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Reducing Protein Content with and without Yeast Probiotic Actisaf Sc 47 Supplementation in the Diet of Dairy Cow: Effects on Milk Performance, Milk Fatty Acid Composition, Digestibility, Feed, and Economic Efficiency

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Abstract

The objective of the present study was to evaluate the effect of reducing CP levels in the diet of dairy cows alone or in combination with a yeast probiotic on milk quantity and quality, milk fatty acid profile, feed and economic efficiency. In total, six cows were included in a 3 × 3 Latin square design with 3 experimental periods of 28 days long (23 days for adaptation and 5 days for data collection). Cows were randomly assigned to one of the three diets according to protein level and supplementation: control diet with 16.5 CP%DM (CTR), a diet with 14.5 CP%DM without Actisaf Sc 47 supplementation (LCP), and a diet with 14.5 CP%DM with Actisaf Sc 47 supplementation at 5 g/cow/day (LCPActisaf). Regarding total milk yield (TMY), a tendency for lower TMY was observed in LCP group compared with the CTR group ($P = 0.1$). No difference was observed between CTR and LCPActisaf groups ($P = 0.90$). The use of Actisaf Sc 47 mitigated the TMY reduction observed in LCP group. Milk fat (MF) and energy-corrected milk (ECM) were unaffected by treatments. Milk protein (MP, kg/d) resulted higher and tended to be higher in LCPActisaf compared to CTR ($P = 0.03$) and LCP ($P = 0.06$) but similar between CTR and LCP ($P = 0.4$). Compared with the CTR group, milk urea nitrogen (MUN) was reduced by both LCP and LCPActisaf (−31.8% and −23.8%, respectively, $P = 0.04$). No difference was observed between LCP and LCPActisaf ($P = 0.49$). Reducing protein alone or in combination with Actisaf Sc 47 did not impact feed efficiency (FE) when compared to CTR ($P = 0.15$ and P = 0.39, respectively) but

reducing protein in combination with Actisaf Sc 47 increased FE compared to reducing protein alone ($P = 0.02$). No differences in milk fatty acid (FA) profile were observed with the exception of a tendency for a greater concentration of linoleic acid in LCPActisaf group compared with the CTR group ($P = 0.09$) and LCP group ($P = 0.08$). Linoleic acid concentration was similar between CTR and LCP groups ($P = 0.93$). *De novo* fatty acid concentration resulted higher ($P = 0.13$) in LCPActisaf (28.90%) compared to CTR (27.75%) and LCP (27.9%). Health indexes explained by Atherogenic index, Thrombogenic index and omega 6/omega 3 were similar between the three treatments. From economic perspective, compared with the CTR and LCP, net revenue/kg of milk was increased and tended to increase with LCPActisaf ($P = 0.05$ and $P = 0.1$, respectively). No difference was observed between CTR and LCP groups ($P = 0.77$). The use of Actisaf Sc 47 increased economic efficiency (EE) by 10% and 8.24% compared with the CTR and LCP groups, respectively. Supplementing reduced-protein diets with Actisaf Sc 47 maintained milk yield, enhanced milk protein output, improved feed efficiency, and increased economic returns, supporting its use as a strategy to optimize dairy production under lower dietary protein conditions.

Keywords

Yeast Probiotic, Actisaf Sc 47, Dietary Crude Protein, Milk Performance, Fatty Acids, Economics

1. Introduction

Maximizing milk production and feed efficiency has long been, and remains, a primary objective for dairy farmers, as these factors directly influence economic profitability and quality of life [1]. To this end, several international feeding systems have been developed to provide evidence-based nutritional guidelines that enable dairy cattle to efficiently realize their genetic potential [2] [3]. Among all nutrients, protein occupies a central role in every nutritional framework as it directly affects milk yield, feed efficiency, production costs, and environmental sustainability, factors that represent a major challenge for all stakeholders across the dairy sector [4]. Dairy cows are notably characterized by low nitrogen use efficiency (NUE) [5]. This inefficiency can negatively affect milk production and feed efficiency, ultimately reducing farm profitability.

Economically, protein management is crucial to farm viability, as dietary protein typically accounts for the largest proportion of feed costs in a lactating cow's ration [6]. Consequently, optimizing NUE through CP reduction has emerged as a strategic approach to improve productivity while mitigating environmental emissions. Although reducing dietary protein shows promise for reducing nitrogen losses, as highlighted in several studies [7]-[9], its implementation must be carefully managed to avoid compromising animal performance. Indeed, research investigating the effects of reduced crude protein (CP) levels on milk yield, feed ef-

ficiency, and nitrogen excretion has yielded variable results. For example, reducing CP from 16% to 15% and from 17% to 15% decreased milk yield (MY) by 1.4 kg and 0.6 kg, respectively [10]. Similarly, other studies reported declines in MY when CP levels were lowered from 17.4% to 15.4% [11] and from 17.3% to 14.4% [12]. Likewise, decreasing dietary CP from 177 to 141 g/kg DM reduced milk yield while improving nitrogen NUE [13]. Conversely, a positive correlation between dietary CP levels and MY, milk fat (MF), and milk protein (MP) content has also been reported [14]. In contrast, reducing CP from 18% to 16% had no significant effect on MY [15]. Although dietary CP reduction presents a promising strategy, it must be implemented cautiously to balance productivity, efficiency, and environmental sustainability.

Inadequate CP levels or the proportion of rumen degradable protein (RDP) and rumen undegradable protein (RUP) can impair rumen microbial growth and fermentation, ultimately compromising both animal performance and metabolic efficiency [16]. To mitigate these risks, rumen fermentation efficiency can be enhanced by minimizing feed protein proteolysis and increasing the supply of rumen-undegradable protein (RUP) to the small intestine [17]. Yeast-based probiotics are particularly effective for this purpose, as they have been shown to support these mechanisms [18] [19]. To the best of our knowledge, no previous study has evaluated the combined effects of dietary CP reduction and yeast probiotic supplementation on dairy cow performance, specifically regarding milk production, milk fatty acid composition, and feed and economic efficiency. Therefore, the present study was designed to address this gap.

2. Materials and Methods

2.1. Animals, Experimental Design and Diets

The experiment was conducted at the Phileo by Lesaffre research station (The Farm, Chemin de Vallesvilles, Seysses, F-31600, France) between December 2024 and May 2025. All animal procedures strictly adhered to European Union guidelines on the protection of animals used for scientific purposes (Directive 2010/63/EU). The experimental protocol received approval from the French Ethical Committee for Animal Experimentation, Animal Sciences and Health N˚115 under the code #38931-2022102016565789.

Six healthy, multiparous Holstein dairy cows in their third lactation were selected for this study (mean ± SD: 92.5 ± 2.59 days in milk; 690.8 ± 79.11 kg body weight; body condition score: 2.7 ± 0.3; milk yield: 38.7 ± 5.92 kg/d). Cows were housed in a free-stall barn equipped with individual feed intake monitoring systems, except during milking and sample collection. The study followed a 3 × 3 Latin square design. Two cows per treatment were grouped in squares based on milk yield and day in milk. The study consisted of 3 experimental periods and each period lasted 28 days (23 days of dietary adaptation and 5 days of measurement and sampling). To avoid any bias and carryover effects due to a sudden change in rations and the potential effect of the treatments offered during each period, which may

impact our results, a 15-day transition period (washout) during which all cows received a common basal ration (16.5% CP, 25.3% starch, 34.4% NDF on a DM basis) was used between each period (Figure 1). The data at the end of each 15-day transition period were compared to identify if there was any difference between the three treatments at the beginning of each experimental period.

Cows were randomly allocated to three treatment groups based on dietary crude protein (CP) level and *Saccharomyces cerevisiae* CNCM I-4407 (Actisaf Sc 47) supplementation: control group (CTR): 16.5 CP%DM, no Actisaf Sc 47 supplementation; positive control group (LCP): 14.5 CP%DM, no Actisaf Sc 47 supplementation; experimental group (LCPActisaf): 14.5 CP%DM with Actisaf Sc 47 supplementation. The recommended yeast probiotic dose of 5 g/cow/d (10^{10} cfu/g of DM, Actisaf® CNCM I-4407, Lesaffre Feed Additives, Marcq en Baroeul, France) was top-dressed on the TMR. Cows were allocated to groups based on milk yield, DIM, and body weight. To ensure balance, cows were rotated through the treatments such that each cow received each diet once over the three periods (e.g., cows starting on CTR in Period 1 moved to LCPActisaf in Period 2 and LCP in Period 3). Health and behavior were monitored daily, including checks for mastitis, lameness, and reductions in dry matter intake (DMI) or milk yield. Inclusion criteria required cows to weigh between 600 and 850 kg, have a BCS between 2.5 and 3.0, and show no signs of health issues such as mastitis or lameness. Exclusion criteria included a prolonged decrease in DMI (≥15% for 3 consecutive days); weight loss (+70 kg) or body condition score (less than 2).

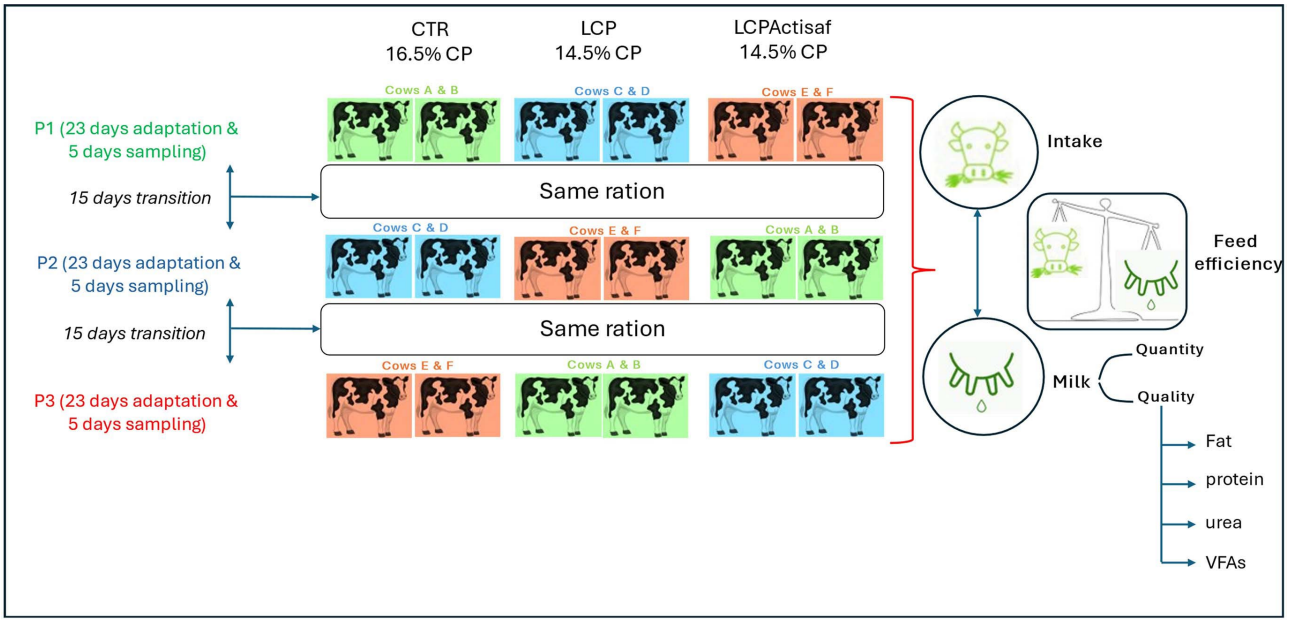

Figure 1. Experimental design used during the trial. LCP: low crude protein without Actisaf (14.5%CP); CTR: control without Actisaf (16.5% CP); LCPActisaf: low crude protein with Actisaf Sc 47 (14.5% CP).

At the start of the trial, cows in the CTR group were 93 ± 2.42 days in milk with 38.64 ± 4.99 kg of milk, cows in the LCP group were 91 ± 1.41 days in milk with

38.41 ± 0.31 kg of milk, and cows in the LCPActisaf group were 93 ± 4.24 days in milk with 39.25 ± 9.48 kg of milk. During the measurement periods, cows were housed in tie stalls and milked twice daily. The barn was equipped with mobile feed carts to enable individual feeding. Diets were offered as total mixed rations (TMRs) comprising corn silage, protein concentrate, energy concentrate, alfalfa hay, and a mineral-vitamin pre-mix (ingredients and chemical composition are detailed in Table 1). All cows received a basal ration formulated at 14.5% CP (DM basis). For the CTR group, an additional quantity of protein-rich concentrate was provided during milking to achieve a final dietary CP level of 16.5% (DM basis). The raw materials composing the protein concentrate are soybean meal, rapeseed meal, corn distillers' grains and soluble, sunflower meal and sodium chloride. Protein concentrate is a source of rumen-degradable proteins. The control diet was formulated to meet the energy and metabolizable protein (MP) requirements for maintenance and production of dairy cow producing 39 kg milk/day with 4 g/kg milk fat and 3 g/kg milk true protein and consuming 25.5 kg DM at the beginning of the experiment [2]. The LCP and LCPActisaf diets were formulated to meet energy and metabolic protein requirements, but the 2% reduction in CP reduced metabolic protein by 8 points. The two diets were isoenergetic. The 2% reduction in CP, made by reducing the quantity of protein concentrate may slightly impact the level of rumen-degradable and undegradable proteins between CTR and both LCP and LCPActisaf diets.

Cows were fed once daily *ad libitum*, with feed allowances adjusted to target ~5% orts (refusals). Feed offered and refused was weighed daily to determine individual dry matter intake. Fresh water was available *ad libitum* throughout the study period. Before the start of the trial, all ingredients composing the rations were analyzed in an external laboratory to determine their chemical composition and formulate the ration accordingly.

Table 1. Ingredients, chemical composition and nutritional value of the total mixed diets fed to lactating dairy cow.

Groups	CTR (16.5% CP)	LCP (14.5% CP)	LCPActisaf (14.5% CP)
Composition of the Diet (%DM)			
Corn silage	64.6	68.1	68.1
Alfalfa hay	9.5	10.0	10.0
Energy concentrate	8.0	8.5	8.5
Protein concentrate	12.5	13.2	13.2
Protein concentrate[1]	5.2		
Mineral	0.2	0.2	0.2
Actisaf Sc 47[2] (g/d)			5
Nutrional Values			
DM (%)	48.10	46.80	46.80

Continued

CP (%DM)	16.50	14.50	14.50
NDF (%DM)	37.40	38.20	38.20
ADF (%DM)	19.10	19.40	19.40
Crude fiber (%DM)	18.40	18.90	18.90
UFL (/kgDM)	0.875	0.874	0.874
PDIE (g/kg DM)	103	95.70	95.70
PDIN (g/kg DM)	108	94.50	94.50
PDI (g/UFL)	118	110	110
Starch (% DM)	25.3	26.70	26.70
Digestible lysine (%PDIE)	7.01	6.55	6.55
Digestible methionine (%PDIE)	1.97	1.85	1.85

[1]Protein concentrate added during milking; [2]Actisaf Sc 47 mixed with 50 g of concentrate. CTR: control group (16.5% CP without Actisaf); LCP: 14.5% CP without Actisaf; LCPA: 14.5% CP with Actisaf Sc 47; UFL: forage unit for lactation; PDIE: true protein absorbable in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen; PDIN: true protein absorbable in the small intestine when degradable nitrogen is limiting microbial protein synthesis in the rumen.

2.2. Measurements and Sampling

Diets were offered once a day at 09:00 h. For each cow, the quantity of diet offered and refused was weighed every morning before the morning feeding, to determine individual daily intake. Samples of each offered total mixed ration and of individual orts (refusals) were collected daily during each 5-day sampling and measurement period. Samples were dried at 60˚C for 48 h in a forced-air oven to determine DM content, ground through a 1-mm screen using a hammer mill and stored until further analysis. Cows were milked twice a day at 07:00 h and 17:00 h, and the total quantity of milk was recorded daily. Throughout the measurement period (5 days), milk samples (100 mL) were collected from individual cow during morning and afternoon milking. The two samples were mixed and treated by preservative and stored at 4˚C until analyzed for milk fat, true protein, and urea by infrared analysis (CIALSO, Auch, F-32000). Energy-corrected milk (ECM) was calculated using Equation (1) of [20].

$$\mathrm{ECM}\left(\frac{\mathrm{kg}}{\mathrm{d}}\right)=\frac{\mathrm{Milk}\left(\frac{\mathrm{kg}}{\mathrm{d}}\right)\times\left[0.383\times\mathrm{fat}(\%)+0.242\times\mathrm{protein}(\%)+0.7832\right]}{3.1138} \quad (1)$$

Fatty acids were estimated from the mid-infrared (MIR) spectrum generated by the analyzer (Bentley for OLA) according to the methodology developed within the European Milk Recording (EMR, https://www.milkrecording.eu/emr.site/). Initially, the spectra were standardized in accordance with [21], and then the equations developed within the Optimir program were in accordance with [22]; https://www.estel-numerique.com/portfolio/optimir/. Atherogenic (A index) and

thrombogenic (T index) indexes were calculated according to Equation (2) and (3), respectively [23]:

$$\text{A index} = \frac{\left(\text{C12:0} + 4 \times \text{C14:0} + \text{C16:0}\right)}{\left(\text{MUFA} + \text{FA omega 6} + \text{FA omega 3}\right)} \quad (2)$$

$$\text{T index} = \frac{\left(\text{C14:0} + \text{C16:0} + \text{C18:0}\right)}{0.5 \times \text{MUFA} + 0.5 \times \text{FA omega 6} + 3 \times \text{FA omega 3} + \left(\frac{\text{FA omega 3}}{\text{FA omega 6}}\right)} \quad (3)$$

Determining digestibility traditionally requires total fecal collection using balance cages, which is laborious and may compromise animal welfare. Therefore, during the measurement period (days 22, 23, 24, and 25), we employed a spot fecal sampling technique as described by [24]. This method involves collecting feces at specific time points over four consecutive days. Six samples were collected to represent a 24-hour cycle at the following intervals after diet distribution: 4, 8, 12, 4, 8, and 24 hours. Specifically, sampling occurred at 13:00 h on day 22; 01:00 h and 17:00 h on day 23; 05:00 h and 21:00 h on day 24; and 09:00 h on day 25. Each sample was oven-dried at 60˚C for 72 h in a forced-air oven to determine dry matter (DM) content, ground through a 1 mm screen using a hammer mill and stored in 150 g plastic containers until further analysis. To ensure consistent drying times and avoid bias, all samples were removed from the oven simultaneously. After drying, samples from each cow were pooled, hand-mixed, and a representative 100 g subsample per cow was sent to the laboratory for analysis. Acid-insoluble ash (AIA) was used as an internal marker to estimate apparent digestibility, as described by [25]. Rations and fecal samples were analyzed for identical constituents to estimate nutrient digestibility using Equation (4):

$$\text{digestibility}\left(\%\right) = 100\left(1 - \frac{\%\ \text{AIA diet}}{\%\ \text{AIA feces}} \times \frac{\%\ \text{nutrient feces}}{\%\ \text{nutrient diet}}\right) \quad (4)$$

Economic analysis was performed according to feed intake and price. The input values in the calculation were as follows: 115 €/ton of corn silage; 375 €/ton of alfalfa hay; 551 €/ton of energy concentrate; 422 €/ton of protein concentrate, and 954 €/ton of minerals. The price of milk was estimated at 0.48 €/kg. Cows were healthy throughout the trial, and no veterinary treatment was administered. The difference between the three groups indicated the economic gain linked to feed.

2.3. Statistical Analysis

Data were tested for normality using the Shapiro-Wilk test and then analyzed using a mixed effects model in Minitab statistical software (version 19). The statistical model included treatment, period, and their interaction as fixed effects, and cow as a random effect. Data were analyzed using the PROC MIXED procedure as follows:

$$Y_{ijk} = \mu + T_i + P_j + T_iP_j + Ck + e_{ijk}$$

where Y_{ijk} is the explained variable; μ, the general mean; T_i = fixed effect of treatment (CP level with and without Actisaf Sc 47 supplementation); P_j = fixed effect

of the period (P_1, P_2 and P_3); T_iP_j = fixed effect of the interaction between diet treatment and period; C_k = the random effect of the cow; and e_{ijk} = the residual error. Differences were considered significant at $P \leq 0.05$ and trends were discussed at $0.05 < P \leq 0.10$. Differences between treatment effects were assessed by pairwise comparisons (Tukey's test).

3. Results

3.1. Effect of Actisaf Sc 47 on Intake, Milk Performance and Efficiency

Milk quantity, milk quality and efficiency are presented in Table 2. Dry matter intake, MF, ECM and average milk produced during the 5 days of measurements did not differ between treatments. Reducing protein alone tended to reduce TMY collected during the 23 days of adaptation and the 5 days of measurements ($P = 0.1$), but the addition of Actisaf mitigated this reduction. Total milk yield did not differ between CTR and LCPActisaf ($P = 0.90$) and between LCP and LCP Actisaf, even if the addition of Actisaf Sc 47 resulted in higher TMY ($P = 0.15$).

Protein yield was significantly higher and tended to be higher in LCPActisaf compared to CTR ($P = 0.03$) and LCP ($P = 0.06$), respectively. No difference was observed between CTR and LCP ($P = 0.4$). Milk urea nitrogen was similar between LCPActisaf and LCP ($P = 0.47$) but significantly lower in both groups compared to CTR ($P = 0.04$).

Feed efficiency was significantly higher in LCPActisaf compared to LCP ($P = 0.02$) but similar to CTR ($P = 0.39$). No difference was observed between CTR and LCP ($P = 0.15$).

Table 2. Effect of reducing CP with and without Actisaf Sc 47 supplementation on milk performance and feed efficiency.

	CTR	LCP	LCPActisaf	SEM	*P*-value		
					T	P	T × P
DMI (kg/d)	24.69	24.87	23.76	0.67	0.29	0.33	0.79
MY (kg/d)	37.30	36.29	37.12	3.66	0.37	0.001	0.19
Total MY (kg)[1]	1005.3^{a*}	956.3^{b*}	1002.4^{ab}	81.4	0.26	0.09	0.74
Protein (%)	3.13^{a}	3.17^{ab}	3.27^{b}	0.07	0.05	0.71	0.98
Protein (kg/d)	1154.2^{a}	1131.1^{a*}	1197.6^{b*}	83.6	0.06	0.01	0.14
Fat (%)	3.93	3.92	4.08	0.26	0.47	0.13	0.53
Fat (kg/d)	1144	1372	1475.6	57	0.35	0.11	0.31
MUN (mg/dL)	15.45^{a}	11.72^{b}	12.48^{b}	1.26	0.04	0.001	0.55
ECM (kg/d)	36.12	34.80	36.8	2.53	0.27	0.01	0.37
FE (ECM/DMI)	1.46^{ab}	1.39^{a}	1.51^{b}	0.10	0.09	0.03	0.18

^{a,b}Mean values in the same line with different superscripts differ significantly ($P < 0.05$). *Mean values in the same line with a tendency of significant difference ($P < 0.1$). SEM: standard error of the mean, T: treatment effect; P: period effect; T × P: treatment and period interaction effect; [1]Total milk yield calculated for each cow during each period; ECM: energy corrected milk; MUN: milk urea nitrogen; CTR: control group (16.5% CP without Actisaf Sc 47); LCP (14.5% CP without Actisaf Sc 47); LCPActisaf (14.5% CP with Actisaf Sc 47).

3.2. Effect of Actisaf Sc 47 on Milk Fatty Acid Profile

Milk fatty acid profile is presented in Table 3. No differences in milk fatty acid profile were observed, with the exception of a tendency for a greater concentration of linoleic acid in LCPActisaf group compared with CTR ($P = 0.09$) and LCP ($P = 0.08$). Compared with CTR and LCP, reducing protein with Actisaf Sc 47 increased linoleic acid concentration by 16% and 17%, respectively. No difference was observed between CTR and LCP ($P = 0.93$).

The supplementation with Actisaf Sc 47 numerically increased *de novo* fatty acids, with cows fed CTR and LCP having a lower concentration of *de novo* FA when compared with cows fed LCPActisaf (27.75%, 27.9%, and 28.90% fat, respectively; $P = 0.13$). Health indexes explained by Atherogenic index, Thrombogenic index and omega 6/omega 3 were similar between the three treatments.

Table 3. Effect of treatment diets on milk FA composition (% fat).

	CTR	LCP	LCPActisaf	SEM	*P*-value		
					T	P	T × P
C4:0	3.19	3.05	2.9	0.17	0.14	0.01	0.57
C6:0	1.98	2.02	1.98	0.134	0.92	0.06	0.11
C8:0	1.23	1.30	1.43	0.47	0.70	0.08	0.02
C10:0	2.53	2.79	3.03	0.386	0.47	0.13	0.024
C12:0	3.34	3.63	3.93	0.46	0.46	0.10	0.021
C14:0	14.35	14.03	14.21	1.24	0.82	0.24	0.36
C14:1	1.14	1.15	1.13	0.074	0.98	0.08	0.71
de Novo FA	27.75	27.90	28.90	2.84	0.13	0.10	0.18
C16:0	31.63	33.18	33.49	2.03	0.62	0.10	0.35
C18:0	11.03	10.62	10.52	0.67	0.77	0.04	0.58
C18:1 9 cis	13.67	14.52	14.40	1.70	0.77	0.12	0.47
C18:2 *cis*-9,12	0.96[a]	0.97[a]	1.13[b*]	0.15	0.10	0.07	0.6
C18:2c9t11	0.86	0.79	0.78	0.24	0.93	0.12	0.59
18:3n-3	0.59	0.52	0.53	0.08	0.62	0.05	0.23
C18:1 trans	3.73	3.32	3.20	0.61	0.74	0.07	0.15
Total SFA	74.03	75.13	75.73	2.42	0.79	0.29	0.14
Total MUFA	21.99	21.83	20.90	2.12	0.85	0.24	0.23
Total PUFA	3.71	3.28	3.33	0.45	0.66	0.10	0.09
Omega 3	0.68	0.62	0.63	0.076	0.77	0.03	0.21
Omega 6	2.28	2.03	2.12	0.163	0.41	0.17	0.04
Omega 6/omega 3	3.36	3.25	3.36	0.31	0.84	0.015	0.50
Atherogenic index	11.30	10.22	10.15	2.18	0.60	0.12	0.22
Thrombogenic index	4.34	4.22	4.21	0.48	0.84	0.20	0.23

[a,b]Mean values in the same line with different superscripts differ significantly ($P < 0.05$). *Mean values in the same line with a tendency of significant difference ($P < 0.1$). SEM: standard error of the mean, T: treatment effect; P: period effect; T × P: treatment and period interaction effect; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; *de novo* = fatty acids C4 to C14; CTR: control group (16.5% CP without Actisaf Sc 47); LCP (14.5% CP without Actisaf Sc 47); LCPActisaf (14.5% CP with Actisaf Sc 47).

3.3. Effect of Actisaf Sc 47 on Digestibility

Table 4 presents the effect of reducing CP level alone or in combination with Actisaf Sc 47 on dry matter (DM) digestibility, cellulose digestibility and acid detergent fiber (ADF) digestibility. Dry matter digestibility was not affected by treatment ($P = 0.17$). Acid detergent fiber digestibility tended to be higher in LCPActisaf group compared to CTR ($P = 0.09$). No difference was observed between CTR and LCP ($P = 0.17$) and between LCP and LCPActisaf ($P = 0.25$) even the use of Actisaf Sc 47 increased ADF digestibility by 14% compared to LCP. Cellulose digestibility tended to be higher in LCPActisaf group compared to CTR ($P = 0.07$) and was numerically higher compared to LCP group ($P = 0.13$). No difference was observed between CTR and LCP groups ($P = 0.17$).

Table 4. Effect of reducing protein alone or in combination with Actisaf Sc 47 on digestibility.

	CTR	LCP	LCPActisaf	SEM	*P*-value		
					T	P	T × P
DM	59.28	62.37	65.10	1.74	0.17	0.32	0.86
Cellulose	32.52^{a*}	39.18^{ab}	47.14^{b*}	3.94	0.10	0.56	0.89
ADF	28.14^{a*}	37.17^{ab}	42.33^{b*}	3.58	0.11	0.47	0.83

^{a,b}Mean values in the same line with different superscripts differ significantly ($P < 0.05$). *Mean values in the same line with a tendency of significant difference ($P < 0.1$). SEM = standard error of the mean, T = treatment effect; P = period effect; T × P = treatment and period interaction effect; DM = Dry matter; ADF = Acid detergent fiber; CTR = control group (16.5% CP without Actisaf Sc 47); LCP = (14.5% CP without Actisaf Sc 47); LCPActisaf = (14.5% CP with Actisaf Sc 47).

3.4. Effect of Actisaf Sc 47 on Economic Gain

Data on economic gain are presented in Table 5. Daily feed cost/kg of milk was lower ($P = 0.04$) and tended to be lower ($P = 0.08$) in LCPActisaf group compared with CTR and LCP groups, respectively. Net revenue/kg of milk was higher ($P = 0.05$) and tended to be higher ($P = 0.1$) in LCPActisaf group compared with CTR and LCP groups, respectively. No difference was observed between CTR and LCP groups ($P = 0.77$).

Table 5. Average feed cost and economic efficiency for lactating cows fed different experimental rations.

	CTR	LCP	LCPActisaf	SEM	*P*-value		
					T	P	T × P
Daily feed intake as fed (kg/d)	55.75	56.32	53.91	1.54	0.31	0.30	0.8
Daily MY (kg/d)	37.30	36.29	37.11	3.66	0.37	0.001	0.19
ECM (kg/d)	36.12	34.8	36.8	2.53	0.27	0.01	0.37
Cost of feed intake (€/cow)	9.94^{a}	9.53^{a}	9.12^{a*}	0.26	0.05	0.32	0.81

Continued

Selling market price of milk (€/kg)	0.48	0.48	0.48				
Selling market price of MY (€/cow)	17.9	17.41	17.81	1.79	0.37	0.001	0.2
Daily feed cost/kg milk	0.281^{a}	0.278^{ab*}	0.25^{b*}	0.096	0.04	0.001	0.36
Net revenue (€/cow)	7.96	7.89	8.69	1.71	0.15	0.001	0.27
Net revenue/kg milk (€)	0.19^{a}	0.20^{ab*}	0.22^{b*}	0.03	0.04	0.001	0.36
Economic efficiency	1.79^{a*}	1.82^{a}	1.97^{a*}	1.36	0.10	0.07	0.59
Improvement of economic efficiency compared to CTR	100%	101.7%	110%				

^{a,b}Mean values in the same line with different superscripts differ significantly ($P < 0.05$). *Mean values in the same line with a tendency of significant difference ($P < 0.1$). SEM: standard error of the mean, T: treatment effect; P: period effect; T × P: treatment and period interaction effect; CTR: control group (16.5% CP without Actisaf Sc 47); LCP (14.5% CP without Actisaf Sc 47); LCPActisaf (14.5% CP with Actisaf Sc 47).

4. Discussion

4.1. Effect of Reducing Protein with and without Actisaf Sc 47 on Intake, Milk Performance and Efficiency

Reducing CP level with and without Actisaf Sc 47 supplementation did not impact dry matter intake (DMI). Previous studies reported no difference in DMI by lactating dairy cow offered diets with different levels of CP (16.5 *versus* 15.5% CP [17]; 16.2 *versus* 14.2% CP [26]; 17.0 *versus* 15.0% CP) [27]. In the same way, by comparing diets with different rumen degradable protein and rumen undegradable protein levels with and without live yeast supplementation, [19] reported no difference in intake. In contrast, [28] and [29] reported that lowering CP level by 2% decreased DMI.

Regarding total milk yield (TMY), over the 23-day dietary adaptation period and subsequent 5-day measurement phase, cows fed either the 16.5% CP diet or the 14.5% CP diet supplemented with Actisaf Sc 47 produced more milk than those fed the 14.5% CP diet alone. The effect of reducing CP level on MY has been extensively studied, yet findings remain inconsistent largely due to variations in experimental design, target of protein levels, and the genetic merit or production potential of the cows evaluated. Our results agree with those of [12] and [13] who reported decline in MY when dietary CP was reduced from 17.3% to 14.4% and from 17.7% to 14.3%, respectively. Conversely, reducing protein alone or in combination with essential oils had no effect on MY [17]. Notably, in our study, supplementing the 14.5% CP diet with Actisaf Sc 47 tended to mitigate the decline in TMY associated with feeding the non-supplemented low CP diet. To the best of our knowledge, no previous study has investigated the combined effect of dietary crude protein reduction and supplementation with the yeast probiotic Actisaf Sc 47. However, the positive impact of Actisaf Sc 47 on milk yield (MY) has been extensively documented. Our findings align with the meta-analysis of [30] and [31] who reported increased MY in dairy cows supplemented with Actisaf Sc 47 compared to non-supplemented cows. The observed improvement in MY may be at-

tributed to enhanced feed utilization and nutrient digestibility, likely resulting from optimized ruminal fermentation and a more favorable microbial environment [32]-[34].

Regarding FE, in the absence of Actisaf Sc 47 supplementation, reducing dietary protein slightly decreased feed efficiency, calculated as the ratio of energy-corrected milk (ECM) to milk yield. This finding contrasts with [10] [17], who reported no effect of protein reduction on FE. However, Actisaf Sc 47 supplementation mitigated this decline. Indeed, the supplement improved FE at the standard dietary protein level (14.5%) and sustained this benefit even at the highest protein level (16.5%). Previous studies have demonstrated that yeast probiotics can improve feed efficiency in dairy cattle [31]. This beneficial effect may be attributed to Actisaf Sc 47's ability to optimize rumen environment and function by stabilizing rumen pH, scavenging oxygen, and promoting the growth of cellulolytic and fiber-degrading bacteria, ultimately enhancing feed utilization and nutrient valorization [33] [34].

4.2. Effect of Reducing Protein with and without Actisaf Sc 47 on Milk Composition and Fatty Acid Profile

Milk urea nitrogen (MUN), a metabolic byproduct of protein catabolism, serves as a practical on-farm indicator of protein status and nitrogen use efficiency (NUE) in dairy cows. In the present study, cows fed the 16.5% CP diet exhibited higher MUN values (15.45 mg/dL), compared to cows fed 14.5 % CP alone (11.72 mg/dL) and cows fed 14.5% CP in combination with Actisaf Sc 47 12.48 mg/dL, reflecting improved nitrogen utilization. Previous study reported a positive correlation between CP level and MUN [10] [35]. The elevated MUN value (15.45 mg/dL) observed in the 16.5% CP group suggests inefficient nitrogen utilization, with excess dietary nitrogen being excreted as urinary urea, a pattern consistent with the inefficiencies described by [36].

Milk fat (MF) was unaffected by the treatments. Our results agree with those of [10] and [17] who observed no effect of reducing CP level on MF. Similarly, [37] reported no changes in milk fat following supplementation of Actisaf Sc 47. In contrast to MF, Actisaf Sc 47 supplementation increased MP content. The positive effect of yeast probiotics in MP has been observed in many studies. For example, [38] synthesized data from 14 studies and reported a trend for increased MP in dairy cows supplemented with yeast probiotics. A similar trend was observed by [39] who demonstrated elevated MP concentrations in cows supplemented with live yeast. The beneficial effect of Actisaf Sc 47 on MP may be driven by its ability to improve rumen environment, thereby enhancing microbial protein synthesis [32] [40].

To the best of our knowledge, very few studies have evaluated the effect of dietary protein reduction alone on the milk fatty acid profile, and none have investigated the combined effect of protein reduction and yeast probiotic supplementation. Milk fatty acid (FA) profile was unaffected by the treatments, except for lin-

oleic acid. Linoleic acid tended to be higher in LCPActisaf group. Previous study in dairy cows reported no effect of reducing CP level on milk fatty acid profile [41]. The higher linoleic acid concentration in LCPActisaf agrees with the findings of [42]. This shift in fatty acid composition may be attributed to altered feed selection. Previous research suggests that yeast supplementation encourages dairy cows to selectively consume fewer long forage particles, which are typically lower in linoleic acid. This feeding behavior likely increases the relative proportion of linoleic acid in the overall diet, subsequently elevating its concentration in milk.

De novo fatty acid concentration was numerically higher in LCPActisaf group compared to both CTR and LCP groups. *De novo* fatty acids (≤C16:0) serve as key biomarkers of rumen health, as they are synthesized directly by the mammary gland from rumen-derived substrates, primarily acetate produced by cellulolytic bacteria. The modest increase in *de nov*o fatty acids observed with Actisaf Sc 47 likely reflects enhanced fiber and starch digestion, leading to greater availability of these precursors. Importantly, the *de novo* fatty acid concentrations recorded in our study exceeded the critical threshold of 21.1 g/100 g of milk fat, above which animal health monitoring is recommended. For context, [43] reported a 7.2-fold higher risk of disease or removal (culling or death) in cows with *de novo* fatty acid concentrations below this threshold. Furthermore, [44] documented a negative correlation between *de novo* fatty acid concentration and the duration of rumen pH < 5.8—a key indicator of subacute ruminal acidosis (SARA) risk, suggesting that higher *de novo* synthesis is associated with improved rumen stability. A limitation of this study is the small sample size (n = 6), which may have reduced the statistical power required to detect significant differences in different estimated parameters. Nevertheless, the present methodology and findings provide a solid foundation for future large-scale trials with increased statistical power.

4.3. Effect of Reducing Protein with and without Actisaf Sc 47 on Digestibility

Reducing protein level alone or in combination with Actisaf Sc 47 did not affect dry matter digestibility and our result is consistent with previous studies, which showed no effect of reducing CP level on DM digestibility [13] [45]. However, it appears to contradict some previous studies. For instance, [46] reported quadratic increases in DM digestibility as dietary CP increased from 13.5% to 15.0%, 16.5%, 17.9%, and then to 19.4% CP. The addition of Actisaf Sc 47 numerically increases DM digestibility, which may be due to its positive effect on rumen environment and function, as explained by [32].

Regarding Acid detergent fiber digestibility, reducing protein level without Actisaf Sc 47 supplementation has no significant effect. This finding aligns with [13] and [17] who reported no effect of 14.1% CP diet compared to diets containing 15.1%, 17.7% or 20.1% CP and 16.5% CP diet compared to diet containing 15.5%CP alone or combined with essential oil. Supplementation with Actisaf Sc 47 tended to increase ADF digestibility. Regarding cellulose digestibility, we observed higher

cellulose digestibility in cows supplemented with Actisaf Sc 47. The positive effect of Actisaf Sc 47 on both ADF and cellulose digestibility is likely related to the probiotic's ability to stimulate the growth and enzymatic activity of fibrolytic and cellulolytic bacteria, thereby enhancing fiber degradation [47] [48]. A limitation of the present study is the small sample size (n = 6), which may have reduced statistical power. Nevertheless, the methodology and findings presented here provide a foundation for future large-scale trials with increased animal numbers and enhanced statistical robustness. Also, during our study, we used the spot fecal sampling technique as described by which potentially limits the accuracy and the precision estimation of our results compared to the total collection of feces. Crude protein, neutral detergent fiber and organic matter digestibility were also measured during the trial but published recently in another journal [32].

4.4. Effect of Reducing Protein with and without Actisaf Sc 47 on Economic Gain

From an economic perspective, when considering milk volume alone, the combination of reduced dietary protein and Actisaf Sc 47 supplementation yielded the highest net revenue and economic efficiency. Previous study reported greater net revenue and economic efficiency in lactating buffaloes supplemented with yeast probiotic compared to non-supplemented cows [49]. The same tendency was reported by [50] who confirmed that feeding live yeast to dairy cow increased overall farm margins by 1.4%. The improved economic performance is likely to stem from enhanced feed efficiency coupled with reduced feed costs associated with lower protein inclusion. We hypothesize that the economic advantage of this strategy would be further amplified if milk payment systems accounted for both fat and protein components, rather than volume alone, as Actisaf Sc 47 also increased total milk protein yield in our study.

5. Conclusion

Overall, compared with CTR and LCPActisaf, reducing protein alone tended to decrease TMY. The 2 percentage-unit reduction in CP alone or in combination with Actisaf Sc 47 had no effect on MF and ECM. However, Actisaf Sc 47 supplementation tended to increase and significantly increase MP when compared with CTR and LCP. Reducing protein alone or in combination with Actisaf Sc 47 reduced MUN. The 2 percentage-unit reduction in CP alone or in combination with Actisaf Sc 47 had no effect on FE. However, Actisaf Sc 47 supplementation at 14.5% CP increased FE relative to non-supplemented 14.5% CP diet. Compared with the CTR and LCP, the supplementation with Actisaf Sc 47 tended to increase linoleic acid and showed higher *de novo* fatty acid concentration. The use of Actisaf Sc 47 reduced daily feed cost, increased and tended to increase net revenue/kg of milk compared with the CTR and LCP. The use of Actisaf increased EE by 10% and 8.24% compared with the CTR and LCP, respectively. In conclusion, lowering crude protein content in combination with Actisaf Sc 47 seems to be an interesting

approach to optimize MY, improve linoleic acid concentration, reduce feed cost and increase EE. Despite these results, further research with more animals is needed to design diets with lower protein content or different proportions of rumen-degradable and undegradable protein in combination with Actisaf Sc 47 that confirms our results.

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Conflicts of Interest

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

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