

## Estrus response in dairy cows surviving foot and mouth disease given Superbooster and Immunobooster

Rizky Asrin Zulfanisa<sup>1</sup>, Rahmi Sugihartuti<sup>2</sup>, Jola Rahmahani<sup>3</sup>,  
Mohammad Anam Al Arif<sup>4</sup>, Tita Damayanti Lestari<sup>5</sup>, Ali Agus<sup>6</sup>,  
Mohammad Sofi'ul Anam<sup>6</sup>

<sup>1</sup> Large Animal Veterinary Professional Interest Group, Jl. Dr. Ir. Soekarno, Surabaya, Indonesia

<sup>2</sup> Division of Basic Veterinary Science, <sup>3</sup> Division of Veterinary Microbiology,

<sup>4</sup> Division of Animal Husbandry, <sup>5</sup> Division of Veterinary Reproduction,  
Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia

<sup>6</sup> Department of Animal Nutrition and Feed Science, Faculty of Animal Husbandry,  
Gadjah Mada University, Yogyakarta, Indonesia

\* Corresponding author, e-mail: rizkyasrin1004@gmail.com

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### ABSTRACT

This study aims to determine the effect of adding Superbooster and Immunobooster on the estrus response of dairy cows after foot and mouth disease (FMD) infection. This study used 18 dairy cows that recovered from FMD based on veterinarian examination. Cows were divided into three groups (T0, T1 and T2). In the control group (T0), cows were fed standard feed. Standard feed consisted of forage (10% of body weight, daily) and concentrate. In the T1 group, cows were fed standard feed added with Superbooster (40 g daily), and in the T2 group cows were fed standard feed added with Superbooster (40 g daily) and Immunobooster (800 g daily). This treatment was carried out for 42 days. The results showed that the estrus rate in T1 and T2 were both 100%, which was higher compared to the control (16.67%). The onset of estrus was shorter ( $p < 0.05$ ) compared to the duration of the last estrus until the start of treatment. Meanwhile, the duration of the last estrus until the day of treatment and onset of estrus between treatment groups were not significantly different ( $p < 0.05$ ). It could be concluded that Superbooster and Immunobooster could stimulate estrus on dairy cows after FMD.

**Keywords:** anestrus, concentrate, mix minerals, onset of estrus

### INTRODUCTION

Foot-and-Mouth Disease (FMD) is a viral disease that attacked cloven-hoofed animals, including cattle, goats, sheep, pigs, and various wildlife species. This highly contagious disease mainly attacked beef and dairy cattle, buffalo, sheep, and goats. Clinical signs of FMD included lethargy, elevated body temperature reaching up

to 41°C, hypersalivation, decreased appetite, reluctance to stand, lameness, weight loss, and reduced milk production in dairy livestock (Adjid, 2020). Given its broad-impacts, FMD raised important economic problems (Permatasari *et al.*, 2024), not only resulting in high mortality rates in young animals but also causing a decline in production of milk and other animal products, which could subsequently

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restrict international trade for countries affected by this disease (Compston *et al.*, 2022).

Indonesia had been free from FMD since 1986; nevertheless, an outbreak reoccurred in early April 2022. On May 7, 2022, the Ministry of Agriculture announced an outbreak of contagious disease in livestock in Indonesia (Susila *et al.*, 2022). The spread of FMD cases in Sidoarjo district occurred in 18 sub-districts. As of July 20, 2022, data from the Sidoarjo Food and Agriculture Office showed that the animals affected by FMD included cattle, sheep, goats, and buffalo, with 1,035 infected, 1,113 recovered, and 52 death (Dispaperta, 2022).

In addition to its immediate health effects, FMD outbreaks had also been shown to reduce fertility in dairy cattle. Infected cows tended to experience a later age at first calving compared to the general population, and the conception rates might decline due to the increased frequency of artificial inseminations performed before the cows could achieve pregnancy (Chaters *et al.*, 2018). As many as 61.9% of cows infected with FMD experienced reproductive disorders (Susilo *et al.*, 2024) which affected the estrus cycle in dairy cows.

To mitigate the effects of an FMD outbreak, maintaining livestock immunity was essential, which could be achieved through the provision of high-quality feed that included complete feeds and concentrate. Nutrition played a critical role in meeting the essential needs of livestock and supporting their immune systems (Zhao *et al.*, 2015). Macrominerals were vital components of nutrition that contributed to growth, health, production, reproduction, and immune function in animals. Ruminants required essential macrominerals such as calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), chloride (Cl), and sulfur (S) (Kegley *et al.*, 2016).

Calcium, in particular, was crucial for reproductive processes. Study by Ali *et al.* (2014) demonstrated that adequate calcium levels in Cholistani cattle enhanced reproductive performance, including increased conception rates and reduced calving intervals. Conversely, calcium deficiency could lead to reproductive

issues, such as delayed postpartum ovulation, failure to conceive, and abortion in cattle. Similarly, phosphorus (P) was essential for reproduction; its deficiency could result in reproductive disorders, including anestrus, low conception rates, prolonged calving intervals, embryonic death, stillbirths, and delayed sexual maturity (Mahen *et al.*, 2018).

Immunobooster is a concentrated nutrient supplement, while Superbooster is a mixture of essential minerals. Azis *et al.* (2023) reported that administering both Immunobooster and Superbooster enhanced the metabolite profile in repeat breeder cows. However, there had been no published research on the use of these supplements to alleviate anestrus in dairy cows recovering from FMD. Therefore, this study aims to evaluate the impact of incorporating Immunobooster and Superbooster into the feed on restoring estrus in dairy cows following their recovery from FMD.

## MATERIALS AND METHODS

This study was conducted at the Kampoeng Ternak farmer cooperative located in Taman district, Situbondo regency, East Java, Indonesia.

### Study Samples

The samples used in this study were Holstein Friesian dairy cows that had recovered from FMD based on veterinarian examination. These cows were lactating and had not exhibited estrous cycles since FMD infection, as evidenced by inseminator records. A total of 18 dairy cows who survived FMD were used in this research. Cows were divided into three groups, each group consisting of six individuals, and the parameters measured included the rate of estrus, the onset of estrus and the duration of the last estrus until treatment. All groups received the same treatment, consisting of standard feed supplemented with a 10 mL dose of vitamin ADE (Injectamine®) and a 20 mL dose of long-acting antibiotic (Vet-Oxy LA®) given once during the study period.

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Cows in the T0 group were given standard feed; cows in the T1 group were given standard feed supplemented with Superbooster (40 g dissolved in 100 mL of water), while cows in T2 group were given standard feed along with Immunobooster (800 g mixed into the concentrate feed, per day). Standard feed consisted of elephant grass and concentrate feed (Jabfeed, produced by KAN Jabung Syariah Jatim, Malang, Indonesia).

### Elephant grass nutrition

Proximate analysis showed that elephant grass contained 11.40% dry matter, 2.01% ash content, 5.47% crude protein, 0.69% crude fat, 1.81% crude fiber, 1.43% nitrogen-free extract, and 8.39% total digestible nutrients.

### Concentrate nutrition

Concentrate contained 14% water, 19% crude protein, 7% crude fat, 0.8-1.3% calcium, 0.4-0.8% phosphorus, and 69% total digestible nutrients (TDN). The ingredients included soybean meal (SBM), corn gluten feed (CGF), wheat bran, wheat pollard, copra meal, minerals and vitamins.

### Superbooster administration

Superbooster is a powder supplement containing calcium, iron, magnesium, sodium, phosphorus, manganese, zinc, potassium, copper, sulfur, cobalt, and selenium, as well as essential oils derived from eucalyptus, orange, lavender, soybeans, walnuts, sesame seeds and olives (Anam et al., 2022). Superbooster was dissolved in 100 mL of water and given to livestock by mixing it into their feed. The dose given was 40 g, divided into two portions of 20 g each, given each in the morning and evening. This protocol was followed for 42 days (Agus et al., 2022).

### Immunobooster administration

Immunobooster is a concentrate consisting of corn, corn gluten meal (CGM), pollard, soybean meal (SBM), as well as essential vitamins (A, D, E, and K) and probiotics such as *Lactobacillus acidophilus*, *Enterococcus*

*faecium*, and *Saccharomyces cerevisiae*, along with with a mixture of essential oils. Immunobooster had a nutritional composition of 89.24% dry matter, 20.82% crude protein, 8.56% crude fiber, 3.64% ether extract, 5.58% ash, and 86% digestible nutrients (TDN) (Gading et al., 2020). Immunobooster was given by mixing it into concentrate feed as much as 800 g or 0.8 kg per head, given twice, 400 g in the morning and 400 g in the evening for 42 days. Determination of the 800 g dose was based on calculations by Agus et al. (2022) who recommended providing Immunobooster should reach 20% of the total daily concentrate feed.

Estrus detection was carried out based on visual observations (Reith and Hoy, 2018) in the morning and evening when cleaning the pen, bathing the cow, feeding and milking. A dairy cow was said to be in estrus if it showed signs of mooing more often than usual, the vaginal mucosa was redder, the vulva was swollen and clear mucus came out (Ramadhana et al., 2022; Hafizuddin et al., 2024). In this study, cows were caged and tied with ropes so that cows in estrus did not show standing estrus. Estrus detection was carried out by the farmer and confirmed by the inseminator. The onset of estrus was defined as the duration (days) of estrus after the first day of treatment. The duration (days) of the last estrus until the start of treatment was determined based on the inseminator's records.

### Data Analysis

Data on estrus rate were analyzed using the Chi-Square test, while data on onset of estrus and duration of last estrus to treatment were tested using independent T with a significance level of 95% ( $p < 0.05$ ) using SPSS version 27 for Windows.

## RESULTS

All dairy cows in the group that received Superbooster (T1) and Immunobooster (T2) exhibited estrus, while in the control group (T0) only one cow showed signs of estrus (Table 1). Based on records from the inseminator, all cows in this study had experienced anestrus for six

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months, measured from their last estrus to the time of treatment. The onset of estrus (the duration of estrus after the start of treatment) was shorter ( $p < 0.05$ ) than the duration of the last

estrus until the start of treatment. Meanwhile, the length of last estrus until the day of treatment and the onset of estrus between treatment groups were not significantly different ( $p > 0.05$ ).

**Table 1** Rate of estrus (%), Means  $\pm$  SD), onset of estrus (days, Means  $\pm$  SD) and duration of last estrus until the start of treatment (days, Means  $\pm$  SD) in FMD survivor dairy cows after Superbooster and Immunobooster administration

	estrus rate	onset of estrus	last estrus - treatment
T0	16.67% (1/6) <sup>a</sup>	23 <sup>*</sup>	180.33 $\pm$ 35.85
T1	100% (6/6) <sup>b</sup>	30.00 $\pm$ 7.95 <sup>c</sup>	175.67 $\pm$ 45.02 <sup>d</sup>
T2	100% (6/6) <sup>b</sup>	21.33 $\pm$ 10.65 <sup>c</sup>	178.00 $\pm$ 20.07 <sup>d</sup>

<sup>a,b</sup>: different superscripts in the same column indicate significant differences ( $p < 0.05$ ) in the Chi-Square test; T0: cows were given standard feed; T1: cows were given standard feed and 40 g Superbooster; T2: cows were given standard feed, 40 g Superbooster, and 800 g Immunobooster; <sup>\*</sup>: only one cow exhibited estrus; <sup>c,d</sup>: different superscript in the same row indicate significant differences ( $p < 0.05$ ).

## DISCUSSION

FMD had a significant impact on the health of livestock, particularly in cattle, giving rise to various reproductive challenges (Chaters *et al.*, 2018). When cows were infected with the FMD virus, their immune system responded by activating various defense mechanisms, including the production of cytokines and chemokines and the recruitment of immune cells, such as macrophages and neutrophils, to the site of infection (Zhu *et al.*, 2022). This immune response often produced ROS, which could act as a protective signal but could also cause oxidative damage if not managed properly (Zhi *et al.*, 2020).

Increased ROS production due to FMDV infection could cause damage to lipids, proteins and DNA in reproductive cells. For example, lipid oxidation could disrupt the integrity and fluidity of all cell membranes (Sachdev *et al.*, 2021), including cells in the ovaries, which was followed by disruption of folliculogenesis. Damage to cell membranes in reproductive endocrine cells (hypothalamic-pituitary-ovary axis) also harmed reproductive endocrine cells. Thereby, the FMD virus and the immune response it causes could interfere with the secretion of reproductive hormones. Imbalances

in hormones, such as estrogen and progesterone, could cause irregularities in the estrus cycle, negatively affecting fertility of dairy cows (Shaban *et al.*, 2022).

During FMD outbreaks, cattle were often confined indoors due to movement restrictions. This confinement not only adversely affected the physical health of the animals but also diminished their nutritional intake, which was essential for maintaining reproductive health (Majid *et al.*, 2023). Deficiencies in key micronutrients and macronutrients, including vitamins and minerals, could negatively impact hormone production and reproductive metabolism (Sammad *et al.*, 2020). Furthermore, clinical symptoms associated with FMD, such as lethargy, fever, and loss of appetite, contributed to an overall decline in body condition (Bayoumi *et al.*, 2021). Poor reproductive health could hinder the estrous cycle by affecting the reproductive control centers in the brain and disrupting reproductive functions at the cellular level (Chaters *et al.*, 2018).

The incidence of estrus in dairy cows diagnosed with FMD was influenced by the provision of dietary supplements, particularly Superbooster and Immunobooster that contain essential minerals. Minerals were crucial for reproductive processes, functioning as

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antioxidants that could restore endocrine function and stimulate estrus. One significant antioxidant in this context was selenium (Se). Zinc (Zn) also played a vital role in stimulating estrus; its deficiency could lead to reduced levels of steroid hormones, such as progesterone and estrogen, which were essential for reproduction (Van Emon *et al.*, 2020). Additionally, zinc and magnesium (Mg) enhanced the development of Graafian follicles; as the number of mature Graafian follicles increases, so does the number of theca cells that produced estrogen. Consequently, the presence of zinc and magnesium could stimulate estrogen secretion by promoting the increased development of graafian follicles. Elevated levels of zinc, selenium, and magnesium in the blood contributed to the improved development of these follicles, leading to higher secretion of estrogen hormones in livestock (Anchordoquy *et al.*, 2019).

Antioxidants played a critical role in reproductive endocrinology by protecting reproductive cells from oxidative damage. They helped maintaining hormonal balance and supported ovarian health. Oxidative stress occurred when there was an imbalance between free radical production and the body's ability to neutralize their harmful effects. Free radicals could damage hormone-producing cells and impair ovarian function (Amin *et al.*, 2023). Common antioxidants important for reproduction, such as selenium and zinc, functioned by binding to free radicals and minimizing their detrimental effects. In the ovaries, antioxidants ensured follicle quality and reduced disruptions in the estrous cycle (Xiao *et al.*, 2021). Hormonal balance was greatly influenced by oxidative stress, as hormones like estrogen and progesterone were particularly susceptible to oxidative damage. This damage could disrupt the synthesis of these hormones, leading to reproductive dysfunction. The presence of antioxidants helped maintain hormone levels.

In summary, FMD infection induced an immune response that increased the production of ROS, causing oxidative damage to

reproductive cells and disrupting hormonal function. This pathology could lead to anestrus conditions and decreased fertility. Additionally, the confinement of cattle during FMD outbreaks limited their movement and nutritional intake, further exacerbating reproductive health issues due to nutrient deficiencies. Dietary supplements, particularly Superbooster and Immunobooster rich in minerals such as selenium and zinc, could help mitigate these adverse effects. These minerals functioned as antioxidants, restoring endocrine function, stimulating estrus, and maintaining hormonal balance, which was crucial for promoting reproductive health and fertility in dairy cows recovered from FMD.

## CONCLUSION

The administration of Superbooster and Immunobooster to dairy cows recovering from FMD

played a role in restoring the estrous cycle.

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## AUTHOR'S CONTRIBUTIONS

Rizky Asrin Zulfanisa (RAZ), Rahmi Sugihartuti (RS), Jola Rahmahani (JR), Mohammad Anam Al Arif (MAA), Tita Damayanti Lestari (TDL), Ali Agus (AA), Mohammad Sofi'ul Anam (MSA)

RAZ: conceived the idea, designed the mainframe of this manuscript, acquisition, analysis and interpretation of data, and manuscript drafting under the supervision of RS. JR, MAA TDL, AA, and MSA: critically read and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

## CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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