

Field Study of the Impact of Supplementation with Probiotic Yeast (*Saccharomyces cerevisiae* Sc47-CNCM I-4407) on Reproductive Performance in Dairy Cows

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Abstract

Probiotic yeasts are used widely today in the diets of dairy cows with a high production potential. Various studies have researched the effects of live yeasts, in particular *Saccharomyces cerevisiae*, on digestion, metabolism and performance in dairy cows. The present study aims at evaluating the beneficial effects of using probiotic yeast in dairy cattle farms as a means of improving the breeding performance of milking cows. A group of 14 dairy cattle farms, with at least 40 dairy cows, was selected for the study, all of them using artificial insemination (AI) and that had not used probiotics in the two milking years preceding the study. On these farms, the lactating cows received probiotic yeast supplementation (5 g/cow/day, Actisaf[ ], *Saccharomyces cerevisiae* Sc47-CNCM I-4407, 1.10¹⁰ CFU/g, Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France) over 13 months including a 4-week period of adaptation to the product and then the observation period lasting a full year. The analysis was therefore based on 2421 Holstein females inseminated with a total of 4230 doses of conventional Holstein semen at 14 farms over three consecutive years: the period of observation and the two preceding years stated as reference period. Use of daily supplementation with probiotic yeast resulted in a significant improvement of 4 points on average in the success rate of artificial insemination and of 5 points in the success rate of first artificial insemination in multiparous dairy cows. The number of inseminations required to obtain a pregnancy was therefore reduced from 3.1 in the reference period to 2.7 in the probiotic yeast supplementation period (p = 0.007). In parallel, the fat and protein yield by these same animals tended to improve (+5%, p = 0.07) between these two periods, demonstrating that it is possible

to combine milk performance and maintenance of an effective reproductive system.

Keywords

Probiotic Yeast, Dairy Cows, Reproduction

1. Introduction

Agriculture, and in particular farming of ruminants, is today raising not only serious challenges for society in terms of the environment and animal welfare, but also challenges to keep farming economic. The balance between productivity, quality and economic viability is constantly shifting. Farmers need to set objectives with this in mind in order to guide the strategic and therefore technical choices used when running their farms. At the herd level, the farmer will first and foremost want to manage their diet to the best possible effect, which must be optimized for ruminants. This approach is effective in improving and managing production, ensuring much research and development has been carried out in this field.

Production and quality in ruminants are determined by the amount and nature of the products derived from ruminal fermentation. Feeding a ruminant is above all feeding its rumen microflora, which lives in symbiosis with the animal. However, while microbial digestion does take place naturally in the rumen, it is possible to improve the efficiency of this. To optimize this system, there is a need to improve rumen fibrolysis and microbial synthesis, and to reduce ruminal proteolysis and amylolysis to promote the arrival of glucose and proteins in the intestines, where they are digested. By choosing the quality and quantity of the feed introduced into an animal's diet, it is possible to adapt the diet to its needs and to the production objectives set by the farmer. Following ruminant rationing recommendations is one way of optimizing the production system at both the animal and the herd level.

In this context, use of feed additives is a key to supplementary control. In Europe, live yeasts, as probiotics, fall within the regulatory scope of feed additives for ruminants [1]. This microscopic fungus, employed for many years in the baking, brewing and wine industries, has also made its mark in ruminant nutrition.

Such products are generally marketed in viable form and have high levels of living cells ($>10^9$ CFU/g). Daily intake of these probiotics has an effect on animal performance; in beef cattle this is reflected by an improvement in body weight [2], and in ADG, while in dairy cows it is primarily the quantity of milk produced [3] [4] [5] fat content [6] that respond positively to live yeast intake. By stabilizing the ruminal pH in animals fed on diets with a high energy content [7], which can cause ruminal acidosis, live yeast is known to improve feed effi-

ciency. However, little research has been carried out to date on the consequences on the reproductive performance of dairy cows, even though efficient utilisation of energy and protein from feed is a determining factor in improving the chances of successful reproduction in high-producing dairy cows.

This study will look at the beneficial effects of using live yeast in dairy cattle farms as a means of improving the breeding performance of milking cows.

2. Materials and Methods

2.1. Choice of Farms

A group of 15 dairy cattle cow farms was chosen for the study. The farms were selected from among farms that are customers of Gènes Diffusion (Douai, France), an animal genetics and reproduction technologies group. The great majority of these farms were therefore already using artificial insemination (AI). Farms with at least 40 dairy cows (at least 47 first AIs or 76 AIs in total over one year) that had not used probiotics in the two milking years preceding the study and located within a 100 km radius of Douai entered the study on a voluntary basis. The study sample selected resulted in a total of 15 farms spread over the Hauts-de-France region (**Figure 1**).

2.2. Study Duration and Observation Period

The dairy cows at these volunteer farms received probiotic supplementation (5 g/cow/day) with yeast (Actisaf[®], *Saccharomyces cerevisiae* Sc47-CNCM I-4407,

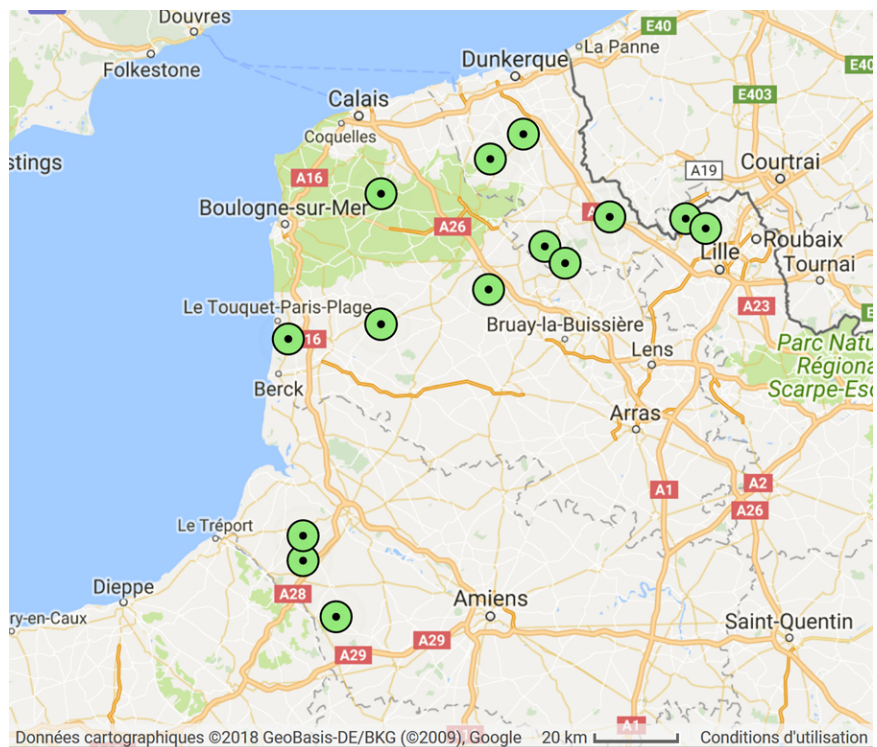


Figure 1. Geographical location of the 15 farms in the study.

1.10¹⁰ CFU/g, Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France) from 1 Sep 2016 to 30 Sep 2017 (observation period) by means of a nutritional feed (Difagri, Saint-Hilaire-de-Loulay, France) included in the animals' daily ration. The distribution period was spread over 13 months. It included:

- A 4-week period of adaptation to the product (the effect of probiotic yeast generally appears in animals after 3 weeks from starting) from 1 Sep 2016 to 30 Sep 2016;
- An observation period from 1 Oct 2016 to 30 Sep 2017, *i.e.* a full year.

2.3. Data Collection and Processing

2.3.1. Continuously throughout the Observation Period

Each farm was monitored by an insemination technician twice a month throughout the observation period and by Phileo on 2 visits during the entire period.

1) Product supply

The amount of product delivered to each farm and the number of dairy cows present at delivery were recorded in order to continuously monitor the correct distribution of the product in terms of quality and quantity throughout the observation year.

2) Feeding of the dairy cow herd

A quantitative report of the composition of the ration given to lactating cows was established by the farmer with the help of the technicians and engineers in charge of monitoring the field study throughout the observation period.

3) Other information

All interventions (prophylaxis, hoof trimming, etc.) and changes in farm practices (buildings, milking, monitoring, etc.) were recorded, based on the farmer's declaration.

2.3.2. At the End of the Observation Period

1) Reproduction data

All the reproduction data were extracted from the Gènes Diffusion databases with the agreement of the farms involved in the study from 1 Oct 2016 to 30 Sep 2017.

The raw data were aggregated by farm and by animal:

- Animal data: breed, parity
- Insemination data: date, parity, type of semen (sexed or conventional), breed
- Calving data: date
- Ultrasound gestation control data (if performed at the farm): pregnant cow, date of gestation control

It should be noted that an AI was considered to have resulted in a pregnancy and was therefore considered a pregnant or fertile AI (AIf) if it resulted in calving within the following 270 - 296 days. Alternatively, if no calving was observed over this period, then it was considered an AIf if the female was declared preg-

nant by ultrasound (from 30 days post-insemination) and confirmed pregnant by palpation at more than 90 days post-insemination.

The data could be used for the individual calculation of:

- Interval of calving to first service (iC-AI1): AI1 date – previous calving date
- Interval of calving to conception (iC-AIf): AIf date – previous calving date
- Number of AI required to achieve an AIf (nAI)

In addition, the criteria listed in **Table 1** were calculated on an annual basis for the 15 farms in the study for both categories of animal (primiparous and multiparous).

2) Production data

Ten herds were enrolled in the individual animal milk recording system over the entire duration of the study, and formed a subsample. The data collected monthly and individually for each of these farms were aggregated:

- Production (kg/d): production of the cow recorded at the date closest to the AI (the performance control most immediately before or after the AI)
- Fat content (FC, g/kg): FC of the milk recorded at the date closest to the AI (the performance control most immediately before or after the AI)
- Protein content (PC, g/kg): PC of the milk recorded at the date closest to the AI (the performance control most immediately before or after the AI)
- FC/PC ratio: FC/PC of the milk recorded at the date closest to the AI (the performance control most immediately before or after the AI)
- Somatic cell count (SCC, cells/mL): SCC of the female at the date closest to the AI (the performance control most immediately before or after the AI)

The individual data were then averaged by parity (primiparous versus multiparous) on an annual basis and by herd.

2.4. Description of the Sample Studied

2.4.1. Reproduction Data

The database for individual cows was triaged to keep only the data relating to:

Table 1. Reproduction criteria calculated for each herd.

Criterion	Abbreviations	Formula
Fertile AI	AIf	$(\text{Total number of AIf} / \text{total number of AI}) \times 100$
Fertile first AI (AI1f)	AI1f	$(\text{Number of first AIf} / \text{total number of AI1}) \times 100$
AI rates greater than or equal to 3	AI3	$(\text{Number of females with 3 or more successive AIs} + \text{number of females with 2 successive AIs with negative consecutive ultrasound}) / \text{number of females with an AI1} \times 100$
Interval calving to 1st AI	iC-AI1	Total iC-AI1/number of animals with an AI1
Interval calving to conception	iC-AIf	Sum iC-AIf/number of animals with an AIf
Number of AIs required to have an AIf	nAI	Total number of AI/number of AIf

- primiparous and multiparous females of known Holstein breed (cow breed code = 66)
- AIs performed with Holstein semen (breed code = 66)
- AIs performed with conventional semen (unsexed)

Note: the data from one farm were removed completely due to a change in management of herd reproduction during the observation period.

Table 2 has taken this into account when presenting the data for each herd (weighted average of the number of AIs performed).

The analysis is therefore based on 2421 Holstein females inseminated with a total of 4230 doses of conventional Holstein semen at 14 farms over 3 consecutive years (2 reference years and 1 year of observation, **Figure 2**).

2.4.2. Feed Tracking Data

The composition of the daily ration given to the lactating cows was monitored twice a month, making it possible to define an average ration over the observation period for each of the 15 farms initially selected.

The feeding system was not a criterion for selecting farms. Despite this, it should be noted that most of the farms were generally intensive livestock farms using corn silage (77% of total forage DM on average), beet pulp included as a feed concentrate (18% of total feed concentrate DM) and a little grass forage (hay, grazing, grass silage) (**Table 3**).

2.5. Data Processing and Analysis

All the production and reproduction data were collected over a period of 3 years, *i.e.* 3 successive milking cycles (**Figure 2**):

- A reference period (Control) from 1 Oct 2014 to 30 Sep 2016; the data from these 2 successive years were averaged to characterize the reference period;
- An observation period (Test) from 1 Oct 2016 to 30 Sep 2017, during which time the cows received supplementation with probiotic yeast.

The daily fat and protein yield was defined as the parameter for evaluating milk production performance in order to take into account both the quantitative and qualitative variations in milk production.

The data collected during the observation and reference periods were processed

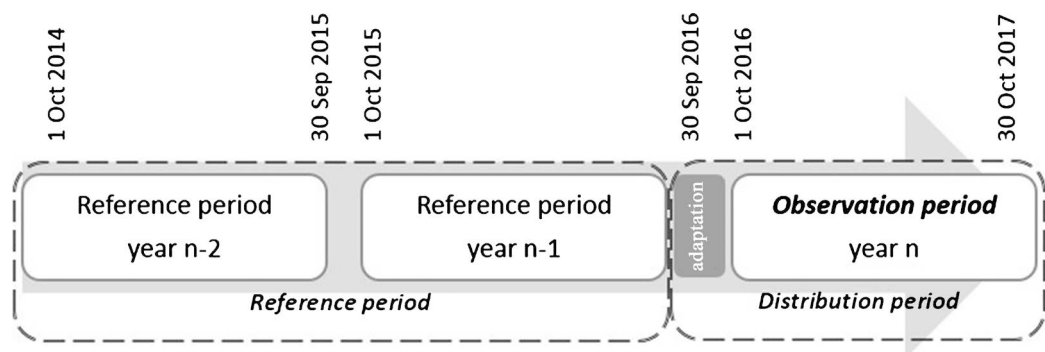
Table 2. Description of the reproduction database.

	Number of females		Number of AIs	
	Multiparous	Primiparous	Multiparous	Primiparous
Total sample (15 farms)	2212	1609	3920	2728
Holstein females	1549	1212	2819	2094
Holstein semen	1546	1212	2816	2094
Conventional semen	1429	1058	2557	1761
14 consistent farms	1397	1024	2513	1717

Table 3. Description of the average dairy cow ration during the observation period.

Herd	Daily ration (% DM)				Corn silage (% of forage DM)	Beet pulp (% of concentrate DM)
	Forage	Feed concentrate	MVF ¹	Other ²		
1	66%	32%	1%	0%	82%	28%
2	79%	19%	1%	1%	77%	0%
3	67%	32%	1%	1%	57%	38%
4	76%	22%	1%	1%	82%	0%
5	78%	20%	1%	1%	74%	0%
6	70%	29%	1%	0%	85%	27%
7	70%	28%	1%	1%	85%	21%
8	73%	25%	1%	1%	68%	43%
9	56%	42%	1%	0%	77%	14%
10	72%	26%	2%	1%	75%	2%
11	63%	36%	1%	1%	77%	35%
12	75%	24%	1%	0%	66%	26%
13	71%	28%	1%	0%	69%	27%
14	70%	28%	1%	0%	90%	8%
15	76%	23%	1%	0%	89%	5%

¹Mineral and vitamin feed; ²Sodium bicarbonate or other buffer substance. Note: beet pulp was included as a feed concentrate.

**Figure 2.** Definition of the chosen analysis periods.

using a mixed model (IBM[®] SPSS[®] Statistics[®], version 23) including the fixed effects of the period, the herd and iC-AI1 as a covariate. The farmers received no specific rules on how the cows should be bred after calving, so this covariate took into account the variability of inter-herd practices. The data, expressed as percentages, were converted (log₁₀) for analysis.

The results from the multiparous and the primiparous cows were processed separately. Nulliparous cows were not included in the analysis because too many of them did not receive the product during the observation period (impossible to implement distribution).

3. Results and Discussion

Probiotic yeasts, in the form of feed additives for animals, are used widely today in the diets of dairy cows with a high production potential [8]. Various studies conducted within the international scientific community have researched the effects of live yeasts, in particular *Saccharomyces cerevisiae*, on digestion, metabolism and performance in dairy cows. Generally speaking, the live yeast *Saccharomyces cerevisiae* increases milk production by 1.2 kg/cow/day on average, as shown by the meta-analysis conducted by Desnoyers *et al.* [6]. In addition, Salvati *et al.* [4] have demonstrated under heat stress conditions the beneficial effects of supplementation with live yeast in terms of more effectively controlling an animal's homeothermy and hence maintenance of milk production.

This improvement in the performance of animals receiving live yeast is explained mainly by the animal's ability to use feed more effectively: Julien *et al.* [9] have observed an increase of between 0.8 and 3.7 points in the digestibility of total organic matter in lactating dairy cows receiving probiotic yeast supplementation. Regarding fibre degradation, Marden *et al.* [7] have reported that probiotic yeast supplementation in dairy cows with digestive disorders such as subclinical ruminal acidosis significantly increased total fibre digestibility from 29.6% to 41.6%. An improvement in the redox (oxidation-reduction potential) values in the rumen is in fact observed in high-producing dairy cows receiving an acidogenic diet supplemented with live yeast [7]: -115 mV for the control diet vs -149 mV for the supplemented diet. According to Pinloche *et al.* [10], this improvement in rumen redox values is linked with the activity of the rumen bacteria [11], in particular lactate-utilizing and fibrolytic bacteria. Unlike the buffer substance sodium bicarbonate, adding live yeast actually induces a lower ruminal lactate concentration. The improvement in rumen reducing conditions is thought to promote the activity of strict anaerobic bacteria such as fibrolytic and lactate-utilizing bacteria, as a result of which the fibre in the ration becomes more digestible [12]. In addition, Jiang *et al.* [13] have confirmed that a certain number of rumen bacterial groups respond to live yeast supplementation, and underline the link between rumen microbial composition and animal performance.

Moreover, although there seems to be a consensus on the nutritional effects and their consequences on milk production, the consequences on breeding performance have yet to be demonstrated. The national analysis of the fertility data of Prim'Holstein livestock (the standard French Holstein) shows that fertility has deteriorated over the past 15 years, although a slight improvement has been observed since 2013 [14]. Everything suggests that farmers' consideration for reproductive indices over the past 5 years has improved reproductive performance in the younger generations of dairy cows. In addition, Chapaux *et al.* [15] argue that for many years the increase in milk production has been accompanied by a decrease in fertility, the first perhaps being the cause of the second. The author nevertheless emphasizes the fact that reproduction performance depends essen-

tially on the quality of the management. Use of feed additives for animals in the diets of ruminants may therefore be considered with this in mind.

In fact, in their study of dairy cows during the peripartum period, Julien *et al.* [16] have demonstrated that the success rate at the first AI is generally better in dairy cows receiving a high dose (10.10^{10} to 20.10^{10} CFU/cow/day) of probiotic yeast over the 6 weeks around calving time; better feed efficiency and hence a lower energy deficit when beginning lactation may therefore improve reproductive function in dairy cows. Production and reproduction compete with each other for the animal's energy intake and cannot easily coexist [17], so use of feed additives for animals may help to control environmental parameters, in particular food efficiency, in order to maximise the animal's full genetic potential. The direct and indirect impact of continuous probiotic yeast supplementation in lactating cows therefore required clarification, and it is the subject of this study.

Overall, no major changes were observed over the observation period in the reproductive parameters of primiparous cows (Table 4), although there was a numerical decrease in the percentage of AIs above parity 3, which was also seen in multiparous cows. On the one hand, it may be assumed that the authors were unable to show any evidence of a difference between the periods studied due to the much smaller number of these primiparous cows (Table 2) compared to those that were multiparous. But what would probably make more sense is that living yeast has a stronger effect on reproductive performance in multiparous cows, which produce more milk than primiparous cows. The same trends are

Table 4. Reproductive parameters over the reference and observation periods for primiparous and multiparous cows.

	Reference period	Observation period	Standard error	<i>p</i> -value		
				cov	herd	period
Primiparous						
AIf	45%	42%	1.1%	0.862	0.519	0.772
AIIf	48%	40%	1.1%	0.958	0.166	0.741
AI3	20%	18%	1.1%	0.243	0.119	0.446
nAI*	2.4	2.5	0.20	0.615	0.85	0.789
Calving to AIf (d)	130.4	133.9	3.02	0.000	0.011	0.441
Multiparous						
AIf	34%	38%	1.0%	0.333	0.112	0.014
AIIf	31%	36%	1.1%	0.084	0.107	0.011
AI3	25%	23%	1.1%	0.514	0.038	0.389
nAI*	3.1	2.7	0.14	0.487	0.097	0.007
Calving to AIf (d)	148.8	143.9	3.64	0.003	0.011	0.323

*This number takes into account the AIs occurring before and after the reference and observation periods used to define the AIf.

observed in terms of milk performance (**Figure 3**). On the other hand, there is an improvement in the AI success rate in multiparous cows of 34% to 38% on average (**Figure 4**). This improvement is linked with an increase in the success rate of the first AI (31% to 36% on average, **Figure 4**).

As a result, the number of inseminations required to achieve pregnancy was significantly lower ($p = 0.007$) when the cows were receiving probiotic yeast compared to the reference period: 3.1 to 2.7 inseminations on average Actisaf[®] Sc 47 (**Figure 5**). However, the difference in the calving to conception interval was not statistically significant, despite an observed numerical decrease of about 5 days. At this stage, it is difficult to give the main reason for this improvement (the general condition of the animal or a direct effect of the probiotic yeast).

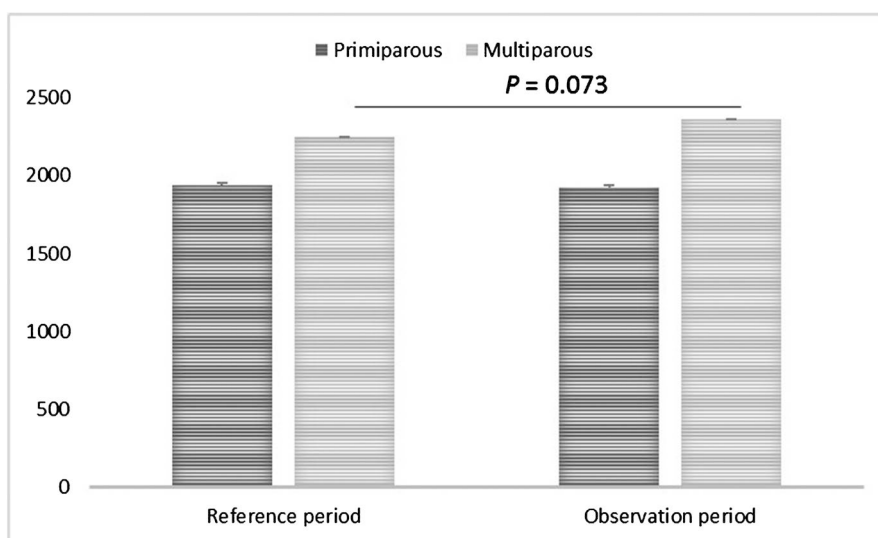


Figure 3. Changes in the average fat and protein yield (g/cow/day) between the reference and observation periods in primiparous and multiparous cows.

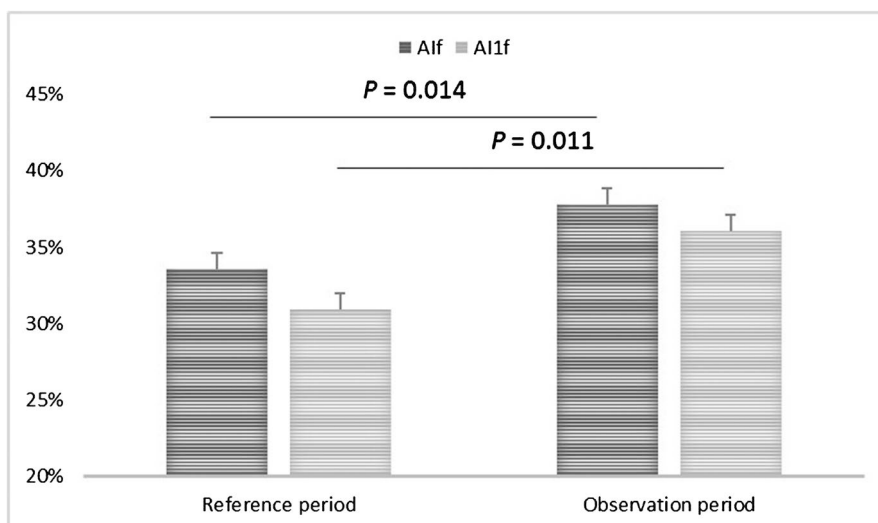


Figure 4. Changes in the fertile AI (AI f) rate and first fertile AI (AI1f) rate between the reference and observation periods in multiparous cows.

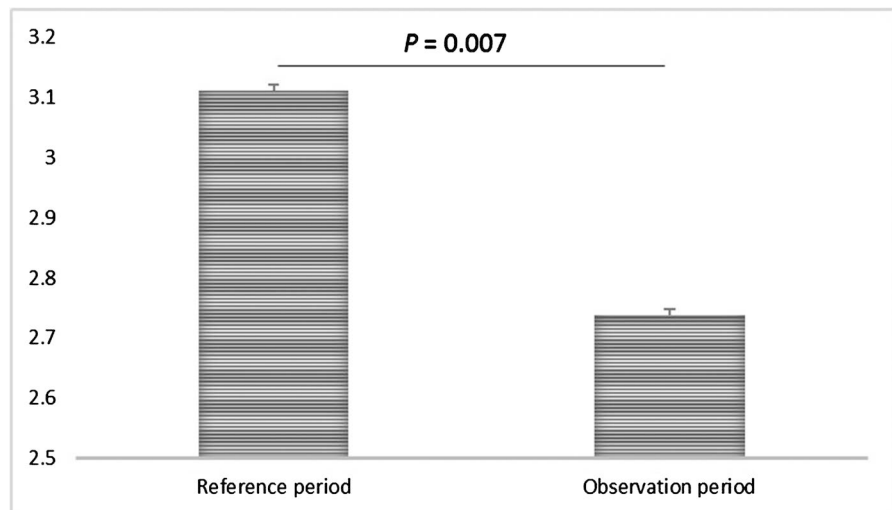


Figure 5. Changes in the number of AIs required for conception between the reference and observation periods in multiparous cows.

Nevertheless, Allbrahim *et al.* [18] have demonstrated that the preovulatory oestradiol peak and the size of the first ovulatory follicle in early-lactation dairy cows tended to increase following supplementation with *Saccharomyces cerevisiae* probiotic yeast. Both direct and indirect effects on breeding performance seem conceivable.

In addition, the improved reproductive performance of multiparous animals went hand in hand with a tendency for better production performance (2 246 and 2 360 g/d, **Figure 3**), which clearly rules out the possibility of the productive function being less demanding on the animal in favour of reproductive function.

4. Conclusion

In conclusion, this field study confirms the beneficial effects of optimizing the feed of dairy cows in production in order to improve their reproductive performance. Use of daily supplementation with live yeast at 14 farms over a year resulted in a significant improvement of 4 points on average in the success rate of artificial insemination and of 6 points in the success rate of first artificial insemination in multiparous dairy cows. The number of inseminations required to obtain a pregnancy was therefore reduced from 3.1 in the reference period to 2.7 in the probiotic yeast supplementation period ($p = 0.007$). In parallel, the fat and protein yield by these same animals tended to improve (+5%, $p = 0.07$) between these two periods, demonstrating that it is possible to combine milk performance and maintenance of an effective reproductive system.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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