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Article

The effects of supplementation timing on stocking rate and milk production per hectare in grazing Holstein dairy cows

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Abstract:

Concentrate supplementation can affect multiple parameters in grazing dairy systems. In mixed pastures (*Medicago sativa* L. and *Dactylis glomerata* L.) grazed by New Zealand Holstein cows, a study was done of the effects of concentrate supplementation timing on individual production, stocking rate and milk production per hectare. Two experiments were done, one in winter and another in spring-summer. Experimental design was 3x3 crossover with treatments defined by concentrate (5.0 kg DM cow^{-1} d^{-1}) supplement administration times: after morning milking (AM), after afternoon milking (PM), and equally divided between both milkings (AM-PM). The experimental units were batches of six (winter) or five cows (spring-summer), which received the treatments, and their respective grazing areas. The rotational grazing management criterion was 8 cm residual forage height in all treatments, which allowed estimation of the effects of the treatments on stocking rate. Stocking rate did not differ (P>0.05) between treatments. Milk production per cow in the AM treatments was an average of 10.2 % higher than the other two treatments, both in winter (8.6 %, P=0.0002) and spring-summer (11.7 %, P<0.0001). The increase in milk production per hectare (9 %) was due to individual response and not to differences in stocking rate. Use of a uniform residual forage height was a simple way of estimating the response in stocking rate and thus milk production per hectare.

Key words: Concentrate, Individual production, Grazing Management, Crossed design.

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Introduction

In research models, improvements in management of grazing dairy production systems have driven increases in milk production per hectare[1]. Two of the main interrelated factors responsible for this increased productivity are stocking rate[2] and use of concentrate supplements[3]. Though already thoroughly studied[4], use of concentrate supplements continues to receive broad attention[5,6,7].

Concentrate supplementation is commonly used in grazing systems. It increases energy intake, which helps to optimize animal nutritional status and body condition[8], as well as individual milk production[9]. Supplementation can lead to changes in milk composition in terms of nutraceutical feed[10]. Animals consuming supplements generally reduce forage intake in the pasture, allowing greater forage utilization efficiency through increased stocking rates (SR). This in turn raises milk production per unit area, and improves milk composition[11]. In grazing systems, SR is vital to calculating system efficiency[12]. Increasing forage utilization efficiency leads to greater milk production per hectare, a principal goal in maximizing profitability per grazed area[13].

Rises in milk production per hectare in response to the higher SRs allowed by supplementation[14], are more affected by changes in SR than by changes in individual production[15]. Responses to supplementation can also be influenced by the timing of concentrate administration because this variable influences pasture forage substitution[16], fiber digestion, and other ruminal fermentation variables[17]. Since ruminal environment, forage composition, and cattle feeding behavior follow circadian cycles, the effects caused by supplementation timing are attributed to changes in the diurnal routine[17].

Cows graze more intensively before sunset, regardless of the supplement provided in their diet[16]. Afternoon or evening grazing is therefore longer and more important for forage intake, due to the effect of circadian rhythms in photosynthesis, and accumulation of dry matter (DM), carbohydrates, and fatty acids, which facilitates forage particle decomposition during the initial phases of digestion[18]. Considering this, it was hypothesized that morning supplementation would result in greater individual milk production than afternoon supplementation or morning/afternoon supplementation (the most common practice). The study objective was to evaluate the impact of concentrate supplementation timing in a dairy grazing system as a tool to increase individual productive performance, SR, and consequently milk productivity per hectare.

Material and methods

Study location

In 2022, two grazing experiments were done at the Grazing Module of the Universidad Autónoma Chapingo, Texcoco, Estado de Mexico (19º 29’ N, 98º 54’ W; 2,240 m asl). The first ran from February 4 to March 26 (winter), and the second from June 6 to July 26 (spring-summer). Regional climate is temperate subhumid with summer rains, 636 mm average annual precipitation, and 15.2 ºC average annual temperature[19].

The experimental units were batches of six (winter) or five (spring-summer) lactating New Zealand Holstein cows in 17-d periods, and their respective grazing areas. The batches were homogenized based on initial live weight, number of births, days lactation and milk production during the two weeks prior to batch creation.

Pastures and grazing management

Ten pastures in a 4.5 ha total area were used. Forage was alfalfa (*Medicago sativa L.*) associated with orchard grass (*Dactylis glomerata L.*) of between two and three years age. Grazing was intensive and rotational, with an average of five days grazing followed by 42 days' rest in winter and 40 days' rest in spring-summer. Each pasture was divided into three equal sections corresponding to the three experimental treatments. A residual forage height (RFH) of 8 cm was maintained in the three treatments by controlling access to specific pasture areas for each batch of cows using a mobile electric fence. Forage was measured with a descending disc. Controlling RFH was essential because SR was a response variable.

Experimental design and treatments

The experimental design was 3 x 3 crossed[(20)], using three treatments corresponding to three post-milking supplementation timings: divided between morning and afternoon (AM-PM), morning (AM) and, afternoon (PM). A twenty-day, pre-experimental adaptation period was implemented during which the batches were formed, and the animals accustomed to grazing management practices and concentrate composition. Each experiment lasted 51 d, divided into three, seventeen-day periods. Each period was divided into two phases: a) a twelve-day adaptation to treatment concentrate level, and b) a five-day response variable data-recording phase. Based on previous results from the same site[(15)], a 5.0 kg DM cow^{-1} d^{-1} concentrate supplementation level was administered. Concentrate composition was calculated considering previously reported average forage composition[(15)], and prepared in the Grazing Module. The formulation was based on rolled corn, ground sorghum, gluten meal, bypass fat, molasses and minerals; average concentrate chemical composition in both experiments was 16.65 % crude protein (CP), 20.43 % neutral detergent fiber (NDF), and 4.98 % acid detergent fiber (ADF).

Measurement and experimental procedure

Pasture access was controlled using mobile electric fencing. Following a previously reported pasture management method[(15,21,22)], every day each batch of cows was initially given access to a 12 × 4 m area. Residual forage height (RFH) was measured frequently in 12 m-wide strips. When RFH reached 8 cm, an additional 2 m-wide area was opened, and RFH measurement continued. This required that during a single day a batch of cows be moved several times within the same pasture unit.

Each pasture was divided into thirds of equal width, ensuring that grazing progress was similar between the batches of cows. Grazed area was measured before (night progress) and after (day progress) the daytime grazing period. The daily area grazed per experimental unit was used to calculate SR (Equation 1).

The concentrate supplement was administered in individual feeders in a separate pen, after each milking, and at the times corresponding to each treatment. When grazing in the pastures, the animals had free access to water in mobile containers located at one end of the grazing area (Table 1).

Table 1: Average times of dairy cow management activities in a grazing system with concentrate supplementation at different times of day

Activity	Time
Morning milking	06:00-07:00
Morning period in supplementation pen	06:30-07:30
Grazing (daytime)	08:00-15:00
Afternoon milking	15:00-16:00
Afternoon period in supplementation pen	15:30-16:30
Grazing (nighttime)	17:00-06:00 (+1 day)

Samples of offered forage (OF) and residual forage (RF) were collected from each experimental unit. Samples were collected by mowing five strips averaging 0.50 x 5 m to an 8 cm height using a mower (Truper®, Mexico)[23]. Residual forage samples were paired with the corresponding OF samples. All samples were dried in a circulating air oven at 50 °C to constant weight.

Composition of consumed forage was estimated based on samples collected by experimental unit. Each sample consisted of ten subsamples collected using the simulated grazing technique[24]; this was modified in that samples were collected at 8 cm above ground level. For measurement of nutritional composition, the forage samples and the concentrate were first dried at 55 °C to constant weight, then ground to 1 mm in a mill (Thomas model 4 Wiley®, USA). Following AOAC methods[25], CP was measured using the Micro-Kjeldahl method; NDF and ADF were estimated using a fiber analyzer (ANKOM 200, Ankom Technology, USA); and acid-insoluble ash (AIA) was also determined.

During the five-day measurement phase, milk production was recorded per cow using Alfa Laval® automatic meters during the morning and afternoon milkings. Milk composition was quantified individually using samples taken with an Alfa Laval® automatic sampler and plastic vials. Fat, protein and total solids in milk samples were measured with a milk analyzer (MilkoScan®, Foss, Denmark). Milk production per hectare was estimated using individual milk production and grazing cycle SR per pasture. Stocking rate (SR) was estimated using the weighted mean of the instantaneous stock density for occupied and rest periods, with Equation 1.

$$SR = ISD * OP/(OP + RP) \qquad \text{(Equation 1)}$$

Where: SR= stocking rate [cows^{-1} ha^{-1} (grazing cycle)]; ISD= instantaneous stock density (cows^{-1} ha^{-1}); OP= occupied period (days); RP= rest period (days).

Cow live weight (LW) was measured using an electronic scale (TruTest®, New Zealand; 1 kg accuracy, 1,000 kg capacity) after morning milking, for 2 d at the beginning and two days at the end of each experimental period. After each weighing, body condition (BC) was estimated by two trained observers using a 1-to-5 scale(26). Changes in LW and BC were calculated as the difference between the measurements taken at the beginning and end of each period, for each variable.

Statistical analysis

The statistical model includes the effects of period, batch and treatment:

$$Yijk = \mu + Period_i + Batch_j + Treatment_k + Eijk \qquad \text{(Equation 2)}$$

Where: Yijk is the average value of LW, BC, SR, individual production, and fat, protein and total solids content across cows and measurement days. Period, batch and treatment were fixed effects. The experimental error was the interaction between these three factors, and it was assumed that this interaction was not of biological or practical importance. Due to differences in climate, forage growth and, in some cases, lactation stage, analyses were run within each experimental period. Response variable analysis was done using the GLM procedure in the SAS package(27). The means were compared between treatments with LSMEANS using the Tukey-Kramer test.

Results and discussion

Residual forage height (RFH) did not differ between treatments (*P*>0.05), and averaged 8.4 cm in both experiments. This allowed forage utilization to remain at the same efficiency in all treatments. It also served as the basis for estimating the effect of concentrate administration timing on SR, and thus on milk production per hectare. Assigned areas averaged 210 m^2 per day per batch in winter and 233 m^2 in spring; no differences were observed between treatments.

No differences (*P*>0.05) between treatments were observed for OF, either in winter (average = 2,183 kg DM ha^{-1}) or in spring-summer (average= 2,630 kg DM ha^{-1})(Table 2). The lower figure in winter is to be expected since environmental conditions in winter such as low temperatures, frost, and low solar radiation and shorter photoperiod, result in a lower rate of forage accumulation[28,29]. In a study of an alfalfa-orchard grass association, decreased growth rates in winter were caused by low temperatures (<10 °C)[30]. In contrast, forage accumulation is greater in spring-summer because the forage growth season begins in late April and ends in mid-October. One study reported an accumulation 2,333 kg DM ha^{-1} in spring-summer[31], which is comparable to the 2,630 kg DM ha^{-1} in the present results.

Neither were differences observed in RF (*P*>0.05), which is due to the 8 cm residual height criterion used here. Using this criterion, grazing efficiency was 76 % in winter and 80 % in spring-summer. Average grazing efficiency was 78 %, similar to the 75.3 % reported for smaller pastures.

Table 2: Offered and residual forage (kg DM ha^{-1}) above 8 cm height, in pastures grazed by cows administered concentrate supplementation at three different timings, in winter and spring-summer experiments

Experiment	Parameter	Treatment	Mean	SE
Winter	OF	AM-PM	2140	34
		AM	2201	
		PM	2206	
	RF	AM-PM	495	14
		AM	527	
		PM	528	
Spring-summer	OF	AM-PM	2624	35
		AM	2672	
		PM	2655	
	RF	AM-PM	512	10
		AM	506	
		PM	517	

OF = Offered forage, RF = Residual forage, AM-PM, AM, PM = concentrate supplement administration timing, SE= standard error; € (P<0.05) = significance level.

The OF nutritional composition results showed decreases in NDF, ADF and CP between morning and afternoon measurements (Table 3). In the winter experiment, NDF decreased by 7.5 %, ADF by 3.8 % and CP by 7.7 %. In the spring-summer experiment, NDF decreased by 9.6 %, ADF by 8.1 % and CP by 10.4 %.

Table 3: Nutritional composition (% DM) of forage offered to grazing dairy cows administered a concentrate supplement at different timings during the day

	Winter			Spring-summer		
	Forage		SE	Forage		SE
Component	**AM**	**PM**		**AM**	**PM**	
DM	$22.3^{bß}$	25.5^{a}	0.41	$21.6^{y\mu}$	24.8^{x}	0.44
NDF	39.4^{a}	37.9^{b}	0.33	49.5^{x}	45.5^{y}	0.87
ADF	29.2^{a}	27.0^{b}	0.33	34.4^{x}	31.1^{y}	0.72
Ash	9.7	9.4	0.20	9.9	9.4	0.17
CP	19.5^{a}	18.0^{b}	0.26	20.3^{x}	18.2^{y}	0.11

AM, PM= sample time; DM= dry matter; NDF= neutral detergent fiber; ADF= acid detergent fiber; CP= crude protein; SE= standard error; (P<0.05) = significance level. ß µ Different letter superscripts in the same row and season indicate significant difference (winter= ^{ab}, spring-summer= ^{xy}) (P<0.05).

The decreases observed in NDF, ADF and CP between the morning and evening OF samples (Table 3) may be due to dilution of these components caused by diurnal fluctuations in soluble carbohydrates concentration as a product of plant photosynthetic activity[32]. These levels are similar to those reported previously. For example, in *Lolium perenne*, between 08:00 and 19:00 h, non-structural carbohydrates (not studied here) were found to increase by 30 % while NDF decreased 8.7 % and CP by 6.1 %, the latter in response to the rise in non-structural carbohydrates[32]. This coincides with reported higher ADF and CP contents in alfalfa forage harvested in the morning than in that harvested in the afternoon[33]. Fluctuations in photosynthetic product concentrations exhibit higher diurnal fluctuations in the leaves than in the stems and pseudostems[18].

Changes in LW were minor over the experimental period, the highest being 45 g cow^{-1} d^{-1} in the winter/AM-PM treatment (Table 4). This is greater (P<0.05) than in the winter/AM and winter/PM treatments. In contrast, the spring-summer/AM-PM treatment exhibited greater (P<0.05) LW gain than in the spring-summer/PM treatment but did not differ (P>0.05) from LW in the spring-summer/AM treatment. Changes in LW caused by supplementation are a function of lactation period; during the first third of lactation, supplementation can reduce weight loss, while later in lactation it can lead to increased weight gain[21]. No changes in body condition were observed between treatments (P<0.05).

Table 4: Changes in live weight and body condition in grazing dairy cows administered concentrate supplements at different times

Experiment	Parameter	Treatment			Mean	SE
		AM-PM	AM	PM		
Winter	Change LW	2.3[a†]	1.8[b]	1.8[b]	2	0.17
	Change BC	0.01[a]	0.003[a]	-0.006[b]	0.002	0.005
Spring-Summer	Change LW	1.9[x€]	1.7[xy]	1.2[y]	1.6	0.20
	Change BC	-0.004	0.008	-0.004	-0.00002	0.004

LW= live weight (kg); BC= body condition (units); AM-PM, AM, PM= concentrate supplementation timing; SE= standard error; [† €] Different letter superscripts in the same row indicate significant difference (winter= [ab], spring-summer= [xy]) (P<0.05).

In both experiments, milk production in the AM treatment was higher than in the other two treatments (Table 5): 6.1 % in winter (P=0.0002) and 8.5 % in spring-summer (P<0.0001). The overall average increase in milk production (both experiments) was 7.3 %.

Table 5: Milk production and composition in grazing dairy cows administered concentrate supplements at different times

Experiment	Parameter	Treatment			Mean	SE
		AM-PM	AM	PM		
Winter	Milk production, L cow^{-1} d^{-1}	$21.7^{b†}$	23.8^{a}	21.8^{b}	22.5	0.24
	Protein, %	3.5	3.6	3.4	3.5	0.02
	Fat, %	3.9	4.0	3.9	3.9	0.03
	Total solids, %	13.3	13.5	13.2	13.3	0.04
Spring-summer	Milk production, L cow^{-1} d^{-1}	$20.3^{y€}$	22.6^{x}	19.6^{y}	20.8	0.29
	Protein, %	3.6	3.5	3.5	3.6	0.03
	Fat, %	4.0	3.9	3.9	4.0	0.03
	Total solids, %	13.5	13.2	13.3	13.3	0.07

AM-PM, AM, PM= concentrate supplementation timing; SE= standard error; $^{† €}$ Different letter superscripts in the same row indicate significant difference (winter= ^{ab} [*P*=0.0002], spring-summer= ^{xy} [*P*<0.0001]).

In a similar study (same study site, cow type, pastures, concentrate supplement level and grazing management criteria)[15], a winter/AM-PM treatment had 21.4 L cow^{-1} d^{-1} production, slightly higher than the 20.3 L cow^{-1} d^{-1} in the same treatment in the present study. However, the present spring-summer/AM treatment produced 23.8 L cow^{-1} d^{-1}, 10 % higher than in the previous study. This coincides with a similar study in which morning administration of concentrate increased (*P*<0.07) individual milk production by 0.5 L cow^{-1} d^{-1} compared to other timings[16]. In another study, milk production was 2.1 L cow^{-1} d^{-1} higher (*P*<0.001) when grazing dairy cows were administered concentrate in the afternoon than in the morning[34].

Milk protein, fat and total solids contents did not differ (*P*>0.05) between treatments. In the literature, milk composition results vary widely. For instance, one study found that milk protein content was higher (*P*<0.05) with evening supplementation than with morning supplementation or none at all[35]. This may have been caused by a restricted daily forage allowance, since a reduction in diet forage proportion in dairy cows increases milk production volume and protein concentration[35]. Another study reported that milk fat content was lower when supplements were administered only in the morning[36]. Finally, a third study found no differences in milk production, milk solids production or fat and protein concentration and amount were observed with treatments with and without corn silage supplementation, and different grazing time allowances[37].

Concentrate administration timing had no effect (P<0.5) on SR in either experiment (Table 6); average SR in winter was 6.7 cows ha^{-1} and in spring-summer it was 5.1 cows ha^{-1}. Stocking rate is normally described as the number of cows per surface unit and time (SR_{Annual} = $cows^{-1}$ ha^{-1} $year^{-1}$, or SR_{Daily} = $cows^{-1}$ ha^{-1} d^{-1})[15]; however, in the present study it was quantified as $SR_{Grazing\ cycle}$.

Table 6: Stocking rate (cows ha^{-1} / grazing cycle) in grazing dairy cows administered concentrate supplements at different times

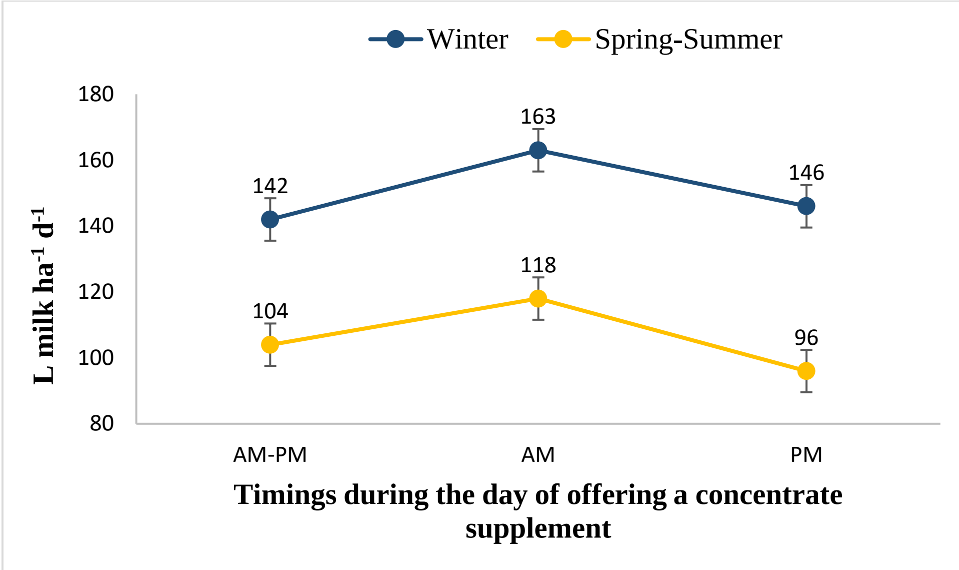
Experiment	**AM-PM**	**SE**	**AM**	**SE**	**PM**	**SE**
Winter	6.5	0.39	6.9	0.05	6.7	0.29
Spring-summer	5.1	0.38	5.2	0.23	4.9	0.22

AM-PM, AM, PM = concentrate supplementation timing; SE = standard error; (P<0.5).

The substitution effect caused by supplementation can allow increases in SR and consequently milk production per hectare[22]. At higher supplementation levels, the substitution effect reduces forage consumption in the pasture and, as a result, forage utilization efficiency[21]. If SR did not respond to changes in supplementation timing, no differences occurred in the substitution effect. This may be due to the fact that total supplementation level did not differ between the three treatments[38]. Treatments can cause changes in circadian rhythms in grazing activity and thus in forage intake[39]; only this type of effect could have caused differences in the substitution effect in the present study, but none were detected.

Milk production per hectare (Figure 1) was the result of individual production per SR. In the AM treatment of both experiments, this parameter exceeded the average of the other two treatments by 8.4 % during winter and 11.3 % during spring-summer. These differences were similar between experiments and originated in differences in individual production, since SR did not differ between treatments.

Figure 1. Milk production per hectare in grazing dairy cows administered concentrate supplements at different times



Offering concentrate once daily in the morning improved system productivity. This approach took advantage of changes in forage composition throughout the day such that, as mentioned elsewhere[40], maximum use of forage occurs in the afternoon, when its nutritional value is highest[35]. The improved milk production in the AM treatments was due to increased individual production, without changes in SR. This contradicts previous reports indicating that increases in milk production per hectare when SR varies respond to increases in SR rather than improvements in individual production[2,15,21].

The advantage of quantifying responses to supplementation in terms of milk production per hectare instead of milk production per cow is that this approach includes this technology's impact on the production system (production unit)[14,15], allowing more accurate estimation of its effect on system economic performance.

Conclusions and implications

Use of concentrate supplementation timing helped in attaining uniform forage utilization efficiency in the pasture, and consequently estimating stocking rate and milk production per hectare. Providing concentrate supplementation only after the morning milking increased individual milk production an average of 10.2 % and improved milk production per hectare by 9.9 %. This strategy raised production system efficiency without increasing inputs because it was based on circadian changes in forage composition and forage intake behavior.

Acknowledgements and conflicts of interests

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Literature cited:

1. McCarthy B, Delaby L, Pierce KM, Journot F, Horan B. Meta-analysis of the impact of stocking rate on the productivity of pasture-based milk production systems. Animal 2011;5(5):784-794

2. Macdonald KA, Penno JW, Lancaster JAS, Roche JR. Effect of stocking rate on pasture production, milk production, and reproduction of dairy cows in pasture-based systems. J Dairy Sci 2008;91(5):2151-2163.

3. Bargo F, Muller LD, Delahoy JE, Cassidy TW. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. J Dairy Sci 2002;85(7):1777-1792.

4. Leaver JD. Effects of supplements on herbage intake and performance. In: Frame J. editor. Grazing. Occasional Symposium No. 19. British Grassland Society. 1985:79-88.

5. John AJ, Garcia SC, Kerrisk KL, Freeman MJ, Islam MR, Clark EF. The effect of temporal variation in feed quality and quantity on the diurnal feeding behavior of dairy cows. Animal 2019;13(11):2519-2526.

6. Bica GS, Pinheiro LC, Teixeira DL, De Sousa KT, Hötzel MJ. Time of grain supplementation and social dominance modify feeding behavior of heifers in rotational grazing systems. Front Vet Sci 2020;1-7.

7. Cahill L, Patton D, Reilly B, Pierce KM, Horan B. Grazing season length and stocking rate affect milk production and supplementary feed requirements of spring-calving dairy cows on marginal soils. J Dairy Sci 2023;106(2):1051-1064.

8. Stockdale CR. Effects of body condition score at calving and feeding various types of concentrate supplements to grazing dairy cows on early lactation performance. Livestock Sci 2008;116(1-3):191-202.

9. Albarrán M, Balocchi OA, Noro M, Wittwer F, Pulido RG. Effect of the type of silage on milk yield, intake and rumen metabolism of dairy cows grazing swards with low herbage mass. Anim Sci J 2016;87(7):878-884.

10. Ramírez-Mella M, Hernández-Mendo O, Ramírez-Bribiesca EJ, Améndola-Massiotti RD, Crosby-Galván MM, Burgueño-Ferreira JA. Effect of vitamin E on milk composition of grazing dairy cows supplemented with microencapsulated conjugated linoleic acid. Trop Animal Healt Prod 2013;45:1783-1788.

11. Bargo F. Suplementación en pastoreo: conclusiones sobre las últimas experiencias en el mundo. Facultad de Agronomía, Universidad de Buenos Aires 2003;1:1-21 https://www.agro.uba.ar/sites/default/files/catedras/bargo.pdf. Consultado 30 Nov, 2023.

12. Fales SL, Muller LD, Ford SA, O'sullivan M, Hoover RJ, Holden LA, *et al*. Stocking rate affects production and profitability in a rotationally grazed pasture system. J Prod Agr 1995;8(1):88-96.

13. Dillon PAT, Hennessy T, Shalloo L, Thorne F, Horan B. Future outlook for the Irish dairy industry: a study of international competitiveness, influence of international trade reform and requirement for change. Inter J Dairy Tec 2008;61(1):16-29.

14. Baudracco J, Lopez-Villalobos N, Holmes CW, Macdonald KA. Effects of stocking rate, supplementation, genotype and their interactions on grazing dairy systems: A review. N Z J Agr Res 2010;53(2):109-133.

15. Castro JC. Suplementación con concentrado sobre producción de leche individual y por hectárea de vacas Holstein en pastoreo [Tesis Maestría]. Texcoco, Estado de México; Universidad Autónoma Chapingo, 2020.

16. Sheahan AJ, Gibbs SJ, Roche JR. Timing of supplementation alters grazing behavior and milk production response in dairy cows. J Dairy Sci 2013;96(1):477-483.

17. Gekara OJ, Prigge EC, Bryan WB, Nestor EL, Seidel G. Influence of sward height, daily timing of concentrate supplementation, and restricted time for grazing on forage utilization by lactating beef cows. J Animal Sci 2005;83(6):1435-1444.

18. Gregorini P. Diurnal grazing pattern: its physiological basis and strategic management. Animal Prod Sci 2012;52(7):416-430.

19. García E. Modificaciones al Sistema de Clasificación Climática de Köpen. 5ta ed. México, DF: Instituto de Geografía-UNAM; 2004.

20. Herrera Haro JG, García Artiga C. Bioestadística en ciencias veterinarias: Procedimientos de análisis de datos con SAS. Universidad Complutense de Madrid: Madrid: área Ciencias de la Salud; 2010.

21. Amendola RD. A dairy system based on forages and grazing in temperate Mexico [PhD Thesis]. The Netherlands. Wageningen Universitiet, 2002.

22. Ramírez-Mella M, Hernández-Mendo O, Améndola-Massiotti RD, Ramírez-Bribiesca EJ, Mendoza-Martínez GD, Burgueño-Ferreira JA. Productive response of grazing dairy cows to fresh chopped maize supplementation under a small farming system in the Mexican Highlands. Trop Animal Health Prod 2010;(42):1377-1383.

23. Jiménez-Rosales JD, Améndola-Massiotti RD, Burgueño-Ferreira JA, Ramírez-Valverde R, Topete-Pelayo P, Huerta-Bravo M. Herbage intake of dairy cows in mixed sequential grazing with breeding ewes as followers. Trop Animal Health Prod 2018;(50):531-536.

24. Bonnet O, Hagenah N, Hebbelmann L, Meuret M, Shrader AM. Is hand plucking an accurate method of estimating bite mass and instantaneous intake of grazing herbivores? Range Eco Manag 2011;64(4):366-374.

25. AOAC. Official Methods of Analysis. 18th ed. Washington, DC; USA Association of Official Agricultural Chemists. 2005.

26. Garcia A, Hippen A. Feeding dairy cows for body condition score. Dairy Sci 2008;137.

27. SAS. SAS/STAT:9.3 User's Guide (3rd ed.) Cary, NC, USA: SAS Inst. Inc. 2010.

28. Esparza JZ, Hernández-Garay A, Pérez JP, Herrera JG, Haro FOG, Hernández PAM, *et al.* Análisis de crecimiento estacional de una pradera asociada alfalfa-pasto ovillo. Tec Pecu Mex 2009;47(2):173-188.

29. Cullen BR, Eckard RJ, Callow MN, Johnson IR, Chapman DF, Rawnsley RP, *et al*. Simulating pasture growth rates in Australian and New Zealand grazing systems. Aust J Agr Res 2008;59(8):761-768.

30. Álvarez FG, López OR. Rendimiento agronómico, digestibilidad *(in vitro*) y contenido de proteína cruda de nueve variedades de alfalfa (*Medicago sativa* L.) en Nazareno, Etla, Oaxaca [Tesis Profesional]. Texcoco, Estado de México. Universidad Autónoma Chapingo, 1992.

31. Lemus V, Guevara A, García JA, Gaspar D, García JG, Pacheco D. Producción de leche de vacas en pastoreo de alfalfa (*Medicago sativa*) en el altiplano mexicano. Rev Mex Cien Pecu 2020;11(1):1-18.

32. Delagarde R, Peyraud JL, Delaby L, Faverdin P. Vertical distribution of biomass, chemical composition and pepsin—cellulase digestibility in a perennial ryegrass sward: Interaction with month of year, regrowth age and time of day. Animal Feed Sci Tec 2000;84(1-2):49-68.

33. Putnam D, Mueller S, Marcum D, Frate C, Lamb C, Canevari M, *et al*. Diurnal changes in alfalfa forage quality. In: Proc, 28th Alfalfa Symposium. California, NV. 1998:31-39.

34. Trevaskis LM, Fulkerson WJ, Nandra KS. Effect of time of feeding carbohydrate supplements and pasture on production of dairy cows. Live Prod Sci 2004;85(2-3):275-285.

35. Jenkins TC, McGuire MA. Major advances in nutrition: impact on milk composition. J Dairy Sci 2006;89(4):1302-1310.

36. Abrahamse PA, Tamminga S, Dijkstra J. Effect of daily movement of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. J Agr Sci 2009;147(6):721-730.

37. Al-Marashdeh O, Gregorini P, Edwards GR. Effect of time of maize silage supplementation on herbage intake, milk production, and nitrogen excretion of grazing dairy cows. J Dairy Sci 2016;99(9):7123-7132.

38. Bargo F, Muller LD, Kolver ES, Delahoy JE. Invited review: Production and digestion of supplemented dairy cows on pasture. J Dairy Sci 2003;86(1):1-42.

39. Gregorini P. Estado interno. Estímulos que motivan el consumo y ciertas conductas ingestivas de rumiantes en pastoreo. Producción Animal en Pastoreo. Buenos Aires, Ediciones INTA 2011;513-65.

40. Soca P, González H, Manterola H, Bruni M, Mattiauda D, Chilibroste P, Gregorini P. Effect of restricting time at pasture and concentrate supplementation on herbage intake, grazing behaviour and performance of lactating dairy cows. Liv Sci 2014;(170):35-42.