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Immunomodulatory Effects of Prebiotic, Probiotic, and Synbiotic Supplementation on Immune Response and Hematological Profile in Lactating Rabbit Does

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ABSTRACT

Lactation compromises immune function through metabolic stress and nutrient partitioning toward milk production, increasing disease susceptibility. This study investigated immunomodulatory effects of prebiotic (inulin), probiotic (*Lactobacillus* spp.), and synbiotic supplementation on immune parameters and hematological profile in lactating does. Forty-eight New Zealand White does (2.8-3.2 kg, second parity) were allocated to four groups (n=12): control, prebiotic (3 g/kg inulin), probiotic (1×10^9 CFU/g *Lactobacillus acidophilus* + *L. plantarum*), and synbiotic (prebiotic + probiotic) from day 1 through 28 post-partum. Parameters included immunoglobulins (IgG, IgA, IgM), cytokines (IL-6, IL-10, TNF-α, IFN-γ), lysozyme activity, complete blood count (CBC), and differential leukocyte count. Results showed synbiotic supplementation significantly elevated serum IgG by 38% (2486±246 mg/dL vs. 1802±186 mg/dL in control, $P<0.001$), "IgA by 42% ($P<0.001$), and IgM by 34% ($P=0.002$), indicating enhanced humoral immunity. Anti-inflammatory cytokine IL-10 increased 56% ($P<0.001$), while pro-inflammatory IL-6 decreased 32% ($P=0.004$) and TNF-α reduced 28% ($P=0.008$), demonstrating a modulation of the immune response toward a more balanced state. However, IFN-γ showed non-significant changes ($P=0.186$). Lysozyme activity increased by 48% with synbiotic treatment ($P<0.001$), confirming enhanced innate immunity. Hematological analysis revealed significant improvements: total leukocyte count increased 24% ($P=0.006$), lymphocyte percentage elevated 18% ($P=0.012$), while neutrophil:lymphocyte ratio decreased 26% ($P=0.018$), indicating reduced inflammation. Red blood cell parameters (RBC, Hb, HCT) remained unchanged ($P>0.05$), confirming erythropoiesis stability. A strong positive correlation was observed between IgA and lysozyme activity ($r=0.74$, $P<0.001$). Probiotic alone showed intermediate immunomodulatory effects, while prebiotic demonstrated milder benefits. This study demonstrates synbiotics enhance both innate and adaptive immunity, modulate inflammatory balance, and improve leukocyte profile in lactating does, supporting disease resistance during this vulnerable period.

1.Introduction

The lactation period is a period of high immune susceptibility in mammals. The excessive metabolic need of milk production (150-250 g/day in rabbits) causes competitive nutrient access between mammary gland and immune system [1]. The prioritization of energy and protein to lactation may undermine the lymphocyte proliferation, synthesis of antibodies and cytokines [2]. Moreover, cortisol increases with lactation stress, and it inhibits immune response by several mechanisms: diminished lymphocyte circulation, disabled cytokine receptor and decreased immunoglobulins [3]. This weakens the immune system, making the doe and kits vulnerable to mastitis, respiratory and gastrointestinal infections [4]. The humoral defense mechanism is mainly represented by immunoglobulins (Ig). The most common type of serum antibody (IgG) is used to offer systemic protection against bacterial and viral pathogens [5]. IgA prevails on the mucosal surfaces, and milk and it offers passive immunity to nursing kits and defends respiratory and gastrointestinal tract of doe [6]. The initial response to novel antigens is provided by IgM [7].

The synthesis of immunoglobulins during lactation competes with milk protein synthesis in terms of amino acids and energy. Reduced serum Ig levels in lactating do accompany high rates of infections [8]. The immune responses are coordinated by cytokines into intricate networks. The pro- inflammatory cytokines (IL-6, TNF- 2, IFN- 7) stimulate the cells of the immune system and have a beneficial effect, increasing the possibility of getting rid of pathogens, but they also provoke tissue damage when used in large amounts [9]. The anti- inflammatory cytokines (IL-10) regulate the responses of the inflammatory process and inhibit immunopathology [10]. The IL-6 functions in two directions; the activation of acute phase responses and the differentiation of B-cells [11]. TNF- alpha, which is generated by the activated macrophages, causes fever and activates neutrophil bactericidal effects but also plays a role in the pathology of mastitis [12]. T-helper 1 and NK cells secrete IFN- 7 which activates macrophages and improves cell- mediated immunity [13]. The main anti-inflammatory cytokine - IL-10 inhibits the production of pro-inflammatory mediators and enhances tissue repair [14].

Lactation stress tends to imbalance the cytokines to overinflammation and impairs immune control and tissue balance [15]. Lysozyme is a bacteriolytic enzyme that is found in serum, milk and secretions and, which hydrolyzes the bacterial cell wall peptidoglycan, especially in Gram positive bacteria.

[16]. An example of innate immunity biomarker is the activity of the enzyme, lysozyme. The lactating animals often exhibit lower levels of lactose because of specific secretion into the milk, undermining the systemic protection against bacteria [17].

Complete blood count (CBC) is an all-inclusive immune status examination. TLC represents the mobilization ability of immune cells [18]. Differential counting shows the pattern of immune response, high lymphocytes signify the activation of adaptive immunity, high neutrophils signify either bacterial infection or inflammation, and high eosinophilic response signifies parasitic challenge or allergy [19]. A biomarker of stress and inflammation is the neutrophil: lymphocyte (N:L) ratio. High levels of N:L ratios are the signs of chronic stress, cortisol upsurge, and inflammatory problems [20]. Increased N:L ratio is generally brought out by lactation hence cortisol-induced neutrophil and lymphopenia [21]. Parameters of red blood cell (RBC) determine oxygen carrying capacity required during high metabolic needs. Periconceptual anemia decreases productivity and health [22].

There are several ways that Probiotics have immunomodulatory effects:

a. **Gut barrier enhancement:** Strengthened tight junctions prevent pathogen translocation and inflammatory molecule absorption [23]
b. **Pattern recognition receptor activation:** Probiotic cell wall components stimulate toll- like receptors, priming immune responses [24]
c. **Regulatory T-cell induction:** Certain strains promote Treg differentiation, enhancing immune tolerance [25]
d. **Cytokine modulation:** Probiotics shift cytokine profiles toward balanced Th1/Th2 responses [26]
e. **IgA secretion stimulation:** Gut-associated lymphoid tissue activation increases mucosal IgA [27]

Lactobacillus species demonstrate particular efficacy in immune enhancement, with documented effects on immunoglobulin synthesis, cytokine production, and phagocyte activity [28].

The prebiotic indirectly regulates the immunity by stimulating the selectivity of the microbiota. The short-chain fatty acids (SCFA: acetate) are the result of fermentation.propionate, butyrate) with immunomodulatory properties:

- Butyrate nourishes colonocytes, strengthening gut barrier [29]
- Propionate modulates dendritic cell function [30]
- Acetate influences systemic immune responses [31]

Inulin, a fructan prebiotic, selectively stimulates *Bifidobacterium* and *Lactobacillus* growth while inhibiting pathogen colonization [32]. Although probiotic immunomodulatory effects have been confirmed in normal animals [33], their effects on the physiological phenomena in lactating does, which encounter special physiological stresses, have not been well delineated. Such connections of probiotic supplementation, immunoglobulin profiles, cytokines balance, markers of innate immunity, and hematologic parameters during lactation need to undergo extensive research [34]. Knowledge of these processes may guide approaches to preserve immune competence in lactation to decrease the occurrence of diseases and antimicrobial consumption [35]. This study aims to: - Evaluate effects of prebiotics, probiotics, and synbiotics on humoral immunity (immunoglobulins). -Assess cytokine profile modulation and inflammatory balance. -Determine innate immunity changes (lysozyme activity). - Characterize hematological profile responses. - Establish correlations between immune parameters.

H1: Synbiotic supplementation enhances immunoglobulin production, particularly IgA. **H2:** Synbiotics modulate cytokine balance toward anti-inflammatory phenotype. **H3:** Lysozyme activity increases with synbiotic treatment. **H4:** Leukocyte profile improves with reduced N:L ratio. **H5:** IgA correlates positively with lysozyme activity.

2. Materials and Methods

This study utilized the same animals and design as the companion study [36]. Briefly: 48 second-parity New Zealand White does allocated to four groups (n=12): control, prebiotic (3 g/kg inulin), probiotic (1×10^9 CFU/g Lactobacillus acidophilus + L. plantarum), and synbiotic from day 1-28 post-partum [37].

Blood Sampling

Blood samples (5mL) were collected via marginal ear vein at days 7, 14, 21, and 28 post-partum [38]. Samples divided:

2 mL in EDTA tubes for CBC

3 mL in serum separator tubes for immunological analyses

Immunoglobulin Assays

were measured by ELISA using rabbit-specific kits [39].

IgG: Sandwich ELISA (Abcam, ab157691, sensitivity 1.5 ng/mL, CV <7%). Serum diluted 1:20,000, results expressed as mg/dL [40].

IgA: Sandwich ELISA (Abcam, ab157692, sensitivity 0.8 ng/mL). Serum diluted 1:5,000, expressed as mg/dL [41].

IgM: Sandwich ELISA (MyBioSource, MBS2507447, sensitivity 2.0 ng/mL). Serum diluted 1:10,000, expressed as mg/dL [42].

All assays performed in duplicate following manufacturer protocols with incubation at room temperature and washing with PBS-Tween [43].

Cytokine Measurements

Cytokines quantified using rabbit-specific ELISA kits [44].

IL-6 (Interleukin-6): Quantitative sandwich ELISA (RayBiotech, ELR-IL6, sensitivity 3 pg/mL, range 15.6-1000 pg/mL). Pro-inflammatory cytokine involved in acute phase response [45].

IL-10 (Interleukin-10): Sandwich ELISA (RayBiotech, ELR-IL10, sensitivity 5 pg/mL). Anti-inflammatory cytokine, regulatory function [46].

TNF-α (Tumor Necrosis Factor-alpha): Competitive ELISA (Abcam, ab100785, sensitivity 4 pg/mL). Pro-inflammatory cytokine, macrophage product [47].

IFN-γ (Interferon-gamma): Sandwich ELISA (MyBioSource, MBS355253, sensitivity 8 pg/mL). Th1 cytokine, macrophage activator [48].

Serum samples analyzed undiluted or at specified dilutions. Results expressed as pg/mL [49].

Lysozyme Activity

was measured using a turbidimetric assay with Micrococcus lysodeikticus as substrate [50].

Procedure: Serum (20 µL) added to 3 mL M. lysodeikticus suspension (0.2 mg/mL in 0.05 M phosphate buffer, pH 6.2) in cuvette. Absorbance at

450 nm monitored for 5 minutes at 25°C using spectrophotometer (Shimadzu UV-1800). Absorbance decrease rate compared to hen egg-white lysozyme standard curve (0-100 µg/mL). Activity expressed as µg lysozyme equivalents/mL [51].

Complete Blood Count (CBC)

Hematological parameters analyzed using automated veterinary hematology analyzer (Mindray BC-5000 Vet) within 2 hours of collection [52].

Parameters measured:

Total leukocyte count (WBC, $\times 10^3/\mu L$)

Red blood cell count (RBC, $\times 10^6/\mu L$)

Hemoglobin (Hb, g/dL)

Hematocrit (HCT, %)

Mean corpuscular volume (MCV, fL)

Mean corpuscular hemoglobin (MCH, pg)

Mean corpuscular hemoglobin concentration (MCHC, g/dL)

Platelet count (PLT, $\times 10^3/\mu L$)

Quality control performed daily using commercial controls (Streck Laboratories) [53].

Differential Leukocyte Count

Differential count performed on Wright-Giemsa stained blood smears [54]. Minimum 200 leukocytes classified under oil immersion (×1000) as:

Lymphocytes (%)

Neutrophils (%)

Monocytes (%)

Eosinophils (%)

Basophils (%)

Neutrophil:lymphocyte (N:L) ratio calculated as neutrophil %/lymphocyte % [55].

Absolute counts calculated as: Absolute count = (cell

%/100) × total WBC [56].

Statistical Analysis

Data analyzed using SPSS 28.0 and GraphPad Prism 10.0 [57]. Normality tested with Shapiro-Wilk, homogeneity with Levene's test [58].

One-way ANOVA with Tukey's HSD for parametric data; Kruskal-Wallis with Dunn's test for non-parametric data [59]. Repeated measures ANOVA for temporal changes [60]. Pearson correlation for relationships [61].

Data: mean ± SEM. Significance: $P<0.05$. Power: 85% ($n=12$, $\alpha=0.05$, $f=0.35$) [62].

3. Results

3.1 Humoral Immunity - Immunoglobulins

3.1.1 Serum IgG

IgG, the predominant serum immunoglobulin, increased significantly with synbiotic supplementation (Table 1). At day 21, synbiotic group exhibited 38% higher IgG (2486±246 mg/dL) compared to control (1802±186 mg/dL, $P<0.001$), demonstrating enhanced

systemic humoral immunity [63]. Probiotic alone achieved 28% elevation (2308±228 mg/dL, P=0.002), while prebiotic showed 18% increase (2126±212 mg/dL, P=0.024) [64]. Temporal analysis revealed IgG progressively increased through lactation in all groups (P<0.001), with synbiotic maintaining significantly higher concentrations throughout (repeated measures ANOVA, P<0.001) [65].

3.1.2 Serum IgA

IgA, critical for mucosal immunity and passive kit protection via milk, showed the most dramatic response to synbiotic treatment (Table 1). At day 21, synbiotic group demonstrated 42% higher IgA (486±52 mg/dL) versus control (342±38 mg/dL, P<0.001), representing substantial mucosal immune enhancement [66]. Probiotic increased IgA by 32% (P<0.001), prebiotic by 22% (P=0.008) [67]. The pronounced IgA response reflects probiotics' preferential effect on gut-associated lymphoid tissue (GALT), the primary IgA synthesis site [68].

3.1.3 Serum IgM

IgM, the first-line humoral response, elevated 34% in synbiotic group (384±42 mg/dL vs. 286±32 mg/dL in control, P=0.002), indicating enhanced primary immune responsiveness (Table 1) [69]. Probiotic showed 24% increase (P=0.012), prebiotic 16% (P=0.048).

Table 1. Serum Immunoglobulin Concentrations at Day 21 Post-Partum

Immunoglobulin	Control	Prebiotic	Probiotic	Synbiotic	P-value
IgG (mg/dL)	1802±186^{c}	2126±212^{b}	2308±228^{ab}	2486±246^{a}	<0.001
IgA (mg/dL)	342±38^{c}	417±46^{bc}	451±48^{ab}	486±52^{a}	<0.001
IgM (mg/dL)	286±32^{c}	332±36^{bc}	355±38^{ab}	384±42^{a}	0.002
Total Ig (mg/dL)	2430±248^{c}	2875±292^{b}	3114±316^{ab}	3356±338^{a}	<0.001

Different superscripts indicate significant differences (P<0.05).

3.2 Cytokine Profile

3.2.1 Anti-Inflammatory Cytokine IL-10

IL-10, the primary anti-inflammatory cytokine, increased dramatically with synbiotic supplementation (Table 2). At day 21, synbiotic group showed 56% higher IL-10 (124.8±14.2 pg/mL) versus control (80.2±9.8 pg/mL, P<0.001), indicating enhanced regulatory immune function [70]. Probiotic elevated IL-10 by 42% (P=0.001), prebiotic by 28% (P=0.012) [71]. This IL-10 enhancement demonstrates synbiotics' capacity to induce regulatory immune responses, preventing excessive inflammation [72].

3.2.2 Pro-Inflammatory Cytokine IL-6

IL-6, elevated during acute phase responses and chronic inflammation, decreased significantly with synbiotic treatment (Table 2). Synbiotic group exhibited 32% lower IL-6 (86.4±10.2 pg/mL) compared to control (127.2±14.8 pg/mL, P=0.004), indicating reduced inflammatory stress [73]. Probiotic reduced IL-6 by 24% (P=0.018), prebiotic by 16% (P=0.064, trend) [74].

3.2.3 Pro-Inflammatory Cytokine TNF-α

TNF-α, a key inflammatory mediator, decreased 28% with synbiotic supplementation (94.6±11.4 vs. 131.4±15.6 pg/mL in control, P=0.008), confirming anti-inflammatory modulation (Table 2) [75]. Probiotic showed 20% reduction (P=0.028), prebiotic 12% (P=0.096, trend).

3.2.4 Th1 Cytokine IFN-γ

IFN-γ, produced by Th1 cells and NK cells, showed only numerical increases without statistical significance (P=0.186), suggesting treatments did not substantially alter Th1-mediated cellular immunity (Table 2) [76]. This pattern indicates synbiotics primarily modulated inflammatory balance rather than shifting Th1/Th2 paradigm.

Table 2. Serum Cytokine Concentrations at Day 21 Post-Partum

Cytokine	Control	Prebiotic	Probiotic	Synbiotic	P-value
IL-10 (pg/mL)	80.2±9.8^{c}	102.6±12.2^{bc}	113.8±13.6^{ab}	124.8±14.2^{a}	<0.001

IL-6 (pg/mL)	127.2±14.8[a]	106.8±12.6[ab]	96.7±11.4[b]	86.4±10.2[b]	0.004
TNF-α (pg/mL)	131.4±15.6[a]	115.6±13.8[ab]	105.1±12.4[b]	94.6±11.4[b]	0.008
IFN-γ (pg/mL)	142.8±16.4	152.6±17.8	158.4±18.2	164.2±19.6	0.186
IL-10:IL-6 ratio	0.63±0.08[c]	0.96±0.12[bc]	1.18±0.14[ab]	1.45±0.18[a]	<0.001

3.3 Innate Immunity - Lysozyme Activity

Lysozyme activity, a key innate defense enzyme, elevated significantly with synbiotic supplementation (Table 3). At day 21, synbiotic group demonstrated 48% higher lysozyme activity (46.8±5.2 μg/mL) compared to control (31.6±3.8 μg/mL, P<0.001), indicating enhanced antibacterial capacity [77]. Probiotic increased activity by 36% (P=0.001), prebiotic by 24% (P=0.014) [78]. The substantial lysozyme enhancement confirms synbiotics improve innate immunity, providing rapid antibacterial defense independent of adaptive responses [79].

Table 3. Lysozyme Activity at Day 21 Post-Partum

Parameter	Control	Prebiotic	Probiotic	Synbiotic	P-value
Lysozyme (μg/mL)	31.6±3.8[c]	39.2±4.6[bc]	43.0±4.8[ab]	46.8±5.2[a]	<0.001

3.4 Complete Blood Count

3.4.1 Leukocyte Parameters

Total leukocyte count increased significantly with synbiotic supplementation, rising 24% from 7.24±0.82 $\times 10^3/\mu L$ in control to 8.98±0.96 $\times 10^3/\mu L$ (P=0.006), indicating enhanced immune cell mobilization capacity (Table 4) [80]. Probiotic elevated WBC by 18% (P=0.024), prebiotic by 12% (P=0.068, trend) [81]. This leukocyte elevation reflects enhanced hematopoietic activity and immune cell production, supporting improved disease resistance [82].

3.4.2 Erythrocyte Parameters

Red blood cell count, hemoglobin, and hematocrit showed only numerical variations without statistical significance (P=0.428, P=0.352, and P=0.286, respectively), indicating treatments did not affect erythropoiesis or oxygen-carrying capacity (Table 4) [83]. This stability confirms treatments maintained normal RBC homeostasis despite increased immune activity. RBC indices (MCV, MCH, MCHC) remained unchanged (P>0.05), confirming normocytic normochromic status [84].

3.4.3 Platelet Count

Platelets showed non-significant numerical increase (P=0.194), suggesting treatments did not substantially alter thrombocytopoiesis (Table 4) [85].

Table 4. Complete Blood Count at Day 21 Post-Partum

Parameter	Control	Prebiotic	Probiotic	Synbiotic	P-value
WBC ($\times 10^3/\mu L$)	7.24±0.82[b]	8.11±0.88[ab]	8.54±0.92[ab]	8.98±0.96[a]	0.006
RBC ($\times 10^6/\mu L$)	5.68±0.62	5.82±0.64	5.94±0.66	6.08±0.68	0.428
Hb (g/dL)	12.4±1.2	12.8±1.3	13.0±1.4	13.4±1.5	0.352
HCT (%)	38.6±3.8	39.8±4.2	40.6±4.4	41.8±4.6	0.286
MCV (fL)	67.9±6.4	68.4±6.8	68.4±6.6	68.8±7.0	0.892
MCH (pg)	21.8±2.2	22.0±2.4	21.9±2.3	22.0±2.5	0.976
MCHC (g/dL)	32.1±3.0	32.2±3.2	32.0±3.1	32.0±3.3	0.998
PLT ($\times 10^3/\mu L$)	286±32	304±36	318±38	332±42	0.194

3.5 Differential Leukocyte Count

3.5.1 Lymphocyte Percentage

Lymphocyte percentage, reflecting adaptive immune cell predominance, increased significantly with synbiotic supplementation (Table 5). Synbiotic group showed 18% higher lymphocytes (64.2±6.4%) compared to control (54.4±5.6%, P=0.012), indicating enhanced adaptive immunity [86]. Probiotic increased lymphocytes by 14% (P=0.032), prebiotic by 8% (P=0.124, non-significant) [87].

3.5.2 Neutrophil Percentage

Neutrophil percentage, elevated during stress and inflammation, decreased with synbiotic treatment, dropping from 32.6±3.4% in control to 26.8±2.8% (18% reduction, P=0.028), demonstrating reduced inflammatory stress (Table 5) [88].

3.5.3 Neutrophil:Lymphocyte Ratio

The N:L ratio, a sensitive stress and inflammation biomarker, decreased significantly by 26% in synbiotic group (0.42±0.06 vs. 0.60±0.08 in control, P=0.018), confirming reduced physiological stress and inflammatory status (Table 5) [89]. Probiotic reduced N:L by 20% (P=0.038), prebiotic by 12% (P=0.186, non-significant) [90]. This N:L reduction indicates synbiotics effectively mitigated lactation-induced stress responses [91].

3.5.4 Other Leukocytes

Monocytes, eosinophils, and basophils showed only minor numerical variations without statistical significance (P=0.324, P=0.428, and P=0.752, respectively), indicating treatments primarily affected lymphocyte and neutrophil populations (Table 5) [92].

Table 5. Differential Leukocyte Count at Day 21 Post-Partum

Parameter	Control	Prebiotic	Probiotic	Synbiotic	P-value
Lymphocytes (%)	54.4±5.6^{b}	58.8±6.0^{ab}	62.0±6.2^{ab}	64.2±6.4^{a}	0.012
Neutrophils (%)	32.6±3.4^{a}	30.2±3.2^{ab}	28.4±2.9^{b}	26.8±2.8^{b}	0.028
N:L Ratio	0.60±0.08^{a}	0.51±0.07^{ab}	0.46±0.06^{b}	0.42±0.06^{b}	0.018
Monocytes (%)	8.2±1.2	7.8±1.0	7.4±0.9	7.0±0.8	0.324
Eosinophils (%)	3.6±0.6	3.2±0.5	2.8±0.4	2.4±0.4	0.428
Basophils (%)	1.2±0.2	1.1±0.2	1.0±0.2	0.9±0.2	0.752

3.6 Absolute Leukocyte Counts

Absolute lymphocyte count increased dramatically by 47% in synbiotic group (5.77±0.64 vs. 3.94±0.46 $\times 10^3/\mu L$ in control, P<0.001), combining effects of increased total WBC and higher lymphocyte percentage (Table 6) [93]. This substantial elevation indicates enhanced adaptive immune cell availability. Absolute neutrophil count remained relatively stable (P=0.186), confirming percentage decreases reflected proportional shifts rather than absolute neutrophil depletion—maintaining adequate innate immune capacity [94].

Table 6. Absolute Leukocyte Counts at Day 21 Post-Partum

Parameter	Control	Prebiotic	Probiotic	Synbiotic	P-value
Abs. Lymphocytes ($\times 10^3/\mu L$)	3.94±0.46^{c}	4.77±0.54^{bc}	5.30±0.60^{ab}	5.77±0.64^{a}	<0.001
Abs. Neutrophils ($\times 10^3/\mu L$)	2.36±0.28	2.45±0.30	2.43±0.29	2.41±0.28	0.186
Abs. Monocytes ($\times 10^3/\mu L$)	0.59±0.08	0.63±0.09	0.63±0.08	0.63±0.09	0.824

3.7 Correlations

Strong positive correlation existed between IgA and lysozyme activity (r=0.74, P<0.001), demonstrating coordinated mucosal and innate immunity

enhancement (Table 7) [95]. IgG correlated positively with total WBC (r=0.68, P=0.002), linking humoral immunity to leukocyte mobilization [96]. IL-10 correlated negatively with IL-6 (r=-0.72, P<0.001) and TNF-α (r=-0.66, P=0.002), validating anti-inflammatory regulatory function [97]. N:L ratio correlated negatively with lymphocyte percentage (r=-0.84, P<0.001), confirming stress-immune relationship [98]. IgA showed positive correlation with absolute lymphocyte count (r=0.64, P=0.006), suggesting B-cell proliferation drives IgA synthesis [99].

Table 7. Pearson Correlation Coefficients - Key Immune Parameters

Variable 1	Variable 2	r	P-value
IgA	Lysozyme Activity	0.74	<0.001
IgG	Total WBC	0.68	0.002
IL-10	IL-6	-0.72	<0.001
IL-10	TNF-α	-0.66	0.002
N:L Ratio	Lymphocyte %	-0.84	<0.001
IgA	Absolute Lymphocytes	0.64	0.006

4. Discussion

4.1. Enhanced Humoral Immunity

The 38-42% increase in immunoglobulins is observed and this increase supports the significant boosting of the humoral immunity through synbiotic supplementation [63, 66]. The high level of IgA (42 percent in particular) response is especially indicative of a specific action on the gut-associated lymphoid tissue (GALT), the major location of IgA production in the body and the largest immune organ [68]. This enhancement of immunoglobulin is supported on a mechanistic level by a combination of factors: B-lymphocyte activation by probiotic stimulation of pattern recognition receptors [100], B-cell differentiation under the influence of cytokines [101], enhanced antigen presentation via dendritic cells [102], better availability of amino acids to support antibody production because of a combination of nutrient absorption [103], and metabolic reallocation to support immunoglobulin production [104]. These increases are clinically significant; the 38% rise of IgG makes the system more immune against circulating pathogens, which is necessary to reduce the threat of sepsis [105], and the 34% increase in IgM strengthens the initial response of periparturient does to new environmental factors, which is essential under new environmental conditions [106].

4.2. Cytokine Balance Modulation

The combined 56% increase in the anti-inflammatory cytokine, IL-10, and extensive declines in the pro-inflammatory cytokines, IL- were induced by the synbiotic treatment, which resulted in a superior immunomodulatory profile.

6 (32%) and TNF-α (28%) [70, 73, 75]. This

pattern represents an optimal re-tuning of the immune system, preserving the protective ability and avoiding the adverse consequences of the chronic inflammation. The rise in IL-10 may be explained by various processes such as the development of the regulatory T-cells by particular means.

The programming of dendritic cells with a tolerogenic phenotype [108], conditioning of dendritic cells in the presence of butyrate via GPR43 receptors [109], and direct production of IL-10-inducing metabolites by bacteria [110]. Simultaneously, the decrease of pro-inflammatory cytokines was attained by blocking the NF- 8B pathway [111], suppressing the toll-like receptor 4 (TLR4) [112] as well as a reduction in inflammatory stimuli as a result of a tightening of the gut barrier [113]. The

subsequent 130% increase in the IL-10:IL-6 ratio is a strong reaction of better functionality in the immune regulation [114]. In addition, the non-significant increase of IFN-7 (P=0.186) can indicate that the vital Th1-mediated cellular immunity was maintained without being over-stimulated pathologically [76].

4.3. Lysozyme Activity and Innate Defense Enhancement

The The 48 percent rise in the activity of lysozyme substantiates the significant enhancement of the natural, non-specific antibacterial capability [77]. It is an enzyme that offers an essential initial defense response against Gram- positive mastitis pathogens including.

Staphylococcus [115]. This improvement is probably due to the probiotic-based enhancement of the macrophage activation [116], the possible redistribution of milk to circulation induced by stress [117], and the enhanced hepatic synthesis that would be ensured by an improvement in the metabolic status [118]. The strong positive

observed correlation between IgA and lysozyme (r=0.74) highlights a concerted increase in the mucosal defense system with IgA neutralizing pathogen and lysozyme only breaking down bacterial cell walls [95]. [95].

4.4. Leukocyte Profile Optimization and Stress Reduction

The 24 percentage point rise in total count of white blood cell (WBC) shows an increase in their mobilization, probably as a result of bone marrow stimulation [119], leukopoesis mediated by cytokines [120] and a decrease in immune cell apoptosis because of an increased antioxidant status [121]. A closer examination by way of a qualitative optimization indicates that the percentage of lymphocytes increased by 18 percent, and the absolute lymphocyte count had grown by 47 percent that indicated a significant expansion of the adaptive immune arm, which offered a greater number of B and T cells to respond to specific antigens [86, 93]. The neutrophil-to- lymphocyte (N: L) ratio decreased 26% [89], which is one of the most indicative signs of low physiological stress. This normalization, which tends to be increased during lactation under the influence of cortisol [21], is an indication of the transition to a biologically more balanced and functional immune picture, provided by lower cortisol and decreased inflammatory chemotaxis [122, 123, 124].

4.5. Stability of Erythropoiesis and Broader Physiological Integration

The insignificant alterations in the parameters of red blood cells confirm that the strong stimulation of the immune system did not rely on the damage of the erythropoiesis [83]. This is an important discovery, in that it proves that the synbiotic approach does not cause the anemia of inflammation which can be caused by certain immune stimulants [125], maintaining the oxygen-carrying capacity required by a high metabolic rate. Combined with the results of companion metabolism, a complete view of physiological optimization is created. The decrease of cortisol mentioned in the record of 28% leads directly to the increase of lymphocyte count and N:L ratio [140] by 18%.

glucose is essential in the growth of immune cells [141], and malondialdehyde (MDA), which is decreased by 42 percent, preserves immune cells against oxidative stress [142]. This has shown that the synbiotics help to cater to the health of the doe as it simultaneously enhances the endocrine, metabolic, and immune systems in the demanding lactation process.

5. Conclusion

In this holistic research, synbiotic supplementation (prebiotic + probiotic) was shown to significantly boost both the adaptive and innate immunity in lactating rabbit does. The 38-42% immunoglobulin increase is a sign of strong humoral immune response, and the increase in IgA (42) is significant, which is an indicator of the activation of the gut-immune axis. The balance of immunoregulation is evidenced by the cytokine modulation in the direction of anti-

inflammatory phenotype (56% IL-10 rise, 28-32% pro- inflammatory cytokines decreatement). The increase in lysozyme by 48 percent is a confirmation that there is an increase in the inbuilt antibacterial potential. Hematological aspects increase in WBC 24% and in lymphocytes 18% and N:L ratio decrease 26% demonstrates optimized immune cell profile with decreased stress. The levels of IgA and lysosome show a strong IgA-lysozyme correlation (r=0.74), which is an indication of coordinated mucosal defense. Constitutive RBC parameters are an affirmation of treatments- sustained erythropoiesis and an improvement of immunity. The non-significant changes in IFN- 7 maintained sufficient cell-mediated immunity. Synergies are proven through Synbiotic superiority to its individual components. These immune enhancements in combination with metabolic optimizations with companion study assist wholesome physiological enhancement during lactation. The results provide evidence-based nutritional supplement regimens of boosting immunological capability and mitigating susceptibility to diseases in nursing rabbit does within this susceptible phase.

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