Effects of Weaning Stress on the Immune System of Young Ruminants and Its Mechanisms: Post-print

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Abstract

During the early weaning period, the digestive and immune systems of young ruminants remain incompletely developed. Weaning stress can induce alterations in hormone levels and immune function, resulting in immune suppression, triggering inflammatory responses, impeding growth and development, and elevating disease susceptibility. This article elucidates the effects of weaning stress on the immune system of young ruminants and its underlying mechanisms from four perspectives: glucocorticoids, immune cells, acute phase proteins, and related cytokines, with the aim of providing a scientific foundation for future research.

Full Text

Effects and Mechanism of Weaning Stress on the Immune System in Young Ruminants

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Abstract: At weaning, the digestive and immune systems of young ruminants remain underdeveloped. Weaning stress triggers hormonal and immunological changes that suppress immune function, induce inflammatory responses, impede growth, and increase disease susceptibility. This review examines the effects and underlying mechanisms of weaning stress on the immune system of young ruminants, focusing on glucocorticoids, immune cells, acute phase proteins, and related cytokines to provide a scientific foundation for future research.

Keywords: young ruminants; weaning stress; immune system; glucocorticoid; cytokine

Introduction

Weaning in young ruminants involves disruption of maternal-offspring bonds, changes in feed composition and physical form, social restructuring, and environmental shifts—all of which induce psychological, physiological, and immunological stress. Behavioral changes include increased vocalization, restlessness, walking frequency, and reduced feeding and rumination time. Because neurohumoral regulation is not yet fully developed in early life, homeostasis is easily disrupted and slow to recover, leading to abnormal heart rate and blood pressure, elevated rectal temperature, and potentially impaired growth. Weaning stress affects both innate and adaptive immunity, altering hormone levels and immune function that result in autoimmune dysregulation. Moreover, weaning stress correlates with increased incidence and severity of respiratory disease. While numerous studies have addressed weaning stress in ruminants, most have focused on production performance, gastrointestinal development, and microbial communities, with limited investigation of immune function, particularly at the molecular level. This review synthesizes current knowledge on how weaning stress impacts the immune system of young ruminants and explores underlying mechanisms to clarify immune regulation during weaning and provide theoretical guidance for mitigation strategies.

1. Effects of Weaning Stress on Immune Function

1.1 Impairment of Acquired Immune Barrier Function During weaning, the immune system of young ruminants remains immature, with innate immunity predominating before adaptive immunity is fully established. After weaning, animals lose access to maternal immunoglobulins and enzymes such as glutathione peroxidase and lysozyme from milk, compromising acquired immune barrier function and increasing susceptibility to respiratory disease. Notably, approximately 44% of calf mortality is associated with respiratory disease occurring at weaning. Therefore, enhancing innate immune function during this critical period is key to reducing disease risk.

1.2 Damage to Immune System Function Weaning induces acute immune stress and physiological changes that may progress to chronic stress, both of which damage immune function and compromise animal health [Figure 1: see original paper]. Significant alterations occur in blood lymphocyte, neutrophil, erythrocyte, and platelet counts, though monocyte changes are rarely reported. O' Loughlin et al. found that lymphocyte counts in calves decreased from 7.2$×10^{3}$ cells/ L pre-weaning to 6.8$×10^{3}$ cells/ L at 2 days

post-weaning, continuing to decline to 6.5$×10^{3}$ cells/ L by day 11 without recovery. Neutrophil counts rose from 2.3$×10^{3}$ cells/ L pre-weaning to 3.5$×10^{3}$ cells/ L at 24 hours post-weaning, remaining elevated for 7 days before returning to baseline by day 11. Erythrocyte counts dropped from 10.6$×10^{6}$ cells/ L to 6.6$×10^{6}$ cells/ L by day 11, while platelet counts fell from 815.9$×10^{6}$ cells/ L to 495.6$×10^{6}$ cells/ L. Weaning stress also alters glucocorticoid levels, affecting cytokine secretion. Expression of IL-1, IL-8, IFN-γ, TNF-α, TLR4, glucocorticoid receptor α (GRα), and apoptosis factors (e.g., Fas) increased significantly, triggering systemic inflammation. Elevated glucocorticoids suppress immunity and increase disease risk; GR expression tripled within 24 hours post-weaning and remained more than double baseline levels throughout the study period, indicating that weaning stress both induces glucocorticoid secretion and upregulates receptor expression. While normal glucocorticoid levels have limited effects on T lymphocytes and $CD4^+/CD8^+$ T cell regulation, elevated levels inhibit leukocyte function and induce premature apoptosis of immature T and B lymphocytes, causing thymic atrophy—a phenomenon observed in both mice and calves.

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2. Mechanisms of Weaning Stress on Immune Regulation

2.1 Glucocorticoid-Mediated Immune Suppression

2.1.1 Effects on Cytokine Secretion Weaning stress activates the hypothalamic-pituitary-adrenal (HPA) axis through internal and external sensory inputs, stimulating sympathetic nervous system activity. The hypothalamus releases corticotropin-releasing hormone (CRH) and vasopressin (VP), which synergistically regulate glucocorticoid secretion from the adrenal cortex. Glucocorticoids then modulate immune function by binding to GR [Figure 2: see original paper]. Research shows that weaning stress suppresses NF- B activity in bovine cells via glucocorticoids, inhibiting expression of target genes including IL-1, IL-8, TNF-α, and IFN-γ, thereby impairing inflammatory responses. Following glucocorticoid treatment, the stress-induced elevation of pro-inflammatory cytokines IL-1, IL-6, TNF-α, and IFN-γ is delayed by 30–120 minutes, while IFN-γ signaling is suppressed for minutes to hours. Similar results demonstrate that glucocorticoids reduce inflammatory cytokine secretion to attenuate inflammation while simultaneously suppressing immunity.

2.2.2 Effects on Neutrophil and Lymphocyte Counts Weaning stress causes significant lymphopenia and neutrophilia [3,13–20], with these leukocyte changes serving as strong evidence of weaning-induced inflammation [41]. Lymphocyte reduction occurs because glucocorticoids inhibit thymocyte maturation and differentiation while inducing premature apoptosis [26]; additionally, lymphocytes exit circulation to infiltrate inflamed or infected tissues. Conversely,

when glucocorticoids suppress expression of the adhesion molecule L-selectin (CD62L), neutrophil margination weakens, preventing adhesion to vascular walls and migration into inflammatory sites [42], resulting in elevated blood neutrophil counts. Inflammation also stimulates stem cell differentiation in bone marrow, releasing mature neutrophils into circulation [43]. Neutrophil counts typically return to pre-weaning levels within 7–14 days [13,20], likely reflecting the immune system' s self-regulatory capacity to control inflammation and prevent excessive tissue damage. Overall, animals undergo an adaptation process to weaning stress during which immune impacts are unavoidable.

2.2.3 Regulation of Monocyte Count Stability During weaning-induced inflammation, monocytes initiate immune responses by non-specifically phagocytosing pathogens and secreting various cytokines that broadly regulate immunity [42,44]. Monocytes also participate in antigen processing and presentation to T cells, with surface adhesion molecules binding co-stimulatory receptors on T cells to generate activation signals. However, because ruminant monocytes lack receptors sensitive to stress hormones [45] and exit circulation within 36–48 hours after bone marrow release to enter peripheral tissues, post-weaning monocyte changes are difficult to detect, leading most studies to overlook monocyte function. It remains unclear whether weaning-induced immune responses contain factors that inhibit monocyte differentiation and proliferation; future research should investigate relevant signaling pathways to address this question.

2.3 Immune Responses Involving Erythrocytes and Platelets Erythrocytes and platelets serve as sensitive indicators of pathophysiological responses and can identify subclinical disease in ruminants [45]. Both cell types exhibit "immune adherence," where antigen-antibody complexes bind complement receptors on their surfaces before macrophage phagocytosis, likely explaining the observed reductions in erythrocyte and platelet counts post-weaning. Erythrocytes also present antigens, enhance T cell activity, and possess peroxidase activity for direct pathogen killing along with some phagocytic capacity [46]. Platelets regulate immune responses and inflammation [47–49]; when immune complexes adhere to platelet surfaces, platelets undergo functional changes, extend dendrites to vascular walls, and undergo adhesive deformation, ultimately causing inflammatory vascular injury [50].

2.4 Abnormal Plasma Glucose and Insulin Concentrations Some studies report significantly elevated plasma glucose in calves post-weaning, likely due to increased catecholamine and glucocorticoid secretion promoting hepatic glycogenolysis [51]. However, another study found glucose rose from 3.2 mmol/L pre-weaning to 3.7 mmol/L at 2 days post-weaning and remained elevated throughout a 35-day trial without significant cortisol changes [16], suggesting insulin resistance (IR) [52]. Weaning-induced inflammation reduces insulin sensitivity in target organs, causing pancreatic compensatory hyperplasia and excessive insulin secretion. Persistent inflammation increases resistance, eventu-

ally decreasing insulin secretion and raising glucose concentrations [53]. Additionally, reduced post-weaning feed intake increases fat mobilization, lowering plasma triglycerides (35.0 vs 18.3 mg/dL) while elevating β-hydroxybutyrate (0.29 vs 0.39 mmol/L) and non-esterified fatty acids, increasing ketosis risk [54] and directly affecting growth and production performance [55]. Monitoring plasma glucose and insulin can guide nutritional strategies to alleviate weaning stress.

2.5 Effects on Acute Phase Proteins Acute phase proteins (enzymes, protease inhibitors, coagulation proteins, fibrinogen, transport proteins) regulate immunity by activating macrophages and participating in tissue repair [56–58]. Normally stable, their secretion increases dramatically during weaning stress as pro-inflammatory cytokines (IL-1, TNF-α, IL-6) stimulate hepatic production, reducing body protein deposition and indirectly affecting growth [56–58]. While acute phase proteins have been proposed as immune stress indicators [60,61], lack of standardized criteria and potential confounding from liver development or damage limit their utility. The most studied proteins in ruminants are haptoglobin and fibrinogen [13–14,16–19,60]: fibrinogen rises from 408 mg/dL pre-weaning to 458 mg/dL at 2 days and 493 mg/dL at 21 days, while haptoglobin increases from 0.33 mg/dL to 0.43 mg/dL at 2 days and 0.72 mg/dL at 14 days, both remaining elevated throughout 35-day trials [16]. However, the linkage mechanisms between acute phase proteins and stress responses remain unclear [58]; future research should classify these proteins and identify the immune signaling pathways initiating their expression.

2.6 Cytokine Responses to Weaning Stress

2.6.1 IFN-γ IFN-γ exhibits antiviral and immunomodulatory functions. Its expression increases nearly threefold within 24 hours post-weaning and remains elevated, enhancing cell-mediated inflammatory responses [20]. IFN-γ antagonizes IL-4, with each cytokine regulating T helper cell differentiation; upregulation of one reduces secretion of the other [62]. IFN-γ enhances innate cellular immunity, activates neutrophils and monocytes, and stimulates $CD4^+$ T cell differentiation toward Th1 cells while suppressing Th2-derived IL-4 secretion [63], thereby exacerbating inflammation and compromising health. Limited research on IFN-γ in young ruminant weaning stress represents a promising future direction.

2.6.2 Interleukins Interleukins play crucial roles in weaning-induced inflammation and immune regulation. IL-8 is a key neutrophil chemoattractant, with expression doubling within 24 hours post-weaning [20], helping explain neutrophilia. IL-8 elevation first appears in macrophages at inflammatory sites, suggesting inflammation may begin earlier than 24 hours post-weaning [64]. Future studies should examine cytokine changes at earlier time points.

2.6.3 Tumor Necrosis Factor and Apoptosis Factors Weaning stress upregulates TNF-α and Fas expression. TNF-α increases significantly within 24 hours, while Fas expression rises nearly fourfold [20], with both remaining elevated longer than anticipated. TNF-α mediates acute inflammatory responses and plays a key role in weaning-induced inflammation [65]. It binds target cell receptors to form TNF-R trimers, inducing death domain formation, binding death domain proteins, and activating caspases to promote apoptosis. Fas similarly regulates apoptosis; membrane Fas protein binding to its ligand triggers premature cell death, inflammation, and disease. IFN-γ and TNF-α upregulation enhances Fas expression across multiple cell types [66], while increased Fas promotes secretion of other pro-inflammatory cytokines [67]. Additionally, cell cycle negative regulator P21 is upregulated during stress-induced inflammation [68–70], participating in apoptosis through cell cycle arrest and Fas signaling pathway activation in T lymphocytes [71–72]. Accelerated apoptosis during weaning stress may represent an immune response to physiological disruption, though its potential harm to animal health and effects on growth or mortality require further investigation.

2.6.4 Toll-like Receptor Family Toll-like receptors (TLRs) are crucial proteins linking innate and adaptive immunity, expressed in monocytes, T cells, B cells, and NK cells [73]. TLR4 recognizes lipopolysaccharide and heat shock proteins from necrotic cells, playing key regulatory roles in antigen presentation and antibody recognition. TLR4 expression doubles by 7 days post-weaning [20], and chronic stress also significantly upregulates TLR4 [75–76]. TLR4 activation upregulates multiple pro-inflammatory cytokines [77], potentially causing severe inflammation and chronic disease [78] while contributing to non-infectious inflammatory conditions [79]. TLR-mediated inflammation may persist longer than expected, suggesting chronic post-weaning inflammation could harm animal health. However, most weaning studies are limited to 7–14 days [3,15,18–20]; future research should extend trial durations for more accurate impact assessment.

2.6.5 Cell Adhesion Molecules CD62L is critical for neutrophil margination and migration to infection sites, participating in immune cell recognition, naive lymphocyte homing, and intercellular adhesion [80]. Increased glucocorticoid secretion during weaning stress suppresses CD62L expression [15,43,81], impairing immune cell margination and migration, thereby reducing normal immune function and disease resistance. Nutritional interventions to relieve CD62L expression inhibition may enhance immunity and disease resistance in weaned animals.

Conclusion

Elevated plasma glucocorticoid concentrations in young ruminants after weaning affect multiple cytokine secretions, and altered cytokine production represents

the primary mechanism underlying weaning stress-induced immune dysregulation. Current research has focused mainly on organ development and production performance, with limited investigation of immune function and animal health, primarily at hormonal and cellular levels. Future studies should integrate hormonal, immune cell, and cytokine perspectives to elucidate mechanisms and provide scientific guidance for nutritional strategies to alleviate weaning stress and safeguard animal health.

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Note: Figure translations are in progress. See original paper for figures.