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Research Report

Systemic expression of galectin genes in periparturient goats

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Abstract: Galectins constitute an evolutionarily conserved family of β-galactoside-binding proteins. They regulate innate and adaptive immunity and homeostasis. Expression of Galectins may regulate periparturient immune suppression. Galectin gene expression was studied in goat blood during the periparturient period. Body weight, body condition and FAMACHA scores, and fecal and blood samples were collected from Five BoerXSpanish goats at 14 days and 7 days after parturition. Fecal samples were used to assess parasite load. Total RNA was isolated from blood using Trizol and converted to cDNA for real-time PCR using specific primers for goat LGALs-1, -2, -3, -4, -7, -8, -9, -11, -12, -14, -15, -16, and ligand Gal3bp, T-cell immunoglobulin domain, and mucin domain 3(TIM-3). Beta-actin and GAPDH housekeeping genes were used as internal controls. Fold changes in transcript abundance were compared to non-pregnant goats and calculated using the Livak method. Secretion of GALS-1, -3 and -9 in plasma was detected using ELISA. Data were analyzed using SAS 9.4 and Pearson correlations (p<0.05). Galectins were expressed and correlated to changes in leukocytes and fecal egg counts. Secreted GALS-1 decreased and GALS-3 and -9 increased (p<0.05) postpartum. Differential expression of Gal may have functional implications in animal health and homeostasis and needs further study.

Keywords: galectins, goats, periparturient

1. Introduction

Goat population has been on the increase and is estimated to be about 1 billion [1]. Different breeds have various advantageous characteristics which aid adaptation to harsh environmental conditions, resistance to diseases, and the capacity to convert poor quality fibrous feedstuff into animal proteins. Goat production is negatively challenged by infectious diseases such as mastitis [2]. Gastrointestinal (GI) nematode infection is considered the most critical limiting factor in goat production systems around the world and results in substantial economic losses to producers [3]. This increase in disease and infection occurrence is usually evident during the periparturient period when there is a temporary impairment in immune function.

The periparturient period is defined as the period from 3 weeks prepartum to 3 weeks postpartum and is marked by several changes [4]. During the periparturient period, increased incidence of health problems is observed and is partly attributed to suboptimal immune responses [5]. Numerous studies have reported the increase in metabolic and infectious diseases during this period [6, 7]. Goats are faced with infection with nematode parasites such as Haemonchus spp. which impairs weight gain and increases mortality [8]. The periparturient period is associated with relaxation in immunity and a rise in parasitic counts in fecal samples [9]. Due to increase in anthelmintic resistance and climate change producers are faced with increasing difficulties stabilizing

herd health during the periparturient period. The ability of goats to resist the establishment of diseases during this period is related to the efficiency of their immune system which consists of a variety of biological components that protects them.

Galectins constitute an evolutionarily conserved family of β-galactoside-binding proteins [10-12] that acts as both pathogens associated molecular pattern and pathogen recognition receptors [11, 13, 14]. Galectins are widely distributed in organisms from lower vertebrates to mammals [14]. There are several types of galectins which have been classified according to their respective carbohydrate recognition domain(s) (CRD). All galectins contain either one or multiple CRDs, distinguishing homo-dimeric from hetero-dimeric galectins. Galectin-1, -2, -5, -7, -10, -11, -13, and -14 contain one CRD and are classified as the "proto type." Galectin-4, -6, -8, -9, and -12 have two separate CRDs connected by non-conserved amino acid sequences and are referred to as the 'tandem repeat types.' Galectin-3 is the only galectin classified as a 'chimeric type'; one CRD and an N-terminal. Recent advances have facilitated their use as biomarkers in metabolic and infectious diseases.

Galectin activity has been well reported across many tissues, and their differential regulation is essential for maintaining cellular functions [15]. They are also expressed at the maternal–fetal interface which serves as an important protein involved in maternal-fetal interactions [16]. Galectins have diverse effects on cells involved in innate immune responses, including macrophages and dendritic cells, neutrophils, eosinophils, and mast cells [17]. They bind to the surface of parasitic helminths, as well as other pathogens, initiating host immune response [14, 18-20]. They contribute to critical biological events occurring during mammalian gestation, immune cell tolerance, inflammation, implantation, and angiogenesis [21].

Galectins have been studied in goats. Galectin-11, -14, and -15 have been studied in relation to pregnancy and parasitic infection [14, 22]. Studies have also reported the expression of goat-heart Galectin-1 as a tool for the detection of post-malignant changes in glycosylation pattern [23]. Goats have also been vaccinated with recombinant galectins of male and female H. contortus (rHco-gal-m/f) which induced partial protection against H. contortus [24] as well as regulating cell maturation and function [14, 25, 26]. Previous studies conducted by our research group has reported the expression off galectins in ruminants [27].

So far there are no studies on the expression of galectins and possible role the play during the periparturient period. The objective of this study was to evaluate the expression of galectins as well as addressing the concerns regarding the incidence of goat parasites and host resilience during the periparturient period.

2. Results

2.1 Physiological Parameters

Several parameters measured were selected to determine parturient immunosuppression in goats. Our results indicate that there was a significant difference in body weight and body condition scores before and after birth (Table 1). There was no significant difference in FAMACHA scores which indicates that the animals were not anemic. Packed cell volume remained within the range of a healthy goat. There was a significant difference in fecal egg rise observed between prepartum and postpartum. The highest increase was observed 7 days before kidding.

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Table 1. Mean, Standard Error and P-value of Body Weight (BW), Body Condition (BC), FAMACHA, Packed Cell Volume and Fecal Egg Count of Goats during the Periparturient Period.

Parameter	14 days before	7 days before	7 days after	14 days after	*p*-value
Body Weight	161±9.52^{ab}	169.6±9.00^{a}	149.6±8.03^{ab}	146.4±11.43^{a}	0.0139
Body Condition	1.4±0.24^{b}	1.8±0.2^{ab}	2.0±0.32^{a}	2.0±0.32^{a}	0.0162
FAMACHA	2.0±0.00	2.0±0.00	2.0±0.00	2.0±0.00	ns
Strongyle	1020±359.37^{ab}	1790±906.55^{a}	590±221.58^{b}	460±106.53^{b}	0.0039
Coccidia	130±68.19^{b}	730±416.71^{a}	50±27.38^{b}	40±18.70^{b}	<0.0001
PCV	25.2±0.86^{b}	25.7±1.18^{b}	29.2±0.90^{a}	27.2±1.45^{ab}	<0.0001

Note. a, b Values within each row with different subscripts differ significantly at $p < 0.05$; ns=non-significant. Error lines represent the ± standard deviation of the mean.

2.2 Total Fecal Egg Count

Our results indicate a high fecal egg count 7 days before kidding (Figure 1). There was a reduction in fecal egg count 7 days after kidding.

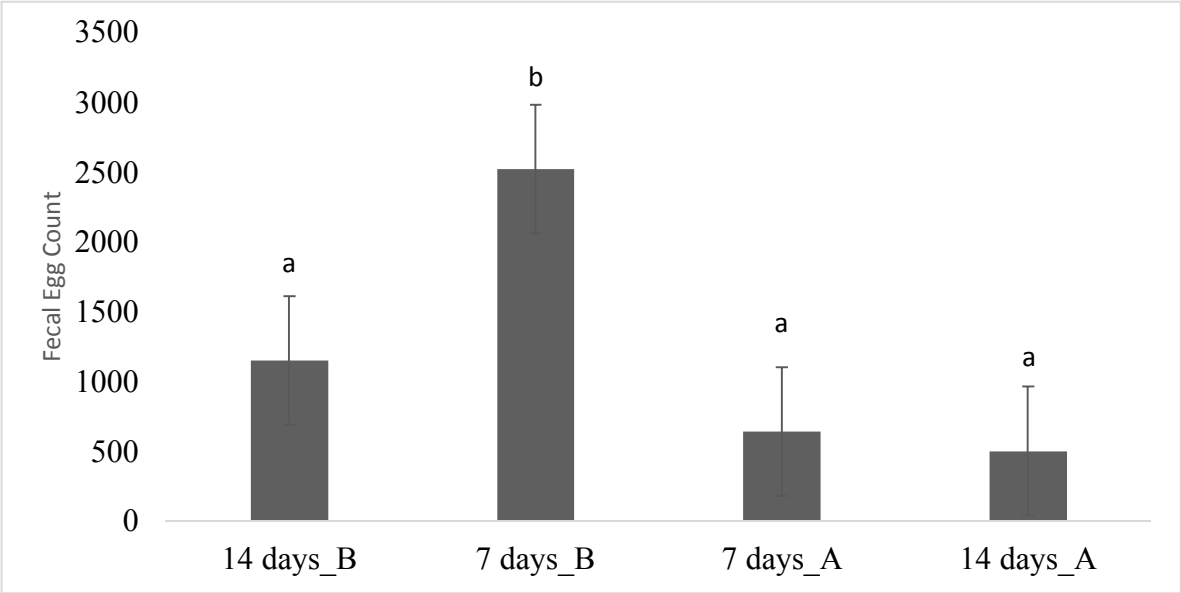

Figure 1. Total fecal egg count from goats during the periparturient period. A – After, B – Before. Means with the same letter are not significantly different from each other (P>0.05). Error lines represent the ± standard deviation of the mean

2.3 White Blood Cell Differential Count

There was a significant difference in neutrophil and lymphocyte cell count during the periparturient period ($p<0.05$). There was an increase in lymphocyte cell count 7 days before kidding and a decrease in neutrophil cell count 7 days after kidding (Figure 2).

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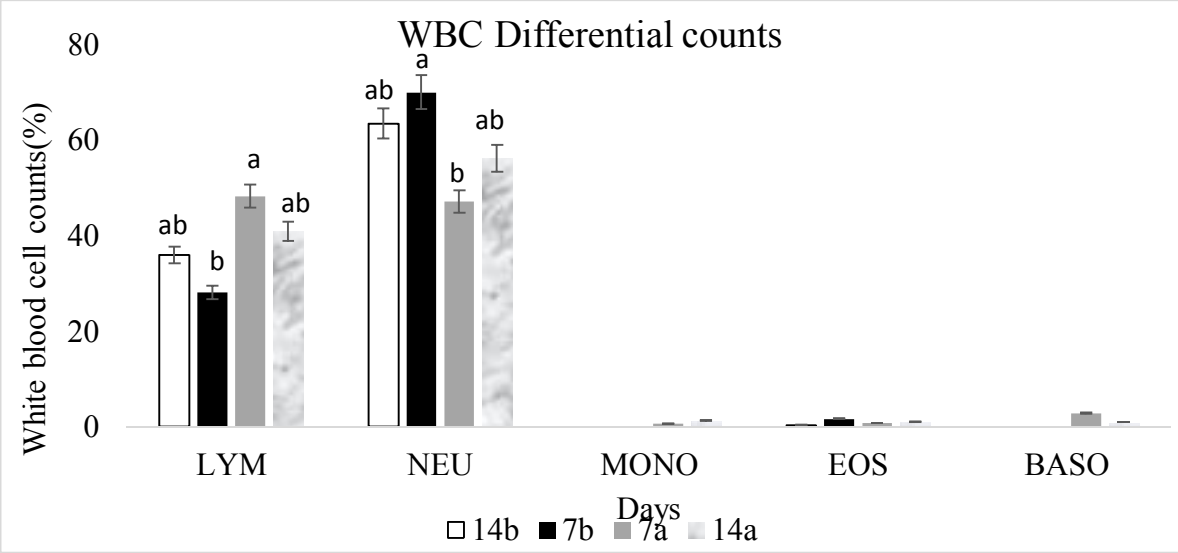

Figure 2. White blood cell count during the periparturient period in goats. a – After, b – Before. Means with the same letter are not significantly different from each other (P>0.05). Error lines represent the ± standard deviation of the mean

2.4 Total Protein Concentration

There was no significant difference in the total protein concentration in plasma during the periparturient period (p>0.8271). There was an observable change in the total plasma concentration during the periparturient period (Figure 3). There was an increase in total protein concentration 7 days before birth and a decrease in total protein concentration 7 days after birth.

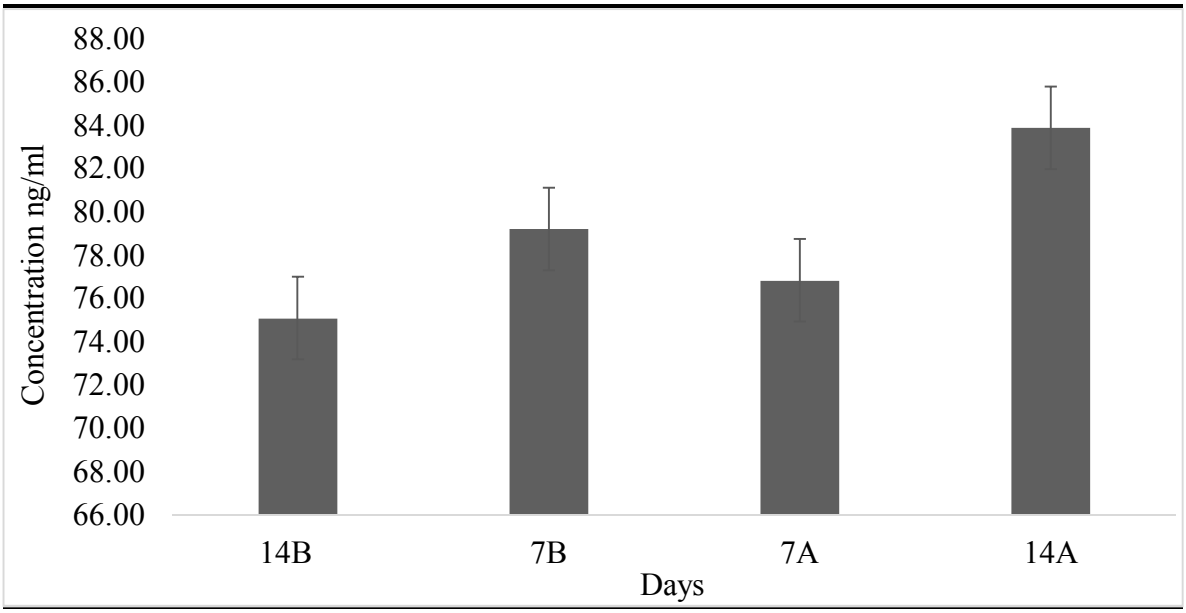

Figure 3. Total protein concentration measured in plasma samples following treatments of whole blood during the periparturient period. A – After, B – Before. Error lines represent the ± standard deviation of the mean.

2.4.1 Galectin Concentration

Galectin-1 secretion decreased significantly over time (p<0.001) (Figure 4). There was a difference in Galectin-3 concentration during the periparturient period (p<0.001). Galectin-3 concentrations increased over time. The concentration of Galectin-3 in plasma increased after birth (Figure 5). There

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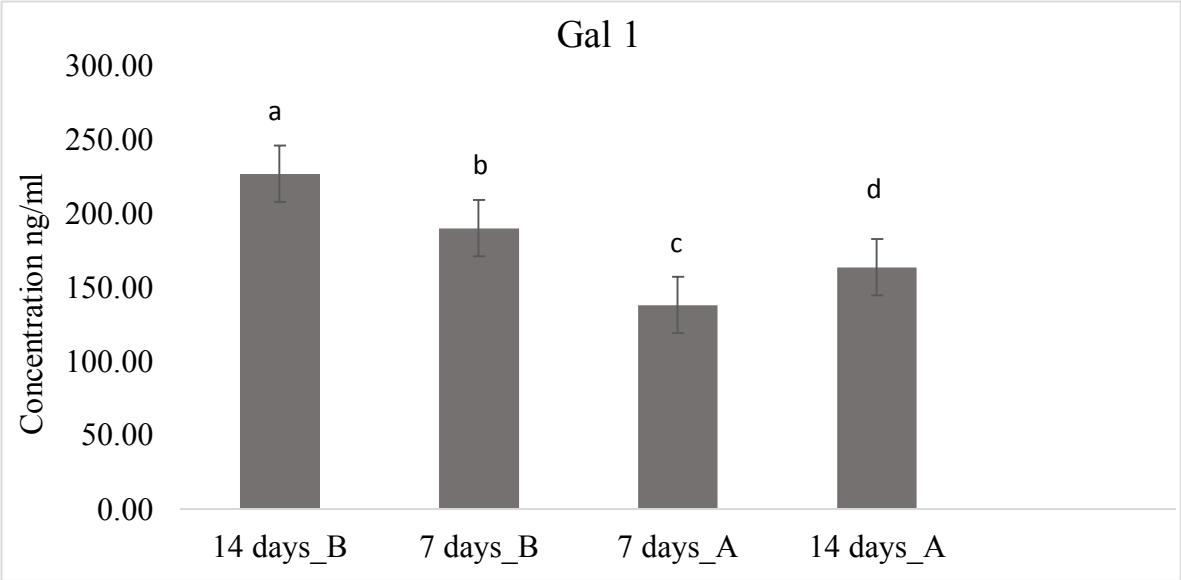
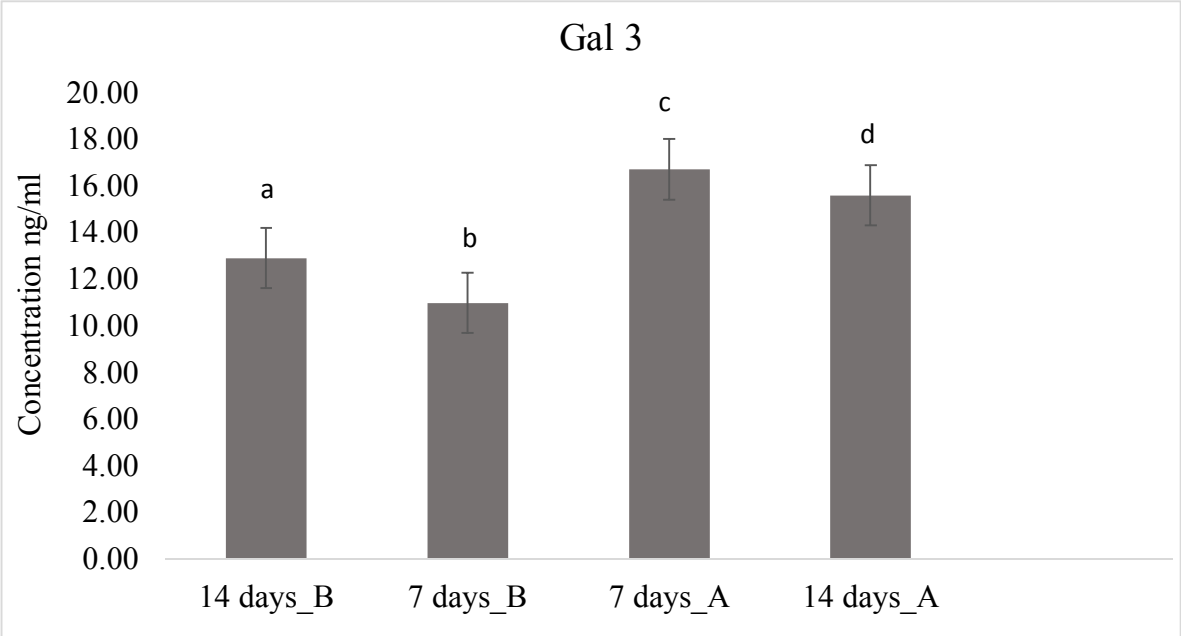
121 was a difference in Galectin-9 concentration during the periparturient period (p<0.001). The
122 concentration of Galectin-9 increased and was highest 7 days after birth (Figure 6).



123

124 **Figure 4.** Galectin-1 concentration measured in plasma samples during the periparturient period.
125 Means with the same letter are not significantly different from each other (P>0.05). Error lines
126 represent the ± standard deviation of the mean.



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128 **Figure 5.** Galectin-3 concentration measured in plasma samples during the periparturient period.
129 Means with the same letter are not significantly different from each other (P>0.05). Error lines
130 represent the ± standard deviation of the mean.

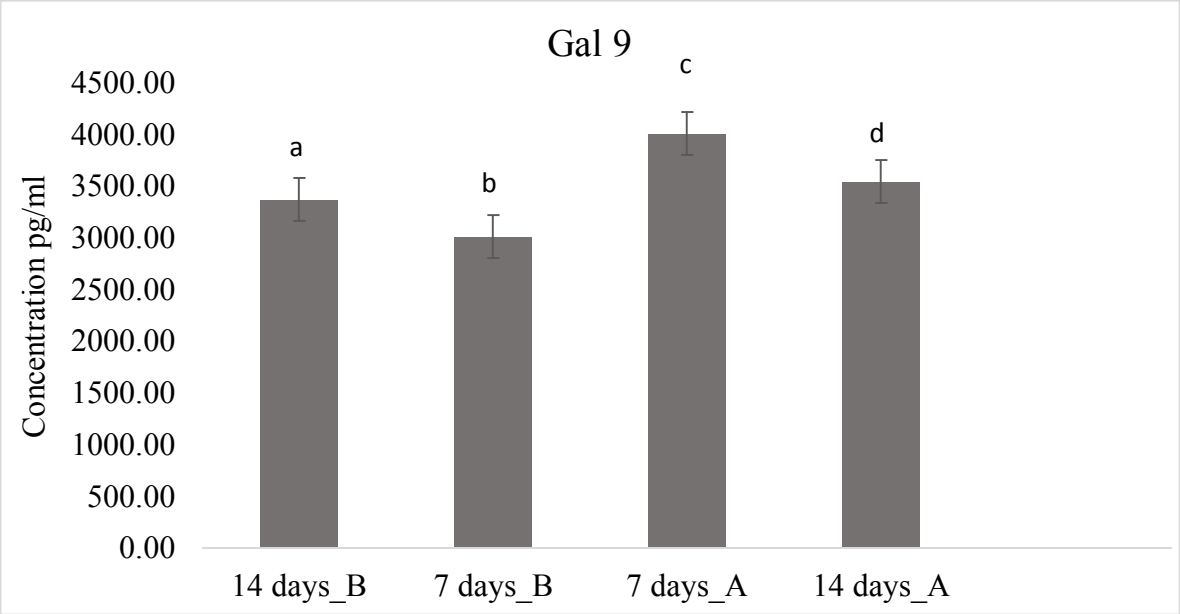

Figure 6. Galectin-9 concentration measured in plasma samples during the periparturient period. Means with the same letter are not significantly different from each other (P>0.05). Error lines represent the ± standard deviation of the mean.

2.4.2 Expression of Galectins (LGALS) and Their Ligands LGALS-3bp and TIM-3 During the Periparturient Period

All galectins and ligands tested were differentially expressed in goat blood in pregnant and non-pregnant goats. Their expression was differentially regulated during the periparturient period (Figure 7). Some galectins were increased, while some galectins were decreased at different time points. Some galectins were also not expressed at a particular time point. Galectin-3 binding protein and TIM-3 which are ligands for Galectin-3 and Galectin-9, respectively, were differentially expressed and regulated.

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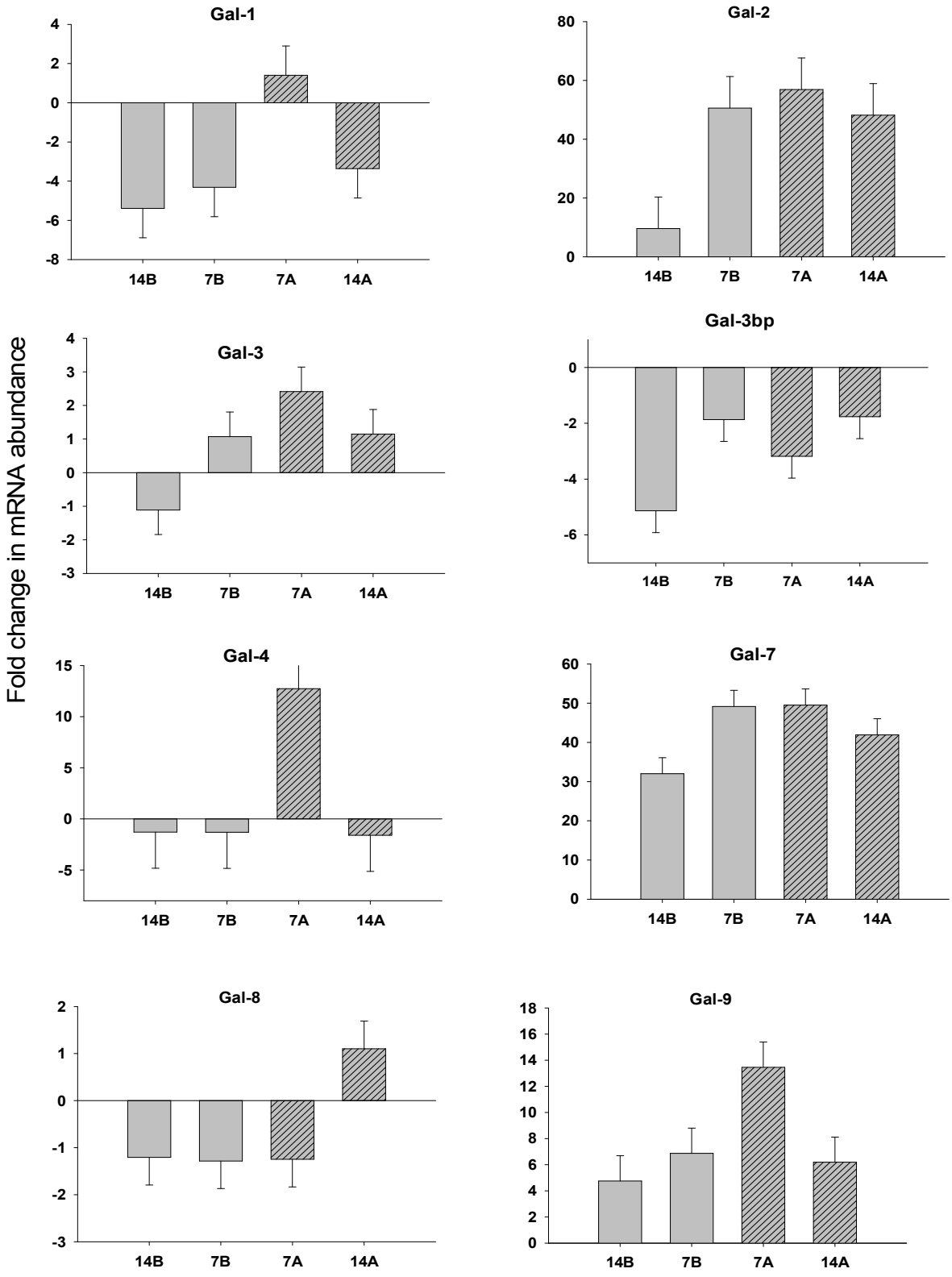

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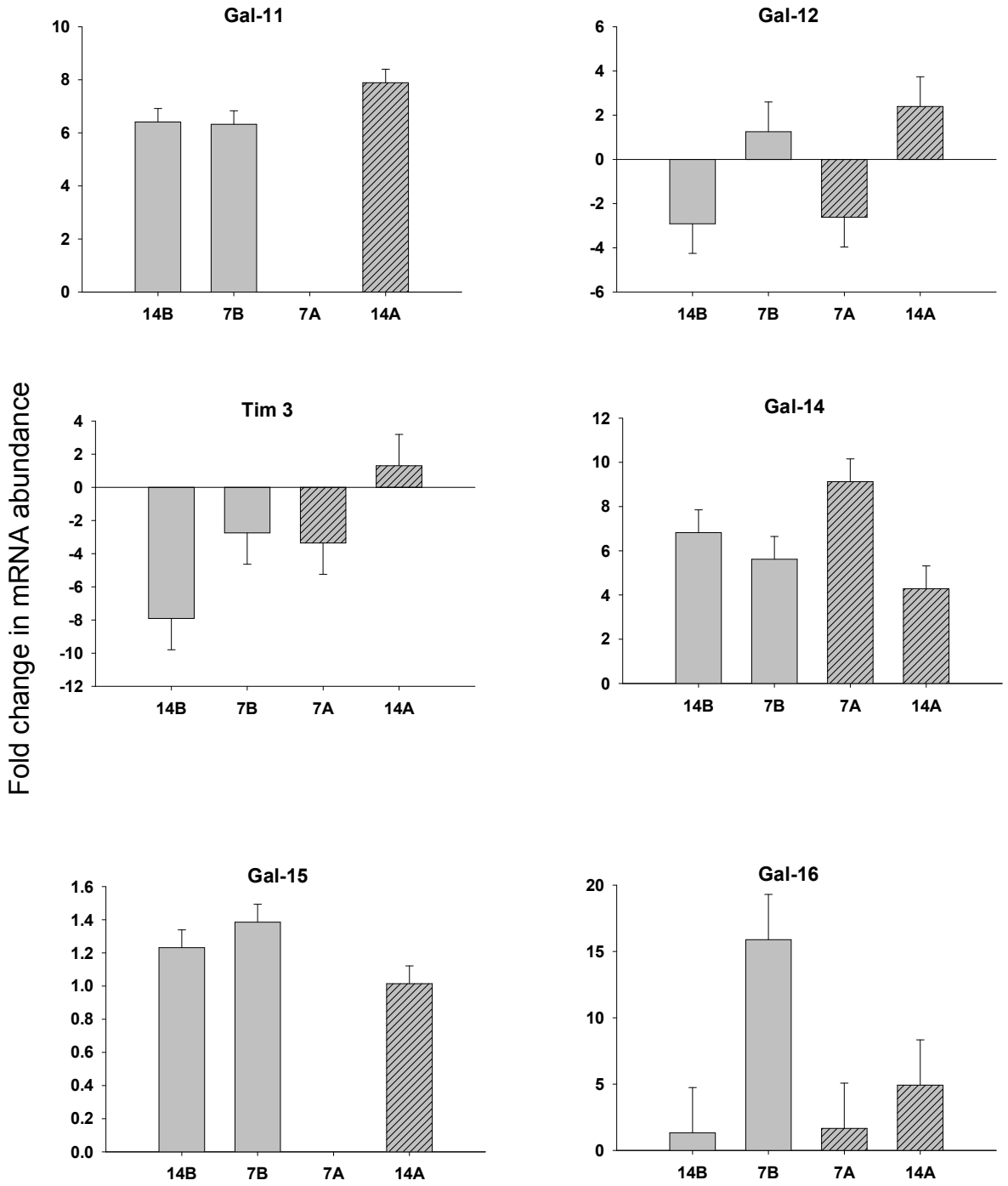

Figure 7. Galectin expression measured in goats during the periparturient period.

2.5 Correlation between Galectins and Phenotypic Parameters

LGAL-1 gene expression correlated positively (p<0.05) with PCV and lymphocyte cell count (r=0.5524 and r=0.5368, respectively). LGAL-3 gene expression correlated positively (p<0.05) with PCV, GALS-1, -3, and -9 plasma concentration (r=0.4875, r=5979, r=0.5409, and r=0.5251, respectively). LGAL-4 gene expression correlated positively (p<0.05) with PCV, lymphocyte cell counts, GAL-1, -3, and -9 plasma concentrations (r=0.5103, r=0.8232, r=0.6694, and r=0.8371, respectively) and negatively with neutrophil count (r=-0.56335). LGAL-7 gene expression correlated positively (p<0.05) with GALS-1 concentrations (r=0.6386) in plasma. LGAL-8 gene expression correlated positively (p<0.05) with monocyte count (r=0.5002). LGAL-9 gene expression correlated positively (p<0.05) with PCV, lymphocyte count, GALS-1, 2 and 3 concentrations (r=0.5145, r=0.4628, r=0.7728, r=0.6274 and r=0.7624 respectively) in plasma and negatively with neutrophil count (r=-0.5290). LGAL-11 gene expression correlated positively (p<0.05) with neutrophil count (r=0.4841) and negatively with PCV, lymphocyte cell count, GALS-1, -2, and -3 concentration (r=-0.4378, r=-0.4367, r=-0.7138, r=-0.5293 and r=-0.7417, respectively) in plasma. LGAL-12 gene expression correlated negatively (p<0.05) with GALS-9 concentration (r=-0.4473) in plasma. LGAL-14 gene expression correlated positively (p<0.05) with GALS-1 and -9 concentrations (r=0.5707 and r=0.6374, respectively) in plasma. LGAL-15 gene expression correlated positively (p<0.05) with neutrophil cell count (r=0.6516) and negatively with PCV, lymphocyte count, GALS-1, -3, and -9 concentrations (r=-0.5381, r=-0.5992, r=-0.9432, r=-0.8421, and r=-0.9450, respectively) in plasma. LGAL-16 gene expression correlated positively (p<0.05) with coccidia egg count and lymphocyte count (r=0.5295 and r=0.4957, respectively) and negatively with GALS-1, -3, and -9 plasma concentrations (r=-0.5162, r=-0.7082, r=-0.7209, and r=-0.7647, respectively). TIM-3 gene expression correlated positively (p<0.05) with monocyte count. There was no correlation of gene expression with body weight, body condition, FAMACHA, and strongle egg count.

3. Discussion

The periparturient period for ruminants is defined as approximately 3 weeks pre-partum through 3 weeks postpartum [4]. During this period there is an increased incidence of several economically important diseases which cause significant production losses in the goat industry and decreases the availability of safe and nutritious food for a growing global population. The body undergoes great changes in order to adapt and maintain homeostasis. Phenotypic criteria can be used to indirectly estimate resistance to parasites and diseases. Body weight is one of the important physiological parameters that helps determine the health status of an animal. In our study, body weight was significant (p<0.0139). As expected, Mbayahaga et al. and Otaru et al. [28, 29] observed a postpartum change in body weight which was corroborates with our study. Our results indicated a decrease in body condition score, which was expected (p<0.0162). Body condition was scored on a 5-point scale of from 1 to 5 based on a precise description of the body region employed according to the amount of fat cover and the thickness of the longissimus dorsi muscle, which are used as a guide for subjective scoring. During pregnancy and after kidding, body condition score remained within the range of a healthy goat.

Fecal egg count is a technique used to determine parasitic infection in goats [30]. Levels of parasitic burden was determined using the McMaster egg counting technique. Our results indicate a significant periparturient rise in fecal egg count. There was an increase in strongle egg count 7 days before kidding and a decrease after kidding (p<0.0039). Strongyliodes are considered to be one of the most economical gastrointestinal nematode in goats [31]. Goats infected with H. contortus with FEC greater than 1000 epg indicate substantial infection while 500 epg signifies mild infection [32]. There was also an increase in Coccidia oocyte count 7 days before kidding. Oocyte counts of 5000 per gram or more in feces of host suspected of coccidia infection are indicative of clinical infection [33]. Studies have shown that during the periparturient goats and sheep experience a rise in fecal egg count [34-38]. This results in a decrease in host innate immunity. These results correlate with the results from our study. Galectins have been reported to be secreted and bind to parasites during parasitic infection [39] which may suggest their role in parasitic infection.

The FAMACHA system was developed in South Africa for the classification of animals into categories based on levels of anemia caused by the gastrointestinal parasites such as Haemonchus contortus [30]. It is also very important to evaluate PCV levels periodically to assess anemia [40]. A significant increase in PCV was observed 7 days after kidding and a decrease occurred 14 days after kidding (p<0.001). Azab and Abdel-Maksoud [41] reported a decrease in PCV post-partum. Also, Mandonnet et al. [36] reported a decrease in PCV during the periparturient period which was similar to our results. Throughout our study FAMACHA scores and PCV values remained within the range for healthy goats.

Total protein concentration is a useful indicator of animal health status. Although there were observable trends total plasma protein concentration did not change significantly during the periparturient period. The trends observed include an increase in plasma protein concentration 7 days before kidding. Previous studies conducted by Tóthová et al. [42] reported an increase in plasma protein concentration 7 days before parturition in cow which was similar to our findings. This increase in plasma protein level during the periparturient period may tend to improve immune status of goats.

3.1 Galectin(LGAL) Expression in Periparturient Goats

Both secretion of galectins-1, -3, and -9 in plasma as well as differential expression of LGALs-1, -2, -3, -3bp, -4, -6, -7, -8 -9, -11, -12, -14, -16, and TIM-3 in blood was observed in periparturient goats. Galectins, a growing family of carbohydrate-binding proteins, have recently attracted the attention novel regulators of immune cell homeostasis. Galectins are expressed by different types of cells and tissues, have diverse functions, and play important roles in host responses to infections of parasites and other pathogens [14].

Galectin-1, which is a prototype galectin, has been reported to be localized in the placenta, macrophages, and most organs [43]. They are widely expressed among different tissues of various species. Studies have shown that they display anti-inflammatory activities by blocking or attenuating signaling events that lead to leukocyte infiltration, migration, and recruitment [44]. They also display effects on innate immunity, including cell surface exposure of phosphatidyl-serine in activated neutrophils, a process that leads to neutrophil removal by phagocytic cells without causing apoptosis, and activation/deactivation of macrophages on a concentration-dependent manner [11, 45]. Galectin-1 may have pro- or anti-apoptotic effects on T cells depending on the developmental stage and activation status of the cell and the microenvironment in which the exposure takes place [11]. In our study there was a decrease in concentration and fold change 7 days before kidding. There was increase in GALS-1 7 days after kidding which may suggest their role during infection.

Galectin-2 is also a prototype galectin that has been reported to be localized in the gastrointestinal tract and placenta [43, 46, 47]. Studies have identified them as one of the main gastric mucosal proteins that is proposed to have a protective role in the stomach which plays a protective function in the gastrointestinal tract [48]. In our study there was an increase in fold change of LGAL-2 which could suggest their role in infection.

Galectin-3 is the only family member that is composed of a glycine/prolinerich N-terminal repeated sequence and a C-terminal carbohydrate-binding domain [49]. Galectin-3 is a chimera-type galectin and is normally expressed in various epithelia and inflammatory cells, such as activated macrophages, dendritic cells, neutrophils, and is upregulated during inflammation, cell proliferation, and cell differentiation [11, 50]. It shows pro-inflammatory activity, enhances macrophage survival, and positively modulates macrophage recruitment and antimicrobial activity [51]. Their role in immunity and immunity and inflammation have been extensively studied [52, 53]. In our study, there was an increase of LGAL-3 7 days after kidding which may suggest their role in inflammation. Previous study conducted by our research group has reported the expression of Galectin-3 in sheep during the periparturient period [27]. In our study, LGAL-3pb which is a receptor for LGAL-3 was also expressed but was decreased during the periparturient period. LGAL-3bp promotes cell-cell adhesion through bridging between galectin molecules bound to extracellular components [54].

Galectin-4 is a tandem-repeat type of galectins that is found in the gastrointestinal tract digestive tract [43, 55] and also expressed at the maternal-fetal interface during placentation in rat [56]. Previous study has shown their function in immune modulation [57]. In our study, LGAL-4 was reduced prepartum but increased 7 days after kidding. Previous studies have reported the role of galectins in the modulation of maternal immune response during pregnancy in cow [58] which may suggest their role in immune modulation during pregnancy.

Galectin-7 is a prototype galectin that is found on the skin and tumors of epidermal origin [43]. Galectin-7 is also found in the cytosol, in mitochondria and the nucleus, but its function in the nucleus is largely unknown [59]. Studies have also shown their expression in mammals [60]. Among the galectins, Galectin-7 presents a unique tissue-specific expression pattern and participates in diverse biological processes, notably in the regulation of epithelial homeostasis [59, 61]. Studies have shown their role in cell growth, cell differentiation, adhesion, migration and apoptosis [62]. In our study, LGAL-7 expression increased during the periparturient period.

Galectin-8 is a mammalian β-galactoside-binding lectin, endowed with proinflammatory properties [63]. They are localized in the liver, kidney, lung, and brain [43]. It has been reported that Galectin-8 is also highly expressed in the human placenta and fetal membranes attached to maternal decidua [16]. Upon secretion, Galectin-8 acts as a physiological modulator of cell adhesion [64]. Studies have shown that Galectin-8 induces firm and reversible adhesion of peripheral blood neutrophils in vitro which plays a role in innate immunity to bacterial infection [65]. Our results show that LGAL-8 was reduced prepartum and 7 days postpartum. This may suggest their role in innate immunity.

Galectin-9 is a tandem-repeat type of galectin that is highly expressed in various tissues of the immune system such as bone marrow, the spleen, thymus, and lymph nodes [11, 43]. Studies have reported that Gal-9 may regulate the immune function of NK cells during pregnancy depending on the activation threshold, stage of pregnancy, inflammatory stimuli, and relative expression of cellular receptors [66, 67]. They induce the transcription of pro-inflammatory cytokines [68]. Studies have suggested a critical role of Galectin-9 in the initiation of innate immune responses through the interaction T-cell immunoglobulin- and mucin domain-containing molecule-3 (TIM-3), which acts as a receptor for Galectin-9, is expressed on innate immune cells, and promotes tissue inflammation [25, 69]. In our study, there was an increase of GALS-9 in concentration and expression after kidding. Among several identified receptors of Galectin-9, TIM-3 has been studied most extensively. Our results also showed that LGAL-9 and its receptor TIM-3 were expressed in goat blood during the periparturient period. Studies have shown that Galectin-9 expressing regulatory T cells and TIM-3 could play an important role in the maintenance of healthy pregnancy as wells as regulation of maternal immune tolerance toward the fetus and may be a potent regulator of the adaptive and innate immune responses [67].

Galectin-11 is a prototype of galectin that is to the nucleus and cytoplasm of epithelial cells lining the gastrointestinal tract and bile ducts and is also found in the mucus of the abomasum and small intestines of infected animals [39, 70, 71]. Studies have shown that they are inducible and highly upregulated in tissues infiltrated by eosinophils after an H. contortus infection in sheep, suggesting that its expression was induced by the inflammatory response [70, 72]. They are also protective against H. contortus infection [18]. The higher rate of expression during a secondary challenge suggests that Galectin-11 might be involved in both the innate and adaptive immune response to gastrointestinal parasite infection [72]. Young and Meeusen [20] detected of high levels of Galectin-11 mRNA in helminth infected goats, but not control. In our study, there was an increase in LGAL-11 expression. There was no expression of LGAL-11 7 days postpartum. The expression of LGAL-11 in our study may suggest their role in mediating resistance to gastrointestinal infection and also their involvement in both the innate and adaptive immune response to gastrointestinal parasite infection.

Galectin-12 is a tandem-repeat type of galectin that is predominantly expressed in adipose tissue but is also detected in macrophages and peripheral blood leukocytes [73]. Other studies have also detected It is also detected low levels in the heart, pancreas, spleen, thymus, and peripheral blood leukocytes [74]. Studies have shown their regulation of cell growth and apoptosis [73]. Yang et al.

[75] reported that Galectin-12 is a major regulator of adipose tissue development. They also regulate macrophage polarization as well as enhancing inflammatory responses. In our study, LGAL-12 was decreased 14 days before kidding and 7 days after kidding. There was an increase in expression 14 days after kidding. Our results may suggest the role they play during inflammation.

Galectin-14 is a prototype galectin whose expression is restricted to eosinophils where it plays a role in allergic inflammation [76]. Studies have associated their function to inflammation induced by allergies and helminth infection [18-20], although their roles in parasitic immunity is still being elucidated. In our study, there was an increase of LGAL-14 with a highest increased in expression 7 days after kidding. Souza et al. [77] reported an increase in Galectin-14 in sheep after infection of H. contortus. This result correlates with the finding from our study and may suggest their role in inflammation and gastrointestinal parasitic infection during the periparturient period.

Galectin-15 also known as OVGAL 11 is a prototype galectin that is expressed specifically by the endometrial luminal epithelium and superficial ductal glandular epithelium of the ovine uterus [14, 78, 79]. Studies have shown that they were identified in ovine intestinal epithelium as being induced in response to infection by Haemonchus contortus, a nematode parasite [80]. They play a role in cell adhesion, chemoattraction, and migration as well as cell growth, differentiation, and apoptosis [81] which are important for peri-implantation blastocyst growth and differentiation [82]. In our study, we detected the expression of LGAL-15 in whole blood from goats during the periparturient period. Galectin-15 was expressed 14 days, 7 days prepartum, and 14 days postpartum. There was no detection of LGAL-15 7 days postpartum. This may suggest their role in response to infection during the periparturient period. Farmer et al. [78] reported the expression of LGAL-15 in goats which also collaborates with our study.

Galectin-16 has not been well studied. It is predominantly and highly expressed in the placenta, endothelia of fetal vessels, and in the amnion and chorionic trophoblasts in fetal membranes [79, 83]. Studies have also shown the expression of Galectin-16 in relation to the differentiation and syncytialization of the villous trophoblast, which is very important in the production of placental hormones in immune proteins [79]. In our study, we report the expression of LGAL-16 in blood from goats during the periparturient period. In our study, LGAL-16 was increased 7 days before kidding and decreased afterward. This may suggest their role in as immune surveillance agents that cross-link and interact with immune cells.

4. Materials and Method

4.1 Animals and Housing

Five female BoerXSpanish goats were used from North Carolina Agricultural and Technical State University Farm. Animals were clinically healthy and not under any treatment. All experiments were approved and performed according to the guiding principles for the Institutional Animal Care and Use Committee (IACUC ID: 15-006.0).

4.2 Sample Collection

Samples were collected at 14 days and 7 days before birth, and 7 days and 14 days after birth. Samples were also collected from non-pregnant goats. The body weight of each goat was measured on a portable weighing scale in kilograms before feeding in the morning. The color of the conjunctival mucosa membranes of each animal was evaluated as classified into five categories according to the FAMACHA eye color chart [30] as previously described by [84]. Body condition score was evaluated as described by [85]. Fecal samples were collected and evaluated once a week throughout the experiment. Individual fecal egg counts were determined using the modified McMaster's technique [86]. The numbers of strongyle eggs per gram and coccidia oocyst were counted as described by [30].

Blood samples (10 mL) were collected from the jugular vein aseptically into tubes containing EDTA for cell count analysis, Gel and Lithium Heparin (BD, Franklin Lakes, NJ) for serum collection and acid citrate dextrose for RNA isolation. Packed Cell Volume (PCV) was evaluated using an aliquot of blood in micro-capillary tubes and centrifuged for 5 min at 14,000 rpm in an IEC MB Micro

Hematocrit centrifuge (Damon/IEC Division). White blood cell differential counts have been described previously [84].

4.3 Blood Plasma Assays

Plasma was analyzed for total protein concentration (Thermo Scientific Pierce, Rockford, IL) following the manufacturer's protocol as previously described [87, 88]. Galectin concentration was detected using a commercial ELISA (ABclonal Biotechnology, Woburn, MA) following the manufacturer's protocol. Results were analyzed following the manufacturer's manual. Plasma concentration was expressed as ng/ml and pg/ml.

4.4 RNA Extraction

Total RNA was isolated from whole blood using TRIzol (Molecular Research Centre, Inc. Cincinnati, OH) following extraction as previously described [87]. The quantity and quality of RNA were measured with ND-1000 UV/VIS Nanodrop (NanoDrop Technologies) spectrophotometer (260 nm and 260/280 nm, respectively).

4.5 Real-time PCR

Reverse transcription of RNA was performed using Oligo (dT) primers with 2 ug of the total RNA from each treatment group using a cDNA RETRO script Kit (Ambion Inc., Austin, TX) as previously described [89]. The cDNA products were measured for purity and concentration using the Nanodrop spectrophotometer. Primers specific for galectins were designed using Primer 3 online tool (v. 0.4.0) and are shown in Table 2 (Eurofins Genomics, Louisville, KY). Each PCR was performed in triplicates and normalized using the housekeeping gene Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and β-actin. Fold change in gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method [90].

Table 2. Primer Sequences for Selected Genes Used for Real-time PCR.

Gene	Primer	Sequence	Expected Product Size (bp)
LGALs 1	Forward	TTCAACCCTCGTTTTGAAGC	170
	Reverse	GGCAGCTTGATGGTTAGGTC	
LGALs 2	Forward	CATCGTGACCTTCGAGAACA	219
	Reverse	TGATCCCACATGAAGAGCAG	
LGALs 3	Forward	TCCACTTTAACCCACGCTTC	151
	Reverse	TCAGGTTCAACCAGCACTTG	
LGALs 3bp	Forward	CATCCGTCCCTTCTACCTGA	220
	Reverse	CCAGGGAAGTCTGCAGTAGC	
LGALs 4	Forward	AGCGAGCACATGAAGAGGTT	163
	Reverse	GCATGCTCATTTTCCTCTCC	
LGALs 7	Forward	TCTACGTGAACCTGCTGTGC	237
	Reverse	ACCGGAAGTGGTGGTATTCA	
LGALs 8	Forward	CAGCCTGGAGTACAAGCACA	156
	Reverse	ACCAAGGCCAGTGTTACAGG	
LGALs 9	Forward	GTGCCCAGGCTTCCTACATA	153
	Reverse	GGTCGTTATAGCCGGTCTGA	

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LGALs 11	Forward	CGAACCCCTATCAGCAGTCT	161
	Reverse	TCCCTTCACCTTCAGCATTT	
LGALs 12	Forward	GTGAACAAAAGAACCCCCGC	171
	Reverse	CTAAGCAGAGAGGGCGATGG	
LGALs 14	Forward	ATTCCTGTTGCAGAAGTCTACCTGG	252
	Reverse	GAACATCTTCCACACGGTAGGGGT	
LGALs 15	Forward	GCGACATTCCATTTCGTTTC	188
	Reverse	CTGGCAGATGGGCTTGTTAT	
LGALs 16	Forward	TTGAGCTGCAGTTCTTGGTG	158
	Reverse	CGCCCCTTATAACGTATCCA	
β-actin	Reverse	TCTTCCAGCCTTCCTTCCTG	172
	Forward	ACCGTGTTGGCGTAGAGGTC	
GAPDH	Reverse	CTCCATGGTGGTGAAGAC	198
	Forward	GTCTTCACCACCATGGAG	
Tim-3	Reverse	AAACGGCACCTAAACAGAGC	101
	Forward	GACAACACCAAGCCCCTAGA	

4.6 Statistical Analysis

All data were analyzed using PROC GLM model in SAS 9.4 version (SAS Institute, Cary, NC). A Pearson's correlation analysis was utilized to evaluate relationships between galectins and other parameters at each of four time points. Statistical significance was set at a $P<0.05$. Mean separation was done using Tukeys. Data are presented as mean ± standard error of the mean (SEM).

5. Conclusion

This study has described the expression of galectins and their ligands in goat blood in non-pregnant and pregnant goats. There was distinct pattern of galectin expression during the periparturient period. There was also a decrease in body weight, body condition, and rise in fecal egg count during the periparturient period. It has been shown that these galectins play an important role in immunity and maintain homeostasis. Galectin signatures may be used for breeding of naturally resistant and resilient livestock, production of better diagnostics, preventives and targeted treatment for improved animal management. Further studies would be required to determine the role of each galectins during the periparturient period. This study will provide interesting new possibilities in the diagnosis and treatment of diseases and help delineate novel therapeutic strategies in inflammatory and infection.

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Conflicts of Interest. The authors declare no conflict of interest.

Author contributions: Kingsley Ekwemalor performed the experiment, analyzed data and prepared the manuscript. Mulumebet Worku designed and supervised experiment. Sarah Adjei-Fremah and Emmanuel Asiamah statistically analyzed the data. Bertha Osei and Egbogoye Eluka-Okoludoh contributed to sample collections and preparations.

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