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Research article

GLYCOSYLATION CHANGES IN DAIRY CALF IgG HEAVY CHAINS DURING PRE-RUMINANT TO RUMINANT TRANSITION: PILOT STUDY

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Although IgG heavy chain glycans play a crucial role in mediating immunoglobulin G (IgG) effector functions, data on their structure in cattle remain limited. This study investigated whether pre-ruminant to ruminant transition in dairy calves is associated with changes in the expression of terminal galactose (Gal) and sialic acid (SA) on their IgG heavy chain glycans.

The study was conducted using pooled serum samples from four groups of 20 calves, with median ages of 48±9, 75±7, 103±7, and 141±8 days (Group I, II, III, and IV, respectively). Gal and SA expression on affinity-isolated serum IgG heavy chains was analyzed by *Ricinus communis* agglutinin I (RCA I) and *Sambucus nigra* agglutinin (SNA) lectin blots.

Gal and SA were expressed on IgG heavy chains of all age groups. The expression of Gal was highest in the youngest calves (Group I) and decreased by 40%, 53%, and 62% in Groups II, III, and IV, respectively. In contrast, SA expression was lowest in Group I, increased sharply in Group II (2.9 times higher than in Group I), remained stable in Group III, and slightly decreased in Group IV. The Gal-to-SA ratio in Group I was 5 to 6 times higher than in the older groups.

This study highlights the dynamic changes in glycosylation of calf IgG molecules during preruminant to ruminant transition. Remains to be determined whether observed age-dependent increase in SA expression might represent an adaptation to the evolving gastrointestinal microbiome in this period, when shifting toward sialylated, anti-inflammatory IgG that may help protect immature tissues from inflammatory damage.

Keywords: Calves, IgG, glycans, galactose, sialic acid

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INTRODUCTION

Cattle have five classes of immunoglobulins (Ig): IgG, IgM, IgA, IgE, and IgD, [1-3] each with its specific effector function. Although IgG shares fundamental biological properties with IgG of other mammals, its exceptionally high molecular polymorphism – highest among mammalian Ig, a long time ago classified bovine IgG as an immunoglobulin infraclass [1]. The bovine IgG has three subclasses (IgG1, IgG2, and IgG3) and a greater number of allotypes (IgG1a/b/c, IgG2a/b, IgG3a/b) [2-4] and numerous glycoforms [4-11]. In addition, bovine IgG molecules differ in lengths of third complementarity-determining region of the heavy chain (CDRH3) – the most variable part of the heavy chain variable domain: canonical with 5-10 ammino acids and ultralong with 48-80 amino acids in antigen-binding domains [12]. All this, together with numerous (more than 40) IgG glycoforms [4-11], contribute to this huge heterogeneity of bovine IgG molecules. In cattle IgG is the predominant immunoglobulin class found in colostrum and on mucosal surfaces [13]. Since there is no transplacental transfer of immunoglobulins in cattle, calves are born agammaglobulinemic, or might have IgG in traces [14,15]. They derive passive immune protection by absorbing immunoglobulins, but also leukocytes, from the colostrum [14,16,17]. It is well known that in the first day of life of calves, the concentration of peripheral blood IgG is highest after colostrum ingestion and then gradually decreases reaching the minimum value in the 10th week of age [18,19]. In this period maternal IgG molecules are almost totally catabolized (half-life of colostral IgG was 28.5 days [20]) and production of calf's own antibodies is low due to immaturity of their immune system.

All immunoglobulins are glycoproteins, and the glycans (oligosaccharide moieties) attached to their heavy chains play a crucial role in modulating their effector functions in response to natural infection or vaccination, but also in their pathogenic potential by modulating immunoglobulin binding to receptors for their crystallizable fragment (Fc receptor) or complement proteins [21-27]. All IgG molecules contain N-linked glycans covalently attached to the Fc region of the heavy chain at a conserved asparagine residue within the canonical N-glycosylation tripeptide motif (Asn–X–Ser/Thr, where X ≠ Pro) located in the CH2 domain of the Fc region. IgG molecules have no conserved N-glycosylation site in their Fab fragment. However, at least for humans, it was shown that somatic mutations in the variable region might create this triplet sequence for attaching carbohydrates in 15-25% of IgG molecules [21-24,26,27]. Glycosylation changes in human IgG heavy chains during various physiological conditions (aging, pregnancy, extensive exercise, etc.) and in infectious, inflammatory/autoimmune, and malignant diseases, have been extensively studied [21-24,26,27].

However, as reported Zlatina and Galuska [25], investigations of IgG glycosylation remain very limited in veterinary medicine. Research on the glycosylation profiles of bovine IgG in peripheral blood and milk from healthy animals is relatively scarce [4-11]. In studies focused on changes in IgG glycosylation during early lactation, it was shown

that changes in sialylation are identified as key changes in bovine colostrum/milk IgG glycans [8-11]. Recently it has been reported that similar trend of decreasing sialylated oligosaccharides during the transition from colostrum through transitional to mature dairy cow milk is not limited to IgG but includes total proteins oligosaccharide [28].

To our knowledge, our research group is the only one to have reported disease-induced alterations in cattle IgG glycosylation. We showed that calf bronchopneumonia is accompanied with an increased expression of sialic acid (SA), but not galactose (Gal), N-acetylglucosamine (GlcNAc), and fucose (Fuc), on IgG heavy chains [29].

Given the immaturity of the calf immune system [30], along with the known influence of IgG glycan composition on effector functions [25], this study aims to investigate whether, besides changes in IgG concentrations in the peripheral blood in pre-ruminant to ruminant transition stage, alterations also occur in the expression of two IgG heavy chain's terminal monosaccharides: SA and Gal that precedes SA in most glycan structures. These terminal monosaccharides play an important role in promoting the anti-inflammatory phenotype of IgG [31-33], and modification of their expression might be critical for the calf's adaptation to the gastrointestinal tract (GIT) microbiome.

MATERIALS AND METHODS

Animals

The study involved 80 healthy female Holstein-Friesian calves, aged between 31 and 154 days, born and raised at Vrbovski Farm, Al Dahra, Belgrade. The use of animals was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade (No 05-11/2023), in accordance with the National Regulation on Animal Welfare. On the second day after birth, all the calves were administered a single dose of 1.5 ml of Rexxolade (Tulathromycin, 100 mg/ml; Dehra, UK), 3 ml of Mediprofen (Ketoprofen, 100 mg/ml; Vet Medik, Vršac, Serbia), and 3 ml of E Selen (100 mg Vitamin E, 0.45 mg selenium as sodium selenite in 100 ml volume; Veyx, Germany). Calves included in the study, as well as and their mothers during pregnancy, were not vaccinated. Peripheral blood from all calves was collected on the same day, in November 2023. Before the blood sampling all calves were clinically examined by a veterinarian. All calves were in good general health condition without any obvious clinical signs of disease.

The calves were categorized into four age groups: Group I, with age of 48 ± 9 days (min – max: 31-60 days), Group II, with median age of 76 ± 7 days (min-max: 64-88 days), Group III, with median age of 103 ± 7 days (min-max: 95-118 days) and Group IV, with median age of 141 ± 7 days (min-max: 127-154 days).

Serum Samples

Serum samples were isolated through spontaneous coagulation of whole blood. Individual sera from each age group were pooled into composite samples, with equal volumes taken from each sample. The concentration of proteins in serum was determined using the Bicinchoninic Acid Assay (BCA) assay with the "BCA Protein Assay" kit (Pierce, Rockford, IL).

Isolation of IgG

IgG from the pooled serum samples was isolated using affinity chromatography on protein G (Dynabeads™ Protein G, Thermo Fisher Scientific) [34]. A 40 μL volume of the beads were added in a 120 μL volume of six times diluted serum samples. After 40 min mixing on orbital shaker at 22 °C, the beads were precipitated by centrifugation and washed four times with phosphate buffered saline pH 7.2–7.4 (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4). Bound proteins were released by the sorbent boiling in 100 μL of 2× nonreducing (125 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 0.02% bromophenol blue) or 100 μL 2× reducing (125 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 0.2 M 2-mercaptoethanol, 0.02% bromophenol blue) sample buffer. The beads were precipitated by centrifugation, and the aliquots of the supernatants with isolated IgG were stored pending analysis at – 20 °C, no longer than one month.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Pooled calf serum samples were analyzed with SDS-PAGE under non-reducing conditions, while affinity isolated calf IgG was analyzed under both non-reducing and reducing conditions [34]. Electrophoresis was carried out in SE 260 Mighty Small II Vertical Slab Electrophoresis Unit (GE HealthCare LifeScience, NJ). For non-reducing conditions, 8% running and 4% stacking gels were used; for reducing conditions, 10% running gels were used. Separation was done at 40 mA with cooling. Protein bands were visualized using Coomassie brilliant blue R-250, C.I. 42660 (Sigma) and imaged with ChemiDoc 2.0 (Bio-Rad, Hercules, CA). Densitometry was performed using the ImageMaster Total Lab TL 120 software (GE HealthCare LifeScience, NJ, USA). As markers of electrophoretic mobility of serum protein fractions we used rivanol soluble serum proteins containing IgG and transferrin [34] isolated in our laboratory, and commercially purchased bovine serum albumin (Serva, Heidelberg, Germany). The proportion (percentage) of each protein fraction visualized by SDS-PAGE was determined using densitometry. The concentration of protein in each protein fraction was calculated using the formula:

$$\text{Protein fraction (g/l)} = \frac{\text{Total serum proteins (g/l) x protein fraction (\%)}}{100\ \%}$$

Lectin blot analysis

The expression levels of Gal and SA on IgG heavy chains were assessed with lectin blotting based on the reactivity with biotinylated *Ricinus communis* agglutinin (RCA I; Vector Laboratories, Newark, CA), specific for β1-4 linked Gal, and *Sambucus nigra* agglutinin (SNA; Vector Laboratories, Newark, CA), specific for α2-6 linked SA.

After SDS-PAGE under reducing conditions the affinity isolated IgG were transferred from gels to nitrocellulose (NC) membrane, with 0,45 μm pores (PanReac AppliChem ITW Reagents, Darmstadt, Germany) using a semi-wet transfer method (Hoefer TE70X; Cytiva, Marlborough, MA) at 2 mA/cm² for 1 hour. The membrane was stained with 0.1% Ponceau S then washed until pink bands appeared. To prevent nonspecific binding of lectins to the membrane, NC membranes were incubated overnight at 4 °C in a 3% BSA solution in TBS Tween and then incubated with biotinylated RCA I or SNA lectins (1 μg/ml and 2.5 μg/ml, respectively) for 1 hour, followed by incubation with avidin-peroxidase (1:1000). Bands were visualized by enhanced chemiluminescence reagent (Serva, Heidelberg, Germany) and imaged with ChemiDoc 2.0 (Bio-Rad, Hercules, CA). The intensity of the chemiluminescence signals from lectin blotting was quantified using Image Master Total Lab software.

RESULTS

In SDS-PAGE under non-reducing conditions, proteins from all serum samples were separated into four main fractions: albumin (66 kDa) [35], transferrin (78 and 72 kDa) [35], IgG (150–160 kDa) [1] and high molecular weight proteins (HMW, i.e., proteins with molecular weights greater >160 kDa). The IgG fraction in all serum samples was not electrophoretically homogeneous, but consisted of several superimposed sub-fractions (Figure 1A). The concentration of protein in the serum fraction, which according to electrophoretic mobility (i.e., molecular weight) corresponds to bovine IgG, increased with age of calves: In Group I was 9 g/L, then in Group II increased sharply to 19 g/L, remained unchanged in Group III, and only slightly decreased (16 g/l) in Group IV (Figure 1B).

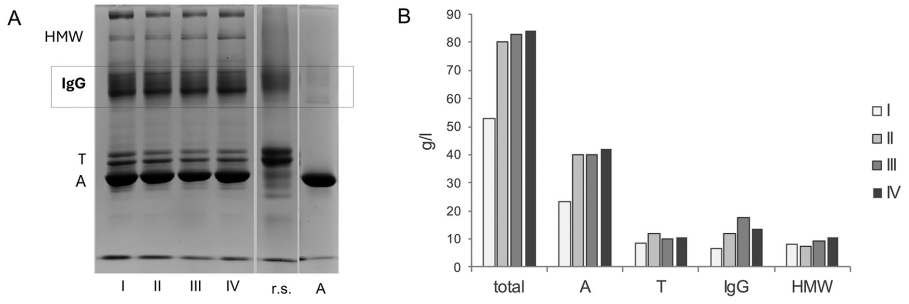

Figure 1. Non reducing SDS-PAGE of pooled serum proteins of healthy calves with age of 48 ± 9, 75 ± 7, 103 ± 7, and 141 ± 8 days (Group I, II, III and IV). **A)** Electrophoretogram; HMW – high molecular weight proteins; T – transferrin; A – albumin; r.s. – rivanol soluble serum proteins. **B)** The concentration of main serum protein fractions calculated based on result of their densitometric quantification.

IgG was isolated from pooled serum samples by affinity precipitation on Protein G sorbent allowing isolation of all molecular form of bovine IgG [36]. Non-reducing SDS-PAGE in all Protein G isolates revealed two major protein fractions, comprising approximately 95% of the total protein content, along with up to five minor protein fractions whose presence depending on calf age (Figure 2A). The proportion of two main protein fractions (Fraction 3 and 4) was influenced by the age of the calves (Figure 2B). The percentage of the higher molecular weight fraction (Fraction 3) was lowest in the youngest calves (Group I) at 31%, increased to 53% in Group II, and remained relatively stable in older calves (Group III – 58%; Group IV – 56%). In contrast, the percentage of the lower molecular weight fraction (Fraction 4) was highest in the youngest calves and decreased with increasing age.

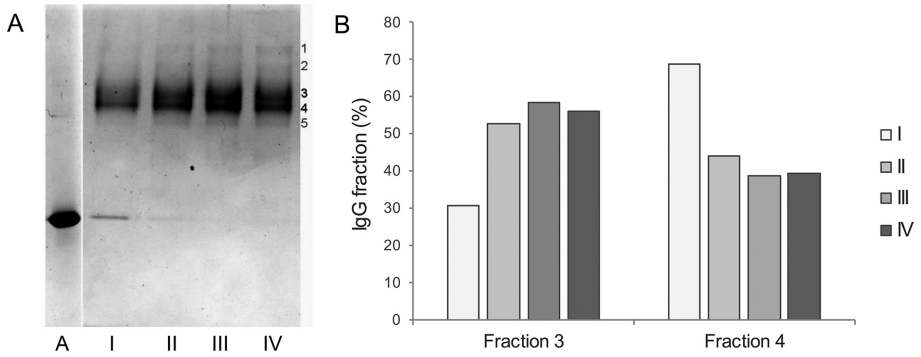

Figure 2. Non reducing SDS-PAGE of Protein G isolated IgG from pooled sera of healthy calves with age 48 ± 9, 75 ± 7, 103 ± 7, and 141 ± 8 days (Group I, II, III and IV). **A)** Electrophoretogram; A – albumin; 1, 2, 3, 4 and 5 – IgG sub-fractions. **B)** Percentage of the two most abundant IgG fractions (fractions 3 and 4) relative to total serum IgG – densitometric analysis.

SDS-PAGE in reducing conditions detected two massive fractions corresponding to IgG heavy and light chains (Figure 3A). Lectin blot analysis using RCA I and SNA lectins revealed that serum IgG heavy chains across all calf age groups exhibited terminal Gal and SA residues (Figure 3B). Relative content (i.e., level of expression) of Gal and SA on IgG heavy chains is present as the ratio of the intensity of their reactivity with specific lectin (RCA I and SNA) after Coomassie blue staining to correct any variation in the amount of IgG applied in SDS-PAGE [29]. The data obtained indicated that Gal expression on IgG heavy chains was highest in the youngest calves (Group I) and decreased progressively with age. Specifically, compared to Group I, Gal expression was reduced by 40%, 53%, and 62% in Groups II, III, and IV, respectively (Figure 3C1). Conversely, SA expression was lowest in Group I and increased sharply in Group II, where it was approximately 2.9 times higher than in Group I. Expression levels remained stable in Group III and slightly decreased in Group IV, maintaining a 2.2-fold increase over Group I (Figure 3C2). Given that that Gal and SA are sequentially

added to the N-linked oligosaccharide of IgG heavy chains their relative content was also present as a ratio of intensity of RCA I and SNA reactivity. The value of Gal/SA ratio was highest in Group I. In older calves the value of Gal/SA ratio was from 5 to 6 times lower than in calves in Group I (Figure 3C3).

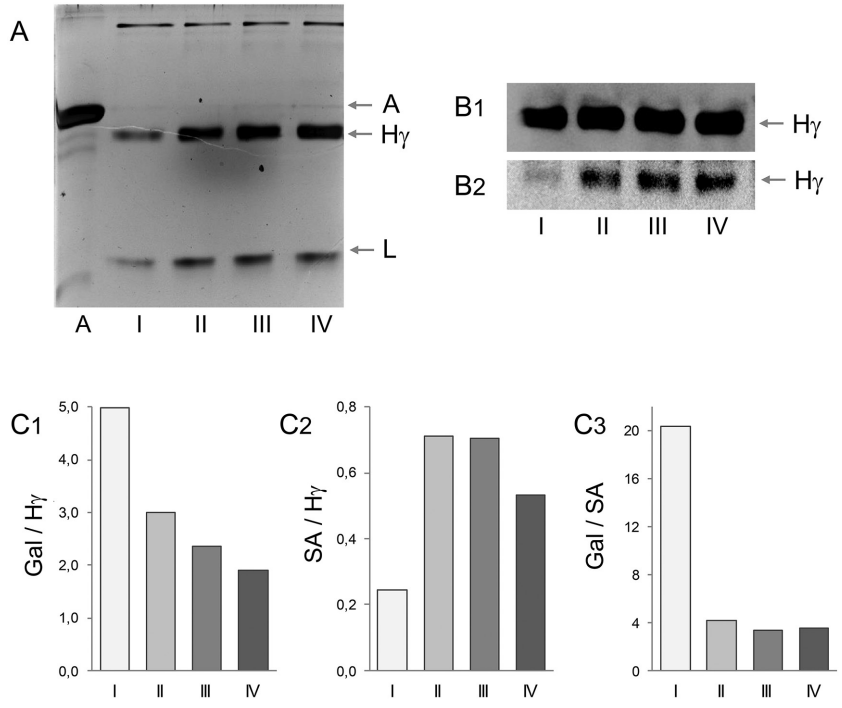

Figure 3. Expression of Gal and SA on Protein G isolated IgG from pooled serum samples of healthy calves with age of 48 ± 9, 75 ± 7, 103 ± 7, and 141 ± 8 days (Group I, II, III and IV). **A)** SDS-PAGE under reducing conditions. Hγ – IgG heavy chains, L – IgG light chains. **B)** Lectin blot with Gal specific RCA I (B1) and SA specific SNA (B2). **C)** The relative amounts of Gal and SA on IgG heavy chain – densitometric analysis

DISCUSSION

Numerous studies have confirmed that IgG glycosylation undergoes permanent changes throughout the lifespan in both humans and experimental animals [22,26,27]. Although analyzing the dynamic changes in oligosaccharide moieties on IgG during early life in domestic animals might be of great importance, to date, only a single study published last year has reported such changes in pigs [37]. To the best of our knowledge, there have been no studies specifically investigating age-dependent alterations in IgG glycosylation patterns in calves.

In this study, we examined the physiological, age-dependent changes in IgG glycosylation in calves during the transition from the pre-ruminant to the ruminant stage.

To ensure that the observed changes in IgG molecules were attributable solely to age – and not to other variables such as sex, genetics, vaccination, or infection in this study we included only healthy, non-vaccinated female dairy calves, from mothers that had not been vaccinated during gestation. In addition, Holstein-Friesians are predominantly homozygous for the IgG2a allotype. Since Holstein-Friesians are predominantly homozygous for the IgG2a allotype, with heterozygous calves being rare and not secreting detectable levels of the IgG2b allotype until around the third or fourth month of age [38], we minimized potential genetic variability. To minimize the impact of inherent bovine IgG's molecular polymorphism [1-3,12,25] on the results of this study, pooled serum samples of each experimental group were analyzed.

Large-scale studies conducted in several research centers have shown that in general human population (not selected based on specific diseases) exhibits high interindividual variability in IgG glycosylation, more precisely in expression of terminal monosaccharides in IgG linked oligosaccharide moieties [27]. In light of this fact, the use of pooled samples for calf IgG glycan analysis might represents a limitation of our study. However, approximately 50% of IgG glycans variability in healthy humans is determined by genetic factors, as further confirmed by interbreeding and inbreeding experiments in mouse strains, while the remaining variation is influenced by physiological factors, including hormonal status, and lifestyle behaviors [27]. In our study, we analyzed IgG from highly crossbred Holstein-Friesian dairy cattle, which likely minimizes the influence of genetic variability on IgG glycosylation. Furthermore, all calves were female, raised on the same farm, and fed an identical diet, reducing the impact of physiological and environmental factors on IgG glycosylation. These considerations justify the use of pooled calf IgG samples in this study.

Inherent polymorphism of bovine IgG may explain the partially overlapping electrophoretic IgG fractions observed following non-reducing SDS-PAGE of both serum and isolated IgG. Similar findings have been reported in our previous studies [34, 39]. While this heterogeneity is undeniable, at this point we cannot explain its precise molecular basis. In cattle, the synthesis of IgG1 precedes that of IgG2. However, the difference in molecular weight between IgG1 (160 kDa) and IgG2 (150 kDa) [1,2] is insufficient to clearly distinguish these two bovine IgG subclasses on 8% non-reducing SDS-PAGE gels and to attribute the observed changes in the proportion of IgG fractions to an increase in IgG2 synthesis. We can hypothesize that the observed increase in the higher molecular weight IgG fraction (Fraction 3, Figure 2A) in Groups II–IV compared with Group I may reflect the synthesis of IgG containing cattle-specific ultralong CDRH3 regions. These antibodies are known to be generated in response to various antigens [12]. Although bovine colostrum is enriched in IgG with ultralong CDRH3 regions [40], maternal immunoglobulins are catabolized by 75 ± 7 days of age (Group II). The earliest occurrence of these antibodies in calves remains unknown. While Zhao et al. [41] showed that five-month-old calves can produce such IgG, evidence for their earlier synthesis is lacking. Therefore, although our findings may suggest this possibility, they cannot be conclusively explained by the synthesis

of IgG containing ultralong CDRH3 regions. It is worth noting that even though IgA monomers have a slightly higher molecular weight than IgG [1,2], serum IgA in cattle exists exclusively in polymeric forms [42]. Therefore, we think that IgA does not contribute to the electrophoretic heterogeneity observed in this study. In addition, we believe that the low-intensity protein fractions observed in the Protein G isolates represent either proteins complexed with IgG or non-specifically bound proteins, as described in our previous studies [29,34]. Based on the results of our current study, we propose that the observed differences in electrophoretic mobility (molecular weight) of the IgG fractions might be, at least partially, attributed to variations in the glycosylation of their heavy chains.

To investigate the glycosylation of IgG, we employed lectin blotting, which leverages lectin-glycan interactions to detect and characterize carbohydrate epitopes on proteins without the need for glycan isolation [43]. This approach might offer several practical advantages over more advanced techniques like mass spectrometry: it is cost-effective, straightforward to perform. Additionally, lectin blotting allows the analysis of glycans in their "natural" conformation [44] providing insights into the significance of terminal monosaccharides in mediating IgG interactions with Fc receptors and complement proteins, and consequently, in their effector functions.

The results of our current study demonstrated that the age-dependent increase in IgG concentration in the peripheral blood of calves was associated with a decrease in Gal expression and a concomitant increase in SA expression on the heavy chains. Similarly, Zlatina et al. [37], found that, during the first months of life in pigs, on intact IgG molecules the proportion of sialylated glycans increased relative to galactose-terminated ones. The expression of glycans on IgG heavy and light chins was not analyzed in this study. In our previous work [29], we showed that IgG light chains of three-month-old healthy calves, but not those of calves with severe bronchopneumonia, failed to bind SNA or RCA I lectins, suggesting that their light chains either lack Gal and SA residues or express them at levels below the detection limit of our analytical method. Consistent with these findings, the present study also shown no reactivity of IgG light chains with SNA or RCA I (*data not shown*).

As mentioned in the Introduction, studies on the glycosylation of IgG in dairy cattle are relatively rare. A key challenge in interpreting and comparing the results from these studies lies in the use of different methods for glycan analysis and the various approaches to isolating IgG from peripheral blood, milk, or colostrum. Despite these challenges, over 40 distinct glycans expressed on bovine IgG have been identified to date. In 1990, Fuji and colleagues [4] reported that bovine IgG contains low levels of SA, with the major glycans being complex, neutral, fucosylated, and biantennary N-glycans, including di – (32%), mono – (27%), and agalactosylated (12%) forms. Similarly, Saba and colleagues, using multiple techniques, found that the predominant N-linked glycans were core-fucosylated, asialyl, biantennary chains with varying galacosylation, along with minor amounts of monosialyl oligosaccharides [6]. Raju et al. reported that, unlike human IgG, bovine IgG glycans contain GlcNac (Neu5Gc)

but lack N-acetylneuraminic acid (Neu5Ac) residues [5]. In 2014, Adamczyk and colleagues [7] identified fucosylated, agalactosylated (12%), di-galactosylated (24%), and mono-galactosylated (29%) neutral glycans as the most abundant in bovine IgG. They also observed that fewer than 5% of bovine IgG glycans expressed SA. Although abovementioned studies reported the absence of Neu5Ac in the glycans of bovine peripheral blood IgG, both Neu5Gc and Neu5Ac were detected in the Fc glycans of bovine colostral and milk IgG [8-11,28].

Age-dependent changes in the glycosylation of peripheral blood IgG in healthy cattle have not yet been explored. To date, research has mainly focused on changes in the glycans of colostral/milk IgG during the early lactation period [8-11]. These studies consistently report that in bovine colostrum, about half of the IgG N-glycans are sialylated and that the sialylation levels decrease significantly by day 10 of lactation. Neu5Ac was identified as a unique feature of bovine colostrum, but it becomes undetectable in IgG from both bovine milk and pooled bovine serum. In contrast, the expression of Neu5Gc remains constant throughout lactation [11]. The biological significance of these changes in IgG sialylation is still unclear. Studies have shown that SA and Gal do not appear to influence IgG binding to the bovine neonatal Fc receptor (FcRn) [8]. While the precise biological role of sialylation in bovine colostral and milk IgG remains uncertain, it is obvious that its function is important in first days of calf life.

In our previous study, we demonstrated that calf bronchopneumonia is associated with an increased expression of SA on the heavy chains of peripheral blood IgG [29]. In the current study, we show that the transition from pre-ruminant to ruminant in healthy calves is characterized by an age-dependent increase in SA expression on the heavy chains of peripheral blood IgG of healthy calves. IgG heavy chain glycans, in both humans and experimental animals, influence conformational stability, electrical charge, and solubility, and they can affect the binding to receptors for IgG Fc fragment (Fcγ receptors) and C1q complement component, as well as catabolism of IgG molecules [21-24,26]. Both SA and Fuc (a predominant monosaccharide in bovine IgG glycans) play a role in stabilizing a "closer" conformation of the Fc fragment [21,22,25]. However, whether these conformations exist in bovine IgG and which IgG characteristics are modulated by SA-containing glycans remain unclear at this point. We can only speculate that, during the pre-ruminant to ruminant transition, SA-containing glycans may promote an anti-inflammatory phenotype of IgG, which could help prevent uncontrolled immune reactions triggered by the colonization of the GIT with microorganisms derived from the dynamic milk microbiome [45] or the environment. However, the role of sialylated glycans might be quite the opposite. For example, the removal of SA from IgE has been shown to reduce effector-cell degranulation and anaphylaxis in various models of allergic disease [46].

The results from the SNA lectin blot analysis should be interpreted with caution. Specifically, SNA recognizes α2-6 linked Neu5Ac, which is the predominant SA linkage found in the glycans of bovine milk IgG during early lactation [8,9]. Several

research groups reported that Neu5Gc is rather than Neu5Ac present in glycans of mature bovine milk IgG [8-11] and peripheral blood IgG of adult cattle [5-7]. However, Isernhagen et al. [28] demonstrated that Neu5Gc (expressed on total milk proteins) was detectable on the day of calving, underwent a gradual decline over the next two days, and became undetectable after this period. The sugar specificity of SNA, we confirmed by inhibitory dot blot assay using 400 mM Neu5Ac (*data not shown*). SubB2M, unique Neu5Gc-specific lectin that could discriminate Neu5Gc – over Neu5Ac-glycoconjugates [47] was not used in this study. We recognize that this limitation may be seen as a potential drawback in our work. We also want to emphasize the need for future research to explore the preference for Neu5Gc versus Neu5Ac expression on peripheral blood IgG across different breeds of domestic cattle (*Bos taurus*), which may be genetically distinct due to artificial selection. This is especially important given that several studies investigating the glycosylation of bovine peripheral IgG [4-8] do not specify the cattle breed or the methods used for isolation of bovine IgG. This omission is critical, as different isolation techniques are known to yield distinct molecular forms of bovine IgG [48].

The results of the study have shown indicate that the postnatal development of calves is characterized not only by an increase in IgG concentration, but also by their structural changes whose significance for effector functions of IgG antibodies remains to be clarified in future research. This should involve more advanced methods for analyzing IgG glycan structures, as well as *in vitro* and *in vivo* studies to assess the biological effects of different IgG glycoforms. Furthermore, given the modulatory potential of immunoglobulin glycans on their effector functions, these findings might be important for predicting the effectiveness of the calves' immune response to infections and to both active (i.e., vaccination) and passive immune protection.

CONCLUSIONS

The results of this study demonstrated that the transition from the pre-ruminant to the ruminant stage in calves is accompanied not only by an increase in IgG concentration and alterations in the proportions of IgG fractions with different molecular weights but also by an elevated ratio of terminal sialic acid to galactose on glycans of the IgG heavy chains.

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Authors' contributions

VLjI and NPF designed the study. DDK, VLjI, BŽR and RŽK performed the laboratory procedures. VLjI and NPF analyzed the data. DDK and VLjI wrote the manuscript. All authors read and approved the final version of the manuscript.

Declaration of conflicting interests

The author(s) reported no potential conflicts of interest concerning the research, authorship, or publication of this article.

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PROMENE GLIKOZILACIJE TEŠKIH LANACA IgG KOD MLEČNE TELADI TOKOM PRELASKA OD PREDPREŽIVARA U PREŽIVARE: PILOT STUDIJA

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Iako glikani imaju značajnu ulogu u ostvarivanju efektorskih funkcija IgG, podaci o njihovoj strukturu kod goveda su malobrojni. U ovom radu smo analizirali da li je kod mlečne teladi prelaz iz faze predpreživara u fazu preživara povezan sa promenama u ekspresiji terminalnih monosaharida, galaktoze i sijalinske kiseline, na teškim lancima IgG molekula.

Studija je rađena sa zbirnim uzorcima seruma četiri grupe od po dvadesetoro teladi starosti 48±9, 75±7, 103±7 i 141±8 dana (Grupe I, II, III i IV). Ekspresija galaktoze i sijalinske kiseline na IgG izolovanim afinitetnom hromatografijom analizirana je lektinskimg blotom, uz pomoć RCA I i SNA lektina.

Galaktoza i sijalinska kiselina su bili eksprimirani na teškim lancima IgG teladi svih starosnih grupa. Ekspresija galaktoze je bila najviša kod najmlađe teladi (Grupa I) i smanjivala se za 40%, 53% i 62% u grupama II, III i IV, respektivno. S druge strane, ekspresija sijalinska kiseline je bila najniža u Grupi I, da bi zatim u Grupi naglo porasla II na vrednost 2,9 puta višu nego u Grupi I, ostala nepromenjena u Grupi III i na kraju blago opala u Grupi IV. Odnos ekspresije galaktoze i sialinske kiseline u Grupi I je bio između 5 i 6 puta veći nego u grupama starije teladi.

Rezultati ove studije su pokazali da se kod teladi u periodu prelaska iz predpreživara u preživare dešavaju dinamične promene glikozilacije IgG molekula. Ostaje da se utvrdi da li porsat ekspresije sijalinske kiseline predstavlja adaptaciju na promene u mikrobiomu gastrointestinalnog trakta u ovom periodu, kada bi pomeranje u pravcu sijalinizovanih, anti-inflamatornih IgG moglo da pomogne u zaštiti nezrelih tkiva od inflamatornih oštećenja.