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Antioxidant injections during the transition period helped in improving total immunoglobulin levels in crossbred cattle plasma

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Abstract
Immunoglobulins (Ig) are important factors in the immunological activity of milk and colostrum from dairy cattle. The primary immunoglobulin in cow colostrum and milk is IgG, which can bind to a wide range of pathogenic bacteria and viruses. Bovine IgG can exert its effects on several levels, including: Direct effects on potential pathogens, Enhancing clearance of pathogens, Influencing intestinal barrier function, and Modulating immune function. The study was conducted to evaluate the effect antioxidant injections on the levels of total immunoglobulins in the crossbred cattle plasma. Four groups T_1, T_2, T_3 and T_4 were created consisting of six crossbred animals each. Group T_1 was used as control group and was fed only basal diet. T_2 group was injected with multi-mineral injections, T_3 was injected with multivitamin injections and T_4 was injected with combination of multivitamin and multimineral injections. They were injected on 7th, 15th, 21st, and 30th day before parturition. T_4 group showed the highest increase in immunoglobulin levels in the plasma ($p<0.05$). T_2 group showed considerable increase also ($p<0.05$). The increase in immunoglobulin levels of T_3 was also there but not very significant. After the completion of study we found that systemic administration of micronutrients can significantly enhance the immunity of animals as well as their health status whereas oral administration also enhances but at lower levels then systemic administration.

Keywords: Immunoglobulin, crossbred, parturition, transition

Introduction

Antioxidants play a crucial role in maintaining the health of cattle by protecting cells from oxidative damage caused by free radicals. Here's how antioxidants affect the health of cattle:
Immune Function: Antioxidants such as vitamins C and E, selenium, and zinc are essential for maintaining a robust immune system in cattle (Whitaker *et al*., 1997) [22]. They help protect immune cells from oxidative stress, ensuring they can effectively fight off pathogens and infections. Adequate levels of antioxidants can enhance the cow's ability to resist diseases and recover from illness more quickly. Reproductive Health: Antioxidants are important for reproductive health in cattle. They protect sperm and egg cells from oxidative damage, which can affect fertility and embryo development. Vitamin E, selenium, and other antioxidants have been shown to improve fertility rates and reduce the incidence of reproductive disorders in cattle (Allahyari *et al*., 2019) [21]. Growth and Development: Antioxidants are necessary for normal growth and development in cattle. They support proper bone formation, muscle growth, and overall development. Vitamin E, in particular, has been linked to improved growth rates and feed efficiency in cattle. Muscle Function: Antioxidants help maintain muscle health and function in cattle. They protect muscle cells from oxidative damage during exercise or stress, reducing the risk of muscle injuries and promoting faster recovery. Vitamin E and selenium are important for muscle health and can help prevent conditions like white muscle disease. Prevention of Diseases: Oxidative stress has been linked to various diseases and health problems in cattle, including respiratory diseases, mastitis, and metabolic disorders (Kellog *et al*., 2004) [23].

Antioxidants help mitigate oxidative stress, reducing the risk of these diseases and improving overall health and well-being. Hoof Health: Antioxidants can also play a role in maintaining hoof health in cattle. They support the growth of strong and healthy hooves, reducing the risk of lameness and hoof-related disorders. Zinc, in particular, is important for hoof integrity and can help prevent conditions like hoof rot. Stress Management: Antioxidants help cattle cope with stress by reducing oxidative damage caused by stress hormones such as cortisol (Krebs, 1998) [24]. Supplementing with antioxidants during periods of stress, such as transportation or weaning, can help minimize the negative effects of stress on cattle health and performance. In summary, antioxidants play a vital role in maintaining the health and well-being of cattle by protecting cells from oxidative damage, supporting immune function, promoting reproductive health, and enhancing growth and development (Cai *et al*., 1994) [25]. Ensuring adequate levels of antioxidants in the diet or through supplementation can help optimize cattle health and performance while reducing the risk of diseases and disorders.

Materials and Methods

The present study was carried out to study the effect of micronutrient administration on crossbred cows and their calves.

Location – Livestock Research Complex NDRI, Karnal Breed – Karan Fries and Frieswal. Twenty four (n=24) pregnant crossbred cows (Karan Fries and Frieswal) in their late gestation (i.e. 30 days before the expected date of calving) were selected from the experimental herd of Livestock Research Centre of NDRI. Animals were injected intramuscularly micronutrients because this route of administration provides better bioavailability of nutrients and reduced incidences of disease. Only animals expected to calve from January, 2021 to March, 2021 were considered for the winter study. Based on their milk yield and parity, the animals will be selected and randomly allocated to one of the three different treatments and one control; each group comprised of six animals. Group 1, without any intervention will act as control. The experimental cows will be injected intramuscularly individually with multivitamins (Group 2), multiminerals (Group 3), and combination of both (Group 4), to study the cumulative effect of all micronutrients. Animals will be administered intramuscularly multivitamins, multiminerals and their combination at -30, -15, -7, 0 before the expected date of calving and 7, 14, 21 days after expected date of calving. The same experiment was again repeated during summer months (May 2021 to July 2021)

The estimation of total immunoglobulins (Ig) in plasma is important for assessing the immune status and health of animals, including cattle. There are several methods available for measuring total immunoglobulins, with some of the most common being:

Immunoglobulin estimation methods

1. Turbid metric or Nephelometric Assay

- **Principle:** This method measures the turbidity or light scattering properties of immune complexes formed when immunoglobulins react with specific antibodies.
- **Procedure:** Plasma is mixed with an antibody specific to immunoglobulins, causing the formation of immune complexes. The degree of turbidity or light scattering is then measured using a spectrophotometer or nephelometer.
- **Advantages:** Simple, rapid, and suitable for high-throughput analysis.
- **Disadvantages:** May lack specificity for certain immunoglobulin classes, and interference from lipids or other proteins can affect accuracy.

2. Radial Immunodiffusion (RID)

- **Principle:** Immunoglobulins diffuse from a well into a gel containing specific antibodies. The diameter of the immunoprecipitin ring formed is directly proportional to the concentration of immunoglobulins in the sample.
- **Procedure:** Plasma samples are placed in wells on an agarose gel containing antibodies against immunoglobulins. After incubation, the diameter of the precipitin ring is measured.
- **Advantages:** Specific for immunoglobulins and relatively simple to perform.
- **Disadvantages:** Requires careful handling, and results may take longer to obtain compared to other methods.

3. Enzyme-linked immunosorbent assay (ELISA)

- **Principle:** This method utilizes specific antibodies and enzyme-linked detection to quantify immunoglobulins in the sample.
- **Procedure:** Plasma samples are added to wells coated with antibodies specific to immunoglobulins. After washing, a detection enzyme linked to a secondary antibody is added. The enzyme reaction produces a color change, which is measured spectrophotometrically.
- **Advantages:** High sensitivity and specificity, suitable for large-scale screening, and can be automated.
- **Disadvantages:** Requires specialized equipment and reagents, and may be subject to interference from contaminants.

4. Immunoturbidimetric Assay

- **Principle:** Similar to the turbidimetric assay, this method measures the turbidity produced when immunoglobulins react with specific antibodies.
- **Procedure:** Plasma samples are mixed with antibodies against immunoglobulins, and the resulting turbidity is measured using a spectrophotometer.
- **Advantages:** Simple, rapid, and suitable for high-throughput analysis.
- **Disadvantages:** May lack specificity for certain immunoglobulin classes.

5. Fluorescence polarization Immunoassay (FPIA)

- **Principle:** This method measures changes in fluorescence polarization when immunoglobulins bind to fluorescently labeled antibodies.
- **Procedure:** Plasma samples are mixed with fluorescently labeled antibodies specific to immunoglobulins. Changes in fluorescence polarization are measured using a fluorescence polarimeter.
- **Advantages:** High sensitivity, specificity, and precision.
- **Disadvantages:** Requires specialized equipment and reagents.

These methods provide options for quantifying total immunoglobulins in plasma, each with its own advantages and limitations. The choice of method depends on factors such as sensitivity, specificity, throughput, and available resources.

Results

The results of total immunoglobulins content (Means±SE) in colostrum of administered and control groups cows of T0, T_1, T_2 and T_3 groups on day 1 was found to be 58.85±4.57, 64.485±6.95, 62.865±3.727 and 81.285±3.38 mg/mL respectively. It was observed that on 1st day total immunoglobulins of T_3 group was significantly ($p<0.05$) higher as compared to other treatment groups. On 2rd day postpartum total immunoglobulins (Means±SE) of T0, T_1, T_2 and T_3 groups were 18.22±0.97, 19.50±2.318, 20.155±0.839 and 26.4425±1.22 respectively. Total immunoglobulins (T_3 group) found significantly ($p<0.05$) higher as compared to other treatment group and control.

On 3rd day postpartum total immunoglobulins (Means±SE) of T0, T_1, T_2 and T_3 groups were 9.18±0.47, 8.985±0.584, 7.58±2.013 and 11.6175^c±0.411 respectively. Total immunoglobulins (T_3 group) found significantly ($p<0.05$) higher as compared to other treatment group and control.

On 7th day postpartum means ± SE of total immunoglobulins of T0, T_1, T_2 and T_3 groups were 0.152±0.025, 0.205±0.033, 0.26±0.006 and 0.4225^a±0.06 respectively. Only the experimental group T_3 revealed significantly ($p<0.05$) higher as compared to control and T_1 group. On 15th day total immunoglobulin levels were found to be 0.165±0.015, 0.235±0.0547, 0.3175±0.0775 and 0.395±0.21 mg/mL respectively. There was no significant difference among groups on 15th day of lactation.

Discussion

Effect of Antioxidant Injections on Immunoglobulin Levels in Cattle. Antioxidants play a crucial role in the immune system of cattle by protecting immune cells from oxidative damage, which can compromise their function. Antioxidant injections are commonly used in cattle management to support immune function and overall health hepatic (Van Saun *et al*., 1989; Patterson *et al*., 1999) [27, 29]. Here, we discuss the potential effects of antioxidant injections on immunoglobulin levels in cattle: Enhanced Immune Function:

Antioxidants, such as vitamins C and E, selenium, and zinc, contribute to the proper functioning of the immune system by neutralizing free radicals and reducing oxidative stress. By protecting immune cells from damage, antioxidant injections may help maintain optimal levels of immunoglobulins, including IgG, IgM, and IgA, which are critical components of the humoral immune response. Improved Colostrum Quality: Colostrum, the first milk produced by cows after calving, is rich in immunoglobulins and provides passive immunity to newborn calves (Van Saun *et al*., 1989) [27]. Antioxidant injections administered to cows during the dry period or prepartum may enhance colostrum quality by increasing the concentration of immunoglobulins transferred to the calf. This can lead to better immunity and disease resistance in newborn calves (Herdt and Smith, 1996) [30]. Reduced Oxidative Stress: Stressors such as calving, weaning, transportation, and environmental changes can increase oxidative stress in cattle, leading to immune suppression and decreased production of immunoglobulins. Antioxidant injections can mitigate this stress by scavenging free radicals and preserving the integrity of immune cells. As a result, cows may maintain higher levels of immunoglobulins even during periods of stress. Prevention of Diseases: Oxidative stress has been linked to various diseases and health problems in cattle, including respiratory diseases, mastitis, and metabolic disorders. By reducing oxidative damage to immune cells, antioxidant injections may help prevent these diseases and maintain higher levels of immunoglobulins, supporting overall health and productivity in cattle. Effect on Vaccine Response: Adequate levels of immunoglobulins are essential for an effective response to vaccination in cattle. Antioxidant injections may enhance the vaccine response by maintaining optimal levels of immunoglobulins and supporting the activation of the immune system. This can lead to better protection against infectious diseases and improved overall herd health. Research Gaps and Considerations: While there is evidence to suggest that antioxidant injections can positively influence immunoglobulin levels in cattle (Goff and Horst, 1997) [26], further research is needed to fully understand the mechanisms involved and optimize their use in cattle management. Factors such as dosage, timing of administration, and individual variability in response should be considered in future studies.

Conclusion

Antioxidant injections have the potential to positively impact immunoglobulin levels in cattle by reducing oxidative stress, enhancing immune function, and improving colostrum quality. By maintaining optimal levels of immunoglobulins, antioxidant injections may help support overall health, disease resistance, and productivity in cattle. However, more research is needed to determine the most effective strategies for incorporating antioxidant injections into cattle management practices.

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