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A preliminary study of the impact of Saccharomyces cerevisiae and Its Cell Wall Supplementation for Dairy cows and the Transfer of passive immunity to calves

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Nadjiba Beldjouhar[1], Mohamed Amine Ayad[2], Mohamed Said Saim[2], Isam Ayad[2], Sofiane Derrar[2], Mustapha Adnane Smadi[3], Saidj Dahia[4]

[1]*Laboratory of the Improvement and Valorization of Local Animal Productions. Veterinary Sciences Institute; Tiaret University, Alger's Road, Pb: 78, 14000 Tiaret, Algeria. ORCID: https://orcid.org/0009-0000-3679-0049*
[2]*Laboratory of Animal Hygiene and Pathologies. Veterinary Sciences Institute; University of Tiaret, Alger's Road, Pb: 78, 14000, Tiaret, Algeria. ORCID: https://orcid.org/0000-0001-7100-9481, ORCID: https://orcid.org/0009-0005-9330-4680, ORCID: https://orcid.org/0009-0006-3051-4792, ORCID: https://orcid.org/0009-0002-5941-7903*
[3]*Animal Biotechnology Laboratory, Center for Research in Biotechnology (CRBt), Constantine, Algeria. ORCID: https://orcid.org/0000-0002-6940-1799*
[4]*Veterinary Sciences Institute, Saad Dahleb University, 1, B.P. 270, Route de Soumâa, 09000. Blida, Algeria. ORCID: https://orcid.org/0000-0001-8380-6437*

Author for correspondence: Nadjiba Beldjouhar – nadjiba.beldjouhar@gmail.com; nadjiba.beldjouhar@univ-tiaret.dz

Abstract: Early detection of failed transfer of passive immunity (FTPI) is crucial to prevent neonatal diseases and mortality. This study evaluates the effect of supplementing dairy cows with Saccharomyces cerevisiae and/or yeast cell walls during the last month of pregnancy on passive immunity transfer to calves. Forty-five Montbeliarde dairy cows were divided into four groups. A control group (CON; n=13) with no supplementation, an SC group (n=12) receiving 5 g/day of S. cerevisiae yeast, a YW group (n=10) receiving 5 g/day of yeast wall (20% mannans, 20% β-glucans), and an SCYW group (n=10) receiving a combination of 5 g of SC and 5 g of YW. At calving, colostrum and blood samples were collected to measure calf serum IgG concentration using radial immunodiffusion (RID). The data were compared using a %Brix refractometer. A one-way ANOVA assessed the effects of supplementation type, colostrum quality, and calf sex. A Spearman correlation test evaluated the suitability of the %Brix refractometer as an indirect tool for IgG estimation. The results showed a significant improvement in passive immune transfer with supplementation ($P < 0.001$), particularly in the SC group, which had an average serum IgG concentration of 35.19 g/L, and the SCYW group, with 37.59 g/L, compared to the control group (20.44 g/L). Moreover, 25% of the calves born to non-supplemented cows exhibited failure of passive immune transfer (FTPI), whereas none (0%) of the calves born to cows supplemented with yeast or with the yeast–yeast cell wall mix showed FTPI. Colostrum quality had a significant effect ($P = 0.017$), whereas calf sex had no impact. A positive correlation was also found between the IgG values obtained using the refractometer (Brix %) and those determined by the RID reference method. In conclusion, supplementing dairy cows with yeast during the last month of pregnancy enhanced passive immunity transfer to calves. The %Brix refractometer is a viable alternative to the RID method for estimating serum IgG concentration and detecting FTPI cases.
Keywords: Dairy cows; Supplementation; FTPI; Immunoglobulin; RID.

1. Introduction

One of the primary concerns of cattle breeders and veterinarians is to enhance the health of calves during the first few days after calving. In cows, the cotyledonary synepitheliochorial placenta serves as a barrier that prevents the transfer of immunoglobulins during pregnancy (Lichtmannsperger et al., 2023). Calves depend on passive immunity derived from immunoglobulins (IgGs) present in colostrum (Godden et al., 2019). Sufficient transfer of passive immunity (TPI) has long been acknowledged as a key factor influencing calf health and survival up to weaning. The failure of passive immunity transfer (FTPI) results in significant economic losses due to higher rates of illness and death. Moreover, it poses a serious concern for animal welfare (Mee et al., 2013). FTPI may be influenced by various factors, including the duration between calving and colostrum harvesting (Morin et al., 2010), the quality of the colostrum, and cow-specific factors such as parity and genetic traits, such as breed (Reschke et al., 2017; Sutter et al., 2019). Environmental conditions, including the season of birth, temperature, and humidity levels, also affect this relation (Cordero-Solorzano et al., 2022). Therefore, most of the enhancing factors are related to colostrum quality (Cordero-Solorzano et al., 2022).

Several types of supplementation have been used in the last month of pregnancy in dairy cows to improve calf health and, consequently, colostrum quality, thereby reducing the incidence of FTPI (Hue et al., 2021a). Probiotics such as yeast and yeast-derived products have gained particular attention due to their ability to modulate rumen fermentation, enhance nutrient utilization, and strengthen the immune system of dairy cows (Broadway et al., 2015). Additives such as *Saccharomyces cerevisiae* and yeast cell wall components rich in mannan-oligosaccharides and β-glucans have been reported to enhance dry matter intake and improve metabolic stability during the transition period. Furthermore, their immunostimulatory properties may contribute to improved colostrum composition, notably higher immunoglobulin concentrations, which are crucial for the passive transfer of immunity to newborn calves. Therefore, probiotic supplementation during the peripartum period is a promising strategy to enhance both maternal and neonatal health and immune status.

FTPI is identified when a calf's serum IgG concentration or total protein levels drop below specific benchmarks during the initial days following birth. Measuring serum IgG and total protein remains a dependable method for assessing the transfer of passive immunity in calves up to 9 days old (Wilm et al., 2018). The most widely accepted technique for verifying TPI is to measure IgG concentrations in calf serum after colostrum consumption using a radial immunodiffusion assay (RID) (Godden et al., 2019).

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Additionally, several indirect methods are available for FTPI detection, including total serum protein measurement, which is widely used due to its strong correlation with IgG levels. One commonly employed tool is the %Brix refractometer (Hue et al., 2021b). The FTPI thresholds for dairy calves vary between studies and methods, but generally accepted FTPI thresholds are less than 10 g/L serum IgG and less than 8.1–8.5% Brix (Hue et al., 2021b).

In recent years, various studies have investigated the impact of yeast and yeast-derived supplements on the immune status of pregnant women and their offspring. This has primarily been achieved by measuring serum IgG levels in sows and piglets (Jang et al., 2012), and in cows and calves (Burdick Sanchez et al., 2021). Although substantial research has been conducted in this area, a comprehensive study specifically addressing the impact of such supplementation on the passive transfer of immunity in newborn calves from supplemented dams remains lacking. Interestingly, similar investigations have been carried out in mares and their foals (Ayad et al., 2017) and in piglets (Jang et al., 2012).

By assessing the calves' serum IgG concentration 24–48 hours after consuming colostrum, this study aims to evaluate FTPI in calves born to dams supplied with yeast *(Saccharomyces cerevisiae)* and yeast wall, or a combination of yeast and yeast wall. The %Brix refractometer and radial immunodiffusion were used to assess serum IgG concentrations.

2. Materials e Methods

2.1. Management of the farm and the animals

The protocol for animal use in this research adheres to the welfare standards outlined in Directive 2010/63/EU, enacted by the European Parliament and the Council on 22 September 2010, which addresses the protection of animals involved in scientific studies (Official Journal of the European Union, 2010).

This study was conducted on a commercial dairy operation located in the Ouamri area of Médéa Province, Algeria. The farm is home to 380 animals, including 170 dairy cows. A total of 45 Montbéliarde dairy cows (8 primiparous and 37 multiparous) were enrolled in the study, 30 days before their expected calving dates, from April 2023 to August 2023. The cows were assigned to four groups:

- Control group (CON; n=13): No supplementation.
- First experimental group (SC; n=12): Was provided a diet enriched with 5 g/day of Saccharomyces cerevisiae (ActiSaf Sc47 STD, a heat-resistant live yeast concentrate from Lesaffre, Phileo, France).
- The second group in the experiment (YW; n=10) Was given a diet enhanced with 5 g/day of yeast wall (SafMannan Premium, Lesaffre, Phileo, France), which includes 20% mannans and 20% β-glucans.
- Third experimental group (SCYW; n=10): Was fed a diet enriched with a blend of 5 g of live yeast (S. cerevisiae) and 5 g of yeast wall (YW).

During the study, the cows were housed in free stalls and provided with 3.5 kg of dry concentrate, 6 kg of hay, and unlimited access to straw and water. All cows stayed in good health, exhibiting no symptoms of illness, and were bred using artificial insemination without the use of hormonal treatments.

2.2. Collection of blood and colostrum samples

After thoroughly cleaning the teats before calving, colostrum samples were routinely collected in sterile containers and stored at -20°C until analysis.

Calves were housed with their dams and had free access to colostrum. Following sex identification, blood samples were drawn from the jugular vein of each calf between 24 and 48 hours of age using blood collection tubes containing lithium heparin. The samples were transported to the laboratory on ice and subsequently centrifuged at 3,500 rpm for 10 minutes. The serum obtained was then pipetted into 1.5 mL Eppendorf tubes and stored at -20°C until analysis.

2.3. Analysis of samples

Radial Immunodiffusion Analysis – The IgG levels in colostrum and calf serum were determined using the IDRing Box-Bovine IgG Test (product code: I-B-IgG-10; lot number: BIgG 1 221731, ID Biotech, Issoire, France), a quantitative radial immunodiffusion assay widely regarded as the benchmark for IgG quantification (Buczinski et al., 2018; Van et al., 2023). This assay employs the Single Radial Immunodiffusion (SRID) technique, utilizing BOV IgG Test Plates with agar gel infused with antibodies specific to bovine IgG. As IgG spreads through the gel, it binds with these antibodies, creating precipitation rings whose size corresponds to the concentration of IgG.

Before testing, colostrum and serum samples were thawed and mixed thoroughly using a vortex. Colostrum samples were diluted in buffer at a 1:750 ratio, while serum samples were diluted at a 1:200 ratio. Each test plate was prepared by adding 15 µL of standard solutions to the first four wells, with the remaining wells filled with sample aliquots. The plates were then placed in a humidity chamber and incubated at 35°C ± 5 °C for 16 to 20 hours. After incubation, the diameters of the diffusion rings were measured using the IDRing viewer digital reader, and an automated standard curve was created in an Excel spreadsheet to calculate the IgG concentration in grams per liter (g/L).

Refractometer analysis – The %Brix refractometer was used as an indirect method to estimate IgG concentration in serum samples and to determine FTPI. The analysis was conducted using a Soplem refractometer C.T type 0–30% H50888 (Soplem, France) (Klaus et al., 1969; Rabaza et al., 2022).

Before analysis, the serum samples were brought to room temperature to thaw and then thoroughly mixed with a vortex to ensure uniformity. The refractometer was calibrated using distilled water, and two drops of serum were applied to the refractometer prism

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with a plastic pipette. The IgG concentration was subsequently measured as a %Brix value. To ensure precision, the refractometer well was cleaned between samples to eliminate any fatty residue, and recalibration was conducted after every ten samples.

3. Statistical analysis

Statistical analyses were conducted using IBM SPSS V. 22.0 (REF), with a significance level set at 0.05. Data normality was assessed using the Shapiro-Wilk test. To evaluate the effect of supplementation type, colostrum quality, and calf sex on passive immunity transfer, a one-way ANOVA was performed.

Passive immunity transfer was classified into two categories:

- Failed passive immunity transfer (FTPI): Serum IgG levels below 10 g/L
- Successful passive immunity transfer: Serum IgG levels at or above 10 g/L

To evaluate the Radial Immunodiffusion method (considered the gold standard) against the %Brix refractometer and to explore the connection between colostrum quality and instances of FTPI, a Spearman rank correlation test was utilized. This non-parametric statistical method examines the monotonic association between variables by ranking the data.

4. Results

4.1. Impact of Supplementation Treatment on Serum IgG Levels and Passive Immunity Transfer

Table 1 presents the summary statistics for IgG concentration values for 24-48 h of calf serum, for the first group, control, the mean± SE was 20.18± 3.12g/l, the second group, yeast, it was 35.19± 3.05, the third group, it was 30.81± 4.61 g/l, and the last one, mixture, was 37.59± 3.44 g/l.

A significant difference ($P < 0.001$) indicates that there were significant differences between at least two of the treatment supplement groups ($P = 0.000$). Significant effects were observed in the yeast group ($P = 0.008$) and the mixture group ($P = 0.007$), indicating statistically meaningful differences within these groups. However, no significant effect was observed in the yeast fraction group (Table 1).

Items	Mean ±SE				p-value
	Control	Yeast SC	YWF	MIX	
serum IgG (g/l)	20.44±3.12 a	35.19±3.05 b	30.81±4.61 b	37.59±3.44 c	0.000*

*. Significant differentiation at 0.05.
Values followed by different letters (a, b, c) within the same row indicate a statistically significant difference.
Table 1 – The concentration of IgG in the serum of calves across various experimental groups.

4.2. Failure of transmission of passive immunity

In the control group, three samples (representing 25% of the calves) exhibited inadequate passive immunity transfer (FTPI), showing serum IgG levels under 10 g/L between 24 and 48h post-birth. No cases of FTPI were observed in the SC and MIX groups, where all samples had serum IgG concentrations above 10 g/L, ranging from a minimum of 20.60 g/L to a maximum of 48.80 g/L. In the YWF group, only one sample (10%) exhibited FTPI. (See Table 2).

Items	Control		Yeast SC		YWF		MIX	
	N	%	N	%	N	%	N	%
FTPI <10 g/l	3	25%	0	0%	1	10%	0	0%
STPI ≥10 g/l	9	75%	13	100%	9	90%	10	100%

Obs. FTPI: Failed transmission of passive immunity. STPI: successful transmission of passive immunity
Table 2 – Classification of Passive Immunity Transfer: FTPI and STPI.

4.3. Impact of Colostrum Quality and Newborn Sex

The findings indicate that higher colostrum quality enhances serum IgG levels in calves and improves the transfer of passive immunity ($P = 0.017$). However, the sex of the newborns did not influence the calves' serum IgG concentrations ($P>0.05$) (Table 3).

Items	Control		Yeast SC		YWF		MIX		P-value
Colostrum IgG(g/l)	26.44±4.66 a		67.85±13.71b		59.24±15.78 b		130.53±14.16 c		0.017*
serum IgG in function of sex-NN	F	M	F	M	F	M	F	M	0.489
	25,9 ±5,84	29,4 ±11,37	29,2 ±3,56	36,6 ±5,64	23,5 ± 9,57	35,7 ± 3,90	16,4 ± 0,50	19,7 ±6,22	

*. Significant differentiation at 0.05. Values followed by different letters (a, b, c) within the same row indicate a statistically significant difference. Obs. M: Male and F: Female.
Table 3 – Impact of colostrum IgG levels and newborn sex on serum IgG concentrations in calves

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4.3.1. Correlation between the serum IgG levels and colostrum IgG concentration

Figure 1 illustrates the relationship between IgG levels in bovine colostrum across different treatments and the corresponding IgG concentrations in calf serum. A moderate yet significant positive correlation was observed between these two parameters (r = 0.312, P = 0.037). This indicates that an increase in colostrum IgG concentration is associated with a rise in serum IgG levels in calves. However, although this relationship is statistically significant, its strength remains moderate, suggesting that other factors may also contribute to the outcome.

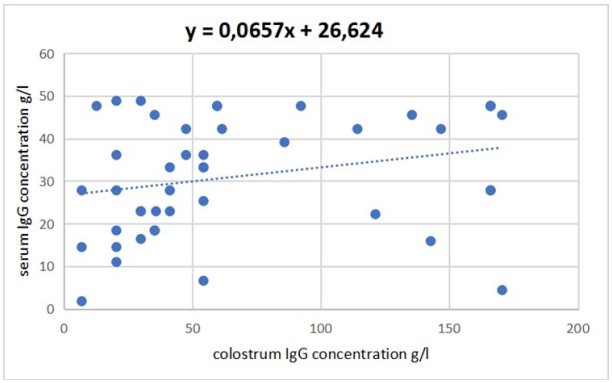

Figure 1 – correlation between IgG concentration in cow colostrum and calf serum

4.4. Correlation between RID and Refractometer Measurements

The correlation coefficient between the RID and refractometer tools, used to assess IgG concentrations in calf serum for evaluating the transfer of passive immunity, showed a positive relationship with a correlation coefficient of r = 0.536 (refer to Figure 2). This suggests a linear relationship between the two methods, where an increase in one corresponds to a rise in the other.

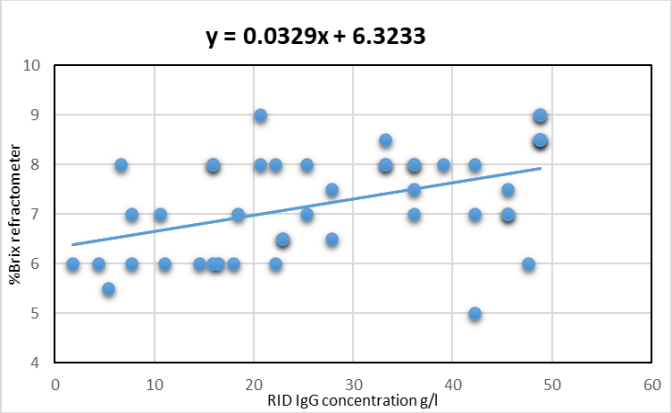

Figure 2 – Correlation between RID and Refractometer tools used on measuring the IgG concentration in serum's calves

5. Discussion

5.1. Failed transfer of passive immunity

Numerous factors significantly influence the failure of passive immunity transfer (FTPI) in calves, such as the timing of their removal from the calving area, the interval between birth and the initial colostrum feeding, and the volume and quality of colostrum provided (Kalus et al., 1969). Newborn calves lack immunoglobulins at birth (agammaglobulinemic) and depend on colostrum consumption to acquire vital nutrients and growth factors, and immunoglobulins—primarily IgG—for passive immunity (Godden et al., 2019). Therefore, colostrum quality is a key factor influencing the successful transfer of passive immunity in calves (Rabaza et al., 2022).

In the first part of this study, conducted by Beldjouhar et al. (2024), which examined the effects of probiotics and prebiotics on colostrum quality, results indicated that the highest immunological quality of colostrum (measured by IgG concentration) was observed in the group supplemented with a combination of the probiotic Saccharomyces cerevisiae and yeast cell wall. Next in sequence was the group treated solely with Saccharomyces cerevisiae, followed by the group receiving yeast cell wall supplementation, and lastly, the control group. The difference in colostrum IgG concentration among groups was statistically significant (P = 0.00).

These findings are further confirmed in the present study (Table 3). This emphasizes how the IgG concentration in colostrum from supplemented cows influences calf serum IgG levels and the process of passive immunity transfer (P = 0.017). Additionally, Figure 2 illustrates a positive correlation between colostrum quality and the transfer of passive immunity in calves (P = 0.037). The

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observed relationship between supplementation and calf serum IgG concentration suggests a reduction in FTPI, as demonstrated in Table 1, which shows a statistically significant difference ($P < 0.001$).

FTPI was diagnosed by measuring calf serum IgG concentrations 24 to 48 hours after birth (Wilm et al., 2018). The standard RID method was used to confirm FTPI (Godden et al., 2019). According to Lotito (2023), FTPI is characterized by a serum IgG level of less than 10 g/L. The frequency of FTPI was notably influenced by treatment supplementation ($P < 0.001$). Notably, no cases of FTPI were recorded in the yeast ($P = 0.008$) and mixture ($P = 0.007$) groups, where the highest serum IgG level reached 45 g/L, indicating excellent transfer of passive immunity (Lopez et al., 2023). In contrast, 25% of calves in the control group experienced FTPI. While no significant difference was observed for the yeast fraction, the results suggest that supplementation improved colostrum quality (IgG levels), thereby reducing FTPI frequency in calves.

Similarly, Van (2023) reported that the quality of colostrum had a greater impact than its quantity in decreasing the occurrence of FTPI in calves. In the present study, colostrum was provided ad libitum and immediately after birth, supporting Godden's (2019) recommendation to focus on high-quality colostrum (IgG > 50 g/L) for FTPI management in calves. Fröhdeová et al. (2014) also reported that calves from cows in the control group had lower serum IgG levels than those from cows supplemented with S. cerevisiae ($P < 0.05$), indicating that even a low level of S. cerevisiae supplementation significantly increased serum IgG concentrations in both cows and their calves. Additionally, Tortora et al. (2013) observed an increase in foal serum IgG levels after mares were injected with glucan, a component of the yeast cell fraction.

Uyama et al. (2022) examined 24 studies exploring the link between colostrum quality and FTPI rates in calves, while Lotito et al. (2023) suggested that manipulating the immunological status of cows could influence colostrum quality and consequently FTPI. Pempek et al. (2018) further highlighted the correlation between improved colostrum IgG concentration and calf health and survival rates.

In this study, the sex of newborn calves did not significantly affect serum IgG concentrations (Table 3), which is consistent with the findings of Trotz-Williams et al. (2018). However, some studies have reported higher IgG concentrations in heifers compared to bull calves (Odde et al., 1988). It remains unclear whether this difference is due to biological factors or differences in rearing practices. In dairy farming, male calves typically hold less economic value than heifers, as they are not used for herd renewal. As a result, they may receive less attention and care from farmers, particularly regarding the intake of colostrum.

5.2. Comparison between RID and the %Brix refractometer tool for estimating IgG concentration in serum and determining the FTPI

To compare the RID tool and the Refractometer method used to measure the serum IgG concentration of calves and determine the FTPI cases. As the first step, IgG concentration in calves' serum is calculated using the RID method. However, time, cost, and limited test range are also limitations of RID, a standard procedure (Röder et al., 2023).

One of the alternatives for on-farm tools used to estimate IgG concentration in calf serum is the %Brix refractometer, a simple and accurate method. This tool assesses the total solids in a liquid, offering an estimate of IgG levels in serum and colostrum through light refraction. Brix refractometry has demonstrated a strong correlation with direct measurements of IgG (Kim et al., 2023).

A moderately positive relationship exists between the RID method and the %Brix refractometer when determining IgG levels in calf serum 24–48 hours post-birth. The Spearman correlation coefficient was ($r=0.536$) in the same range (Akköse et al., 2022), indicating a positive correlation between RID IgG concentration and %Brix ($r=0.885$) with a cut-off <8.4%- 9.6%, between serum RID and %Brix in lamps ($r=0.69$) (Röder et al., 2023), and in goat (Batmaz et al., 2019). Conversely, Thompson et al. (2023) found that the IgG concentration measured by RID had a higher degree of variance for specific serum compared to the values obtained by the refractometer. Based on these results, the %Brix refractometer is a simple indirect method that can be used as an alternative tool to the Radial immunodiffusion method to evaluate or determine the failed transfer of passive immunity in farms. And prevent the neonatal diseases caused by the FTPI.

6. Conclusion

This study highlights the benefits of supplementing dairy cows with *Saccharomyces cerevisiae* yeast, a yeast product called yeast wall fraction, or a combination of both, starting thirty days before their expected calving date. These supplements enhance the transfer of passive immunity to neonate calves within 24-48 hours of birth. This helps breeders and veterinarians to reduce neonatal conditions, such as diarrhea and mortality, within the first week of a calf's life. Moreover, the %Brix refractometer is regarded as a straightforward and precise instrument for identifying Failure of Passive Transfer of Immunity (FTPI) in newborn calves.

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