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Improved immune status by fecal microbiota transplant mediated gut microbiota modulation in late lactation cows in a low land

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ABSTRACT

Dairy cows in late lactation experience compromised immune status. A promising strategy to improve immune health is to manipulate gut microbiota. This study evaluated the effect of fecal microbiota transplant (FMT) on the immunity profile of Friesian Holstein cows during late lactation. Donor cow from the Ciawi, Bogor highlands were selected. Fecal microbiota transplants (FMT) were prepared using donor Friesian Holstein cow feces, mixed with glycerol (1:1), diluted in saline, encapsulated in double-layered capsules, and stored at -20°C. The study used a Latin square design with three Friesian Holstein cows in late lactation, testing one control and two FMT levels (5 g and 10 g per day per cow) in a 3% body weight ration (60% elephant grass, 40% concentrate) over three cycles. Using a shotgun metagenomic approach, we identified key microbial populations that correlated with the maintenance of gut homeostasis and immune function. These microbial communities, including *Bacteroides*, *Bifidobacterium*, and *Prevotella*, produce gut-derived metabolites (acetate, butyrate, and propionate) that influence lymphocytes of T1 (4.02 x 10^3/uL) and T2 (3.87 x 10^3/uL) and monocytes of T1 (0.38 x 10^3/uL) and T2 (0.31 x 10^3/uL), thus modulate adaptive immune responses, aid in the repair of the intestinal barrier, and strengthened immune system. CAZy enzyme analysis revealed diverse carbohydrate-active enzymes, highlighting the microbial contributions to fiber degradation and SCFA production. Our findings provide valuable insights into the role of the microbiota in regulating the digestive and immune systems of dairy cows in lowland climates.

Keywords: Dairy cows, Fecal microbiota transplant, Immune system, Low land, Microbiota

INTRODUCTION

Indonesia is a tropical country with temperatures reaching 36°C and relative humidity levels of 70-90% (Asmarasari *et al.,* 2023). High temperature and relative humidity result in heat stress in dairy cows, causing decreased productivity due to hormonal and metabolic disorders (Soriani *et al.,* 2013). Metabolic disorders and autoimmune diseases are likely caused by an imbalance (dysbiosis) in hindgut microbiota (Xu *et al.,* 2019). The hindgut microbiota of dairy cows is related to various activities and functions such as short chain fatty acid (SCFA) production and

methane and nitrogen emissions. The diversity of intestinal microbiota in dairy cows includes *Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria,* and *Verrucomicrobia* (Rinninella *et al.,* 2019).

Numerous studies have shown that intestinal dysbiosis plays a role in the emergence of illnesses in dairy cows (Ma *et al.,* 2018). Bacteria, such as *Streptococcus*, *Enterobacteriaceae*, *Ruminobacter, Treponema,* and *Bacteroidaceae*, produce higher quantities of lactate, which can cause heat stress in dairy cows (Zhao *et al.,* 2019). Lactate can activate certain pathways in immune cells, which contribute to chronic inflammation or immune responses (Manosalva *et al.,* 2022). Lactate is carried by the monocarboxylate transporter (MCT) as a mediator and interacts with receptors, such as GPR81, which regulates inflammation and the immune response of various immune cells, such as macrophages and T-cells (Wu *et al.,* 2023).

The diversity of the gut microbiome is essential for ruminant health and production, and FMT is gaining recognition as a method of choice to bring about comprehensive and dynamic changes in microbial communities (Elokil *et al.,* 2022; Olivia *et al.,* 2023; Wang *et al.,* 2022). FMT can affect the structure of the gut microbiota in ruminants without causing any significant negative side effects. FMT can be a useful strategy for mitigating low milk yield and improving overall immune responses (Khan *et al.,* 2023). Previous studies have demonstrated that FMT significantly alters the composition of gut microbiota in calves (Wu *et al.,* 2018). Research conducted by Ardiansyah *et al.* (2024) on the administration of FMT to Nellore cattle showed that FMT did not adversely affect animal health, as indicated by haematological values. We demonstrated that FMT from a healthy donor can modulate the gut microbiota, alleviate microbiota dysbiosis, and help maintain immune system homeostasis.

MATERIALS AND METHODS

Preparation of FMT

Healthy, high yielding donor cow was selected for fecal microbiota transplant. Fecal samples were collected and immediately placed in a cooler box containing ice gel packs to maintain a stable temperature during transport to the laboratory. After homogenization, 80 g of fecal material was weighed, transferred to a sterile plastic bag, and clipped. Glycerol was then added to the sample at a 1:1 ratio until total weight reaches160 g. To prepare the solution, 20 litters of saline (NaCl) was added to the mixture, which was stirred thoroughly to ensure uniform dilution and maintain solution balance. The prepared solution was then drawn into a syringe and encapsulated, with each capsule having a volume of 0.9 ml. The capsules were securely sealed and stored in a freezer at -20°C (Figure 1).

Experimental Design

The experimental unit used three Friesian Holstein dairy cows in the 8th month of lactation (late lactation). The encapsulated FMT was administered orally. The feed was given in the form of a ration of 3% of their body weight (370 – 430 kg) consisted of 60% elephant grass and 40% concentrate (Table 1) with dry matter intake T0 = 11.91 kg, T1 = 12.48 kg, and T2 = 11.57 kg is shown in Table 2. This study uses a Latin square design with three cycles. The treatments consisted of three concentrations with different FMT levels: T0 = Ration + FMT 0 g, T1 = T0 + FMT 5 g, and T2 = T0 + FMT 10 g.

Blood Sample Collection

Data collection of blood parameters was carried out using a 10 ml syringe, and blood was taken through the jugular vein and inserted into a 3 ml vacutainer. The vacutainer was placed in a cool box and brought to the Soeparwi Animal Hospital, Gadjah Mada University, for hematology analysis.

Fecal Sample Collection

We collected fecal samples from four (1 donor 3 experimental), donor (FeSCAcow), control (Fe_cowT0), and experimental Friesian Holstein cows (Fe_cowT1, Fe_cowT2), preserved them in RNA/DNA shield tubes, and sent them to the laboratory for processing. Genomic DNA was extracted from 1 g of feces using a Zymo-Biomics stool kit (catalog number D4304) for metagenomic analysis. The DNA quality was assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

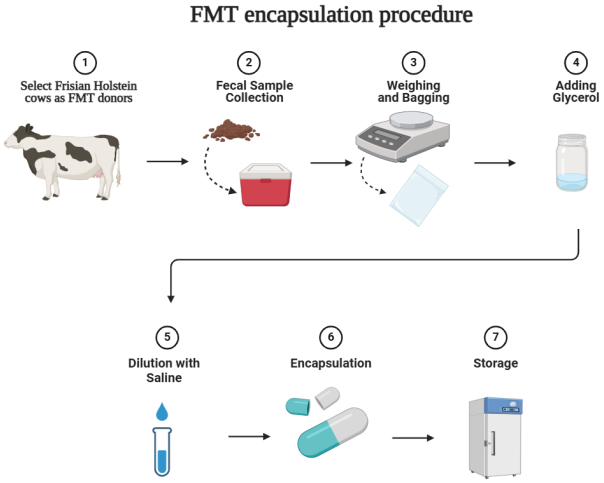

Figure 1. FMT Encapsulation Procedure. Feces were Collected from Donor Cow, Weighed, and Packed. Glycerol was Added to the Mixture and Dilute with Saline Solution, Encapsulated and Stored for Future Use.

Shotgun Metagenomic Sequencing

Genomic DNA was extracted and assessed for integrity using 1% agarose gel electrophoresis to ensure the quality of DNA for downstream analysis. Fragmentation of the DNA to 350 bp was achieved using a Covaris M200 instrument. Subsequently, a paired-end (PE) library was constructed using PCR amplification to enrich the library template. Library quantity was determined by quantitative real-time PCR (qPCR). High-quality libraries that met the specified criteria were processed and sequenced using the Nova Hiseq X-ten platform.

Statistical Analysis

Haematological data analysis was performed using statistical methods, specifically analysis of variance (ANOVA) with an F-test at a 5% significance level. To control for potential variability, a Latin square design was employed as the linear model for analysis to enhance the accuracy of the results.

RESULTS AND DISCUSSION

Relative Abundance of Bacteria in Control, Treated and Donor

The data presented in Figure 2A provides critical insights into the impact of fecal microbiota transplant (FMT) on microbial community composition and establishes a foundation for understanding dose-dependent microbial shifts. At baseline (T0), the untreated control group exhibited low relative abundances of *Alistipes* (0.029%), *Bacteroides* (0.013%), *Prevotella* (0.02%), and *Clostridium* (0.007%), with *Bifidobacterium* being notably absent. Administering 5 g of FMT (T1) led to measurable alterations in the microbial composition. *Bifidobacterium* emerged at a relative abundance of 0.002%, whereas *Alistipes* and *Bacteroides* increased to 0.04% and 0.017%, respectively. Slight shifts were also observed in *Prevotella* and *Clostridium* with relative abundances of 0.02% and 0.009%, respectively. An increase in the FMT dosage to 10 g (T2) further magnified these changes. *The abundance of Alistipes* and Clostridium increased to 0.041% and 0.013%, respectively. However, *Bifidobacterium* showed a minor decline of 0.001%, suggesting a differential response of microbial taxa to higher FMT doses. *Bifidobacterium* species were more abundant at T1 than at T2 or T0. Covian *et al.*, (2013) reported that *Bifidobacterium* produces exopolysaccharides (EPS), which serve as a carbon source for *Bacteroides,* and metabolizes these EPS to generate short-chain fatty acids (SCFAs). Furthermore, *Bifidobacterium* helps improve the growth and development of calves by optimizing the metabolic functions in the digestive tract (Zhuang *et al.*, 2024). *Ruminococcus* are an important category of gut microbes that break down complex polysaccharides by cellulosomes and produce butyrate that maintains host health

Table 1. Feed Composition and Nutrient Content

Feed Ingredients	% Content (DM basis)
Dry Cassava Pulp	30
Brand Polard	21
Palm Kernel Meal	38
[1]Extruded soybean (Soyxyl)	7
[1]Mineral mix (St.Vit)	1
CALCIT	1
Salt	1
[1]Suplement RDP (GoPro)	1
[2]Nutrient Content	%
Dry Matter	85.56
Inorganic Ash	5.28
Crude Protein	17.08
Total Digestible Nutrients	76.83
Crude Fat	8.38
Crude Fiber	15.04
Nitrogen-Free Extract	54.21
[2]Nutrient Content of *Pennisetum purpureum*	%
Dry Matter	19.9
Inorganic Ash	12.76
Crude Protein	10.2
Total Digestible Nutrients	45.99
Crude Fat	1.6
Crude Fiber	42.3
Nitrogen-Free Extract	33.14

Table 2. Daily Feed Intake

Variable	T0	T1	T2
Dry Matter Intake (kg)	11.91	12.48	11.57

(Reau and Suen, 2018; Artzi *et al.,* 2017; El-Sayed *et al.*, 2021). *Prevotella*-derived metabolites, particularly propionic acid, can indirectly influence immune function. However, the activation of antigen-presenting cells (APCs), including dendritic cells, is predominantly driven by *the microbial components of Prevotella*, such as lipopolysaccharide (LPS) and peptidoglycan (Larsen, 2017).

Heatmap data showed that *Bacteroides* was the most dominant (Figure 2B) in T1 compared to that in T2 and T0. Portincasa *et al.* (2022) reported *that Bacteroides* species are correlated with gut-derived bioactive compounds (SFAs), such as propionate and acetate, which help to maintain a healthy intestinal barrier and reduce inflammation. *Bacteroides* plays a major role in the fermentation of complex carbohydrates, production of SCFAs, and protein digestion (Tufail and Schmitz, 2024). SCFAs contribute to strengthening the gut barrier, enhancing glucose and lipid metabolism, modulating the immune system, managing inflammation, and regulating blood pressure (Nogal *et al.,* 2021).

Carbohydrate-Active Enzyme response to FMT treatment

Carbohydrate-Active Enzyme (CAZy) is a specialized database that categorizes and arranges data regarding the enzymes that create, break down, and alter carbohydrates. CAZymes are categorized into six major groups in the CAZy database: Glycoside Hydrolases, Glycosyl Transferases, Polysaccharide Lyases, Carbohydrate

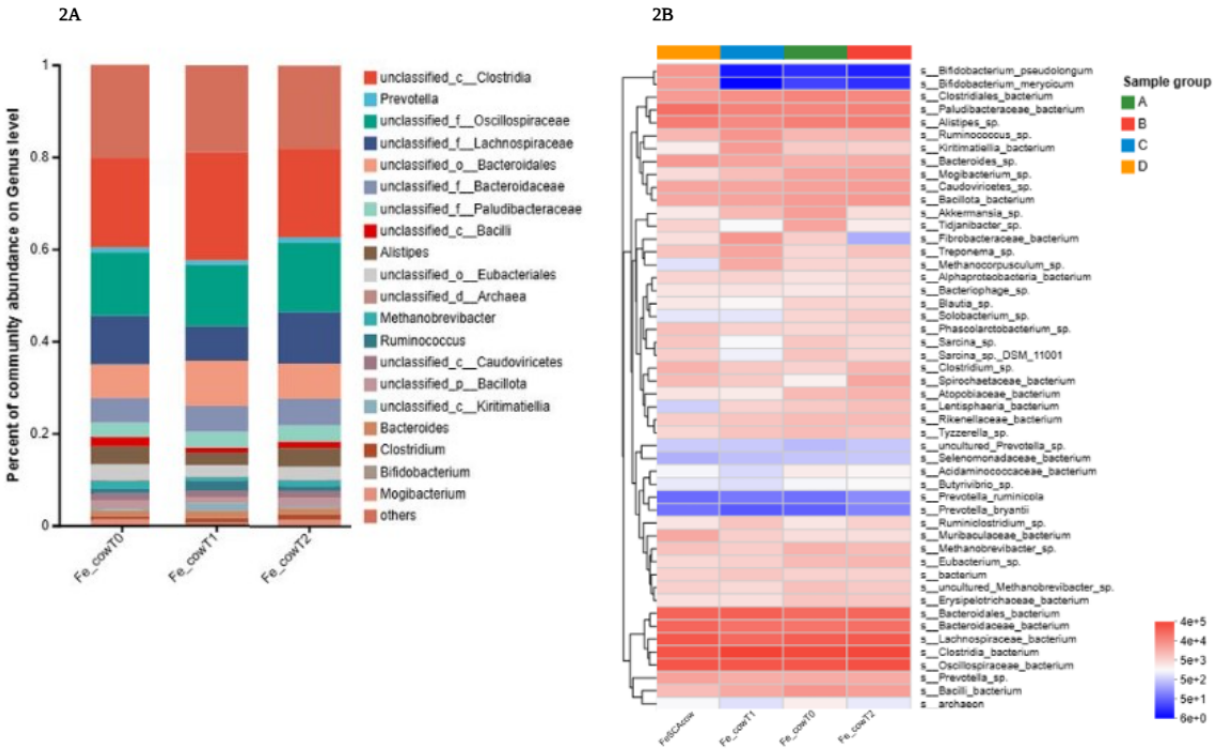

Figure 2A. Bar Graph of the Relative Abundance of Bacterial Communities at Genus Level among the Donor and Treated Groups.

Figure 2B. Heatmap Species level among Donor and Treated Groups.

Esterases, Auxiliary Activities, and Carbohydrate-Binding Modules (Lombart *et al.,* 2014). These CAZymes interact simultaneously to decompose dietary fibers, such as cellulose, hemicellulose, and pectin. In addition, carbohydrate-binding modules (CBMs), which are non-enzymatic components, play a vital role in enhancing enzymatic activity. Studies have shown that CBMs significantly improve the catalytic efficiency of enzymes by specifically binding to polysaccharides, thereby localizing them to their target substrates and optimizing their functionality (Wang *et al.*, 2019). To determine function, the resulting CAZymes were compared to the KEGG pathway database (Al-Shareef, 2024), as shown in (Figure 3A) and (3B).

A total of 19 CAZy enzyme families were detected in fecal microbiome samples (Figure 3A). Eleven GH families (GH2, GH3, GH78, GH109, GH36, GH77, GH25, GH31, GH20, GH97, and GH95) were detected in abundance in our study. GH families, such as GH2, GH3, GH31, GH97, and GH95, are oligosaccharide-degrading enzymes. Previously, oligosaccharide-degrading glycoside hydrolases (oligo-GHs) exhibited the highest bacterial diversity, with *Bacteroides* and *Prevotella* serving as the primary contributors to oligosaccharide breakdown, followed by *Clostridium and Ruminococcus* (Wang *et al.*, 2019). Moreover, GH78 (α-l-rhamnosidase) is involved in debranching and hemicellulose degradation (Mu *et al.* 2021). Five GT families (GT2, GT4, GT41, GT35, and GT5) and four CE families (CE1, CE10, CE4, and CE9) were identified in our study. These enzymes play a critical role in polysaccharide degradation, glycan synthesis, and carbohydrate utilization (Bains *et al.,* 2024). The gut microbiota produces CAZy enzymes that metabolize dietary fibers into short-chain fatty acids (SCFAs), including butyrate and propionate. The metabolism of carbohydrates by microbes in the gut influences immunological development and control (Kim, 2018). Dysbiosis can disrupt immunological tolerance and result in persistent inflammation and autoimmune disorders. *Bacteroides* produce short-chain fatty acids (SCFAs) and interact with gut hormones, such as G protein-coupled

receptors (GPCRs), including GPR41, GPR43, and GPR109A, present on immune cells (e.g., neutrophils, macrophages, and dendritic cells) (Liu *et al.,* 2023). The short chain fatty acids butyrate and propionate mediate Treg cell proliferation through the G protein-coupled receptor pathway and upregulation of the anti-inflammatory pathway due to inhibition of NF-κB activity (Fusco *et al.,* 2023).

Impact of FMT on Immune System

Numerous factors, particularly challenging environments such as heat stress, can adversely affect the health of cattle. Heat stress can disrupt physiological homeostasis and impair immunological processes in dairy cattle by hindering both humoral and cell-mediated immunity (Bagath *et al.,* 2022). Fecal Microbiota Transplant (FMT) is a potential therapeutic intervention to alleviate heat stress by restoring the commensal microbial community within the intestine. This approach involves the transfer of a diverse community of beneficial microorganisms from a screened donor to recipient.

Researchers have identified FMT as a viable treatment option for conditions associated with various common diseases, particularly for patients who have exhausted other therapeutic alternatives. Karimi *et al.* (2024) demonstrated that FMT is both safe and effective. Furthermore, Mahmoudi and Hadi (2023) highlighted that FMT can effectively restore gut microbial communities, emphasizing its potential as a restorative therapy for dysbiosis of gut microbiota.

FMT is associated with enhanced immune tolerance by reshaping gut microbiota, modulating immune cell activity, and influencing cytokine production (Soveral *et al.,* 2022). Immune function is significantly affected by the abundance of gut microbiota and the quantities of specific metabolites they produce, which interact with both the innate and adaptive immune systems. These interactions are crucial for maintaining immune homeostasis and for preventing immune-related diseases. The composition of gut microbiota is influenced by innate immunity in a

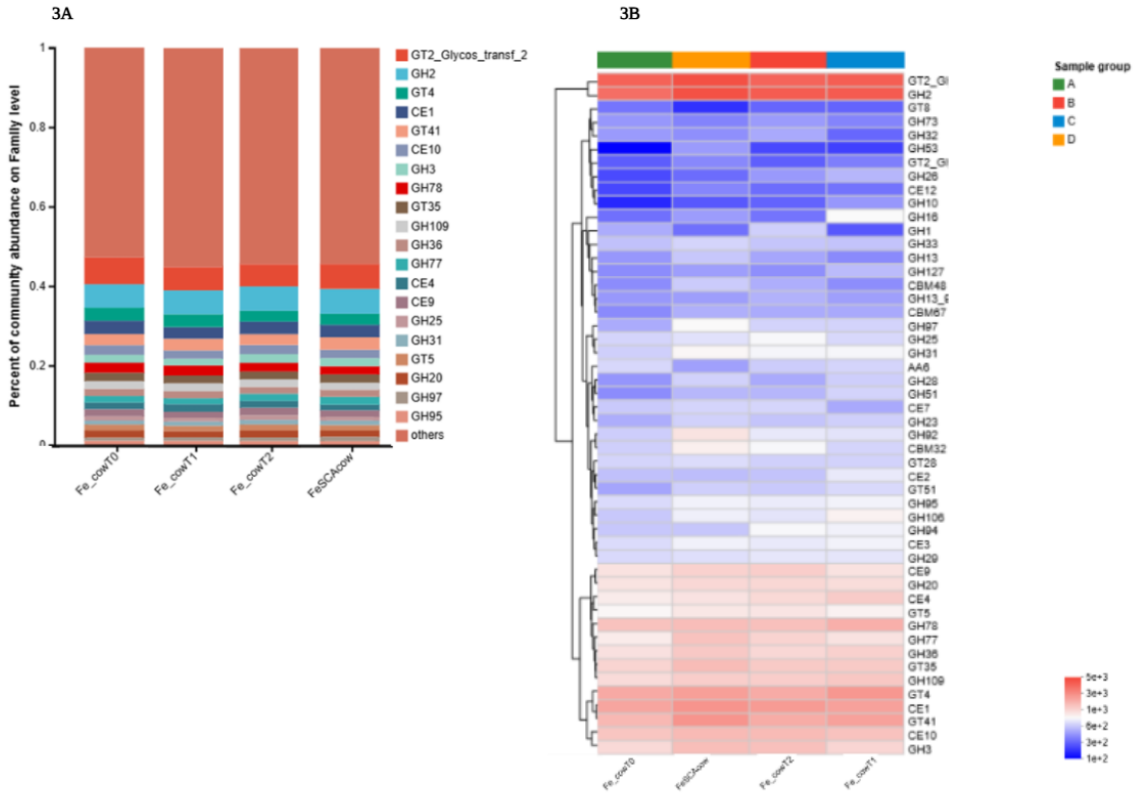

Figure 3A. Percentage Community of CAZy Enzymes at Family Level among the Donor and Treated Groups**.**

Figure 3B. Heatmap of CAZy Enzymes among Donor and Treated Groups.

healthy gut environment (Verberkmoes *et al.,* 2009). Table 3 presents leukocyte profile data that provide additional information regarding the impact of microbial metabolites on the immune system.

The immune system functions through the involvement of leukocytes or white blood cells (Tigner *et al.,* 2022). Table 3 shows the variable leukocyte profiles in the normal range. The results of leukocytes examination, white blood cells (6.26 - 7.20 x 10^3/µL); neutrophils (1.27 – 1.40 x 10^3/uL); eosinophils (0.62 - 0.72 x 10^3/ uL); lymphocytes (3.78 – 4.02 x 10^3/uL) and monocytes (0.31 – 0.45 x 10^3/uL) after administration of FMT. An increased leukocyte count is generally considered a sign of an immune response to infection (Sordillo, 2016). Recently, eosinophils have been shown to be the most abundant leukocyte population in bovine adipose tissue (Bentley *et al.,* 2019). Eosinophils also play a role in maintaining tissue homeostasis and immunity (Broberg *et al.,* 2021). Eosinophils and Neutrophils play an important role in the innate immune system (Dorosz *et al.,* 2020). Neutrophils, a key component of the immune system, are produced in the bone marrow and released into the bloodstream. In the present analysis, neutrophil levels remained within the normal range and were not significantly different between the treatment groups. This observation suggests the absence of bacterial infections or other inflammatory conditions. Neutrophils serve as first responders to bacterial infections and play a critical role in the innate immune response by rapidly targeting and neutralizing pathogens (Alhussien and Dang, 2019). Neutrophils influence adaptive immunity by acting as antigen-presenting cells and activating T-cells. They perform several functions, including pathogen phagocytosis, release of reactive oxygen species (ROS), and secretion of proinflammatory cytokines. Upon detection of infection, neutrophils rapidly migrate to the infection site (Rutkowska *et al.,* 2024).

Lymphocytes are white blood cells that play important roles in the immune system. Lymphocytes have 2 types, namely T lymphocytes and B lymphocytes. T and B lymphocytes cells interact closely within secondary lymphoid organs to elicit an effective immune response against invading pathogens with T lymphocytes attacking infectect cell and B lymphocytes produce vantibodies to neutralize certain phatogens (Langelaar et al., 2020). T cells (T lymphocytes) comprise various subtypes, each with distinct roles in immune functions. The two most prevalent and widely studied subtypes are CD4+ T cells, commonly referred to as helper T cells, and CD8+ T cells, also known as cytotoxic or killer T cells. CD4+ T cells can be further classified as regulatory T cells (Tregs), which play a critical role in main- taining immune tolerance and preventing autoimmunity (Sauls et al., 2023; Corthay, 2009). A key advantage of lymphocytes is their ability to develop immunological memory, which enables them to respond more effectively to subsequent encounters with the same pathogen or foreign molecules (Dachlan, 2019). The increase in lymphocyte parameters in T1 (4.02 10 3 /uL) results may be due to the immunomodulatory effects of microbes present in the gut environment. SCFA-producing microbes, such as Bac-

Table 3. The Leukocyte Profile of Control and Treatment Groups after Administration of FMT.

Variables	T0	T1	T2	Range	Signification
White blood cell ($10^{\wedge}3$/uL)	7.2	6.54	6.26	5.1 – 7.6*	P > 0.05
Neutrophils ($10^{\wedge}3$/uL)	1.40	1.27	1.28	1.0 – 3.5**	P > 0.05
Eosinophils ($10^{\wedge}3$/uL)	0.72	0.70	0.62	0 – 0.9*	P > 0.05
Limphocyets ($10^{\wedge}3$/uL)	3.78	4.02	3.87	1,6 – 5.6*	P > 0.05
Monocytes ($10^{\wedge}3$/uL)	0.45	0.38	0.31	0 – 0.8*	P < 0.05

* George, J. W., J. Snipes and V. M. Lane. 2010. Comparison of bovine hematology reference intervals from 1957 to 2006. Vet Clin Pathol 39:138–148.

**Kraft W and U. M Durr. 2005. Klinische Labordiagnostik in der Tiermedizin [Clinical laboratory dianostics in veterinary medicine], 6th ed. Schattauer, Stuttgart, Germany, In German.

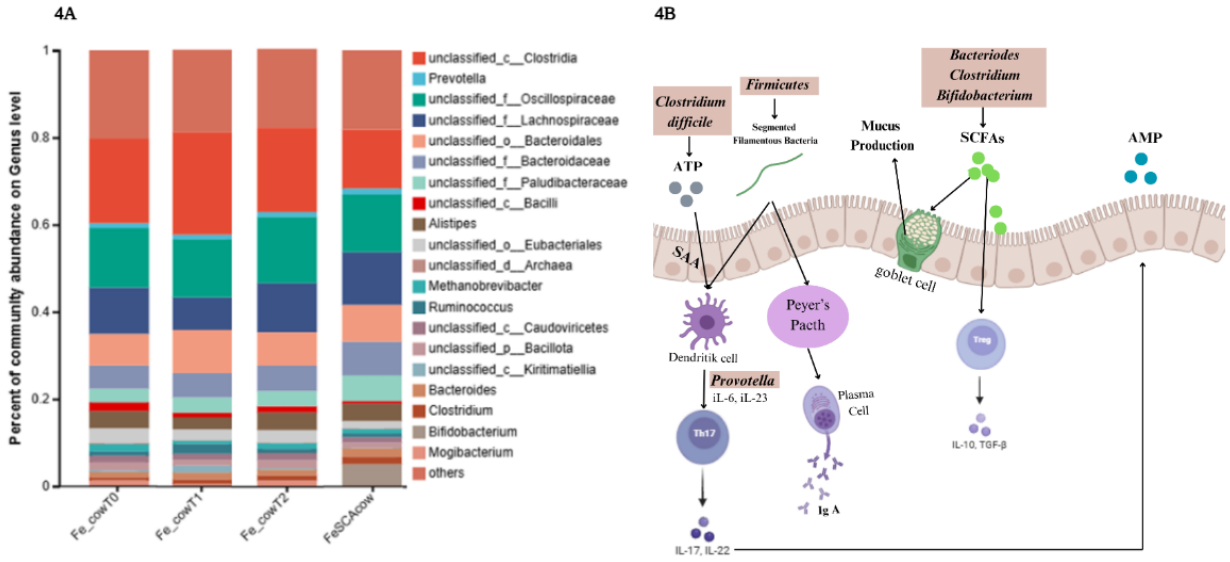

Figure 4A. The Bar Graph Depicts Microbiome Diversity in the Donor (FeSCACow) and Treated Groups T0; T1; T2**.**

Figure 4B. *Prevotella*: Induces Pro-inflammatory Cytokines (IL-6 and IL-23), Leading to Activation of Th17 Cells. *Clostridium difficile*: Produces ATP, which Stimulates the Immune Response and Induces the Production of Serum AmFyloid A (SAA) in Peyer's Patches. *Firmicutes* (Segmented Filamentous Bacteria): Influence the Activation of Immune Cells and Stimulate Immune Cells in Peyer's Patches to Maintain Immune Surveillance and Tolerance, Ensuring Gut Homeostasis with Produce IgA. *Bacteroides, Clostridium, Bifidobacterium:* Produce Short-Chain Fatty Acids (SCFAs) that Play a Regulatory Role in the Immune System and Gut Barrier.

teroides, increase Foxp3 expression through the GPR43 pathways and inhibit HDAC activity, which is important for Treg differentiation (Fusco *et al.,* 2023). Moreover, lactate can significantly affect the lymphocytes. Elevated lactate levels can induce lactate reversal through Monocarboxylate Transporters (MCT), where lactate re-enters the cell and disrupts the glucose metabolism pathway (glycolysis), causing T cells to lose their primary energy source (glucose) to support optimal functioning. (Manosalva et al., 2022).

Monocytes are a type of white blood cell capable of engulfing and defending against pathogens through phagocytosis, as well as producing various cytokines and chemokines to signal the immune system (Hussen and Schuberth, 2017). In the T1 group, monocyte levels were elevated ($0.62 \times 10^3/\mu L$) compared to T2 and T0. This increase suggests that monocytes may contribute to tissue repair, resolution of inflammation, and modulation of immune response. These functions are influenced by the surrounding microenvironment and balance of short-chain fatty acids (SCFAs), which regulate monocyte activity and differentiation (Zheng *et al.,* 2020).

A comparison of lymphocyte and monocyte levels between T0 and T2 revealed significant differences. At T0, the baseline data served as a critical control reference, providing a foundation for assessing the effects of FMT. Zou *et al.* (2019) emphasized the importance of baseline measurements in evaluating the efficacy of FMT in modulating microbiota and improving clinical outcomes. At baseline, the microbiota is likely to be in a stable state, unaffected by stressors, such as infection or inflammation, resulting in a regulated immune response with minimal immune system activation. It is suspected that dominant protective microbes can convert substrates into positive metabolites (SCFA and lactate) that support immunity and inhibit pathogens. Protective microbes compete with pathogens for substrates and living spaces while producing metabolites that inhibit pathogen growth (Ghoul and Mitri, 2016).

SCFA produced by *Bacteroides* play an important role in preserving the integrity of the in-

testinal barrier and in lowering local inflammation (Yoo *et al.,* 2020). Immune cells interact with SCFAs by activating G-protein coupled receptors (GPCRs), such as FFAR2 and FFAR3, on intestinal epithelial cells and immune cells, as well as through inhibition of histone deacetylase (HDAC), which increases the expression of anti-inflammatory genes, including the gene encoding Foxp3, which plays a role in the differentiation of regulatory Treg cells (Ney *et al.,* 2023). *Bifidobacterium* also produces SCFAs that support tight junction formation by promoting epithelial integrity through modulation of immune pathways to maintain a balance between pro- and anti-inflammatory responses (Alessandri *et al.,* 2019). It was previously reported that *Bifidobacterium* enhances intestinal immune function through several mechanisms. These include stimulating intestinal dendritic cells to secrete interleukin-12 (IL-12), elevating plasma levels of interleukin-10 (IL-10) and interferon-gamma (IFN-γ), increasing the IL-4 / IFN-γ ratio within the intestinal mucosa, and promoting the differentiation of thymic T cells into T helper 1 (Th1) cells (Dong *et al.*, 2022). *Similar* to *Bacteroides*, Clostridium produces butyrate, which plays a crucial role in the accumulation of regulatory T cells (Tregs). The formation of Tregs is mediated by transforming growth factor-beta (TGF-β) and interleukin-10 (IL-10) (Guo *et al.,* 2020). In addition, butyrate suppresses the activity of pro-inflammatory cytokines and immune cells, including neutrophils and M1 macrophages, while promoting the activation of anti-inflammatory cells such as regulatory T cells (Tregs) and M2 macrophages (Anshory *et al.*, 2023). In a healthy environment, *Prevotella* microbes support mucosal immunity by optimizing neutrophil recruitment and Th17 response to fight local pathogens (Larsen, 2017). Segmented filamentous bacteria (SFB) have been classified in the phylum *Firmicutes,* belong to the family *Clostridiaceae*, and are found attached to the ileal mucosa (Oemcke *et al.,* 2021). *Clostridium difficile* produces ATP, which binds to the purinergic receptor P2X7 and mediates K+ effusion from dendritic cells. Decreased intracellular K+ levels signal activation of the NLRP3 inflammasome, triggering the production of pro-inflammatory cytokines and increasing the ability of antigen-presenting cells (APCs), including dendritic cells (Figure 4) (Liu *et al.,* 2018).

CONCLUSION

The gut microbial balance in the recipient cows was improved by FMT. Lowland cows experience heat stress in tropical regions, which weakens their immunity over time. The gut microbiota balance, health, and immunity status were improved by FMT administration in healthy donors. Microbial composition under FMT conditions influenced feed fermentation, SCFA production, and inflammation reduction via *Bifidobacterium, Bacteroides,* and *Clostridium.* The modulation of innate and adaptive immunity and intestinal barrier repair is supported by haematological data. The FMT approach shows great promise for coping with the lowered immunity status of dairy cows in tropical regions.

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REFERENCES

Anshory, M., Effendi, R. M. R. A., Kalim, H., Dwiyana, R. F., Suwarsa, O., Nijsten, T. E., ... and Thio, H. B. (2023). Butyrate properties in immune-related diseases: friend or foe?. Fermentation, 9 (3), 205.

Alessandri, G., M. C. Ossiprandi., J. MacSharry., D. van Sinderen and M.Ventura. 2019. Bifidobacterial Dialogue With Its Human Host and Consequent Modulation of the Immune System. Front. Immunol. 10:2348

Alhussien, M. N and A. K. Dang. 2019. Potential roles of neutrophils in maintaining the health and productivity of dairy cows during various physiological and physiopathological conditions: a review. J. 67: 21-38.

Alshareef, S. A. 2024. Metabolic analysis of the CAZy class glycosyltransferases in rhizospheric soil fungiome of the plant

species *Moringa oleifera*. Saudi J Biol Sci. 31(4):103956

Ardiansyah, P., Widiyanto., N. S. Pandupuspitasari and B. W. H. E. Prasetiyono. 2024. Hematology Status of Nellore Crossbreed Through Feed Supplementation Containing Go Pro, Rumen-Protected Nutrients, and Fecal Microbiota Transplantation. J. Animal Production 22(2): 82-87.

Artzi, L., E. A. Bayer and S. Moraïs. 2017. Cellulosomes: bacterial nanomachines for dismantling plant polysaccharides. Nat Rev Microbiol.15(2):83-95.

Asmarasari, S. A., N. Azizah., S. Sutikno., W. Puastuti., A. Amir., L. Praharani., S. Rusdiana., C. Hidayat., A. Hafid., D. A. Kusumaningrum., F. Saputra., C. Talib., A. Herliatika., M. I. Shiddieqy and S. Y. Hayanti. 2023. A review of dairy cattle heat stress mitigation in Indonesia. Veterinary World, EISSN: 2231-0916.

Bagath. 2022. The impact of heat stress on the immune system in dairy cattle: A review. J. Veterinary Sciences. 126 : 94 – 102.

Bains, R. K., S. A. Nasseri., J. F. Wardman and S. G. Withers. 2024. Advances in the understanding and exploitation of carbohydrate-active enzymes. Current Opinion in Chemical Biology 80:102457

Bentley, E. G. 2019. An analysis of the immune compartment within bovine adipose tissue. J. Dev Comp Immunol. 100:103411.

Broberg, L., P. G. Cano., N. Arsic., Y. Popowiych and P. J. Griebel. 2021. Isolation and characterization of eosinophils in bovine blood and small intestine. J. Veterinary Immunology and Immunopathology. 242.

Corthay, A. 2009. How do regulatory T cells work?. Scand J Immunol. 70(4):326-36.

Dong, J., Ping, L., Cao, T., Sun, L., Liu, D., Wang, S., ... and Li, B. (2022). Immunomodulatory effects of the Bifidobacterium longum BL-10 on lipopolysaccharide-induced intestinal mucosal immune injury. Frontiers in Immunology, 13, 947755.

Dachlan, Y. 2019. Immune System. Course Material. Doctoral Program, Faculty of Medicine : Airlangga University.

Dorosz, A., M. Grosicki., J. Dybas., E. Matuszyk., M. Rodewald., T. Meyer., J. Popp., K. Malek and M. Baranska. 2020. Eosinophils and Neutrophils-Molecular Differences Revealed by Spontaneous Raman, CARS and Fluorescence Microscopy. Cells. 9(9):2041.

El-Sayed, A., Aleya, L., and Kamel, M. (2021). The link among microbiota, epigenetics, and disease development. Environmental Science and Pollution Research, 28, 28926-28964.

Fusco, W., Lorenzo, M. B., Cintoni, M., Porcari, S., Rinninella, E., Kaitsas, F., ... and Ianiro, G. (2023). Short-chain fatty-acid-producing bacteria: key components of the human gut microbiota. Nutrients, 15(9), 2211.

Fitria, L and M. Sarto. 2014. Profil Hematologi Tikus (*Rattus norvegicus Berkenhou*t, 1769) Galur Wistar Jantan dan Betina Umur 4, 6, dan 8 Minggu. J. Biogenesis. 2(2) : 94 – 100.

George, J. W., J. Snipes and V. M. Lane. 2010. Comparison of bovine hematology reference intervals from 1957 to 2006. Vet Clin Pathol 39:138–148.

Ghoul, M and S. Mitri. 2016. The Ecology and Evolution of Microbial Competition. Trends Microbiol. 24(10):833-845.

Hussen, J and H. J. Schuberth. 2017. Heterogeneity of Bovine Peripheral Blood Monocytes. Front. Immunol.8:1875.

Jiao, J., J. Wu., C. Zhou., Z. He., Z. Tan and M. Wang. 2024. Ecological niches and assembly dynamics of diverse microbial consortia in the gastrointestine of goat kids, The ISME Journal. 18(1)

Karimi, M., N. Shirsalimi., Z. Hashempour., H. Salehi Omran., E. Sedighi., F. Beigi and M. Mortezazadeh. 2024. Safety and

efficacy of fecal microbiota transplantation (FMT) as a modern adjuvant therapy in various diseases and disorders: a comprehensive literature review. Front. Immunol. 15:1439176.

Khan, F. A., N. S. Pandupuspitasari., C. Huang., W. Negara., B. Ahmed., E. M. Putri., P. Lestari., T. P. Priyatno., A. Prima., V. Restitrisnani., M. Surachman., S. Akhadiarto., IWA. Darmawan., D. S. Wahyuni and H. Herdis. Unlocking gut microbiota potential of dairy cows in varied environmental conditions using shotgun metagenomic approach. BMC Microbiol. 23(1):344.

Kim, C. H. 2018. Immune regulation by microbiome metabolites. Immunology. 154 (2):220-229.

Kraft W and U. M Durr. 2005. Klinische Labordiagnostik in der Tiermedizin [Clinical laboratory dianostics in veterinary medicine], 6th ed. Schattauer, Stuttgart, Germany, In German.

Larsen, J. M. 2017. The immune response to *Prevotella* bacteria in chronic inflammatory disease. Immunology. 151(4):363-374.

Liu X. F, J. H. Shao., Y. T. Liao., L. N.Wang., Y. Jia., P. J. Dong., Z. Z. Liu., D. D. He., C. Li and X. Zhang. 2023. Regulation of short-chain fatty acids in the immune system. Front Immunol.14:1186892.

Liu, Y. H., Y. C. Chang., L. K. Chen., P. A. Su., W. C. Ko., Y. S.Tsai., Y. H. Chen., H. C. Lai., C. Y. Wu., Y. P. Hung and P. J. Tsai. 2018. The ATP-P2X7 Signaling Axis Is an Essential Sentinel for Intracellular Clostridium difficile Pathogen-Induced Inflammasome Activation. Front. Cell. Infect. Microbiol. 8:84.

Lombard, V., R. H. Golaconda., E. Drula., P. M. Coutinho and B. Henrissat. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. PMID: 24270786; PMCID: PMC3965031.

Ma C, Z. Sun., B. Zeng., S. Huang., J. Zhao., Y. Zhang., X. Su., J. Xu., H. Wei and H. Zhang. 2018. Cow-to-mouse fecal transplantations suggest intestinal microbiome as one cause of mastitis. Microbiome 6:200.

Mahmoudi, H and H. Hossainpour. 2023. Application and development of fecal microbiota transplantation in the treatment of gastrointestinal and metabolic diseases: A review. Saudi J Gastroenterol. 29(1):3-11.

Manosalva, C., J. Quiroga., A. I. Hidalgo., P. Alarcón., N. Anseoleaga., M. A. Hidalgo and R. A. Burgos. 2022. Role of Lactate in Inflammatory Processes: Friend or Foe. Front Immunol. 12:808799.

Mu, Y. Y., Qi, W. P., Zhang, T., Zhang, J. Y., and Mao, S. Y. (2021). Gene function adjustment for carbohydrate metabolism and enrichment of rumen microbiota with antibiotic resistance genes during subacute rumen acidosis induced by a high-grain diet in lactating dairy cows. Journal of Dairy Science, 104(2), 2087-2105.

Mariana, E., Sumantri, C., Astuti, D.A., Anggraeni, A. and Gunawan, A. 2019. Thermoregulation, hematological profile and productivity of Holstein–Friesian under heat stress at different land elevations. Bull. Anim. Sci., 43(1): 8–16.

Ney L-M, M. Wipplinger., M. Grossmann., N. Engert., V. D. Wegner and A. S. Mosig. 2023 Short chain fatty acids: key regulators of the local and systemic immune response in inflammatory diseases and infections. Open Biol. 13: 230014

Nogal, A., A. M. Valdes and C. Menni. 2021. The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. Gut Microbes. 13(1):1-24.

NRC. 2001. Nutrient Requirement of Dairy Cattle. 8th Edition. National academic of Science, Washington D. C.

Oemcke, L. A., R. C. Anderson., E. Alter-

mann., N. C. Roy and W. C. McNabb. 2021. The Role of Segmented Filamentous Bacteria in Immune Barrier Maturation of the Small Intestine at Weaning. Front Nutr.18;8:759137.

Olivia, H. M., S. A. Kahn and W. J. Steinbach. 2023. AAP Section on Gastroenteroloy, Hepatology, and Nutrition, Committee on Infectious Diseases. Fecal Microbiota Transplantation: Information for the Pediatrician. Pediatrics. 152 (6):e2023062922.

Portincasa, P., L. Bonfrate, M. Vacca., M. De Angelis, I. Farella, E. Lanza., M. Khalil, D. Q. -H. Wang., M. Sperandio and A. Di Ciaula. 2022. Gut Microbiota and Short Chain Fatty Acids: Implications in Glucose Homeostasis(Review). J. Molecular Sci. 23(3) : 1105.

Reau, L. A. J and G. Suen. 2018. The *Ruminococci*: key symbionts of the gut ecosystem. J Microbiol. 56, 199–208.

Rinninella, E., P. Raoul., M. Cintoni., F. Franceschi., G. A. D. Miggiano., A. Gasbarrini and M. C. Mele. 2019. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. J. Microorganisms. 7, 14.

Rios-Covian, D., S. Arboleya., A. M. Hernandez-Barranco., J. R. Alvarez-Buylla., P. Ruas-Madiedo., M. Gueimonde and C. G. de los Reyes-Gavilan. 2013. Interactions between Bifidobacterium and Bacteroides species in cofermentations are affected by carbon sources, including exopolysaccharides produced by bifidobacteria. Appl Environ Microbiol. 79(23):7518.

Rutkowska, E., I. Kwiecień., A. Raniszewska., R. Sokołowski., J. Bednarek., K. Jahnz-Różyk., A. Chciałowski and P. Rzepecki. 2024. New Neutrophil Parameters in Diseases with Various Inflammatory Processes. Biomedicines. 12(9):2016

Sauls, R.S., C. McCausland and B. N. Taylor. 2023. Histology, T-Cell Lymphocyte. : StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. PMID: 30571054.

Sordillo, L. M. 2016. Nutritional strategies to optimize dairy cattle immunity. J. Dairy Sci.99:4967–4982

Soriani, N., G. Panella., and L. Calamari. 2013. Rumination time during the summer season and its relationships with metabolic conditions and milk production. J. Dairy Sci. 96, 5082–5094.

Soveral, L. F., G. G. Korczaguin., P. S. Schmidt., I. S. Nunes., C. Fernandes and C. R. Zárate-Bladés. 2022. Immunological mechanisms of fecal microbiota transplantation in recurrent *Clostridioides difficile* infection. World J Gastroenterol. 2022 7;28 (33):4762-4772.

Tigner, A., S A. Ibrahim and I. V. Murray. 2022. Histology, White Blood Cell. Treasure Island, FL: StatPearls Publishing.

Tufail, M. A and R. A. Schmitz. 2024. Exploring the Probiotic Potential of *Bacteroides spp*. Within One Health Paradigm. https://doi.org/10.1007/s12602-024-10370-9

Verberkmoes, N. C., A. L. Russell., M. Shah., A. Godzik., M. Rosenquist., J. Halfvarson., M. G. Lefsrud., J. Apajalahti., C. Tysk., R. L. Hettich and J. K. Jansson. 2009. Shotgun metaproteomics of the human distal gut microbiota. ISME J. 3(2):179-89.

Wang, L., Zhang, G., Xu, H., Xin, H., and Zhang, Y. 2019. Metagenomic analyses of microbial and carbohydrate-active enzymes in the rumen of holstein cows fed different forage-to-concentrate ratios. Front. Microbiol. 10, 649.

Wang, M. , Xie, X. , Zhao. S. , Han, W and Zhang, Y. 2022. Global research trends and hotspots of fecal microbiota transplantation: A

bibliometric and visualization study. Front. Microbiol. 13:990800

Wu, Z. H. 2018. Effects of Fecal Microbiota Transplantation on Intestinal Barrier Function and Microbiota Establishment in Calves with Failure of Passive Immune Transfer. [Doctoral's Thesis], China Agricultural University.

Wu, D., K. Zhang., F. A. Khan., Q. Wu., N. S. Pandupuspitasari., Y. Tang., K. Guan., F. Sun and C. Huang. 2023. The emerging era of lactate: A rising star in cellular signaling and its regulatory mechanisms. J. Cellular Biochemistry. 1 –15.

Xu H, M. Liu., J. Cao., X. Li., D. Fan., Y. Xia., X. Lu., J. Li., D. Ju and H. Zhao. 2019. The Dynamic Interplay between the Gut Microbiota and Autoimmune Diseases. J Immunol Res:7546047.

Yoo, J. Y., M. Groer., S. V. O. Dutra., A. Sarkar and D. I. McSkimming. 2020. Gut Microbiota and Immune System Interactions. Microorganisms 8, 1587.

Zhao, S., M. Li., Z. Nan and W. Jiaqi. 2019. Effect of Heat Stress on Bacterial Composition and Metabolism in the Rumen of Lactating Dairy Cows. Animals (Basel). 9(11):925.

Zheng, D., Liwinski, T. and Elinav, E. Interaction between microbiota and immunity in health and disease. Cell Res **30**, 492–506

Zhuang, Y., S. Liu and D. Gao. 2024. The *Bifidobacterium*-dominated fecal microbiome in dairy calves shapes the characteristic growth phenotype of host. J. Biofilms Microbiomes 10:59

Zou, M., Z. Jie., B. Cui., H. Wang., Q. Feng., Y. Zou., X. Zhang., H. Yang., J. Wang., F. Zhang and H. Jia. 2019. Fecal microbiota transplantation results in bacterial straindisplacement in patients with inflammatory bowel diseases. J. Febs Press. 10: 1 https://doi.org/10.1002/2211