Effects of Gastrointestinal Lipopolysaccharide on Dairy Cow Health and Production Performance and Its Prevention and Control Measures: Post-print

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Abstract

Lipopolysaccharide is a major component of the outer membrane of Gram-negative bacteria. Excessively high concentrate proportions or excessively low effective neutral detergent fiber content in dairy cow diets can significantly increase gastrointestinal lipopolysaccharide content. Lipopolysaccharide can act synergistically with gastrointestinal environmental factors such as pH to damage the gastrointestinal epithelium; once damaged, lipopolysaccharide may translocate into the systemic circulation, leading to a series of immune-metabolic responses in dairy cows that subsequently affect their health and production performance. This article reviews the structure and origin of lipopolysaccharide, the impact of gastrointestinal lipopolysaccharide on dairy cow health and production performance, and mitigation strategies, aiming to provide a theoretical basis for safeguarding dairy cow health and improving production performance in modern dairy production.

Full Text

Effects of Gastrointestinal Lipopolysaccharide on Health and Production Performance of Dairy Cows and Prevention Strategies

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Abstract: Lipopolysaccharide (LPS), a primary component of the outer membrane of Gram-negative bacteria, increases significantly in the gastrointestinal tract of dairy cows fed high-concentrate diets or diets with insufficient effective neutral detergent fiber. LPS interacts with gastrointestinal environmental factors such as pH to damage the epithelial lining. Once the gastrointestinal epithelium is compromised, LPS translocates into peripheral circulation, triggering a cascade of immune and metabolic responses that affect cow health and production performance. This review examines the structure and sources of LPS, its effects on dairy cow health and productivity, and mitigation strategies, providing a theoretical basis for safeguarding cow health and improving performance in modern dairy production systems.

Keywords: lipopolysaccharide; dairy cows; inflammatory response; subacute ruminal acidosis; production performance; prevention strategies

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Lipopolysaccharide (LPS), also known as endotoxin, constitutes the main component of the outer membrane of Gram-negative bacteria. Under modern intensive production conditions, diets with excessive concentrate proportions or inadequate effective neutral detergent fiber significantly increase LPS content in the gastrointestinal tract of dairy cows [1-2], leading to structural and functional changes in the gastrointestinal epithelium that impair nutrient absorption and facilitate LPS translocation. When LPS enters peripheral circulation, it induces various immune and metabolic alterations [3], including inflammatory responses and changes in nutrient metabolism. Although immune reactions represent a protective mechanism, prolonged or intense responses compromise animal health and redirect nutrient allocation, with substantial resources diverted to immune functions at the expense of production performance [4]. This review synthesizes current knowledge on LPS structure and sources, the impact of gastrointestinal LPS on dairy cow health and productivity, and strategies for mitigation, aiming to provide theoretical guidance for maintaining cow health and enhancing production efficiency in modern dairy operations.

1.1 LPS Structure

LPS is ubiquitous in the outer membrane of Gram-negative bacteria. While the molecular weight of LPS from different sources ranges from several thousand to tens of thousands, the molecule consistently comprises three distinct regions (Figure [Figure 1: see original paper]). The first region is the O-specific polysaccharide antigen, which exhibits high specificity compared to the other two components and extends outward from the membrane surface as repeating oligosaccharide units. Based on the presence of this O-antigen, LPS can be classified into smooth and rough types [5], with smooth-type LPS demonstrating greater toxicity than rough-type LPS. The second region is the core polysaccharide, composed of heptose, galactose, ketodeoxyoctanoic acid, and other components, and consists of inner and outer core sections. The outer

core connects to the O-antigen, while the inner core links to lipid A. The third region, lipid A, represents the most conserved portion of the LPS molecule and serves as its toxic center, possessing the complete toxic activity of LPS. Its basic structure is illustrated in Figure [Figure 2: see original paper].

1.2 LPS Function and Sources in Dairy Cows

LPS functions primarily as a permeability barrier in the outer membrane of Gram-negative bacteria. The molecular structure of LPS strictly regulates membrane permeability, allowing only small, hydrophilic molecules to pass through. This selective permeability prevents bile salts, lysozyme, and antimicrobial substances in the digestive tract from penetrating the bacterial interior, thereby maintaining bacterial structural integrity [8]. According to Andersen [6], Gram-negative bacteria release LPS during both rapid growth and lysis, with up to 60% of LPS released during active growth phases. When cows consume high-starch diets, microorganisms including Gram-negative bacteria enter a period of rapid proliferation. Newly synthesized proteins become embedded in the outer membrane, causing expansion or turnover of the cell wall in these regions and resulting in LPS shedding into the gastrointestinal tract. Additionally, when diets contain excessive concentrate or insufficient effective neutral detergent fiber, the highly acidic ruminal environment causes massive lysis of Gram-negative bacteria, releasing substantial amounts of LPS into the gastrointestinal tract. This toxic LPS, upon translocating into peripheral circulation, triggers a series of immune and metabolic changes in the host.

2. Effects of Gastrointestinal LPS on Dairy Cow Health and Production Performance

Feeding dairy cows diets with excessive concentrate proportions or inadequate effective neutral detergent fiber accelerates fermentation rates, leading to accumulation of volatile fatty acids (VFAs) and other organic acids. Concurrently, reduced rumination activity decreases saliva secretion, causing rapid pH decline in the gastrointestinal tract and massive lysis of Gram-negative bacteria with subsequent LPS release. Gastrointestinal LPS can act synergistically with low pH to directly damage epithelial structure and function, impairing nutrient absorption, or translocate across the epithelium into peripheral circulation to affect immune and metabolic functions.

2.1 Exacerbation of Subacute Rumen Acidosis (SARA)

Diets high in concentrate or low in effective neutral detergent fiber induce subacute ruminal acidosis (SARA) and significantly elevate LPS content in the gastrointestinal tract [1-2]. Emmanuel et al. [3] demonstrated that high LPS concentrations combined with low pH damage rumen epithelium, while Penner et al. [9] found that SARA-induced rumen epithelial damage and cell cycle alterations cause parakeratosis or hyperkeratosis, reducing VFA absorption and

accelerating VFA accumulation. Parakeratosis facilitates bacterial and LPS access to the outer epithelial layer, causing rumenitis and abscess formation [3], which further impairs VFA absorption. Accumulation of VFAs increases propionate and butyrate concentrations, which promote epithelial cell proliferation in rumen papillae [10]. However, excessive proliferation leads to parakeratosis, severely compromising VFA absorption efficiency and further exacerbating VFA accumulation [11], explaining why SARA conditions tend to persist and worsen.

2.2 Induction of Inflammatory Responses

Barrier function constitutes an essential component of innate immunity in the body' s long-term defense system. The gastrointestinal epithelium serves as a selective barrier that normally prevents bacteria and pathogens from passing through. When this barrier is compromised, LPS permeability increases, allowing translocation into peripheral circulation. LPS crosses the gastrointestinal barrier through two primary pathways: paracellular transport between epithelial cells and transcellular transport via receptor-mediated endocytosis. However, numerous studies have failed to detect LPS in peripheral blood [12-14], possibly because the liver serves as an effective barrier that detoxifies enteric bacterial LPS before it reaches systemic circulation [15]. Bode et al. [16] reported that the primary cytokine receptors reside in hepatic Kupffer cells, suggesting that initial cytokine formation may occur before hepatic detoxification. When LPS penetrates the gastrointestinal epithelium or enters peripheral blood, leukocytes recognize it and activate immune responses that trigger pro-inflammatory cytokine secretion [17]. The CD14-toll-like receptor 4 (TLR4)-myeloid differentiation protein-2 (MD2) pathway represents the most common LPS recognition mechanism, as illustrated in Figure [Figure 3: see original paper].

LPS-binding protein (LBP) exhibits high affinity for LPS and, upon binding, is recognized by the CD14 receptor on cell surfaces [18]. CD14 is a glycosylphosphatidylinositol-anchored membrane protein lacking a transmembrane domain, requiring TLR4 for signal transduction across the membrane [19]. TLR4 must associate with MD2 to activate intracellular pathways [20]. Once these pathways are activated, signals are transmitted to the nucleus, initiating synthesis of pro-inflammatory cytokines that disseminate signals to various tissues and organs, triggering downstream inflammatory responses.

2.3 Reduced Lactation Performance

LPS translocation into peripheral circulation activates immune responses and alters metabolism, causing nutrient repartitioning. Substantial nutrients are diverted to immune functions, reducing the amount available for milk component synthesis in the mammary gland, thereby decreasing milk yield and altering milk composition. As blood LPS concentrations increase, more LPS enters the mammary gland, intensifying local immune activity and further reducing nutrients available for milk synthesis. Khafipour et al. [22] observed that following SARA onset, milk protein percentage increased from 3.29% to 3.42% as rumi-

nal LPS content rose, while milk fat synthesis was suppressed. However, milk protein yield did not increase, indicating that the reduction in milk yield exceeded the reduction in milk protein yield. Zhang [23] infused LPS into the external pudendal artery of dairy cows and found that while milk protein percentage increased significantly, yields of milk protein and casein decreased to varying degrees (though not significantly), suggesting that LPS reduced milk yield more than protein yield and may decrease milk protein and casein synthesis by affecting gene expression pathways. Baldi et al. [24] reported that LPS stimulates mammary epithelial cells to release plasminogen activators, which enter the cistern and milk, enhancing casein hydrolysis by activating plasminogen. Zebeli et al. [13] used high-concentrate diets to induce SARA and found that rumen-derived LPS affected milk fat yield and energy utilization efficiency. López-Soriano et al. [25] proposed that LPS influences milk fat synthesis by regulating key enzymes such as fatty acid synthase in the mammary gland. Zu et al. [26] found that LPS promotes lipid hydrolysis while inhibiting milk fat synthesis. These findings demonstrate that immune and metabolic changes induced by gastrointestinal LPS translocation reduce milk yield and alter milk component yields and proportions.

2.4 Reduced Reproductive Performance

During the estrous cycle, the hypothalamus releases gonadotropin-releasing hormone to increase secretion of gonadotropins, primarily luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH act synergistically on the ovary to promote follicular growth and development and stimulate estradiol (E2) secretion. E2 and FSH cooperate to promote LH and FSH receptor synthesis in granulosa cells, enhancing hormone binding to the ovary and accelerating follicular growth [27]. Elevated LPS concentrations suppress LH release and pre-ovulatory E2 increases. Battaglia et al. [28] observed that intravenous LPS injection in ewes significantly reduced the rate of increase in pre-ovulatory E2, LH, and FSH while increasing progesterone concentrations. Lavon et al. [29] reported that LPS administration to dairy cows decreased or delayed the LH surge. Research indicates that nitric oxide (NO) positively correlates with LPS concentration, and NO-activated guanylate cyclase can downregulate aromatase in bovine granulosa cells, thereby inhibiting E2 production [30]. Consequently, increased LPS reduces E2, LH, and FSH secretion, delaying follicular development.

When gastrointestinal LPS translocates into peripheral circulation and acts on macrophages, it triggers release of cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- (TNF-). IL-1 acts on the thermoregulatory center, causing fever and disrupting follicular development. During follicular maturation, LPS and TNF- compromise oocyte quality, and LPS significantly increases polyspermy rates while reducing oocyte quality [31]. Helen et al. [32] found that high LPS concentrations can cause abortion in dairy cows, primarily by affecting embryo stability.

3. Strategies to Mitigate Gastrointestinal LPS Effects on Dairy Cow Health and Production

High gastrointestinal LPS concentrations act synergistically with low pH to directly impair nutrient absorption by the gastrointestinal epithelium or translocate into peripheral circulation to trigger inflammatory responses and affect production performance. Both pathways require LPS to reach certain concentrations. Therefore, reducing gastrointestinal LPS content and minimizing translocation can mitigate its detrimental effects on dairy cow health and productivity.

3.1 Rational Diet Formulation

Since gastrointestinal LPS can translocate into peripheral circulation and affect cow health and performance, causing substantial economic losses, dietary strategies to reduce LPS production are essential. Numerous studies have shown that compared to low-concentrate hay diets, high-concentrate or alfalfa pellet diets significantly increase ruminal LPS content and decrease ruminal pH [1-2,14,33-34]. Plaizier et al. [33] found that sustained feeding of high-concentrate diets significantly increased LPS content in ileal and cecal digesta. Zebeli et al. [34] demonstrated that high-concentrate diets elevated LPS concentrations in cecal digesta and feces, whereas alfalfa pellet diets had no such effect. These findings indicate that diet composition affects LPS content and pH in both the rumen and hindgut. Appropriately reducing dietary concentrate proportion and increasing effective neutral detergent fiber can slow fermentation rates, reduce accumulation of VFAs and other organic acids, increase pH, and decrease gastrointestinal LPS content. Additionally, increased hay proportion and particle length enhance rumination and saliva secretion, raising ruminal pH and reducing LPS release. Iqbal et al. [35] used lactic acid-treated concentrate to slow starch fermentation, which significantly increased ruminal pH and reduced ruminal LPS concentration.

3.2 Probiotic Supplementation

The gastrointestinal epithelial barrier, as a component of innate immunity, plays a crucial role in long-term defense. Under normal physiological conditions, the intestinal barrier effectively prevents LPS translocation into peripheral circulation. However, epithelial damage enables LPS translocation, triggering immune and metabolic changes. Therefore, improving gastrointestinal epithelial health to block LPS translocation can mitigate LPS-induced damage. Research indicates that increased epithelial cell apoptosis and downregulated tight junction protein expression are primary causes of barrier dysfunction [36]. Mennigen et al. [36] demonstrated in mice that the probiotic mixture VSL#3 maintained tight junction protein expression and reduced epithelial cell apoptosis, thereby protecting barrier function. Studies have also shown that probiotics can adhere to gastrointestinal mucosal surfaces, preventing pathogen contact and enhancing protective mucosal effects [37]. In modern dairy production, appropriate probi-

otic supplementation can strengthen gastrointestinal epithelial barrier function, reduce LPS translocation, and safeguard cow health.

3.3 Stress Reduction

High ambient temperatures during summer induce heat stress in dairy cows. To increase heat dissipation, ruminants divert more blood to subcutaneous vessels, reducing splanchnic blood supply. Additionally, excessive sweating at high temperatures causes water and salt loss, further compromising visceral blood flow. During visceral ischemia, intestinal hypoxanthine cannot be further metabolized and is instead oxidized by accumulated xanthine oxidase, generating reactive oxygen species that damage intestinal epithelial cells and tight junctions, compromising barrier function and increasing LPS permeability [31]. Goff [38] reported that dietary changes cause metabolic and oxidative stress, particularly during postpartum negative energy balance, and that metabolic stress, oxidative stress, and intestinal inflammation increase ruminal LPS permeability through altered tight junctions [39]. Therefore, adequate ventilation should be maintained during high summer temperatures to prevent heat stress, and frequent dietary changes should be avoided to minimize metabolic and oxidative stress. Proper management practices that reduce stress responses can decrease LPS translocation.

Conclusion

Extensive research has documented increased gastrointestinal and systemic LPS concentrations in dairy cows due to dietary and environmental factors, as well as the associated impacts on health and production performance. However, further investigation is needed to develop effective strategies for preventing LPS-induced damage. In modern intensive dairy production, excessive dietary concentrate or insufficient effective neutral detergent fiber decreases gastrointestinal pH and increases LPS concentrations. The synergistic action of low pH and high LPS disrupts gastrointestinal epithelial barrier function, promoting LPS translocation and triggering immune-metabolic changes. Systemic LPS induces inflammatory responses, exacerbates SARA, reduces energy utilization efficiency, and redirects nutrients, impairing lactation performance. LPS translocation delays follicular development by reducing E2, LH, and FSH secretion, compromises oocyte quality through cytokine production, and can cause abortion, severely affecting reproductive performance. Nutritional and management strategies—including controlling dietary ingredient physical form and concentrate-to-forage ratios, feeding appropriate probiotics, and minimizing environmental stress—can enhance gastrointestinal epithelial health and reduce LPS translocation frequency, thereby safeguarding cow health and improving production performance.

References

[1] RODRÍGUEZ-LECOMPTE J C, KROEKER A D, CEBALLOS-MÁRQUEZ A, et al. Evaluation of the systemic innate immune response and metabolic alterations of nonlactating cows with diet-induced subacute ruminal acidosis[J]. Journal of Dairy Science, 2014, 97(12): 7777–7787.

[2] LI S, KHAFIPOUR E, KRAUSE D O, et al. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows[J]. Journal of Dairy Science, 2012, 95(1): 294–303.

[3] EMMANUEL D G V, MADSEN K L, CHURCHILL T A, et al. Acidosis and Lipopolysaccharide from Escherichia coli B:055 cause hyperpermeability of rumen and colon tissues[J]. Journal of Dairy Science, 2007, 90(12): 5552–5557.

[4] AMETAJ B N, ZEBELI Q, IQBAL S. Nutrition, microbiota, and endotoxin-related diseases in dairy cows[J]. Revista Brasileira De Zootecnia, 2010, 39(S1): 433–444.

[5] RIETSCHEL T, SCHADE U, JENSEN M, et al. Bacterial endotoxins: chemical structure, biological activity and role in septicaemia[J]. Scandinavian Journal of Infectious Diseases Supplementum, 1982, 31: 8–21.

[6] ANDERSEN P H. Bovine endotoxicosis-some aspects of relevance to production diseases. A Review[J]. Acta Veterinaria Scandinavica, 2003, 98: 141-155.

[7] 王镜岩, 朱圣庚, 徐长法. 生物化学: 上册 [M]. 北京: 高等教育出版社, 2002.

[8] RIETSCHEL E T, KIRIKAE T, SCHADE F U, et al. Bacterial endotoxin: molecular relationships of structure to activity and function[J]. The FASEB Journal, 1994, 8(2): 217–225.

[9] PENNER G B, STEELE M A, ASCHENBACH J R, et al. Ruminant Nutrition symposium: molecular adaptation of ruminal epithelia to highly fermentable diets[J]. Journal of Animal Science, 2010, 89(4): 1108–1119.

[10] GARRY F B, MCCONNEL C. Indigestion in ruminants[M]. 2008: 829–830.

[11] STEELE M A, CROOM J, KAHLER M, et al. Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis[J]. American Journal of Physiology, 2011, 300(6): R1515–R1523.

[12] GOZHO G N, PLAIZIER J C, KRAUSE D O, et al. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response[J]. Journal of Dairy Science, 2005, 88(4): 1399–1403.

[13] ZEBELI Q, AMETAJ B N. Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows[J]. Journal of Dairy Science, 2009, 92(8): 3800–3809.

[14] ZHOU J, DONG G Z, AO C J, et al. Feeding a high-concentrate corn straw diet increased the release of endotoxin in the rumen and pro-inflammatory

cytokines in the mammary gland of dairy cows[J]. BMC Veterinary Research, 2014, 10(1): 172.

[15] ANDERSEN H. Bovine endotoxicosis: aspects relevance ruminal acidosis[D]. Ph.D. Thesis. Copenhagen, Denmark: The Royal Veterinary and Agricultural University, 2000.

[16] BODE J G, HEINRICH P C. Interleukin-6 signaling during the acute-phase response of the liver[C]//ARIAS I, WOLKOFF A, BOYER J, et al. The liver: biology and pathobiology. 4th ed. Philadelphia: Lippincott Williams Wilkins, 2001: 565-580.

[17] DA SILVA CORREIA J, SOLDAU K, CHRISTEN U, et al. Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. Transfer from CD14 to TLR4 and MD-2[J]. The Journal of Biological Chemistry, 2001, 276(24): 21129-21135.

[18] HAILMAN E, LICHENSTEIN H S, WURFEL M M, et al. Lipopolysaccharide (LPS)-binding protein accelerates binding of LPS to CD14[J]. Journal of Experimental Medicine, 1994, 179(1): 269-277.

[19] HOSHINO K, TAKEUCHI O, KAWAI T, et al. Cutting edge: toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product[J]. Journal of Immunology, 1999, 162(7): 3749-3752.

[20] SAUTER K S, BRCIC M, FRANCHINI M, et al. Stable transduction of bovine TLR4 and bovine MD-2 into LPS-nonresponsive cells and soluble CD14 promote the ability to respond to LPS[J]. Veterinary Immunology and Immunopathology, 2007, 118(1/2): 92-104.

[21] YAMAMOTO M, SATO S, HEMMI H, et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway[J]. Science, 2003, 301(5633): 640-643.

[22] KHAFIPOUR E, KRAUSE D O, PLAIZIER J C. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation[J]. Journal of Dairy Science, 2009, 92(3): 1060-1070.

[23] 张养东. 脂多糖对泌乳奶牛乳脂肪和乳蛋白影响及其机理研究 [D]. 博士学位论文. 哈尔滨: 东北农业大学, 2011.

[24] BALDI A, PECORINI C, REBUCCI R, et al. Effect of Escherichia coli lipopolysaccharide on u-PA activity and u-PA and u-PAR RNA expression in a bovine mammary epithelial cell line[J]. Research in Veterinary Science, 2012, 93(2): 758-762.

[25] LÓPEZ-SORIANO F J, WILLIAMSON D H. Acute effects of endotoxin (lipopolysaccharide) on tissue lipid metabolism in the lactating rat. The role of delivery of intestinal glucose[J]. Molecular and Cellular Biochemistry, 1994, 141(2): 113-120.

[26] ZU L X, HE J H, JIANG H F, et al. Bacterial endotoxin stimulates adipose lipolysis via toll-like receptor 4 and extracellular signal-regulated kinase pathway[J]. Journal of Biological Chemistry, 2009, 284(9): 5915-5926.

[27] GREEN M P, LEDGARD A M, BEAUMONT S E, et al. Long-term alteration of follicular steroid concentrations in relation to subclinical endometritis in postpartum dairy cows[J]. Journal of Animal Science, 2011, 89(11): 3551-3560.

[28] BATTAGLIA D F, KRASA H B, PADMANABHAN V, et al. Endocrine alterations that underlie endotoxin-induced disruption of the follicular phase in ewes[J]. Biology of Reproduction, 2010, 62(1): 45-53.

[29] LAVON Y, LEITNER G, GOSHEN T, et al. Exposure to endotoxin during estrus alters the timing of ovulation and hormonal concentrations in cows[J]. Theriogenology, 2008, 70(6): 956-967.

[30] LI D J, LIU Y F, LI Y F, et al. Significance of nitric oxide concentration in plasma and uterine secretes with puerperal endometritis dairy cows[J]. Veterinary Research Communications, 2010, 34(4): 315-321.

[31] 原利荣, 董国忠, 史自涛, 等. 内毒素对奶牛繁殖性能的影响及其机制 [J]. 动物营养学报, 2014, 26(1): 43-48.

[32] LABURN H P, FAURIE A, MITCHELL D. The fetus and fever[J]. Journal of Thermal Biology, 2003, 28(2): 107-116.

[33] PLAIZIER J C, LI S, LE SCIELLOUR M, et al. Effects of duration of moderate increases in grain feeding on endotoxins in the digestive tract and acute phase proteins in peripheral blood of yearling calves[J]. Journal of Dairy Science, 2014, 97(11): 7076-7084.

[34] ZEBELI Q, DUNN S M, AMETAJ B N. Strong associations among rumen endotoxin and acute phase proteins with plasma minerals in lactating cows fed graded amounts of concentrate[J]. Journal of Animal Science, 2009, 88(4): 1545-1553.

[35] IQBAL S, ZEBELI Q, MAZZOLARI A, et al. Feeding rolled barley grain steeped in lactic acid modulated energy status and innate immunity in dairy cows[J]. Journal of Dairy Science, 2010, 93(11): 5147-5156.

[36] MENNIGEN R, NOLTE K, RIJCKEN E, et al. Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis[J]. American Journal of Physiology: Gastrointestinal and Liver Physiology, 2009, 296(5): G1140-G1149.

[37] MENNIGEN R, BRUEWER M. Effect of probiotics on intestinal barrier function[J]. Annals of the New York Academy of Sciences, 2009, 1165(1): 183-189.

[38] GOFF J P. Major advances in our understanding of nutritional influences on bovine health[J]. Journal of Dairy Science, 2006, 89(4): 1292-1301.

[39] MANI V, WEBER T E, BAUMGARD L H, et al. Growth and development symposium: endotoxin, inflammation, and intestinal function in livestock[J]. Journal of Animal Science, 2012, 90(5): 1452-1465.

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