OPTIMAL MANAGEMENT OF LUCERNE-BASED FEEDING TO MAXIMIZE MILK YIELD AND NUTRITIONAL QUALITY

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ABSTRACT

The quality of forage, a key factor in livestock feed selection, is now a major development objective to ensure food security, nutrition, and human health. It also represents a sustainable intensification of livestock farming systems. Two experiments were conducted to assess the effects of forage type and lucerne growth stage on milk yield and physicochemical composition. In the first experiment, 25 cows were randomly allocated to five dietary treatments (n = 5 cows per treatment) including: (i) green barley + oat hay as the control, reflecting standard farm practice; green lucerne + oat hay; pasture + oat hay; green sorghum + lucerne hay; and green sorghum + oat hay. The second experiment involved a separate group of 10 cows randomly assigned to two treatments (n = 5 cows per treatment) to evaluate the influence of lucerne growth stage on milk production and composition. The treatments consisted of fresh lucerne used at either the budding stage or the flowering stage. The data were analyzed using a one-way ANOVA with feed treatment as a fixed factor. The fresh lucerne–oat hay resulted in the highest milk yield (16.93 kg day^{-1}) compared with sorghum–lucerne hay and the control diet. Milk production decreased by about 50% when cows were fed pasture–oat hay or green sorghum–oat hay. Moreover, feeding fresh lucerne increased milk yield by over 31% compared with lucerne hay. Fresh lucerne improved milk quality indicated by a higher protein (3.66%) and fat (4.19%), lactose and mineral content but it exhibited the lowest Dornic acidity (16°D), compared with lucerne hay and other diets. Markedly, fresh lucerne at the budding stage significantly improved milk yield (+30.8%) compared with the flowering stage which induced a higher crude fiber (25.23%), that may reduce digestibility. In contrast, budding stage lucerne increased the milk quality by enhancing the crude protein (+24.20%), digestible crude protein (+25.34%), mineral content (+45.01%), and feed units for lactation (+22.58%). The findings of this investigation highlighted that an incorporation of the fresh lucerne, particularly at budding stage into ruminant diets could represent a promising strategy to improve forage quality and supports optimal lactation performance due to its high nutritional richness in protein and mineral components.

Keywords: Lucerne forage, Dairy cattle, Milk composition, Milk yield, Forage quality

https://doi.org/10.36899/JAPS.2026.5.0114 Published first online June, 24, 2026

INTRODUCTION

On an average, one-third of the protein consumed by humans comes from livestock products (Thornton and Herrero, 2010). Livestock plays a crucial part in supporting the livelihoods of approximately 600 million impoverished people globally and accounts for almost 40% of agriculture's GDP (Wróbel *et al.,* 2025). Because it contains fat, protein, lactose (a sugar), enzymes, vitamins, minerals, organoleptic qualities, and digestibility, milk is a complete diet. In order to meet the growing demand brought on by population growth, several nations place a high priority on the dairy industry to ensure food security. However, because they vary depending on breed, health status, age, milking stage, season, and milking technique, the quantity and nutritional value of cow's milk may be a barrier to meeting milk requirements. Dairy cows' forage diet also affects its nutritional value. According to Arzani *et al.,* (2012) and Dindová *et al*., (2019), fodder quality is significantly impacted by climate change, plant contaminants and diseases, plant species, plant organs, and plant growth stage. In fact, in the arid and semi-arid regions of Algeria, milk production is highly dependent on climatic conditions, which influence the availability and diversity of forage species (Kalli *et al.,* 2023). By combining grass, non-grass feed, and legume plants, grassland species diversification in prairies is currently of significant interest (Jezequel *et al.,* 2024).

During growth stage, the lucerne (*Medicago sativa* L.) as all legume species, is characterized by its high protein and amino acid content making it an essential plant for animal feed while at maturity, lucerne hay has an average of 20.7% crude protein, 1.5% starches, and 41.1% neutral detergent fiber (NASEM, 2021). Numerous research has demonstrated the advantages of lucerne hay for animal diets, but few or no studies have examined the nutritional value of fresh lucerne at each stage of growth and its usage as pasture for milk production and bovine feeding. The advantages of collecting fodder legumes at an earlier growth phase considerably lower intestinal CH_4 emissions in dairy cows (Warner *et al.*, (2017). We hypothesize that the amount and nutritional value of milk produced to satisfy dairy needs could be enhanced by incorporating protein-rich forages. The objective of this study was to evaluate the impact of fresh lucerne-based forage on milk production and characteristics in cows, compared to a diet based on lucerne hay and other grasses. It also aimed to assess the effects of fresh lucerne, at the budding and flowering stages, on milk yield and its nutritional value, in order to optimize feed quality and contribute to improving the efficiency and sustainability of milk production.

MATERIALS AND METHODS

Location and Period: The experiments were carried out in 2024 from April to May at the experimental farm of the Technical Institute of Livestock Breeding, located at Algiers, Algeria, which is located in a Mediterranean environment typical of northern Algeria. The average annual rainfall is around 500–600 mm, focused largely during the autumn-winter period (October-March), and followed by a dry period in the summer (May-September). The average air temperature in fall is 15.5°C, 11.2 °C in winter, and 21.33°C in spring (ONM, 2024).

Animals and Management: The trial included 35 dairy cows of the Holstein-Friesian breed at 95 ± 7 days in milk and 2^{nd} lactation at the start of the study. The average age was 5 ± 0 years, the average body weight was 564 ± 16.6 kg, and the body condition score (BCS) was 3.1 ± 0.2 (scale 1–5). The baseline milk yield, measured over 15 days prior to the start of the trial, was 9.74 ± 0.26 Kg/day. Cows were housed in free-stall barns that had unlimited access to water. Prior to data collection, a 15days pre-experimental adaptation period was allowed. Each experimental period lasted 30 days following the adaptation period**.**

Experimental Design and Feeding Treatments: After the adaptation period, cows were randomly allocated to two independent experiments conducted under identical environmental, housing, and management conditions. Each experiment was carried out using a distinct group of cows. Random allocation was performed after balancing cows for parity, days in milk, body weight, and initial milk yield to ensure homogeneous experimental groups, following established experimental design principles (Kaps and Lamberson, 2004).

The first experiment included 25 cows randomly assigned to five dietary treatments (n = 5 cows per treatment) to evaluate the effects of different forage combinations on milk yield and on physicochemical composition. The treatments consisted of five dietary treatments: (i) green barley (*Hordeum vulgare* L.) + oat hay as a control diet, (ii) green lucerne (*Medicago sativa* L.) + oat hay (*Avena sativa* L.), (iii) pasture + oat hay, (iv) green sorghum (*Sorghum bicolor* (L.) Moench + lucerne hay, and (v) green sorghum-oat hay. The control diet corresponded to the standard farm feeding practice based on green barley and oat hay. The second experiment included another group of 10 cows (with same characteristics as the first experiment) randomly assigned to two dietary treatments (n = 5 cows per treatment) to investigate the effect of lucerne growth stage (budding vs. flowering) on milk yield and composition. The treatments consisted of two intercropping combinations of (i) green lucerne in the budding stage, and (ii) green lucerne in the flowering stage.

In both experiments, fresh (green) forages were offered *ad libitum*, whereas hay was provided in restricted amounts (5 kg/cow/day) according to the experimental design. The composition and nutritive value of each feed component are presented in Tables 4 and 5. Each experiment was analyzed separately, with each cow considered an independent experimental unit. The first experiment evaluated the effects of five forage combinations, while the second experiment evaluated the effect of lucerne growth stage. Overall, the study included a total of 35 lactating dairy cows, with 25 cows in experiment 1 and 10 cows in experiment 2. In this study, we specify that no cow included in more than one experiment.

Feed Sampling and Chemical Analysis: Samples of alfalfa (*Medicago sativa* L.) forage were collected at different phenological stages to assess their nutritional composition. Representative samples were manually selected from randomly chosen quadrats. Each diet (fresh or hay) was collected, dried at 60 °C for 48 hours, and then analyzed.

Chemical analysis was performed according to official international methods (AOAC 2005a) and included: dry matter (DM, %) (method 930.15), mineral matter (MM, %) (method 942.05), organic matter (OM, %), crude protein (CP, %) (method 984.13), crude fiber (CF, %) (method 978.10), calcium (Ca, %) (method 984.27), and phosphorus (P, %) (Method 965.17 ; AOAC 2005a). Organic matter (OM) content was estimated by subtracting MM from DM. Nutritional values, such as the feed unit for lactation (FUL) and digestible crude protein (DCP), were assessed using the INRA nutrition tables (INRA, 2018).

Milk Sampling and Physico-Chemical Analysis: The cows were milked twice daily, at 6:00 a.m. and 6:00 p.m., using a mechanical milking system. The milking protocol was identical for all cows throughout the experiment. Standard hygiene measures were applied before milking, including cleaning and drying the udder to prevent contamination. Individual milk production was measured at each milking (morning and evening) using calibrated meters. Before milk collection, the udder was thoroughly washed with water and then dried to prevent contamination. Milk samples were collected after milking, five times a week, in sterile 500 ml plastic bottles. Then, they were transported under refrigeration (at 4°C) to the dairy laboratory and analyzed the same day.

Physicochemical analysis: The Gerber method was used to calculate the fat content (Kleyn *et al.*, 2001). The crude protein content of milk samples was determined by using the Kjeldahl method (AOAC, 1995). The lactose concentration of milk was determined by the official AOAC spectrophotometric enzyme method 984.15 (AOAC, 2005a). Total solids content was determined according to the AOAC method (AOAC, 1990a).

Milk density was determined according to the method of Association of Official Analytical Chemists (AOAC, 1990b). The titratable acidity of the milk samples was determined using the AOAC method (AOAC, 2005b). The freezing point of milk was determined using a cryoscope (IDF, 2002).

An automatic milk analyser (Lactostar, Funke Gerber, Germany) was used to measure milk fat, protein, lactose, total solids, milk density, titratable acidity, and freezing point (Funke-Gerber, 2022). Final values for each parameter were computed as the means of the two techniques reducing the analytical bias and improving reliability.

Statistical Analysis: The data were analyzed using the variance technique (one-way ANOVA) with dietary treatment as the only fixed factor. Mean separation was done by least significant difference (LSD) after a significant ANOVA ($p < 0.05$). Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was verified using Levene's test prior to ANOVA. To perform the statistical analyses of the data, SPSS 25 (IBM, 2017) was employed.

RESULTS AND DISCUSSION

Results showed that dietary changes have a significant impact on the milk yield. Interestingly, fresh lucerne and fresh pasture had higher digestible crude protein (DCP), organic matter, and calcium content, whereas lucerne hay and oat hay indicated a higher dry matter, crude fiber, and mineral content compared to control feed. Lucerne is strong in protein nutrients and nutritional quality, which improves animal health and production performance (Wang *et al.*, 2021). Furthermore, the feed unit for lactation (FUL) was significantly lower in lucerne hay (0.51 g/kg) and fresh lucerne (0.61 g/kg) than in other diets (Table 1). The lucerne's high protein content may have a positive impact on rumen fill, ammonia recycling, protein metabolization, and nitrogen efficiency, all of which are required for the rumen diet (Mangwe *et al*., 2024; Rabee *et al*., 2025). Barley, sorghum, and oat hay pastures have high energy values due to their high starch and polysaccharides content, which causes an accumulation of easily fermentable organic acids, possibly predisposing cows to acidosis (Oelker *et al.,* 2009). However, incorporating fresh grass into cow diets presents a challenge in ensuring high milk production while limiting the negative environmental impact of methane emissions. Fresh lucerne is an inexpensive forage rich in organic nitrogen, compared to conserved forages (Delaby *et al*., 2020). Our findings highlighted that lucerne, particularly fresh lucerne, has an interesting nutritional profile for increased productivity and milk quality. According to Suwignyo and Sasongko (2019), feeding fresh lucerne does not necessarily raise body weight, but it may improve feed conversion rate as a nutritional supplement. Improved feed conversion of fresh lucerne could lead to better lactation performance. The high crude fiber content of lucerne hay may slow dietary digestion, affecting the optimal utilization of metabolic energy (Sauvant *et al.,* 2002).

Remarkedly, the fresh lucerne-oat hay produced the highest milk (16.93 kg day^{-1}), followed by sorghum-lucerne hay (11.6 kg day^{-1}) and control (10.37 kg day^{-1}). In fact, the milk yield significantly decreased ($p< 0.001$) by more than 50% 54.34% when cows were fed pasture-oat hay and green sorghum-oat hay, respectively, compared with fresh lucerne feed (Table 2). Recent studies suggested that forage type and quality significantly influence milk yield, composition, and rumen microbial activity in dairy cows (La *et al.*, 2024; Wang *et al.*, 2023; Mangwe *et al.*, 2024). These results are in accordance with those found by Sánchez-Duarte and García (2023) and Wang *et al.,* (2023), who reported that fresh lucerne increased milk protein, minerals, and overall nutritional quality. The use of fresh lucerne and lucerne hay indicated that the fresh lucerne significantly improved ($p < 0.001$) by more than (31%) the milk yield. This increase could be attributed to improving nutritional value of the cows' feed ration through the addition of lucerne. Our results corroborate those of Wang *et al*., (2023) who observed higher crude protein digestibility in cows fed green lucerne than in those fed lucerne hay. This improved digestibility could increase metabolizable protein intake and, consequently, milk production. Our results also indicated that fresh lucerne improved the nutritional value of milk (Table 2). similarly, with recent findings showing that fresh lucerne and high-quality silages can improve milk protein and mineral content (La *et al*., 2024; Rabee *et al.*, 2025). The superior chemical properties of fresh lucerne could contribute to improvement of nutrient digestibility, increasing the absorption of nutrients by animals, as well as promoting microbial growth and fermentative activity. The abundance and diversity of microbial species in rumen fluid varied according to the type of lucerne consumed by the cows, suggesting an impact on the microbial fermentation of crude protein by improving overall nitrogen use efficiency and, therefore, milk production performance Zhao *et al*., (2024). Ruminal fermentation converts ingested food into energy by crushing fibers during the rumination process. Feed is regurgitated, chewed, swallowed, and then carried to the stomach, where bacteria convert it with water into volatile fatty acids, lactic acid, and gas emissions like methane and CO_2. The metabolized nitrogen is converted by the rumen microbe population into ammonia for protein synthesis. These proteins are then digested in the small intestine, providing ruminants with energy and amino acids. Ensuring a balance between rumen degradable and undegradable protein, and energy source is a primordial factor in increasing microbial protein synthesis and improving feed efficiency. In fact, a nutritional strategy based on an adequate supply of metabolizable amino acids and nitrogen derived from amino acids metabolization could ameliorate milk yield and milk protein content and reduce nitrogen losses for sustainability of dairy production systems.

In general, the daily improvement system for milk production is a scientific and technological management method based on many small and continuous daily adjustments, used in dairy farms to increase milk yield and quality and can lead to significant improvements in long-term milk production performance. It involves regularly monitoring the lactation performance and milk composition of dairy cows. Measuring each organic and chemical characteristic and determining each milk parameter provides information that allows for adjusting feeding strategies and could optimize production potential. Interestingly, our findings suggested that milk produced from fresh lucerne indicated a higher nutritional value than milk produced using other feed sources. The milks produced using fresh lucerne as feed is characterized by appreciable protein and fat content by about 3.66% and 4.19% respectively, as well as better supply of lactose and mineral components (Table 3). which could support enhancing the growth of proteolytic and cellulolytic microbial populations in the cecum and colon of animals (Yue *et al.*, 2022). In fact, diets with a high crude protein content have been shown to increase prolactin levels in dairy rats by stimulating mammary gland development and supporting lactation by providing amino acids to the gland via the sodium-coupled neutral amino acid transporter 2 (SAT2) and consequently facilitating the synthesis of milk proteins (Velázquez-Villegas *et al*. 2015). Conversely, the Dornic acidity level was lowest, corresponding to 16 °D of lactic acid when the fresh lucerne was used to feed cows, compared with 17°D of lactic acid measured in milk obtained from lucerne hay, pasture, and fresh sorghum. The combination of chemical and physical parameters such as the freezing point is an indicator of the nutritional value of milk. Although the point freezing values were different between all milks, feed based on the green lucerne did not have any significant effects ($p \leq 0.05$, $p < 0.01$, $p < 0.001$) on the freezing point (-0.51 °C) indicating rapid freezing of the milk produced for its proper preservation as described by Fayziev (2024).

The study of the impact of various growth stages of fresh lucerne on milk yield indicated that the budding stage improved the milk production (30.80%) compared to the flowering stage. The lucerne used in vegetative or budding stages provides higher crude protein and mineral content, which could result in improvement of milk yield and quality (La *et al*., 2024). Figures 1 and 2 indicated the advantage of the physicochemical characterization of the collected milks, as evidenced by a statistically significant increase ($p \leq 0.05$, $p < 0.01$, $p < 0.001$) in milk density (0.48%), fat content (9.47%), protein content (11.17%) and total solids (13.54%) when lucerne at budding stage was used as cow feed. Nevertheless, no significant differences ($p \leq 0.05$, $p < 0.01$, $p < 0.001$) were observed in lactose content between milk obtained from cows fed lucerne at the budding and flowering stages (Table 4). These results could be attributed to the chemical proprieties of fresh lucerne at different stages. In fact, the comparison between both types of lucerne indicated that the flowering stage plants had higher dry matter (20.95%), organic matter (91.81%), and crude fiber contents (25.23%), revealing a high content of structural polysaccharides during plant maturation, which could reduce digestibility and limits nutrient availability to ruminants. In contrast, lucerne harvested at the budding stage exhibited significantly ($p \leq 0.05$, $p < 0.01$, $p < 0.001$) higher crude protein (+24.20%), digestible crude protein (+25.34%), mineral matter (+ 45.01%), and feed unit for lactation (+22.58%) confirming the superiority of nutritive value of lucerne at this development stage (Table 4). During the vegetative and budding stages, lucerne plants develop maximum symbiotic activity with *Rhizobium* bacteria, inducing the formation of a large number of nitrogen-fixing nodules (Mueller and Teuber, 2007), Consequently, the increased nitrogen assimilation could contribute to a greater accumulation of protein in plant tissues, explaining the higher levels of crude protein (CP) and digestible protein (DP) observed at the budding stage. However, the high degradability of the protein fraction may increase the risk of ruminal imbalances or bloat if lucerne is alone as indicated by Wang *et al.*, (2021), who suggested to combine lucerne with complementary forages or fiber sources to ensure optimal rumen function. High-protein diets can increase the risk of rumen bloat due to the rapid digestion of protein, leading to the trapping of fermentation gases, which can compromise milk production and cow health. Practical strategies can mitigate the risk of bloat such as to divide the ration based on fresh lucerne throughout the day in order to attenuate the rumen fermentation. The polysaccharides and fibers metabolization coming from grasses (barley, sorghum and others) could induce a high degree of fermentation, thus, a suitable combination of fresh lucerne (e.g. at the budding stage) with grass or other fiber-rich forages could balance the intake of protein, fiber, and energy, improve the structure of the rumen mucosa and stabilize the fermentation process. Additionally, the use of anti-bloat agents (e.g., poloxalene) or the incorporation of feed additives that modify rumen fermentation can further reduce the incidence of bloat (Nuthall *et al.*, 2000).

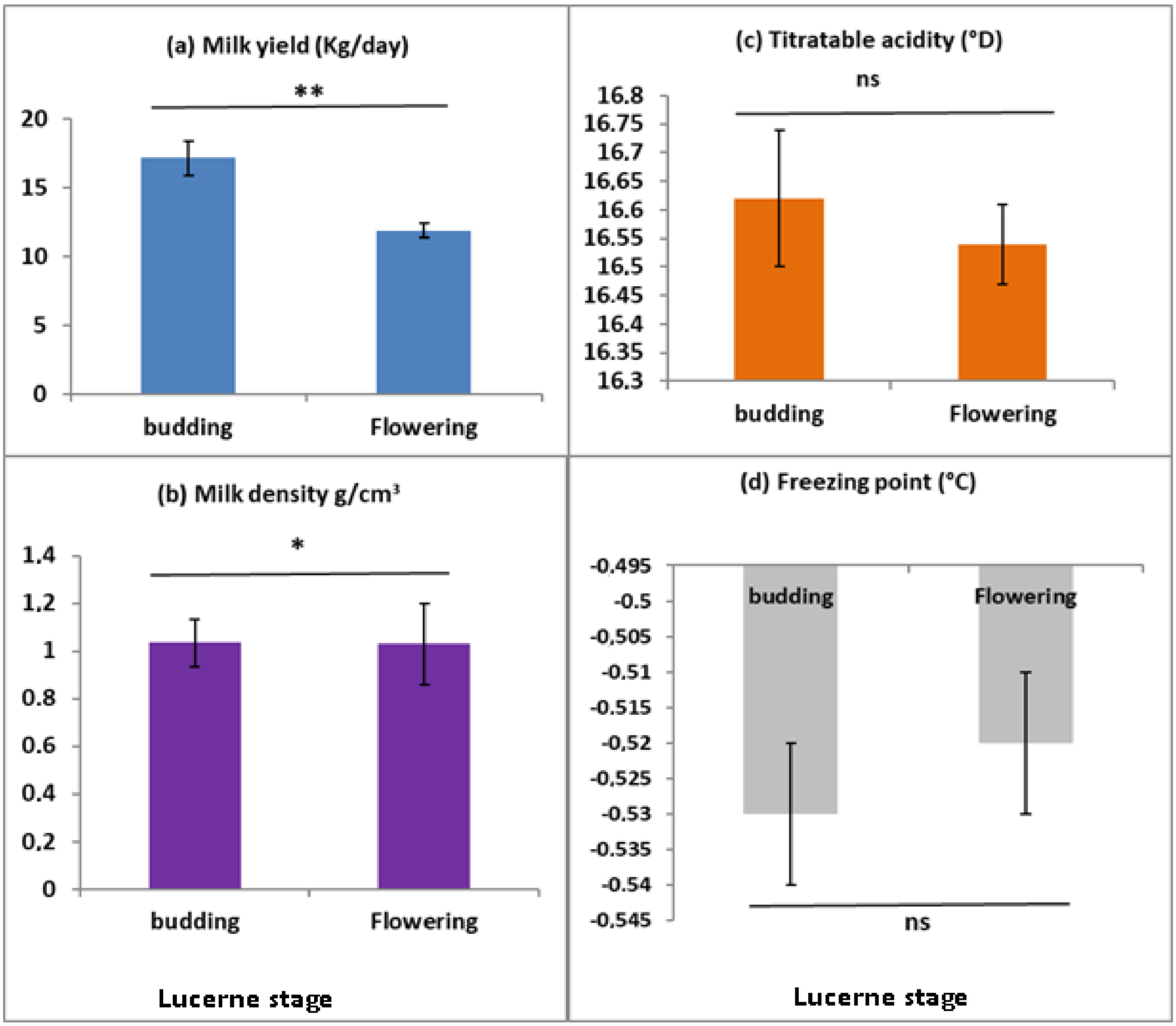

Figure 1. Effect of lucerne stage growth (Budding and Flowering) on (a) Milk yield (Kg/day) and physical composition of milk: (b) Milk density (g/cm^3), Protein (%), (c) Freezing point (°C), (d) Titratable acidity (°D). Values are mean± SE. Error bars represent standard error (SE). Means followed by asterisk (*) indicating significant differences at LSD test p ≤ 0.05 among stage for each parameter. * P<0.05, ** P<0.01 and *** P<0.001.

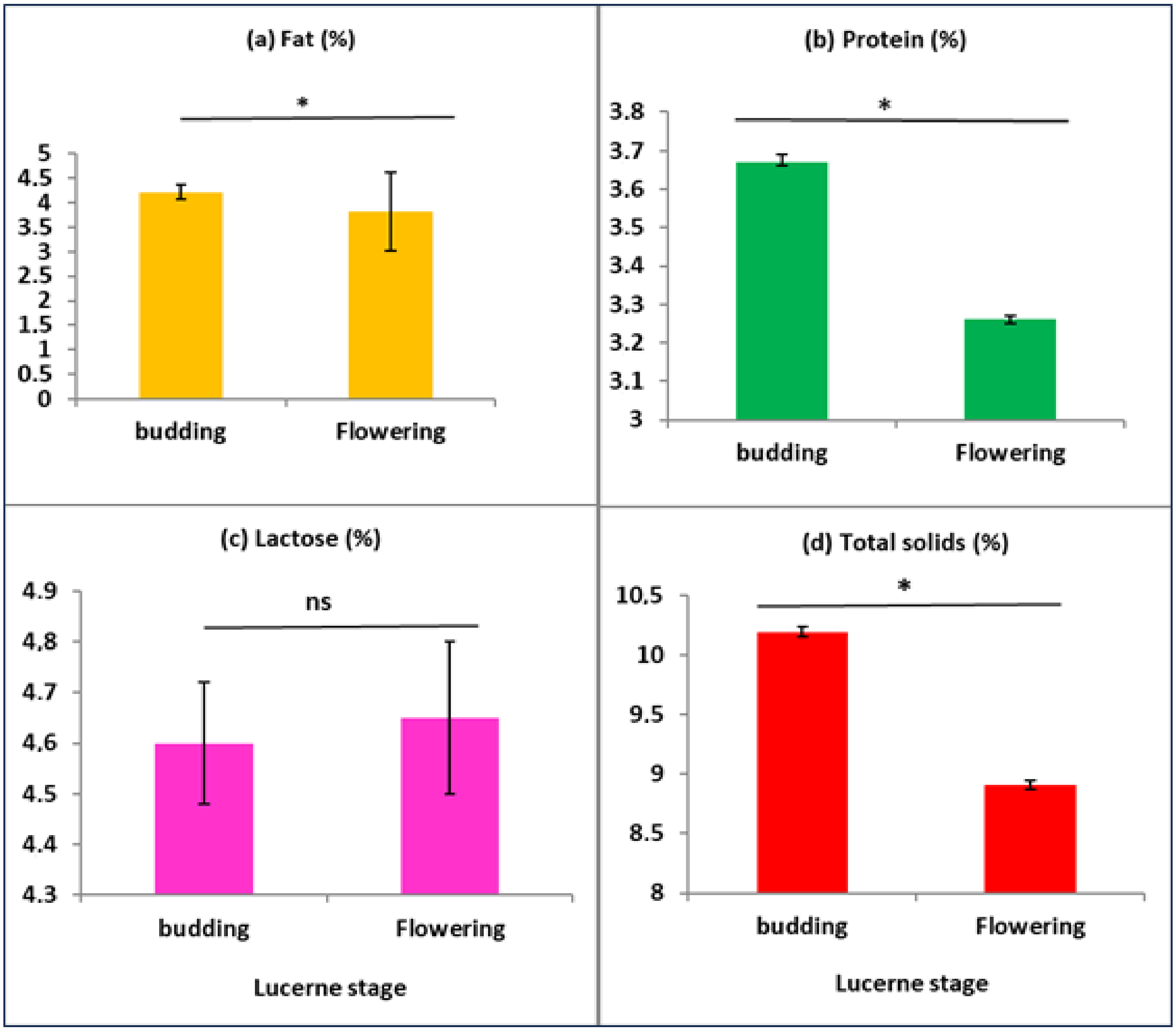

Figure 2. Effect of lucerne stage growth (budding and flowering) on chemical composition of milk: (a) fat (%), (b) Protein (%), (c) Lactose (%), (d) Total solids (%). Values are mean± SE. Error bars represent standard error (SE). Means followed by asterisk (*) indicating significant differences at LSD test p ≤ 0.05 among stage for each parameter. * P<0.05, ** P<0.01 and *** P<0.001.

Table 1. Nutritional value of fresh and hay forage used in this study.

Diets	Chemical composition (%)							Nutritional value	
	DM (%)	MM (%)	OM (%)	CP	CF	Ca	P	UFL	DCP± (g/Kg)
Fresh lucerne	20.05±0.28[d]	8.85±0.33[b]	91.14±0.03[c]	22.33±0.48[a]	26.96±1.39[e]	1.39±0.23[a]	0.36±0.30[c]	0.61±0.02[c]	182.84±0.81[a]
Fresh barley	32.29±0.40[c]	8.27±0.33[c]	91.73±0.26[b]	10.08±0.39[d]	32.99±0.13[c]	0.31±0.23[b]	0.24±0.21[c]	0.81±0.01[ab]	70.64±0.65[d]
Pasture	21.76±0.37[e]	11.41±0.54[a]	88.59±0.27[d]	21.62±0.32[b]	26.63±0.32[e]	1.48±0.35[a]	0.80±0.17[b]	0.83±0.01[a]	174.02±0.54[b]
Fresh sorghum	18.00±0.29[f]	7.57±0.35[d]	92.42±0.12[a]	12.18±0.33[d]	31.41±0.33[d]	0.44±0.36[b]	0.26±0.24[c]	0.50±0.01[d]	35.00±0.33[f]
Oat hay	84.27±0.49[a]	9.21±0.23[b]	90.88±0.43[c]	7.42±0.54[e]	35.74±0.54[a]	1.07±0.26[a]	1.18±0.31[a]	0.80±0.01[b]	37.10±0.33[e]
Lucerne hay	81.74±0.55[b]	8.98±0.11[b]	90.79±0.13[c]	16.46±0.41[c]	34.70±0.41[b]	1.40±0.22[a]	0.15±0.14[c]	0.51±0.01[d]	115.22±0.52[c]
F (5, 24)	2299.7	56.83	82.02	843.43	489.89	13.09	11.21	308.45	0.52
P Value	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$	$P<0.001^{***}$

Abreviations: DM (%): dry matter. MM (%): mineral matter. OM (%): organic matter. CP: crude protein. CF: crude Fiber. Ca: Calcium. P: phosphorus. UFL: feed unit for lactation. DCP: digestible crude protein Values are expressed as mean ± standard error. The values in a column with different alphabetical letter are significantly different according to the LSD test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) among diets for each parameter. ***Overall ANOVA was highly significant (P<0.001). F-values, degrees of freedom, and P-values correspond to one-way ANOVA for each parameter. (DM, MM, OM, CP, CF, Ca, P, FUL, and DCP).

Table 2. Study of effects of different diets on milk yield.

Diets	Milk yield (Kg/day)
Fresh barley+ Oat hay (Control)	10.37±1.18[bc]
Fresh lucerne+Oat hay	16.93±1.16[d]
Pasture+Oat hay	8.45±1.02[ab]
Fresh sorghum + lucerne hay	11.60±0.71[c]
Fresh sorghum +Oat hay	7.73±0.19[a]
ANOVA	$F (4, 20) = 60.87$, $p < 0.001^{***}$

Values are expressed as mean ± standard error. Different lower-case letters within a column indicate significant differences according to the LSD test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). F-values and degrees of freedom are shown in the table. Values are expressed as mean ± standard error. The values in a column with different alphabetical letter are significantly different according to the LSD test ($p \leq 0.05$) among studied diets. ***Overall ANOVA was highly significant (P<0.001). F-values, degrees of freedom, and P-values correspond to one-way ANOVA for each parameter.

Table 3. Effects of different diets on physicochemical characteristics of milk produced by cows.

Diets	Fresh barley + oat hay (Control	Fresh lucerne + oat hay	Pasture + oat hay	Fresh sorghum + lucerne hay	Fresh sorghum +oat hay	P value	F (4, 20)
Fat (%)	3.96±0.06^{a}	4.19 ± 0.31^{a}	4.09±0.24^{a}	4.05±0.06^{a}	4.03±0.02^{a}	0.64	0.65
Protein (%)	3.23±0.01^{a}	3.66 ± 0.02^{d}	3.47±0.02^{c}	3.50±0.02^{c}	3.35±0.01^{b}	P<0.001***	189.75
Lactose (%)	4.51 ±0.10a^{b}	4.68 ± 0.13^{b}	4.42 ± 0.03^{a}	4.53 ± 0.18^{b}	4.48 ± 0.12^{a}	0.05	4.98
Total solids (%)	8.44 ± 0.11^{a}	9.91 ± 0.28^{c}	9.61 ± 0.02^{bc}	9.41 ± 0.03^{b}	8.29 ± 0.03^{a}	P<0.001***	79.27
Milk density g/cm^{3}	1.03±0.002^{a}	1.034 ± 0.005^{c}	1.032±0.005^{b}	1.032±0.003^{b}	1.029±0.001^{a}	P<0.001***	95.25
Titratable acidity (° D)	16.21±0.14^{a}	16.38±0.25^{ab}	17.00±0.37^{b}	17.13±0.01^{b}	16.77±0.41^{ab}	0.01*	5.88
Freezing point (° C)	-0.51±0.01^{a}	-0.51±0.01^{a}	-0.51±0.01^{a}	-0.51±0.02^{a}	-0.51±0.01^{a}	0.84	0.35

Values are expressed as mean ± standard error. The different lower-case letters indicate significant differences according to the LSD test *p≤0.05*. * *p<0.05*, ** *p<0.01 and* *** *p<0.001*. F-values and degrees of freedom are shown in the table. Values are expressed as mean ± standard error. The values in a column with different alphabetical letter are significantly different according to the LSD test (*P < 0.05, **P < 0.01, ***P < 0.001) among studied diets. ***Overall ANOVA was highly significant (P<0.001). F-values, degrees of freedom, and P-values correspond to one-way ANOVA for each parameter studied.

Table 4. Nutritional values at different phenological stages of fresh lucerne.

Form	Stage	Chemical composition (%)							Nutritional value	
		DM (%)	MM (%)	OM (%)	CP	CF	Ca	P	FUL	DCP (g/Kg)
Fresh lucerne	Vegetative	12.40±0.01^{d}	11.46±0.04^{b}	88.49±0.03^{c}	20.19±0.03^{b}	11.46±0.03^{d}	1.18±0.02^{d}	0.26±0.02^{d}	0.50±0.01c	160.00±1.63^{d}
	Budding	16.42±0.01^{c}	12.33±0.10^{a}	87.67±0.12^{d}	23.05±0.90^{a}	18.94±0.18^{c}	1.47±0.02^{a}	0.38±0.01^{a}	0.62±0.01^{a}	194.25±1.70^{a}
	Early flowering	20.95±0.75^{b}	8.17±0.08^{c}	91.81±0.12^{b}	20.00±0.18^{b}	25.83±0.17^{b}	1.36±0.01^{b}	0.35±0.01^{b}	0.58±0.01^{b}	166.20±1.63^{b}
	Full flowering	26.00±0.57^{a}	6.78±0.25^{d}	93.22±0.33^{a}	17.47±0.02^{c}	36.92±0.38^{a}	1.30±0.02^{c}	0.29±0.01^{c}	0.48±0.01^{c}	145.02±0.81^{c}
[2]F (3, 12)		612.58	1358.41	787.60	97.63	8860.56	270.00	102.87	68.24	761.50
P value		P<0.001***	P<0.001***	P<0.001***	P<0.001***	P<0.001***	P<0.001***	P<0.001***	P<0.001***	P<0.001***

Abbreviations: DM (%): dry matter; MM (%): mineral matter; OM (%): organic matter; CP: crude protein; CF: crude fiber; Ca: calcium; P: phosphorus; UFL: feed unit for lactation; DCP: digestible crude protein. $^{a-c}$Values in a column with different alphabetical letters are significantly different according to the LSD test (*P < 0.05, **P < 0.01, ***P < 0.001) among stages for each parameter. ***Overall ANOVA was highly significant (P < 0.001). [1]Values are expressed as mean ± standard error. [2]F-values, degrees of freedom, and P-values correspond to one-way ANOVA for each parameter (DM, MM, OM, CP, CF, Ca, P, UFL, DCP) of Fresh lucerne at different growth stages.

Conclusion: According to the results of this study, the incorporation of lucerne increased milk production, while the most significant decrease was observed when grasses were not combined with lucerne. Although milk production was notable in cows fed lucerne hay, the highest yield was obtained with the incorporation of fresh lucerne into the ration. Furthermore, high milk production, associated with significant nutritional value, was observed with a ration composed of fresh lucerne at the budding stage (vegetative stage), compared to a diet based on mature lucerne. Given the advantages of fresh lucerne, particularly at the budding stage, its integration into dairy farming practices would be both beneficial and potentially cost-effective for milk production.

Conflict of interest: The authors declare that there is no conflict of interest.

Authors' contribution: NF conceived and designed the study, conducted the experiments, and collected milk samples. SAH, KD, ZA and BL assisted in the experimentation and helped analyze and interpret the results. CHA and CM assisted with data analysis and feed preparation. NF and SAH drafted and critically revised the manuscript. All authors read and approved the final version.

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