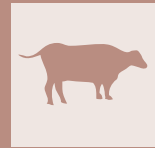


# Effect of probiotic and prebiotic supplementations in dietary dairy cows on colostrum IgG concentration



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## SUMMARY

The aim of this study was to determine the effect of supplementing dairy cows with probiotics, prebiotics and synbiotics to improve the immunological quality of the cow's colostrum and the transfer of passive immunity in their calves, and at the same time to evaluate the Brix refractometer as an effective tool to measure the immunoglobulin (IgG) concentration in colostrum in comparison with the golden test Radial Immunodiffusion (RID). Thirty days before the expected calving date, forty five Montbeliarde cows (8 primiparous, and 37 multiparous) were divided into four groups, Control group (CNT; n=13) received dry period diet (DPD) with no supplementation, yeast group (SC; n=13) received dry period diet supplemented with 5g/day/cow of live yeast *Saccharomyces Cerevisiae* (probiotic), yeast wall fraction group (YWF; n=10) supplemented with 5g/day/cow of yeast wall fraction (mannans and  $\beta$ -glucans; prebiotic) and the Mixture group (MIX; n=10) received dry period diet with a combination of 5g of yeast *Saccharomyces Cerevisiae* and 5 g of yeast wall fraction (mannans and  $\beta$ -glucans) (synbiotic). Colostrum samples were collected in sterile universal containers immediately after calving and frozen at - 20°C until analysis. Statistical analysis was performed using SPSS "IBM SPSS V. 22.0". Significant differences in colostrum IgG concentration (IgG 50g/l) were shown for the yeast group and mixture compared to control group (P 0.01) compared to the control group. There was no effect of body condition score (BCS), age, parity and the sex of neonates (P 0.05) on colostrum IgG concentration. The correlation between Radial immunodiffusion (RID) and refractometer was high and positive (r=0.785) for colostrum. In conclusion, supplementation of dairy cows with probiotics and synbiotics positively improved the immunological quality of colostrum. The Brix refractometer stands as an accessible and cost-effective tool for on-farm use, assisting producers and veterinarians in improving their calf health management programs. Therefore, avoid fatal neonatal diseases in newborns.

## KEY WORDS

Dairy cows; Probiotic; Prebiotic; Colostrum; Immunoglobulin.

## INTRODUCTION

The transfer of immunoglobulins from colostrum into the blood of newborn calves has various technical terms, including «passive transfer of immunity» and more recently, «transfer of passive immunity» which is more precise because immunity is provided passively by ingestion of colostrum rather than by passive transfer of immunoglobulins (1). A part of the successful delivery of passive immunity is the provision of adequate amounts of high quality colostrum to dairy calves as soon as possible after birth and before the onset of "gut closure" (2), which results in poor absorption of macro-molecules including immunoglobulins, and can limit the immune capacity of

dairy calves. Closure of the calf's intestine occurs approximately 24 hours after birth, but this timing can vary (3). If the calf receives insufficient quality or quantity of colostrum before gut closure, failure of passive transfer of immunity (FTPI) may occur, and FTPI is associated with increased calf morbidity and mortality.

During pregnancy, nutrients are transferred to the fetus through the placenta, which consists of the cotyledons (part of the placenta) and the caruncle (part of the uterine horns). The placenta does not allow the passage of antibodies: therefore, the intake of colostrum plays a crucial role for the newborn, which contains 30-200 g/L of protein, mainly in the form of antibodies. IgG, IgA, and IgM account for approximately 85% to 90%, 5%, and 7%, respectively, of the total Ig in colostrum (4). Furthermore, the absorption efficiency of immunoglobulins decreases with time, especially when administered 6 hours after birth, with approximately 66% recovered in plasma, approximately 50% after 12 hours, etc. (5). The immunological

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quality of colostrum can therefore be defined by measuring the amount of IgG. The latter can be measured by different methods. Among these methods, radial immunodiffusion is considered the gold standard for IgG measurement. Although refractometer is practical for rapid estimation IgG levels, compared with RID which is only used for scientific research (2). In recent years, a growing body of literature has investigated the effects of yeast and yeast product supplements on the immunity of the pregnant females and their newborns by measuring IgG serum concentrations in sows and piglets (6), cows and their calves (7,8,9). Despite the large amount of work that has been carried out on this subject, there has been no in-depth study of the effect of this type of supplementation on the quality of colostrum, and consequently on the transfer of passive immunity to the newborn calf. However, such study has been conducted in mares and their foals (10) and in sows and piglets (6).

The aim of this study was to determine the effect of yeast, yeast products and their combination on colostrum concentration on IgG in cows using two methods: the “Gold standard” radial Immunodiffusion and the % Brix measured with a digital refractometer.

## MATERIALS AND METHODS

### Farm and studies animals

The Protocol on the Use of Animals respects animal welfare law as set out in Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Official Journal of the European Union, 2010). The study was conducted on a commercial dairy farm located in Ouamri region in the province of Medea, Algeria, with a total of 380 animals, including 170 dairy cows. The herd was selected because of its willingness to participate in the research project and its high frequency of calving over a short period of time, thus reducing fluctuations between calves and cows that may occur during different seasons (calving in the same season “April to July 2023”).

### The Animals

Cows were housed in free stalls barns with free access to water. They received 3,5 kg of concentrate, 6 kg of hay and free access to straw. The cows received preventive internal and external anti-parasitic treatment (Eprinomectine), at the beginning of the experiment the cows did not show any sign of diseases. Cows didn't receive any hormonal treatment, bred by artificial insemination AI. At the beginning of the experiment, age, BCS and parity were recorded for each cow in the trial. Forty-five Montbeliarde dairy cows; including 8 primiparous and 37 multiparous dry-cow pen and moved to a maternity pen approximately 30 days before expected calving date to be supplemented daily by 5 g of probiotic and/ or prebiotic, the feed additive is mixed manually at a rate of 5g per day with the concentrate. Cows were regrouped into 4 groups according to the type of supplement feed. The cows within each group were assigned randomly to one of four groups, the first group (SC; n=13) 29% supplemented with 5g/day/cow of probiotic which was yeast *Saccharomyces Cerevisiae* ActiSaf® Sc47 STD thermostable live yeast concentrate (Lesaffre, Phileo, France) the second group (YWF; n=10) 22% supplemented with 5g/day/cow of prebiotic which was yeast products-feed materials SafMannan® Premium yeast fraction heat resistant concentrate of yeast fraction

(Lesaffre, Phileo, France) which contain Mannans 20%,  $\beta$ -glucans 20%, Moisture 6% and crude protein 10-25%. The third group was supplemented with a mixture of probiotic (*Saccharomyces Cerevisiae*; SC) and prebiotic (yeast wall fraction) (MIX; n= 10) 22% and the last group was a control group (CNT; n=12) 27%.

### 2.3. Colostrum collection

After calving, the colostrum was routinely collected (by hand). Teats were cleaned, and samples were collected in sterile universal containers. Colostrum samples were placed on ice within one hour of collection, transported to the laboratory and frozen at - 20°C until analysis.

### Samples analysis

#### Radial ImmunoDiffusion measurements

IgG was measured in colostrum and calf serum using the IDRing Box- Bovine IgG Test (code product: I-B-IgG-10; the batch number: BlgG 1 221731, ID Biotech, Issoire, France) kit for the quantitative analysis of bovine immunoglobulin G IgG by radial Immunodiffusion, which is recognized as the gold standard for IgG measurement (2).

The test is based on the Single Radial Immuno-Diffusion (SRID) laboratory method. The BOV IgG test plates consist of an agar gel containing specific antibodies to bovine IgG. During the diffusion in the agar gel, the antibodies react specifically with the bovine IgG and form precipitation rings.

All samples were thawed and vortexed, including colostrum or serum, colostrum samples were diluted in buffer (1:750) and (1:200) for serum. Each plate was then identified with internal references and filled it with 15  $\mu$ l of standards for the first four wells, the remaining wells were filled with samples. The plates were placed in a humidity chamber and incubated at 35  $^{\circ}$ C  $\pm$  5 for 16 to 20 hours. The diffusion zones were measured (diameter) using the IDRing viewer digital reader, and a standard curve was automatically plotted using an Excel spreadsheet:

$$Y = a\sqrt{X} + b$$

Y is the measured diameter

X is the concentration of each standard

a is the slope

b is the intercept

The concentration of each sample was automatically converted on g/l.

#### Brix refractometer measurements

On the same day as incubation of the RID plates, the IgG concentration of the test sample was estimated indirectly using a Soplem Brix refractometer C.T type 0 - 30% H50888 (Soplem, France). (11)

Samples were thawed at room temperature and vortexed before testing and the refractometer was calibrated with distilled water. Two drops of colostrum/serum were applied to the prism of the refractometer using plastic pipette and the colostrum/serum IgG were measured in % brix. To remove any fatty residues, the refractometer well was cleaned between samples and calibration was performed routinely after measurement of 10 colostrum samples.

#### Statistical analysis

Statistical analysis was performed using SPSS “IBM SPSS V. 22.0” (12). All statistical analyses were performed at a pre-set sig-

nificance level of 0.05, which indicates statistically significant difference. Prior to the analysis, all variables were tested for normality using the Shapiro-Wilk test if they were normally distributed, were then subjected to One-way ANOVA to study the effect of the supplement treatment on IgG concentration of colostrum. Alternatively, when the variables were not normally distributed (IgG concentration measured by RID), data were processed using the Kruskal-Wallis non-parametric test, with treatment as the main effect. Added to that, a post-hoc test “Mann-Whitney” was used to estimate the minimal differences between groups to determine the effect of type of supplement treatment on colostrum quality specifically on the IgG concentration. A categorization of the quality of colostrum was elaborated according to the mean of IgG concentration in each group, two categories; poor quality IgG 50g/l and excellent quality IgG ≥50g/l, described with frequencies and percentages. A multiple linear regression model was used to evaluate the impact of several factors: BCS ([1- 3[, [3, ], 3 - 5]) age (2-4 years, 4-6 years and 6-8 years), parity (primiparous, L1, L2, L3, L4 and L5) and newborn’s sex (male and female) on the IgG concentration of colostrum, applied to multiple independent variables (BCS, age, ...) to allow the assessment of the effect of each factor on the dependent variable (IgG concentration). The correlation between IgG concentrations measured by RID (golden standard) and Digital Brix Refractometer was assessed using the Spearman rank correlation test. This non-parametric test evaluates the monotonic relationship between variables by ranking the data.

## RESULTS

### RID and % Brix colostrum analysis

Analysis of the IgG concentration using RID and Brix refractometer revealed 26.44 ± 4.66 g/l (RID) and 16.04 ± 1.38% (% Brix) for the control group CNT and 67.85 ± 13.71 (RID), 22.27 ± 1.50% (Brix) for the yeast group SC, the YWF group was 59.24 ± 15.78 g/l, 21.80 ± 2.52%, the mixture group “Mix”, 130.53 ± 14.16 g/L, 23.90 ± 1.85% which is conceded as excellent quality, Table 1.

**Table 2** shows the categorization of colostrum Immunoglobulin G (IgG) concentration in different experimental groups, including control, yeast, yeast fraction, and mixture. The concentrations are categorized into two groups: those less than 50g/l were qualified as poor quality and those equal to or greater than 50g/l as good quality of colostrum. Eleven (11) samples of the control group (91.7%) fall below 50 g/l, indicating a predominant frequency of lower IgG concentrations, yeast group 7 samples (58.3%) showed good quality with IgG concentrations ≥50g/l, 6 samples (60%) of the yeast fraction group had poor quality with IgG concentrations less than 50g/l.

### Effect of supplement treatment on colostrum IgG concentration

A significant difference was shown on the effect of supplement treatment on the concentration of colostrum IgG between groups (P 0.01) (Table 1), the mixture treatment “yeast and yeast fraction” significantly affected the IgG concentration (P 0.01) compared with the other treatments. Also, for the SC group (P=0.005) compared to the CNT group. No significant difference (P 0.05) was shown for the group “YWF”.

### Digital % Brix refractometer measurements

The %Brix was used for indirect measurement of colostrum IgG concentration, the results show a significant effect of the type of supplementation on the %Brix refractometer (P=0.018) (Table 1), (P=0.020) for the group MIX. Therefore, no significant difference reported for the other groups; SC (P=0.061), YWF (P=0.130) compared to control group.

### The effect of BCS, parity, age and the new-born gender

The result of the linear regression model yielded an R=0.206 (RID) and R=0.323 (the %Brix) indicates a low correlation between the predictors (BCS, parity, Age and the sex of newborns) and the dependent variable «the colostrum IgG concentration». and R<sup>2</sup>=0.043 (RID) and R<sup>2</sup>=0.104 for the %Brix. which mean, only 4.3% (RID) and 10.4% (%Brix) of the variance of the dependent variable «colostrum IgG con-

**Table 1** - Colostrum’s IgG concentration and the %Brix Refractometer in Different Experimental Groups.

| Items                 | Group         |               |                           |                | P value |
|-----------------------|---------------|---------------|---------------------------|----------------|---------|
|                       | Control (CNT) | Yeast (SC)    | Yeast Wall Fraction (YWF) | Mixture (MIX)  |         |
| RID concentration g/l | 26.44±4.66 a  | 67.85±13.71 b | 59.24±15.78 b             | 130.53±14.16 c | 0.000   |
| %Brix refractometer   | 16.04±1.38 a  | 22.27±1.50 ab | 21.80±2.52 ab             | 23.90±1.85 b   | 0.018   |

Values followed by different letters a, b and c on the same line are significantly different.

**Table 2** - Categorization of Colostrum quality in different experimental groups.

| Groups                        | Control   |      | Yeast (SC) |      | Yeast Fraction |     | Mixture   |     |
|-------------------------------|-----------|------|------------|------|----------------|-----|-----------|-----|
|                               | Frequency | %    | Frequency  | %    | Frequency      | %   | Frequency | %   |
| colostrum’s IgG concentration |           |      |            |      |                |     |           |     |
| < 50g/l                       | 11        | 91.7 | 5          | 41.7 | 6              | 60  | 0         | 0   |
| ≥ 50g/l                       | 1         | 8.3  | 7          | 58.3 | 4              | 40  | 10        | 100 |
| Total                         | 12.00     | 100  | 13         | 100  | 10             | 100 | 10        | 100 |

centration» was explained by these factors (BCS, parity, Age and the sex of newborns). None of these factors was showed a significant effect with the type of supplementation on colostrum IgG concentration ( $P=0.776$ ). With ( $P=0.348$ ) for BCS, ( $P=0.472$ ) for parity, ( $p=0.792$ ) for age and ( $P=0.615$ )(Table 3), for calf sex (Table 4).

### Correlation between RID and Digital % Brix Refractometer

Regarding the study of correlation between RID method and Digital % Brix Refractometer method, there is a strong and significant positive correlation ( $P < 0.01$ ), the Spearman correlation coefficient ( $r$ ) between RID and Digital % Brix Refractometer used to measure the IgG concentration of colostrum was  $r = 0.785$  (Figure 1). Suggesting that there is a linear relationship between these two methods when one increases, the other tends to increase, and vice versa.

## DISCUSSION

### Effect of supplementation on colostrum IgG concentration

The immune system responses have shown variability when yeast and yeast products are administered as supplements to animals specifically, particularly cattle and pigs. Burdick Sanchez et al (9) reported in a meta-analysis paper that both of immune responses, “innate response and adaptive response”, were modulated by probiotics supplementation. Several researchers have jointly dedicated their studies to this area (9, 13).

In the same field, the present research was dedicated to the study of the effect of supplementation with yeast, yeast fraction “yeast cell wall; purified cell components” and the combination between them on the immunity system more precisely on the immunological quality of colostrum “IgG concentration” which

has an important role in the transfer of passive immunity. All colostrum immunoglobulin’s concentration values in this study were consistent with normal ranges reported previously (2, 3, 5). Table 1 shows that by supplementing dairy cows with yeast “*Saccharomyces Cerevisiae*, SC” the concentration of IgG in colostrum was significantly increased with an average of  $67.85 \pm 13.71$  g/l (Table 2) and 58.3% qualified as High quality 50 g/l ( $p=0.007$ ), for the cows supplemented with yeast fraction “ $\beta$ -glucan and MOS” was  $59.24 \pm 15.78$  g/l with 40% qualified as colostrum of high quality (Table 2) but the difference wasn’t significant  $p=0.90$ , These results can be probably be linked to the fact that among the cows with poor colostrum quality in this group, 30% were primiparous cows. Primiparous cows generally have a less developed immune system then multiparous cows (25). In addition, 20% of the cows had an IgG concentration in their colostrum close to 50 g/L, with values around of 44.1%, the last group that received a combination between yeast “SC” and yeast fraction a significant increase ( $p 0.001$ ) with  $130.53 \pm 14.15$  g/l, 100% of the colostrum samples were qualified as colostrum of high-quality (Table 2).

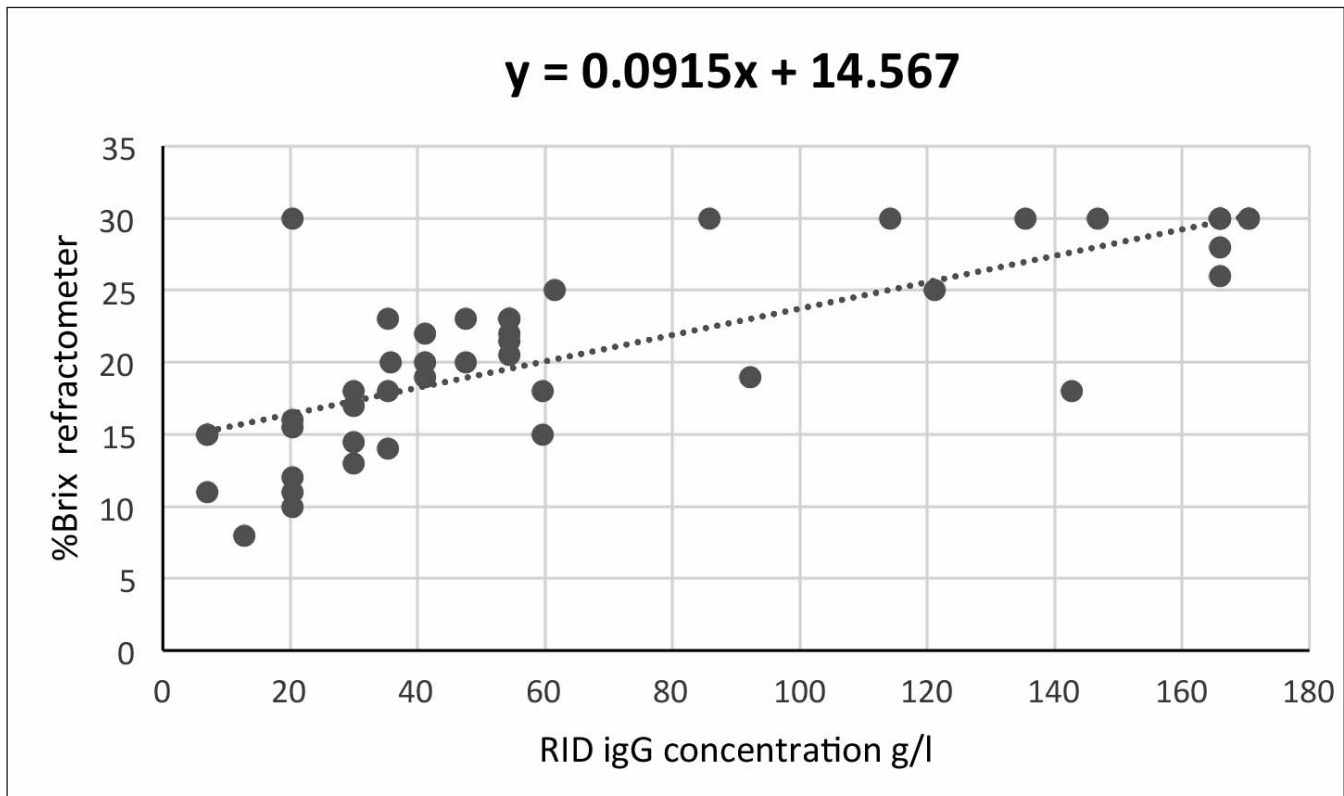
These results are in agreement with previous researchers, Ayad (10) who found that the concentration of IgG in mares supplemented with yeast “SC” during pregnancy was increased  $122.25 \pm 145.59$  g/l compared to control group  $104.51 \pm 157$  ( $P=0.02$ ) and Jang (6) who support the same observations in sows receiving live yeast supplementation the IgG concentration tended to be higher ( $P=0.10$ ) compared with control group, and agree with those observed on serum IgG concentration of cows supplemented with yeast culture 90 days before and after parturition resulted in greater serum concentration of IgG, IgM and IgA (8). Similarly, Fröhdeová (7) reported that supplementation of pregnant cows with yeast culture resulted in higher serum IgG concentration (2 days after calving). The IgG contained in colostrum comes from the mother’s blood and are selectively concentrated in the udder by specific transport

**Table 3** - Effect of BCS, Age and parity on colostrum IgG concentration.

| Groups |             | Control (CNT) |             | Yeast (SC)   |             | (Yeast fraction) YWF |             | Mixture       |            | P value |       |
|--------|-------------|---------------|-------------|--------------|-------------|----------------------|-------------|---------------|------------|---------|-------|
|        |             | IDR           | Brix%       | IDR          | Brix%       | IDR                  | Brix%       | IDR           | Brix%      | IDR     | Brix% |
| BCS    | [1- 3[      | 33.1 ± 9.79   | 17.6 ± 1.84 | 69.4±26.04   | 22.3 ± 2.36 | 72.2±27.06           | 25.0±3.38   | 119.2 ± 20.03 | 22.0 ±2.38 | 0.348   | 0.455 |
|        | [3]         | 23.3 ± 6.54   | 15.4 ± 2.69 | 40.4 ±6.22   | 19.0 ± 1.82 | 32.3±6.19            | 17.0±3.21   | 166.0 ± 0.00  | 29.3 ±0.66 |         |       |
|        | ]3 - 5]     | 23.8 ± 9.98   | 15.0 ± 2.89 | 93.4±28.36   | 30.0 ± 3.07 | 67.3±46.90           | 21.0±9.00   | 92.2 ±0.00    | 19.0±0.00  |         |       |
| Age    | 2-4 years   | 38.5 ± 2.70   | 20.0 ± 0    | 75.7±23.72   | 24.0 ± 2.12 | 39.1±15.72           | 21.0±5.21   | 142.7 ± 0.00  | 18.0 ±0.00 | 0.792   | 0.082 |
|        | 4 -6 years  | 33.2 ± 6.06   | 16.9 ± 2.25 | 71.2±21.35   | 22.6 ± 2.37 | 83.1± 25.96          | 24.4± 2.40  | 199.0 ± 0.00  | 29.0± 1.00 |         |       |
|        | 6 -8 years  | 10.3 ± 3.37   | 12.8 ± 1.31 | 29.9 ±0.00   | 17.5 ±0.50  | 20.4±0.00            | 12.0± 0.00  | 118.7 ± 18.52 | 23.3 ±2.26 |         |       |
| Parity | Primiparous | -             | -           | 52.0± 3.97   | 22.0 ±1.73  | 39.1±31.44           | 21.0 ±10.42 | 142.7 ±0.00   | 18.0 ±0.00 | 0.472   | 0.153 |
|        | L2          | 31.1±11.80    | 16.7 ±6.12  | 146.7 ±0.00  | 30.0 ±0.00  | 20.4± 0.00           | 12.0 ± 0.00 | 166.0 ± 0.00  | 29.0 ±1.41 |         |       |
|        | L3          | 21.1 ±20.08   | 14.5 ±0.71  | 76.675±65.07 | 22.8 ±6.20  | 82.3±76.41           | 23.3 ±6.11  | 121.2 ±0.00   | 25.0 ±0.00 |         |       |
|        | L4          | 22.1±22.37    | 15.9 ±4.33  | 60.675±50.50 | 21.1 ±6.91  | 84.3±42.36           | 26.0 ±5.66  | 108.7 ±53.99  | 22.4 ±7.09 |         |       |
|        | L5          | -             | -           | 29.9 ±0.00   | 18.0 ±0.00  | -                    | -           | 166.0 ±0.00   | 26.0 ±0.00 |         |       |

**Table 4** - Effect of the calves’ sex on colostrum IgG concentration.

| groups | CNT         |              | SC           |              | YWF          |              | MIX           |             | P value |           |
|--------|-------------|--------------|--------------|--------------|--------------|--------------|---------------|-------------|---------|-----------|
|        | RID         | %Brix        | RID          | %Brix        | RID          | %Brix        | RID           | %Brix       | RID*SEX | %Brix*sex |
| Female | 33.59±5.85  | 17.36 ± 1.44 | 64.98 ±15.42 | 22.81 ± 1.28 | 66.85 ±34.83 | 20.25 ± 3.97 | 133.58 ±25.26 | 22.25 ±3.47 | 0.615   | 0.391     |
| Male   | 18.83 ±6.15 | 14.00 ± 3.34 | 72.46 ±28.11 | 21.40 ± 3.53 | 54.17 ±15.78 | 22.83 ± 3.51 | 128.50 ±18.56 | 25.00 ±2.19 |         |           |



**Figure 1** - Correlation between RID IgG concentration and the % Brix of colostrum.

mechanism. So, a greater amount of serum IgG is concentrated in the udder of dairy cows subsequently, colostrum (14). In contrast, (15) found no supplementation effect on immunoglobulin concentration against ovalbumin challenge. Furthermore, the results observed in the treatment supplementation with yeast fraction was beneficial, but the difference was not significant because of the limited content of live yeast that wasn't able to stimulate the adaptive immune response in a similar way compared to "SC".

These findings were consistent with those observed by Yuan et al. (16) in plasma anti-ovalbumin IgG levels against ovalbumin challenge, confirmed that yeast wall products (YWF) alter immune function in animals. This effect could be explained by the fact that the main components of yeast cell wall products YWF are the polysaccharides "β-glucans and Mannans", which interact directly with immune cells (17). Pigs supplemented with β-glucans showed an increased antibody response after stimulation also, Che (18), confirmed the immune effect of β-glucans in weaned pigs. The best results observed in the present study was in the MIX group with highly significant difference compared to CNT group and a very high mean 130.53 g/l (P=0.00). Based on these results, we suspect that the combined supplementation of yeast "SC" and YWF "β-glucans and Mannans" has a synergistic effect on immunity improving the IgG concentration of colostrum. Therefore, this area needs further study with different supplemented doses and different combinations.

### **The effect of BCS, age, parity and neonatal sex on colostrum quality**

For the other factors «BCS, age, parity and the newborn sex», no significant difference was shown (P=0.776) in the effect of BCS on the concentration of colostrum IgG, possibly because

the three types of supplementation treatments had no effect on BCS. This hypothesis was supported by Allbrahim (19); who reported that supplementation with live yeast SC had no effect on BCS (P 0.10). and that supplementation of cows with two different doses of live yeast SC had no effect on BCS (20). According to Allbrahim (19), the lack of effect of live yeast supplementation on BCS could reflect a lack of effect on dry matter intake (DMI), a factor that wasn't addressed in the present study. This is in contrast to Finck (21) who observed an increase in DMI and weight gain in beef cattle supplemented with SC. The same result was reported by (22) with fermentation products of SC supplemented cows.

A positive correlation was showed between age and parity of the experiment cows (P 0.01; r=0.70), that why the effects of this factors on IgG concentration in colostrum are discussing together, the results were somewhat surprising despite the fact that the age and parity (rank of lactation) had no effect or interaction with treatments on the IgG concentration (P 0.05), Godden et al. (3) explained that most studies say that older cows produce better colostrum. In fact, several studies report that the IgG concentration of colostrum tends to increase with parity, especially from the third calving onwards (11). (23) suggested that the older cows are more exposed to antigens and therefore produce more antibodies. Weaver (14) strongly advised against the omission of colostrum from primiparous cows. Avendaño-Reyes et al. (24) found that the IgG concentration of colostrum was affected by parity (p< 0.01) and confirmed that multiparous cows produced high-quality colostrum with higher IgG concentration compared to primiparous cows. Therefore, Godden et al. (3) recommend that primiparous colostrum should not be discarded, but should be tested first. However, the influence of parity on colostrum IgG concentration has not always been found. A study of 120 prim' Hol-

stein cows showed no significant differences between lactation (11). In this study, the majority of cows were multiparous 82.22% and 57.77% had 3 and greater calvings, it is possible why these two factors did not show any effect on IgG concentration. Also, the other factors that could improve colostrum quality were almost homogeneous; farm, genetic factor which was the same bred cows, nutrition in the periparturient period, calving season, length of dry period it was 2 months before the private calving date, delayed colostrum collection which was the first milking for all samples in this study (3). No significant effect of neonatal sex was observed on the IgG concentration ( $P < 0.05$ ). In addition, no study has investigated the effect of neonatal sex on colostrum quality, particularly on IgG concentration.

### Evaluation of %Brix refractometer tool for estimating IgG concentration in colostrum

In the current study, the IgG concentration of colostrum was measured by radial immunodiffusion RID which is still the reference method for measuring IgG concentration. Although the limited test range, time and cost are the disadvantages of RID. It's not feasible for calf health management and monitoring (2). There are several on-farm tests that can be used to assess colostrum quality. However, measuring colostrum quality using on farm tests should be simple and accurate. One of these methods, the %Brix refractometer, used the refraction of a beam of light to determine the degree of dissolved solids are present in the liquid, and the percentage of Brix is then determined (11). Morill (25) showed that it's a reliable tool for the determination of cholesterol IgG.

A positive correlation was observed between RID and % Brix refractometer ( $r=0.785$ ;  $P < 0.01$ ) in the measurement of colostrum IgG, similar to that observed by Coleman (26) who reported that the highest quality colostrum in cows was characterized by a Brix threshold of IgG greater than 22%. Odde (27) shared the same results with Pearson correlation coefficient ( $r=0.71$ ). However, (28) pointed out that the Brix refractometer is not accurate in estimating the IgG concentration of first-milk colostrum. Nevertheless, it can be used to approximate the minimum amount of IgG by using predefined %Brix cut-offs corresponding to first-milk colostrum concentrations of 25, 50, 75, and 100 g/l. Another observation by (29) that the %Brix the performance of the refractometer decreased as the %Brix values increase, especially when the Brix values reach 30% or more. Buranakarl (30) reported that the %Brix refractometer can be an acceptable tool for evaluating only poor quality colostrum; IgG < 18.5%.

In general, the addition of probiotics (yeast: *Saccharomyces Cerevisiae*), prebiotics (yeast wall fraction) and a mixture of probiotics and prebiotics qualified as synbiotics in cattle feed was reported in several studies and was recommended by many authors for its effect on health and immunity (9,16). This study was investigated to study the effect of this supplementation treatment on colostrum quality (IgG concentration) and it reported positive results, especially with the yeast and mixture treatment. (9) This study suggests that supplementation with yeast products could improve energy availability during an immune challenge. This potential improvement could be advantageous, facilitating a quicker resolution. In 2021, the same author Bur-

dick Sanchez (9) added that it seems that yeast supplementation could offer significant advantages to an animal during an immune challenge by enhancing available energy, primarily through increased glucose levels. In vitro studies showed that yeast can produce amino-acids, organic acids and vitamins (22). This makes the IgG concentration in serum calves increase and brings a successful transfer of passive immunity as reported by (10). All other factors (age, parity, BCS and the sex of the newborn) showed no significant effect on the IgG concentration in colostrum. The results showed in this area were not stable possibly, due to the dose used, season of the experiment, breed of cows, nutrient.

Finally, the use of %Brix refractometer to evaluate the IgG concentration in colostrum was positively correlated with the RID tool except that the use of %Brix refractometer was associated with the lowest diagnostic accuracy. However, it remains a quick and easy technique to use in farms for passive immune transfer failure management.

### CONCLUSION

In conclusion, there appears to be convincing evidence that supplementation with probiotics (yeast: SC), prebiotics (yeast wall fraction:  $\beta$ -glucan and mannans), and synbiotics (a mixture of SC and YWF) at a dose of 5g per cow per day, starting 30 days before the expected calving date, can improve colostrum quality. However, yeast SC and synbiotic (SC and YWF) showed better results. The BCS, age, parity, and sex of the newborns had no effect on the IgG concentration, indicating that this type of supplementation can be used at all ages and parities in both lean and overweight cows, which is very important for the prevention of neonatal calf diseases. To assess colostrum quality, the Brix refractometer can be a cost-effective tool for on-farm use, helping producers and veterinarians improve their calf health management programs.

### Statements and Declarations

#### Ethical statement

Formal consent or ethics approval was not required for this study.

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#### Contributions

Beldjouhar Nadjiba, Ayad Mohamed Amine and Saim Mohamed Said contributed to the study conception and design. Beldjouhar Nadjiba collected and analyzed data and prepared initial draft of manuscript. Saidj Dahia reviewed the manuscript. All authors read and approved the final manuscript.

#### Conflict of interest

The authors declare no competing interests.

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