JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Decreased immune response to foot-and-mouth disease vaccination following synbiotic supplementation in Hanwoo (*Bos taurus coreanae*) cows
Running Title (within 10 words)	Synbiotic reduce side effects of FMD vaccination in Hanwoo cows
Author	
Affiliation	
ORCID **(for more information, please visit https://orcid.org)**	
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	
Acknowledgements	It is described in detail in the text.
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	
Ethics approval and consent to participate	

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	
Email address – this is where your proofs will be sent	
Secondary Email address	
Address	
Cell phone number	
Office phone number	
Fax number	

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	
Email address – this is where your proofs will be sent	
Secondary Email address	
Address	
Cell phone number	
Office phone number	
Fax number	

Abstract

Foot-and-mouth disease (FMD) vaccination is known to increase ruminoreticular temperature, acute-phase immune proteins, and white blood cell counts. Recent studies indicate that synbiotic supplementation can help reduce stress by alleviating stress-induced intestinal inflammation. However, there are no studies on the stress of FMD vaccination according to synbiotic supplementation. Therefore, this study aimed to investigate the stress-relieving effects of synbiotic treatment by measuring ruminoreticular temperature, body activity, acute-phase immune protein concentrations, and complete blood count analysis following FMD vaccination. The study included three groups; the positive control group received only synbiotic supplementation for two weeks, the 2W_SB_FMD group received synbiotic supplementation for two weeks prior to FMD vaccination, and the 1W_SB_FMD group received synbiotic supplementation for one week prior to vaccination. Ruminoreticular temperature and body activity were measured before and after FMD vaccination. Blood samples were collected on days 0, 3, 6, and 10 relative to FMD vaccination, and concentrations of haptoglobin (HPT), serum amyloid A (SAA), and white blood cells (WBC) were analyzed. According to data that our research team previously identified body changes and immune responses before and after FMD vaccines in Hanwoo cows, the ruminoreticular temperature was significantly lower in the synbiotic-treated group compared to the FMD-only group 24 hours post-vaccination ($p < 0.0001$). Significant differences in body activity were also observed between the FMD-only group and the synbiotic-treated group ($p < 0.0001$), with the synbiotic-treated group exhibiting stable activity before and after vaccination. Levels of HPT and SAA, which indicate acute immune responses, increased until day 3 post-vaccination but then sharply declined between days 6 and 10. The lymphocyte ratio gradually decreased more in the 2W_SB_FMD group than in the 1W_SB_FMD group. Overall, synbiotic supplementation, which helps relieve stress by reducing intestinal inflammation, significantly influenced ruminoreticular temperature and HPT, SAA, and WBC concentrations depending on the duration of the synbiotic treatment. These findings suggest that synbiotic treatment following FMD vaccination may serve as an effective stress reliever.

Keywords: FMD vaccination, Stress relief, Direct-fed microbials, Ruminoreticular biosensor, Acute-phase immune protein, Hanwoo

Introduction

Foot-and-mouth disease (FMD) vaccination is an effective method for controlling FMD and has the potential to eradicate the disease [1]. Vaccinating all animals provides partial protection to uninfected animals, which can help prevent the further spread of the disease [2]. The most widely used FMD vaccine globally is the inactivated vaccine, which primarily targets the O and A serotypes [3]. The Type O PanAsia-2 (O PA-2) vaccine strain is highly effective against several FMD virus strains from Southeast Asia, East Asia, and the Far East [4]. The A22/Iraq/24/64 (A22 IRQ) strain is widely used in most FMD vaccine banks [5]. Currently, international standards categorize countries into three categories based on the status of FMD: FMD-free, vaccination countries, and infected countries. To prevent disease transmission, strict risk management measures are implemented for animal and animal product trade in vaccination and infected countries.

Although FMD vaccination is a crucial strategy for controlling viral spread, it can cause various side effects. Hanwoo cows had decreased ovulation rates and increased levels of acute phase immune proteins such as haptoglobin and serum amyloid A on days 8-9 post-vaccination [6]. Vaccination in the first trimester (before 45 days) of pregnancy increased early fetal deaths [7]. Increased sperm abnormalities, transiently elevated ruminoreticular temperature, and delayed ovulation have been shown as side effects of FMD vaccination [8, 9].

Numerous recent studies have analyzed reproductive endpoints in cows, such as estrus, pregnancy, and parturition, by evaluating ruminoreticular parameters using implanted biosensors. Similarly, in a previous study, we investigated the physiological response of pregnant Hanwoo cows to FMD vaccination using ruminoreticular biosensors [10].

We found that rumminoreticular temperature and body activity increased after FMD vaccination in both early (<80 days) and late gestation (>210 days) [10]. In addition, rumminoreticular temperature and body activity increased after estrus, with pregnant cows at 250-270 days gestation showing higher rumminoreticular temperature and body activity with a distinct pattern before and after pregnancy [11].

These stress-relieving supplements aim to improve health, strengthen the immune system, and alleviate stress [12]. Common stress relievers include ionophores, microorganisms administered through feed, and β-adrenergic agonists. Moreover, *Lactobacillus plantarum*, a microbe administered directly through the feed, has been shown to improve the feed conversion ratio (FCR) and enhance crude protein and phosphorus digestibility while lowering cortisol levels [13].

Similarly, *Bacillus subtilis* supplementation has been shown to improve the performance and health of Holstein calves [14]. Additionally, monensin and tylosin, used as transitional feed-grade antimicrobials, have been found to reduce coccidia levels and increase average daily gain (ADG) in beef cattle [13].

Previous research suggests that mannan-oligosaccharide and *Bacillus subtilis* supplementation, a combination of yeast-derived prebiotics and *Bacillus subtilis* probiotics, can positively impact livestock productivity and immunity. Specifically, supplementing cattle feed with this combination has been shown to enhance feed intake and improve nutrient utilization. Moreover, the immunological benefits of *Bacillus subtilis* and yeast-derived compounds have been linked to overall improvements in cattle health. The yeast-derived component of this supplementation contains β-glucan and mannan-oligosaccharides, which are known for their immune-boosting properties. β-glucan promotes the proliferation and activation of T cells in response to antigens or cytokines, whereas mannan-oligosaccharides enhance immunity against gram-negative bacteria by presenting weakened antigens to immune cells [15-17].

Based on these findings, stress relievers are expected to improve the rumen environment and modulate inflammatory responses, thereby reducing the side effects of vaccination. However, no previous studies have examined biological changes following FMD vaccination in relation to stress-reliever treatments. To address this gap in the literature, this study aimed to investigate the efficacy of mannan-oligosaccharide and *Bacillus subtilis* supplementation in alleviating the side effects of FMD vaccination.

Materials and Methods

Animals

A total of 15 non-pregnant Hanwoo cows were included in the study (Table 1). During the experimental period, the cows were housed in groups according to their respective experimental conditions. The animals were housed in a well-ventilated shed that met the recommended space requirements for the experiment. Their diet followed the Korean Specification Standard Program, consisting of rice straw, mineral blocks, and ad libitum access to water. Each cow received 7–8 kg of roughage and 3 kg of compound feed per day. The average age of the cows was 84.1 ± 7.1 months, with an average parity of 2.9 ± 0.4.

Synbiotic

The synbiotic supplementation used in this study consisted of mannan-oligosaccharides and *Bacillus subtilis*, formulated with intentionally selected *Bacillus* strains, a yeast culture blend, refined functional carbohydrates, and a unique strain of *Lactobacillus plantarum*. The major probiotics in this formulation were A&H *Bacillus* and *Lactobacillus plantarum1037*. It was top-dressed daily with spoons in blended feed at a dose of 10 mg per cow, because the supplement is powder. In this study, this supplementation is referred to as 'Synbiotic.'

Experimental design

15 Hanwoo cows was used in the experiment. The Hanwoo cows for the experiment were divided into three groups (five cows per group: Positive Control, 2W_SB_FMD, and 1W_SB_FMD) by minimizing the difference between parity and age of months. In this study, 2 ml of DS FMD Vaccine from Daesung Microbiological Labs Co., Ltd. was used as the FMD vaccine. The vaccine was administered intramuscularly into the levator scapulae muscle using a 3.5 cm, 18 gauge needle. The day of FMD vaccination was designated as day 0. The positive control group did not receive the FMD vaccine. This group received synbiotic supplementation for two weeks prior to day 0 and an additional six days after Day 0. The 2W_SB_FMD group received the FMD vaccine on day 0. In this group, synbiotic supplementation was administered for two weeks prior to vaccination and an additional six days after vaccination. The 1W_SB_FMD group also received the FMD vaccine on day 0. For this group, synbiotic supplementation was administered for one week prior to FMD vaccination and for an additional six days after vaccination.

Ovulation synchronization

To ensure accurate comparative analysis, all experimental cows underwent ovulation synchronization using the OvSynch method (Fig. 1). Before day 23, relative to the FMD vaccination date (day 0), a progesterone-releasing intravaginal device was inserted into each cow and removed after seven days. On the same day the device was removed, all cows were administered 25 mg of $PGF_{2\alpha}$, followed by 250 μg of GnRH administration four days later.

Ruminoreticular biosensor

Ruminoreticular biosensors (SmaXtec Classic Bolus) were inserted into 15 Hanwoo cows (five cows per group: Positive Control, 2W_SB_FMD, and 1W_SB_FMD) to monitor ruminoreticular temperature and body activity. The SmaXtec Classic Bolus has been tested and certified by the DLG (German Agricultural Society) for its resistance to rumen fluid.

The sensor was orally inserted by a specialist using a specialized instrument and placed in the rumen and reticulum. The sensor measures 132 × 35 mm (length x diameter) and operates on a battery with an approximate lifespan of five years. Temperature measurements were recorded with an accuracy of ± 0.01 °C. Body activity (V) was calculated as the square root of the sum of $X^2 + Y^2 + Z^2$, using an indwelling 3-axis accelerometer (X, Y, and Z). The sensors recorded these parameters every 10 minutes and transmitted the data in real-time to a base station via the SmaXtec system.

Blood collection, acute immune protein analysis, and complete blood count

Blood samples were collected from all groups on days 0, 3, 6, and 10, in accordance with the ovulation synchronization and FMD vaccination protocols (Fig. 1). For CBC analysis, blood was collected in ethylene diamine tetraacetic acid tubes. To analyze acute immune proteins, serum was collected in serum separator tubes. CBC analysis was performed within four hours of blood collection using an automated hematology analyzer. The following parameters were analyzed: white blood cells (WBCs; 1000 cells/μL), neutrophils (000 cells/μL), eosinophils (1000 cells/μL), basophils (1000 cells/μL), monocytes (MONOs; 1000 cells/μL), and lymphocytes (LYMs; 1000 cells/μL). For acute immune protein analysis, serum samples were stored at −70 °C and analyzed using ELISA kits to measure Haptoglobin (HPT) and serum amyloid A (SAA) concentrations.

Statistical analysis

An analysis of covariance was performed using parity and month as covariates and statistical analysis was conducted using GraphPad Prism and SPSS Statistics (version 29.0.2.0, IBM Inc., USA). Differences in ruminoreticular temperature, body activity, serum concentrations of HPT and SAA and CBC parameters were assessed using ANCOVA. A p value indicates statistical significance; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Results

Ruminoreticular temperature and body activity

For comparison, changes in ruminoreticular temperature and body activity in the FMD-only group were based on results from a previous study [6]. The ruminoreticular temperature was lower in the synbiotic-treated groups compared to the FMD-only group for 24 hours after vaccine administration ($p < 0.0001$). When comparing the 2W_SB_FMD group to the 1W_SB_FMD group, the 1W_SB_FMD group exhibited a greater increase in ruminoreticular temperature, with a significant difference observed at 39 hours post-vaccination ($p < 0.05$) (Fig. 2). Body activity remained more stable in the synbiotic-treated groups compared to the FMD-only vaccination group which did not receive synbiotic treatment. Overall, the 2W_SB_FMD group exhibited stable activity levels similar to those of the positive control (Fig. 3).

Haptoglobin and serum amyloid A concentrations

Three days after FMD vaccination, HPT and SAA concentrations were higher in the 2W_SB_FMD group compared to the 1W_SB_FMD group. However, by day six, the HPT and SAA concentration in the 2W_SB_FMD group exhibited a marked decrease (Fig. 4, 5). The immune response in the 2W_SB_FMD group appeared to normalize faster than in the other groups. There was a significant difference in SAA concentrations between 2W_SB_FMD group and 1W_SB_FMD group on day ten post-vaccination. Further analysis beyond day ten post-vaccination is required to verify whether HPT and SAA concentrations fully return to pre-vaccination levels.

White blood cell concentrations

WBC levels were similar across all synbiotic-treated groups on day three following FMD vaccination. However, a significant decrease in WBC levels was observed in the 2W_SB_FMD group compared to the 1W_SB_FMD group between days 3 and 6 ($p < 0.01$) (Fig. 6). The LYM ratio gradually decreased in the 2W_SB_FMD group after vaccination compared to the 1W_SB_FMD group. However, LYM levels began to rise again on day 10, after synbiotic treatment had been discontinued (Fig. 7). This suggests that for synbiotic treatment to be fully effective, long-term administration may be required following vaccination.

Conclusion

Synbiotic supplements, which combine probiotics and prebiotics, create a synergistic effect, amplifying the individual benefits of each component. Various studies have demonstrated that synbiotics improve the intestinal environment, with intestinal microorganisms playing key roles in the host’s immunological, physiological, nutritional, and protective functions. However, data on synbiotic supplementation in cattle remain limited. Therefore, our study aimed to provide scientific evidence on the use of synbiotics to alleviate the side effects of FMD vaccination.

In this study, there should be a group that was vaccinated with foot-and-mouth disease vaccine without synbiotic, but it was difficult to set up such a group because it is difficult to obtain many Hanwoo cows for such a study due to the nature of large animal experiments, and it is only possible to conduct experiments in April and November when foot-and-mouth disease vaccine is administered. This is because the Republic of Korea's foot-and-mouth disease vaccination policy requires nationwide simultaneous vaccination in April and November of each year. Additionally, a limitation arose because repeating the same experiment could raise ethical concerns with the Institutional Animal Care and Use Committee. Therefore, it was not feasible to set up a control group that neither received the synbiotic nor the foot-and-mouth disease (FMD) vaccine, nor an FMD-only group that received the vaccine without synbiotic supplementation.

We completed this study by citing previous findings from a group that received only the foot-and-mouth vaccine without the synbiotic supplementation [6]. According to data that our research team previously identified body changes and immune responses before and after FMD vaccines in Hanwoo cows, ruminoreticular temperature was significantly lower in the synbiotic-treated group compared to the FMD-only group 24 hours after vaccination [6]. Significant differences in body activity were also observed, with the synbiotic-treated group exhibiting more stable activity before and after vaccination. HPT and SAA levels, which are associated with acute immune responses, increased until day three post-vaccination and sharply declined by days 6–10. The lymphocyte ratio gradually decreased to a greater degree in the 2W_SB_FMD group than in the 1W_SB_FMD group.

According to data that our research team previously identified, HPT and SAA concentrations increased three days after FMD vaccination in all vaccinated groups except the unvaccinated control group [8]. However, by day 10 days post-vaccination, HPT and SAA concentrations returned to normal ranges in all experimental groups.

This study demonstrated that synbiotic supplementation mitigated the adverse physiological and immunological responses associated with FMD vaccination by stabilizing ruminoreticular temperature, body activity, and acute-phase protein levels. In contrast to previous findings where FMD vaccination alone induced immune stress, synbiotic supplementation facilitated a more rapid recovery [6]. These results provide foundational evidence supporting the incorporation of synbiotics into livestock health management and preventive strategies during vaccination programs.

Discussion

Studies on synbiotic supplementation in cattle are still limited. It is difficult to find data on benefits of synbiotic supplementation, especially in vaccinated Hanwoo cows. Therefore, this study was a very challenging study.

According to a study reported by Saha, Sudeb, et al., probiotic (*Bacillus coagulans* MTCC 25250) treatment in dairy cows with subclinical mastitis improved blood parameters, such as LYM and MONO counts, contributing to mastitis symptom relief [18]. Similar studies have reported reductions in immune-related lymphocyte levels with probiotic supplementation, suggesting that synbiotics may also have positive effects as feed additives.

In this study, LYM levels gradually decreased following FMD vaccination in cows treated with synbiotics. However, after synbiotic treatment was discontinued, LYM levels began to increase again by day 10 post-vaccination. This suggests that long-term synbiotic supplementation following FMD vaccination may be necessary to maximize its effects. Importantly, these findings are significant, as no previous research has confirmed changes in ruminoreticular temperature and body activity in cattle in response to synbiotic treatment.

Live yeast (*Saccharomyces cerevisiae*) is one of the most effective probiotics in ruminant nutrition [19, 20]. Studies indicate that under heat stress conditions, increased supplementation of *Saccharomyces cerevisiae* can reduce the concentrations of acute-phase proteins, such as HPT and SAA [21].

In this study, synbiotic treatment administered before and after FMD vaccination initially caused an increase in HPT and SAA concentrations up to day three post-vaccination, followed by a sharp decrease. The more significant reduction in acute immune proteins observed in the 2W_SB_FMD group compared to the 1W_SB_FMD group suggests that synbiotic treatment facilitates a more rapid recovery of immune responses to normal.

Determining whether the combination of probiotics and prebiotics has a direct impact on stress relief poses a significant challenge. However, improvements in overall health, as indicated by improvements in the intestinal environment, were indirectly supported by our findings (e.g., changes in ruminoreticular temperature, body activity, CBC, and acute immune protein levels).

Acknowledgments

This study was supported by the Cooperative Research Program for Agriculture Science and Technology Development (Project No. RE-2024-00351672), Rural Development Administration, Republic of Korea.

Tables and Figures

Table 1. Experimental animals (n = 15) and treatment groups

Age and parity data are expressed as mean ± SEM.

Group	No. of cows	Mean ± SEM	
		Age (months)	Parity
Positive control	5	100.0 ± 8.3	3.0 ± 0.8
2W_SB_FMD	5	90.1 ± 12.1	3.2 ± 0.6
1W_SB_FMD	5	62.2 ± 11.0	2.6 ± 0.8
Total	15	84.1 ± 7.1	2.9 ± 0.4

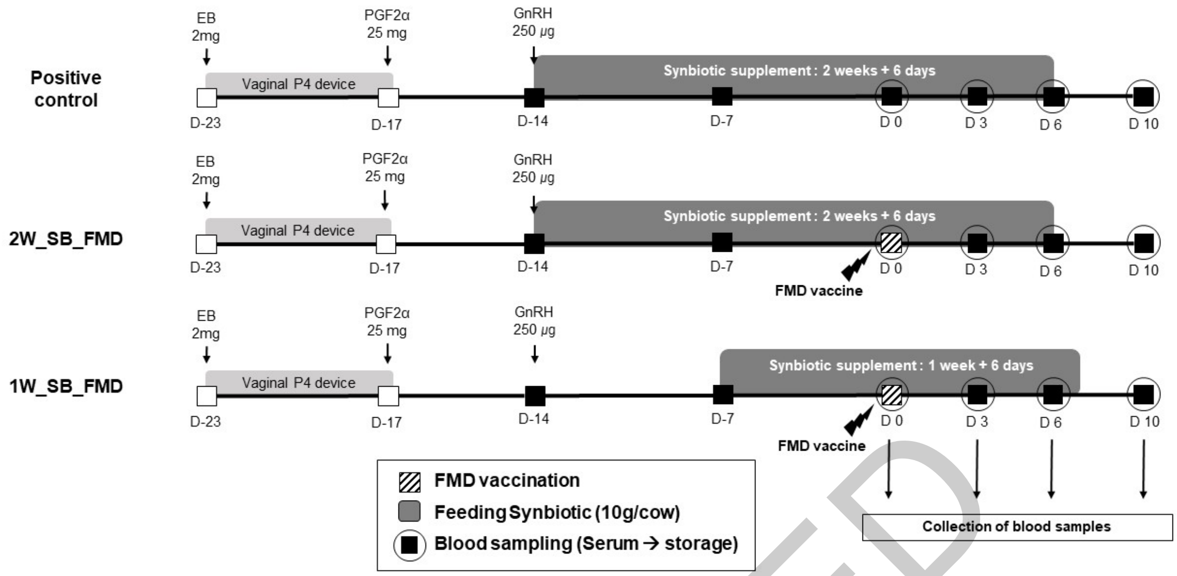


Fig 1. Schematic diagram of the OvSynch protocol for estrus synchronization in the experimental cows.

To compare and analyze ruminoreticular temperature and body activity before and after estrus, all experimental cows underwent ovulation synchronization using the OvSynch method. Ruminoreticular temperature and body activity were measured before and after FMD vaccination. Blood samples were collected on days 0, 3, 6, and 10 relative to FMD vaccination day, and HPT, SAA, and WBC concentrations were analyzed.

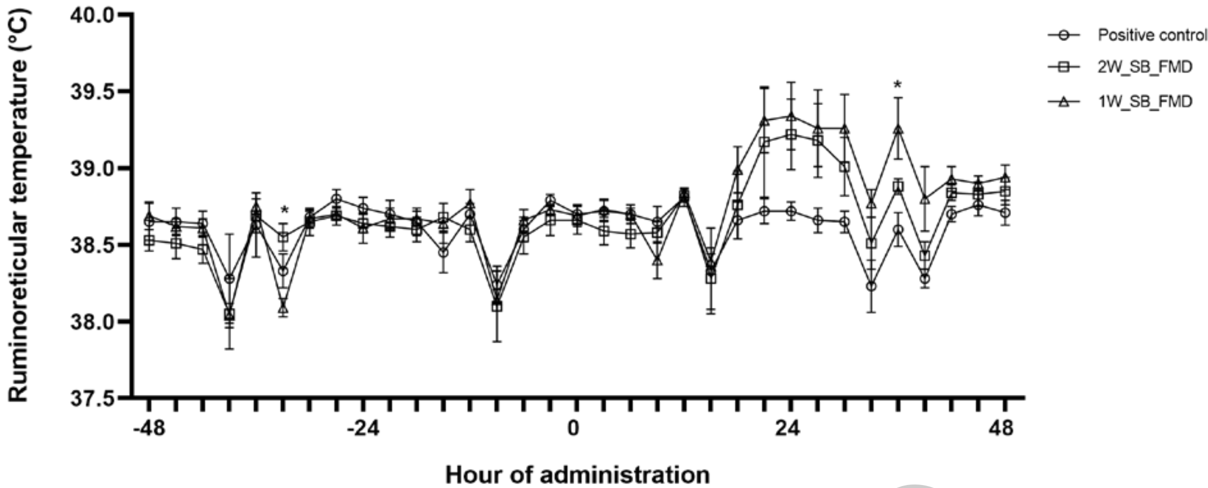


Fig 2. Effects of synbiotic treatment on ruminoreticular temperature before and after FMD vaccination in different experimental groups (n = 15).

Hour 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean. * indicates a significance level of $p < 0.05$.

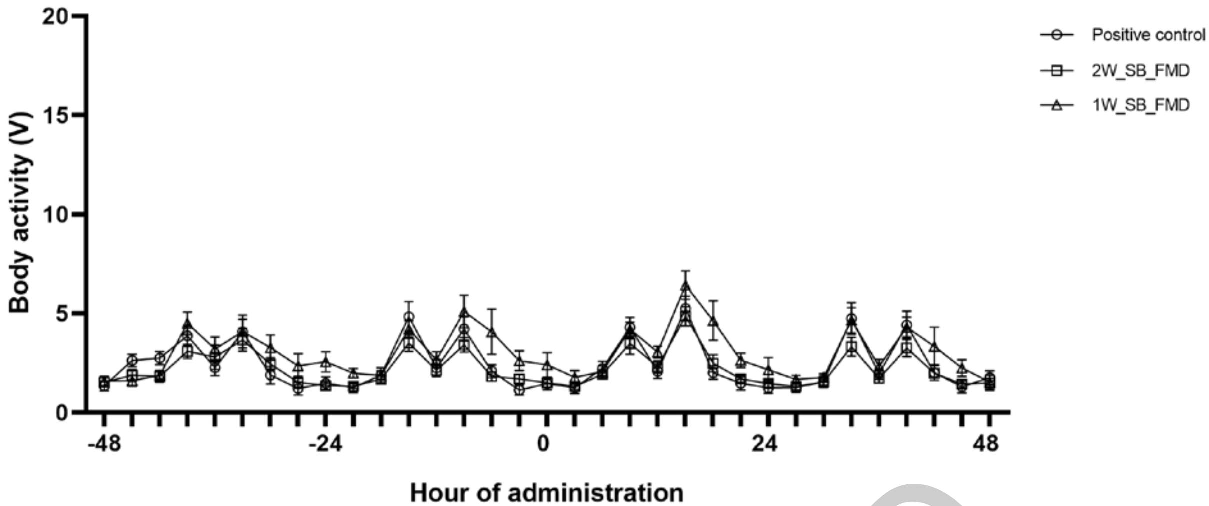


Fig 3. Effects of synbiotic treatment on body activity before and after FMD vaccination in different experimental groups (n = 15).

Hour 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean.

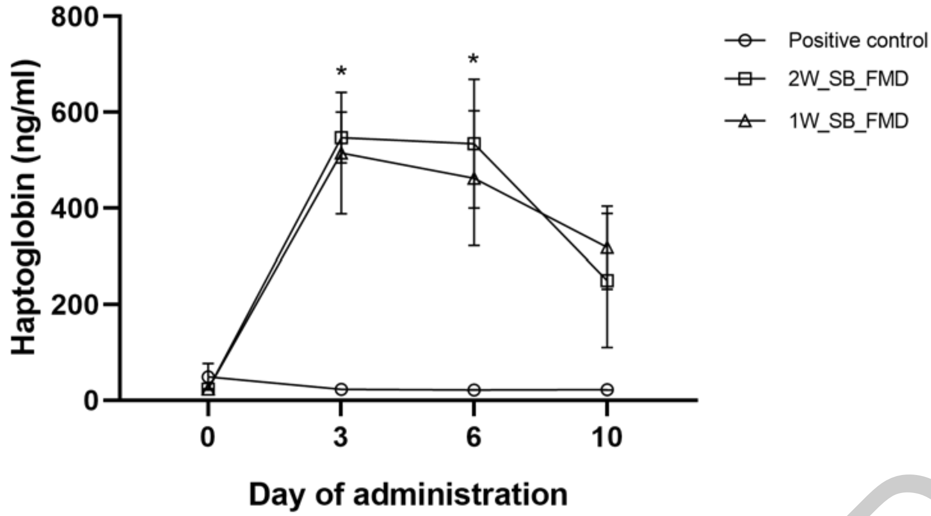


Fig 4. Effects of synbiotic treatment on HPT concentrations following FMD vaccination in different experimental groups (n = 15).

Hour 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean. * indicates a significance level of $p < 0.05$.

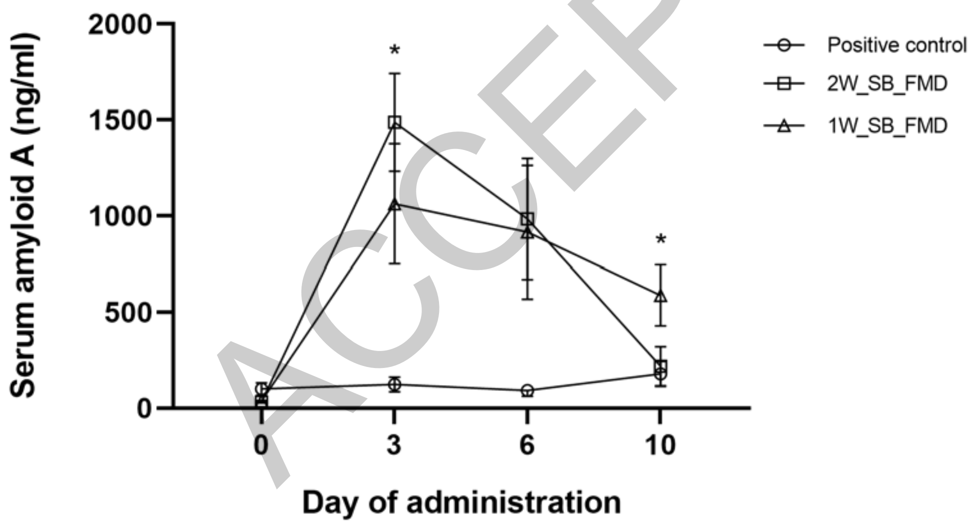


Fig 5. Effects of synbiotic treatment on SAA concentrations following FMD vaccination in different experimental groups (n = 15).

Hour 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean. * indicates a significance level of $p < 0.05$.

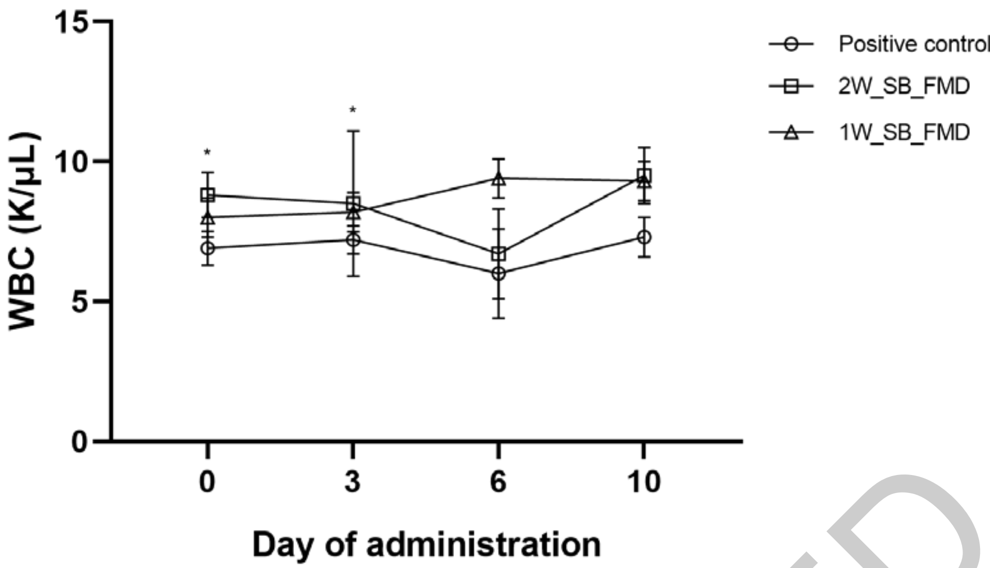


Fig 6. Effects of synbiotic treatment on WBC concentrations following FMD vaccination in different experimental groups (n = 15).

Hour 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean. * indicates a significance level of $p < 0.05$.

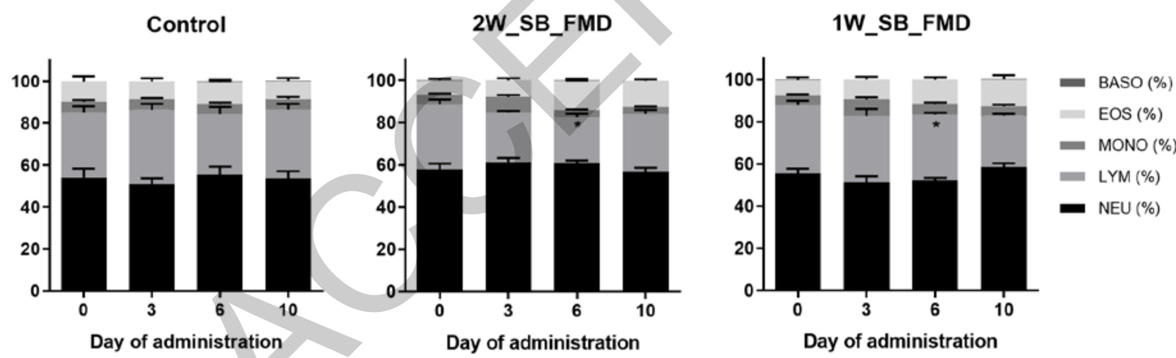
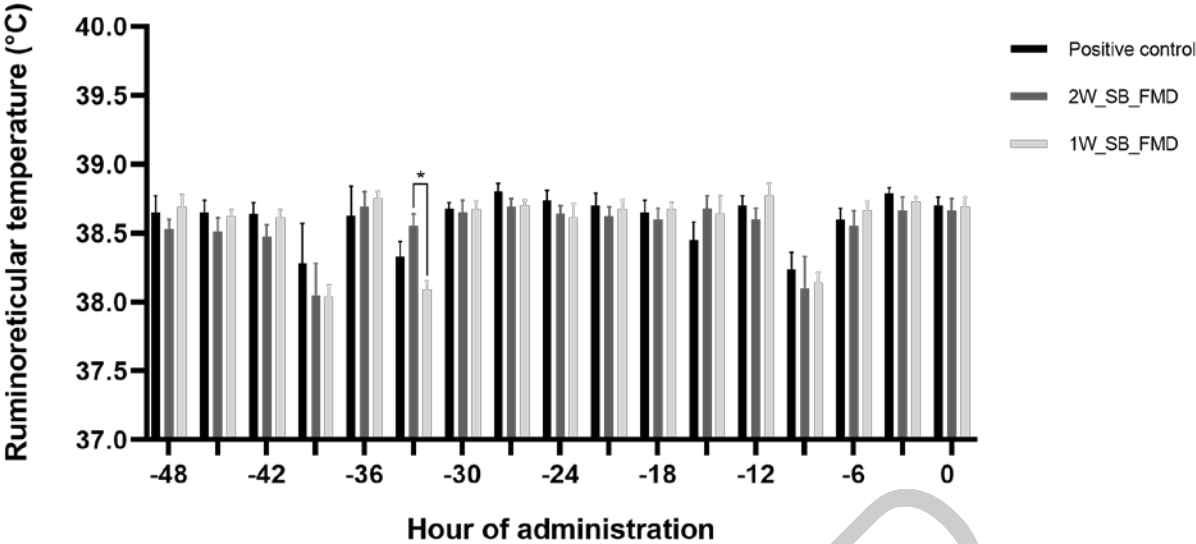
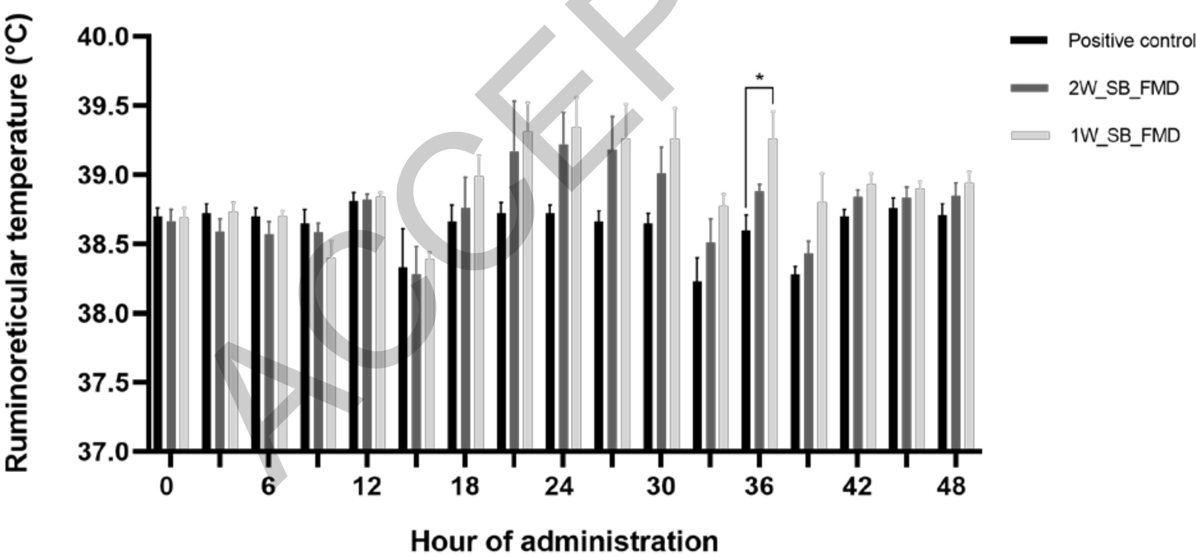


Fig 7. Effects of synbiotic treatment on leukocyte percentages following FMD vaccination in different experimental groups (n = 15).

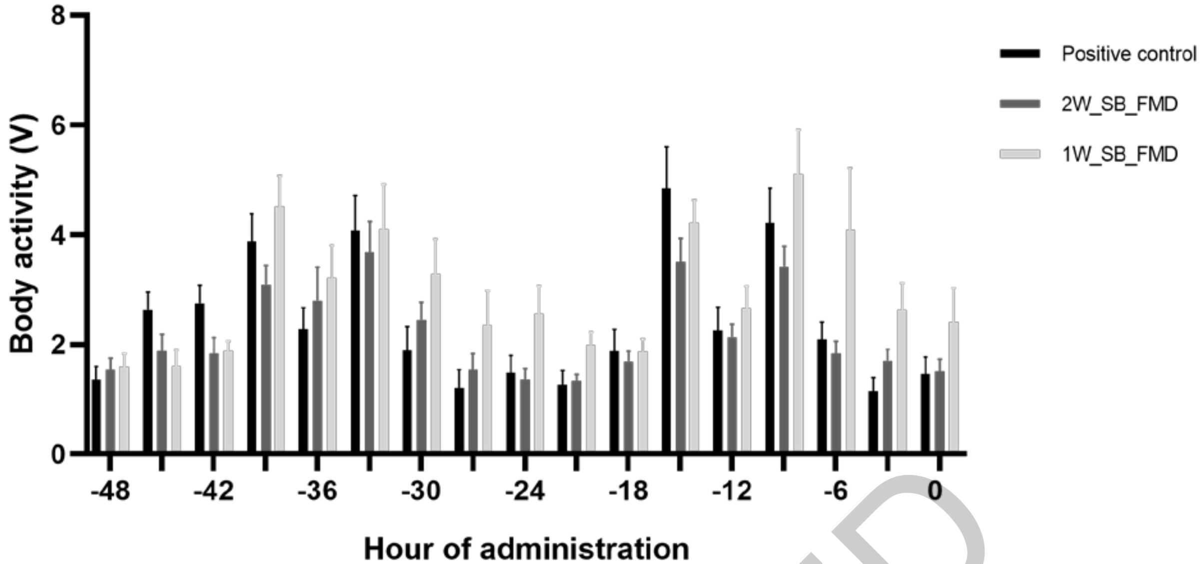
Hour 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean. * indicates a significance level of $p < 0.05$.



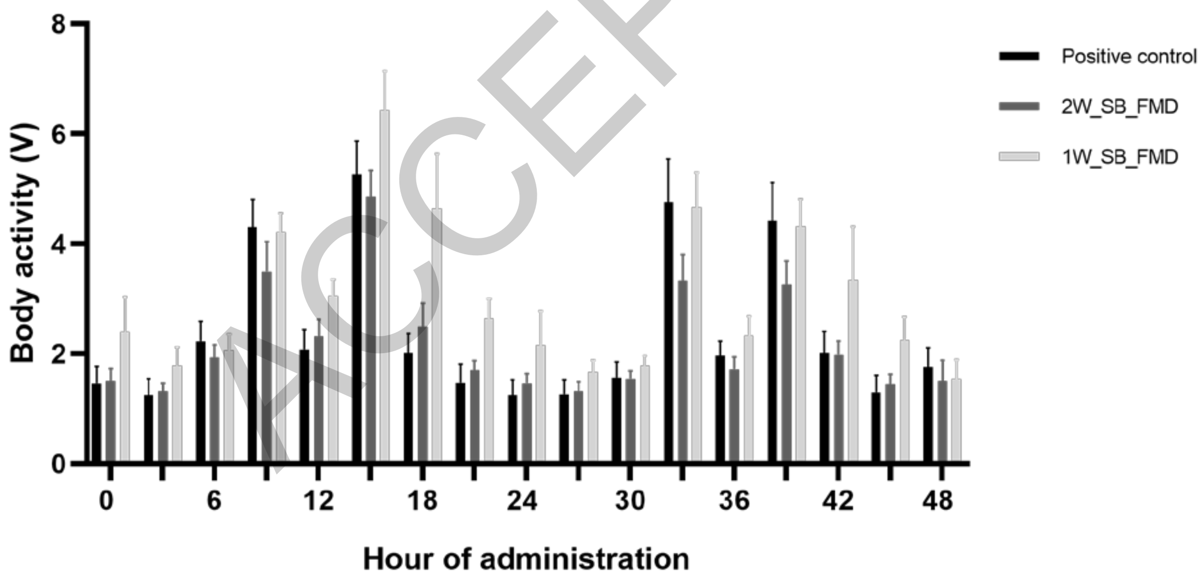
Supplementary fig 1-1. Effects of synbiotic treatment on ruminoreticular temperature before FMD vaccination in different experimental groups (n = 15).
Hour 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean. * indicates a significance level of $p < 0.05$. There is a significant difference between the 2W_SB_FMD group and the 1W_SB_FMD group at -33 hours.



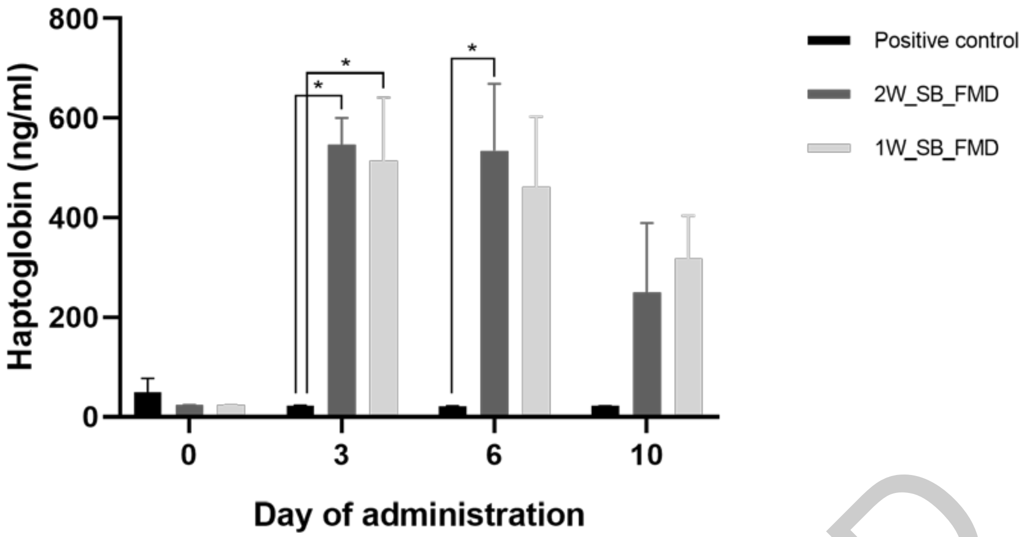
Supplementary fig 1-2. Effects of synbiotic treatment on ruminoreticular temperature after FMD vaccination in different experimental groups (n = 15).
Hour 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean. * indicates a significance level of $p < 0.05$. There is a significant difference between the Positive control and the 1W_SB_FMD group at 36 hours.



Supplementary fig 2-1. Effects of synbiotic treatment on body activity before FMD vaccination in different experimental groups (n = 15).
Hour 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean.

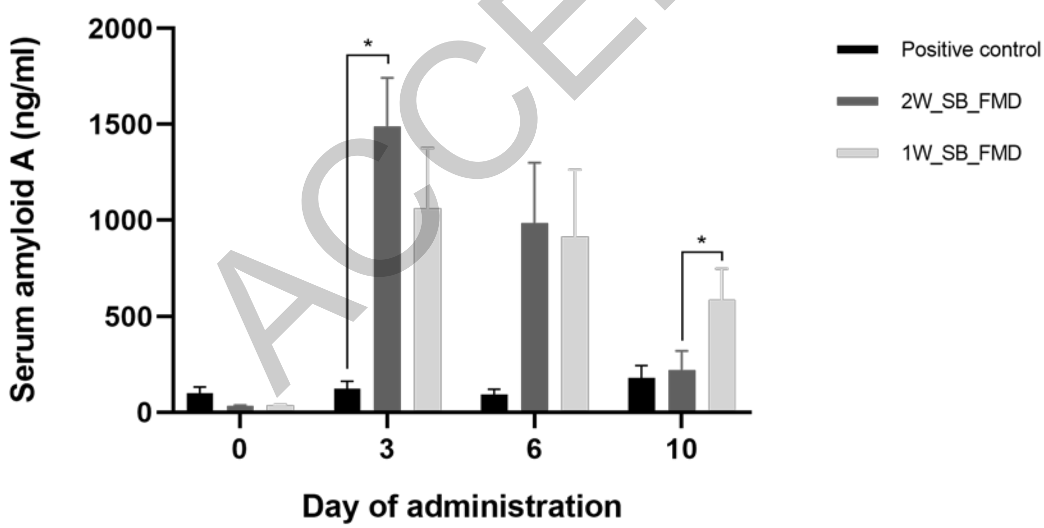


Supplementary fig 2-2. Effects of synbiotic treatment on body activity after FMD vaccination in different experimental groups (n = 15).
Hour 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean.



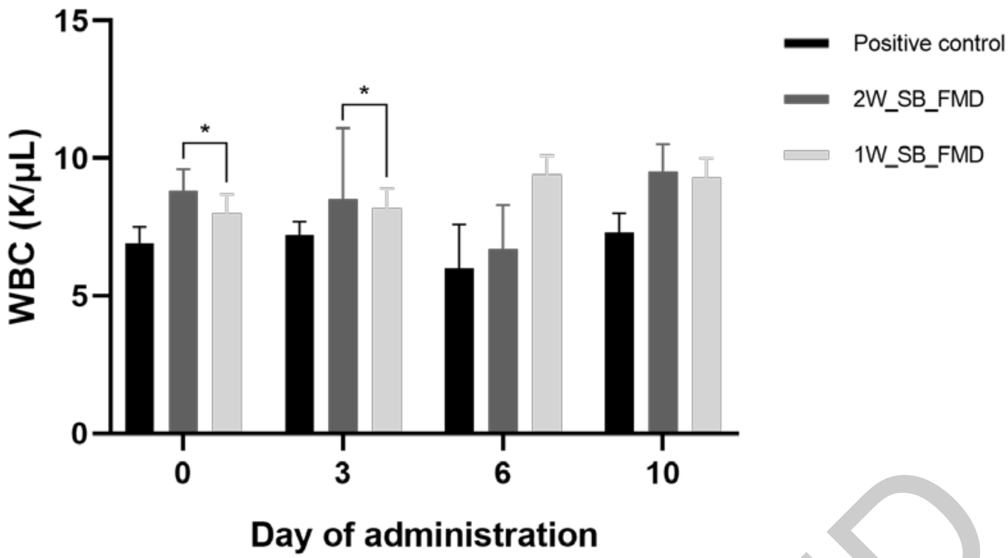
Supplementary fig 3. Effects of synbiotic treatment on HPT concentrations following FMD vaccination in different experimental groups (n = 15).

Day 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean. * indicates a significance level of $p < 0.05$. The Positive control group is individually significantly different from the 1W_SB_FMD group and the 2W_SB_FMD group on day 3. Additionally, there is a significant difference between the Positive control and the 2W_SB_FMD group on day 6.



Supplementary fig 4. Effects of synbiotic treatment on SAA concentrations following FMD vaccination in different experimental groups (n = 15).

Day 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean. * indicates a significance level of $p < 0.05$. The Positive control group is significantly different from the 2W_SB_FMD group on day 3. There is a significant difference between the 2W_SB_FMD group and the 1W_SB_FMD group on day 10.



Supplementary fig 5. Effects of synbiotic treatment on WBC concentrations following FMD vaccination in different experimental groups (n = 15).

Day 0 represents the time of FMD vaccination, and error bars indicate the standard error of the mean. * indicates a significance level of $p < 0.05$. There is a significant difference between the 2W_SB_FMD group and the 1W_SB_FMD group on day 0 and 3.

Figure legends

Fig 1. Schematic diagram of the OvSynch protocol for estrus synchronization in the experimental cows.

Fig 2. Effects of synbiotic treatment on ruminoreticular temperature before and after FMD vaccination in different experimental groups (n = 15).

Fig 3. Effects of synbiotic treatment on body activity before and after FMD vaccination in different experimental groups (n = 15).

Fig 4. Effects of synbiotic treatment on HPT concentrations following FMD vaccination in different experimental groups (n = 15).

Fig 5. Effects of synbiotic treatment on SAA concentrations following FMD vaccination in different experimental groups (n = 15).

Fig 6. Effects of synbiotic treatment on WBC concentrations following FMD vaccination in different experimental groups (n = 15).

Fig 7. Effects of synbiotic treatment on leukocyte percentages following FMD vaccination in different experimental groups (n = 15).

Supplementary fig 1-1. Effects of synbiotic treatment on ruminoreticular temperature before FMD vaccination in different experimental groups (n = 15).

Supplementary fig 1-2. Effects of synbiotic treatment on ruminoreticular temperature after FMD vaccination in different experimental groups (n = 15).

Supplementary fig 2-1. Effects of synbiotic treatment on body activity before FMD vaccination in different experimental groups (n = 15).

Supplementary fig 2-2. Effects of synbiotic treatment on body activity after FMD vaccination in different experimental groups (n = 15).

Supplementary fig 3. Effects of synbiotic treatment on HPT concentrations following FMD vaccination in different experimental groups (n = 15).

Supplementary fig 4. Effects of synbiotic treatment on SAA concentrations following FMD vaccination in different experimental groups (n = 15).

Supplementary fig 5. Effects of synbiotic treatment on WBC concentrations following FMD vaccination in different experimental groups (n = 15).

References

1. Shahriari A, Habibi-Pirkoohi M. Developing Vaccines Against Foot-and-Mouth Disease: a Biotechnological Approach. Arch Razi Inst. 2018;73(1):1-10.
2. Kahn S, Geale DW, Kitching PR, Bouffard A, Allard DG, Duncan JR. Vaccination against foot-and-mouth disease: the implications for Canada. Can Vet J. 2002;43(5):349-54.
3. Singh RK, Sharma GK, Mahajan S, Dhama K, Basagoudanavar SH, Hosamani M, et al. Foot-and-Mouth Disease Virus: Immunobiology, Advances in Vaccines and Vaccination Strategies Addressing Vaccine Failures-An Indian Perspective. Vaccines (Basel). 2019;7(3).
4. Mahapatra M, Upadhyaya S, Aviso S, Babu A, Hutchings G, Parida S. Selection of vaccine strains for serotype O foot-and-mouth disease viruses (2007-2012) circulating in Southeast Asia, East Asia and Far East. Vaccine. 2017;35(51):7147-53.
5. B Singanallur N, Dekker A, Eble PL, van Hemert-Kluitenberg F, Weerdmeester K, Horsington J, et al. Emergency Foot-and-Mouth Disease Vaccines A Malaysia 97 and A(22) Iraq 64 Offer Good Protection against Heterologous Challenge with A Variant Serotype A ASIA/G-IX/SEA-97 Lineage Virus. Vaccines (Basel). 2020;8(1).
6. Kim D, Moon J, Ha J, Kim D, Yi J. Effect of Foot-and-Mouth Disease Vaccination on Acute Phase Immune Response and Anovulation in Hanwoo (Bos taurus coreanae). Vaccines (Basel). 2021;9(5).
7. Garcia-Pintos C, Riet-Correa F, Menchaca A. Effect of Foot-and-Mouth Disease Vaccine on Pregnancy Failure in Beef Cows. Front Vet Sci. 2021;8:761304.
8. Kim D, Kwon WS, Ha J, Kim J, Kim D, Lee W, et al. Effect of oestrus synchronisation through ovulation delay by vaccination against foot-and-mouth disease in Hanwoo (Bos taurus coreanae) cows. Vet Med Sci. 2023;9(2):974-81.
9. P Perumal* KK, C Rajkhowa. Effect of foot and mouth disease vaccination on seminal and biochemical profiles of mithun (*Bos frontalis*) semen. 2013.
10. Kim D, Ha J, Moon J, Kim D, Lee W, Lee C, et al. Increased Ruminoreticular Temperature and Body Activity after Foot-and-Mouth Vaccination in Pregnant Hanwoo (Bos taurus coreanae) Cows. Vaccines (Basel). 2021;9(11).
11. Kim D, Kwon WS, Ha J, Moon J, Yi J. Increased accuracy of estrus prediction using ruminoreticular biocapsule sensors in Hanwoo (Bos taurus coreanae) cows. J Anim Sci Technol. 2023;65(4):759-66.
12. Samuelson KL, Hubbert ME, Galyean ML, Loest CA. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey. J Anim Sci. 2016;94(6):2648-63.
13. Zhang R, Zhou M, Tu Y, Zhang NF, Deng KD, Ma T, et al. Effect of oral administration of probiotics on growth performance, apparent nutrient digestibility and stress-related indicators in Holstein calves. J Anim Physiol Anim Nutr (Berl). 2016;100(1):33-8.
14. Kowalski ZM, Górka P, Schlagheck A, Jagusiak W, Micek P, Strzetelski J. Performance of Holstein calves fed milk-replacer and starter mixture supplemented with probiotic feed additive. J Anim Feed Sci. 2009;18(3):399-411.
15. Colombo EA, Cooke RF, Brandao AP, Wiegand JB, Schubach KM, Sowers CA, et al. Performance, health, and physiological responses of newly received feedlot cattle supplemented with pre- and probiotic ingredients. Animal. 2021;15(5):100214.
16. Silva LGT, Cooke RF, Schubach KM, Brandao AP, Marques RS, Schumaher TF, et al. Supplementing a yeast-derived product to enhance productive and health responses of beef steers. Animal. 2018;12(8):1576-83.

17. Danielo J, McCarty KJ, Tipton JE, Ricks RE, Long NM. Effects of post-weaning supplementation of immunomodulatory feed ingredient on body weight and cortisol concentrations in program-fed beef heifers. Domest Anim Endocrinol. 2020;72:106427.

18. Sudeb Saha1, Jay Prakash Ray3, Apurbo Kumar Mondal4, Md. Irtija Ahsan5, Marina Debnath6, Dilruba Afrin7, Syed Sayeem Uddin Ahmed5, Jayanta Datta Gupta3, Nivedita Datta8, Hugo O. Toledo-Alvarado9, Haruki Kitazawa1. Effect of Probiotic Bacillus coagulans on Performance and Blood Metabolites of Dairy Cows with Subclinical Mastitis.

19. Muhammed A. Arowolo JH. Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review.

20. M I Anjum 1, S Javaid 2, M S Ansar 3, A Ghaffar 1. Effects of yeast (Saccharomyces cerevisiae) supplementation on intake, digestibility, rumen fermentation and milk yield in Nili-Ravi buffaloes.

21. M.C. Perdomo RSM, M.G. Favoreto, A. Adesogan, C.R. Staples, J.E.P. Santos. Effects of feeding live yeast at 2 dosages on performance and feeding behavior of dairy cows under heat stress.