercial cattle diet (about 2.7 kg/d per cow) rich in energy, protein, minerals, and vitamins were provided twice a day to the grazing cows[23].

Table no2: 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

Sample Collection

Serum samples: They were gathered from every cow, both pre- and post-vaccination. Before being employed in the serological tests, the sera were kept at -20°C after being inactivated for 30 minutes at 56°C.

Evaluation of average body weight and body weight gain:

The average weight gain was computed by taking the difference between the initial and final body weights, which were weighted in (kg).

Neutralizing antibody titers: measured on the first day prior to vaccinations, and on the seventh- and fourteenth-days following vaccinations, as per Hong *et al*. [24.]

ELISA test:

According to El-Bagoury *et al.*[25], serum was taken from cows in the first and second groups and examined using ELISA for antibody titers against BVDV, FMDV, IBRV, lumpy skin virus, and BEFV.

Serum mineral concentrations: assessed on the first week before vaccinations, and on the seventh and fourteenth weeks after vaccinations, using the colorimetric method and commercial kits as described by Samanta *et al*[26].

Statistical analysis:

Using the SPSS software program version 23.0[27], independent sample t-tests were used to analyses all of the collected data variables. The acquired result was presented as mean ± standard error (SE), with statistical significance being considered to be represented by P < 0.05.

III. Results

The average of body weights and milk production

In first week; both groups appear to have similar rates, while the first group has less variation in body weight and production. We observed higher average of milk production in 7th week in the Gp2 compared to Gp1, with less variance indicating greater stability. In 14th w Gp2 maintained a slightly higher milk production rate than the first group, with a larger standard deviation, indicating that ITM injections may have a positive effect on body weight and milk production but with some variation. (Table 3, Fig. 1 & 2).

Table no3: Average of body weights and milk production (Kg) during experiment in different groups

Weeks	Groups	BW/Kg (n:20)	Milk/Kg (n:20)	Health case
1st week	Gp1	350±4	27.8	Pregnant cows (3/20)
	Gp2	350±4	25.9	Pregnant cows (3/20)
7th week	Gp1	380±4	31.5	Pregnant cows (4/20)
	Gp2	400±4	33.75	Pregnant cows (5/20) Birth cow (1/20)
14th week	Gp1	400±4	32.1	Pregnant cows (3/20) Birth cows (2/20)
	Gp2	420±4	35.1	Pregnant cows (5/20) Birth cow (1/20)

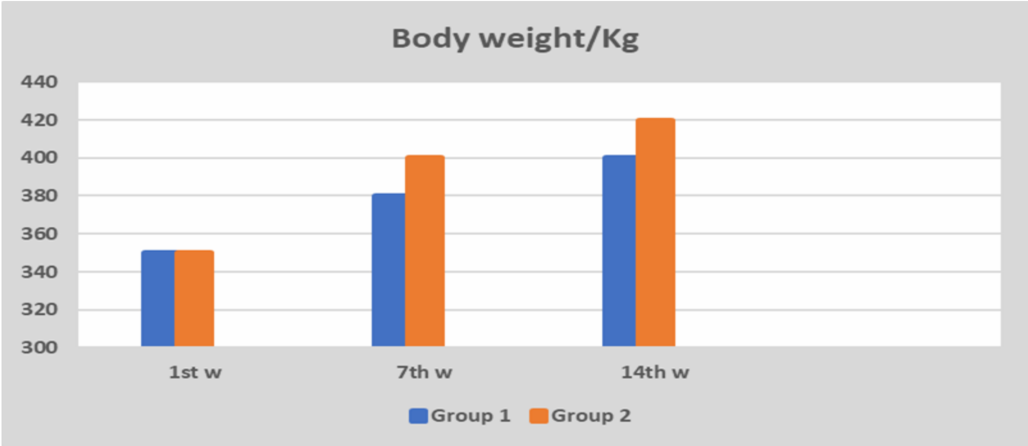

Fig. no1: The average of Body Weight/Kg during 14 weeks in different Groups

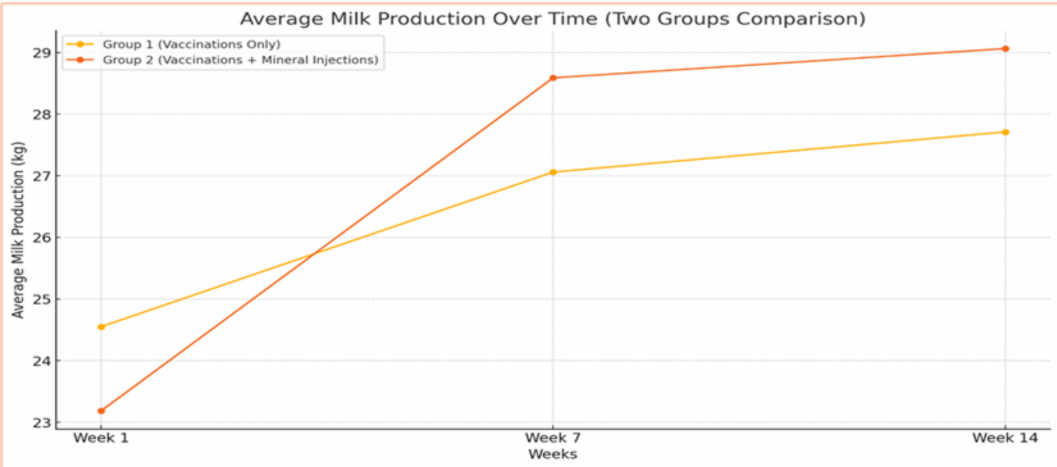

Fig. no2: The average of milk production/Kg during 14 weeks in different Groups

Trace Mineral Concentration in Serum

Cows in two groups had different serum concentrations of trace minerals during the experimental period. Table (4) shows that there are clear increases in the concentration of minerals (Cu, Zn, Se, Mo, Co, Mn, Fe) in Gp2 compared to Gp1, which indicates better absorption of these minerals when they are injection (Fig. 3).

Table no4: The concentration of trace minerals in blood serum (µmol/L) of dairy cows in two groups.

Items	1st week	7th week		14th week	
	Gp1 & Gp2	Gp1	Gp2	Gp1	Gp2
Cu	18.2	19.9	21.6	22.1	29.5
Zn	70.90	71.9	75.2	71.5	79.8
Se	80.5	84.6	87.3	86.2	90.7
Mo	5.72	6.55	6.72	6.60	6.77
Co	0.20	0.25	0.45	0.27	0.55
Mn	8.77	9.60	10.80	11.3	11.6
Fe	1.56	1.65	1.97	1.53	2.58

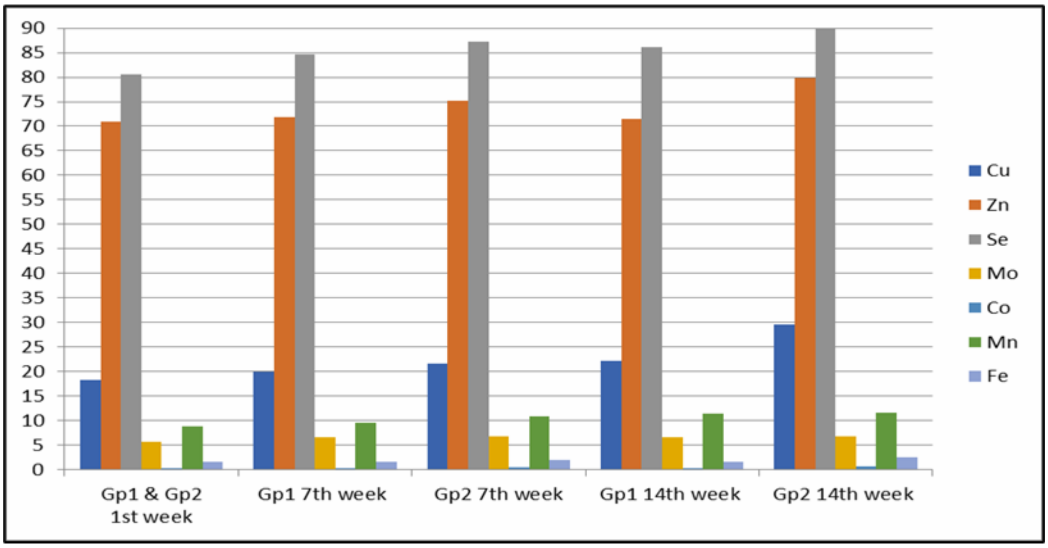

Fig. no3: Concentration of trace minerals in blood serum (µmol/L) of dairy cows in two groups.

Serum neutralizing and ELISA test antibody titers

The humeral immune response to viral vaccines with FMDV, lumpy skin, BEFV, BVD and IBRV performed an increased frequency of antibodies titer for viral vaccines in both groups vaccinated cows. The mean antibodies titers were gradually increased from 7th weeks post-vaccines till reached higher level at 14th weeks post- vaccines for all viral vaccines as measured by SNT and ELISA test. The antibodies peak titer was observed higher at 14th w post-vaccination in Gp2 significantly greater than Gp1(Tables 5 & 6; Fig. 4 & 5).

Table no5: Serum neutralizing antibody titer in sera of dairy cows in two groups.

Viral Vaccines	1st week	7th week		14th week	
	Gp1 & Gp2	Gp1	Gp2	Gp1	Gp2
FMD	0.20	4.0	4.7	4.5	4.9
BEFV	0.0	8	16	32	64
BVD	0.2	1.25	2.35	2.5	2.75
Lumpy skin v	0.1	1.80	1.99	1.85	2.1
IBRV	0.0	2.14	2.28	2.35	2.43

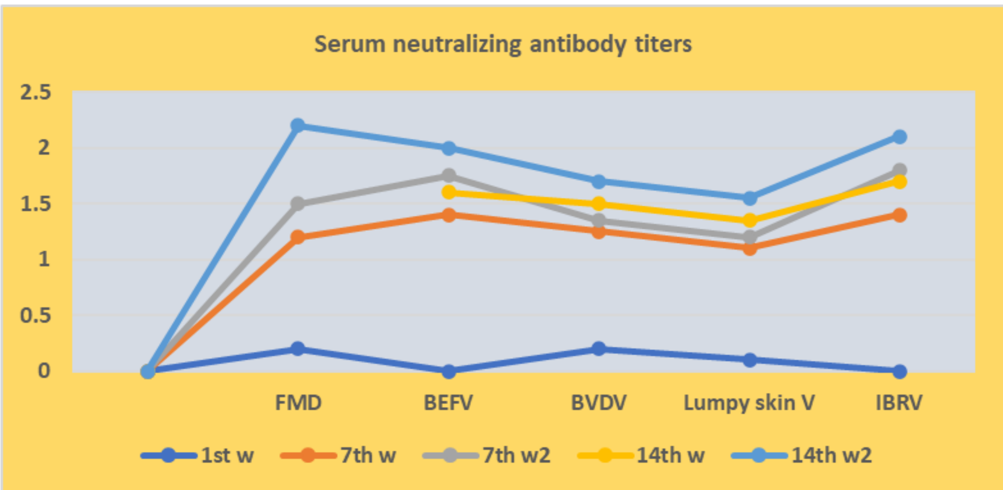

Fig. no4: Serum neutralizing antibody titers in sera of dairy cows following vaccination with viral vaccines (Gp1) and viral vaccines + ITM (Gp2)

Table no6: ELISA antibody titers in sera of dairy cows in two groups after viral vaccines.

Viral Vaccines	1st week	7th week		14th week	
	Gp1 & Gp2	Gp1	Gp2	Gp1	Gp2
FMD	0.20	1.5	1.65	1.75	2.50
BEFV	0.25	1.65	1.75	1.90	2.25
BVDV	0.50	1.55	1.85	2.00	2.10
Lumpy skin v	0.40	1.40	1.65	1.75	1.95
IBRV	0.20	1.50	1.80	1.95	2.25

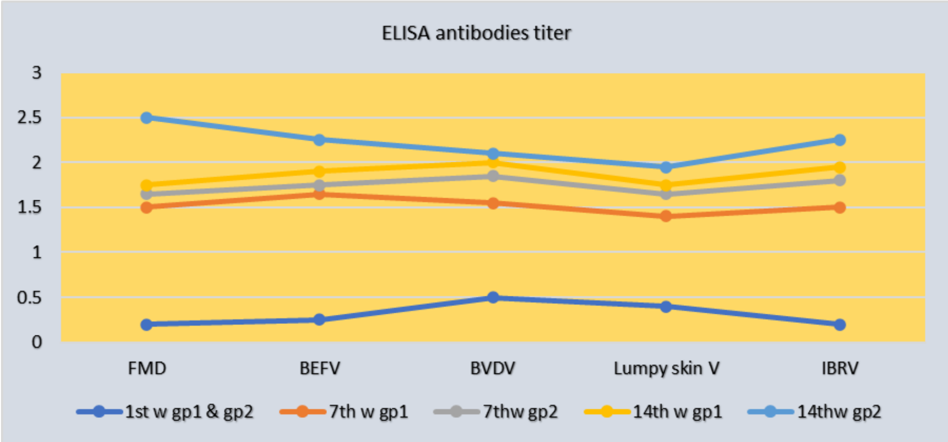

Fig. no5: ELISA antibody titers in sera of dairy cows following vaccination with viral vaccines (Gp1) and viral vaccines + ITM (Gp2)

IV. Discussion

Administering injectable trace minerals (ITM) including zinc (Zn), manganese (Mn), selenium (Se), and copper (Cu) alongside bovine virus vaccinations in dairy calves has been demonstrated to improve humoral and cell-mediated immune responses28. By perhaps boosting the immunological response that the vaccinations elicit, these supplements can enhance the overall effectiveness of vaccination29. A sufficient level of trace minerals can improve resistance to disease, which lowers the risk of infection and the need for antibiotic treatments—a factor that is especially crucial when it comes to diseases affecting cattle30.

Table (3) shows that Gp2 received injections of ITM showed an increase in the amount of milk or a stability in weight compared to the Gp1, which indicates that there is a positive effect of these salts on the humoral immune response. Fig.1 shows a comparison between the average milk production of the two groups over the three weeks (1st w, 7th w and 14th w). During the experimental period, administration of ITM resulted in increased mineral concentrations of Gp2 (at 7th w and 14th w $P < 0.001$) compared with Gp1. These results agree with **Palomares *et al.* (2016)** and previous reports by[31]. The increase in serum concentration after ITM administration could have contributed to enhance the immune response via reduction of reactive oxygen species (ROS) produced during oxidative stress[32]**.**

Table (4) shows that an increase in the concentration of Cu in blood serum was observed from the first week to the fourteenth week in both groups, while Gp2 showed a greater increase compared to Gp1. It may indicate an improvement in general health status or copper metabolism in the second group compared to the first[33]. Serum Zn concentration ranged from 70.9 to 79.8 µmol/L, with a slight increase over the weeks. While the second group showed slightly higher levels. Higher zinc levels indicate good nutrition and better immune function, especially in the second group. Selenium concentration increased over time in both groups, with the second group remaining higher[28]. Selenium is an antioxidant, which indicates a greater improvement in the oxidative stress state in the second group. Other elements (Mo, Co, Mn, Fe) showed slight increases over time, with varying differences between the two groups. These changes may reflect a general improvement in metabolism or response to improved nutrition (Fig. 3). Previous studies have shown an increase in serum copper and zinc concentrations in newly received cows after ITM administration[34].

Table (5) shows that Gp2 higher antibodies in general compared to Gp1 after vaccination against viruses (FMD, BEFV, BVD, Lumpy skin v, IBRV). FMD in Gp2 showed clear increases in antibodies compared to Gp1 at all time periods. The second group may have responded better to vaccination, indicating greater immunity. BEFV, IBRV, BVD and lumpy skin virus are similar increases in antibody titers were observed, especially in the Gp2. Significant increases in titers indicate an overall improved immune response in the Gp2 (Fig. 4). The antibody titers changed differentially in both groups during the experimental period. In Gp1 antibody titers decreased after primary vaccination and rose on 7th w, and 14th w. In contrast, in Gp2 antibody titers significantly increased earlier and more consistently (from 7th w to 14th w) compared with Gp1 ($P < 0.01$). The initial dose of vaccine apparently primed the immune system; therefore, the animals were able to respond more effectively to vaccination[35].

The poor antibody response after primary vaccination observed in this study for lumpy skin vaccine at 9th w followed by booster vaccination 4 weeks apart vaccination to reach a significant humoral immunity These reported before by[36]. The presence of neutralizing antibodies against viral vaccines is considered a major arm of defense that contributes to the prevention of viral diseases positively correlated with enhanced performance in dairy cows[37].

Antibody levels in Gp2 are generally higher than those in Gp1 for all viral types, according to estimates of the averages for each group. An analysis of variance (ANOVA) could be used to determine whether these variations are statistically significant over time. The present study reveals statistically significant differences in antibody levels between the two groups (Gp1 and Gp2) at the significance level of ($p > 0.05$). These differences suggest that the immune responses of the two groups differ significantly, with Gp2 likely to have a stronger immune response over time following viral vaccinations[20].

The findings generally show that the second group's immunological response was superior to the first group and there was a general improvement in mineral concentrations. The observed disparity between the two cohorts could potentially be attributed to variations in dietary habits, medical practices, or additional contextual elements that impact the well-being and defense mechanisms of cows. Additionally, research investigated the use of ITM in conjunction with vaccination as a means of enhancing dairy cows' protective immunological response to viral vaccines[39, 40].

Viral Vaccines are accompanied with ITM in Gp2 that mimic the disease-causing organism. When administered, these antigens stimulate the immune system without causing disease by; B Cell Activation produce antibodies, Antibody Production leads to a quicker and stronger secondary immune response, Long-Term Immunity by memory cells re-exposure to the pathogen, Influence on Overall Health by reduce the incidence of infections, improve disease resistance, enhance overall herd health, productivity, and milk production in dairy cattle[41].

V. Conclusion

A quick defense against viral infection was produced by viral vaccination given concurrently with ITM. Furthermore, ITM injection at the time of vaccination was linked to improved health status and an increased humoral immune response. The strategic use of ITM in conjunction with vaccination procedures is supported by these results, particularly in situations when dairy cows require protection and a quick immune response.

Ethics statement

According to the guidelines in the Care and Use of Agricultural Animals in Agricultural Research and Teaching[22], the dairy cows were taken care of. The University of Cairo Institutional Animal Care and Use Committee, permission number 0000588, authorized the research protocol (cu II F 424/2024).

Conflict of interest

The research was carried out without any financial or commercial ties that might be seen as having a conflict of interest, the authors disclose.

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