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Diet change from a system combining total mixed ration and pasture to confinement system (total mixed ration) on milk production and composition, blood biochemistry and behavior of dairy cows

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Abstract

This study aimed to determine if a diet change from a mixed system to a confinement system affects the milk production and composition, behavior and blood biochemistry of dairy cows. Cows were assigned randomly to one of the two treatments: cows fed with TMR (total-mixed-ration) (confined) throughout the period group fed TMR (GTMR, $n = 15$) and cows that changed their diet from pasture plus TMR to exclusive TMR at 70 ± 14 DIM (GCHD, $n = 15$). GTMR cows produced more milk and greater lactose and protein yield before the change of diet than GCHD cows ($p \le .01$), but these differences disappeared after the change. GCHD cows decreased the frequency of rumination and lying from before to after the change ($p \le .03$), but in GTMR cows no changes were observed. After diet change, GCHD cows had lower frequency of rumination and lying than GTMR cows ($p \le .02$). Before the change, GCHD cows had greater NEFA (non esterified fatty acids) concentrations than GTMR cows ($p = .002$). Abrupt change from a mixed system to a confined system was favorable on blood biochemical and milk variables of dairy cows. However, in relation to behavior, the cows expressed difficulties to adapt quickly to the abrupt change of system.

KEYWORDS
dietary change, housed system, pasture-based system, rumination behavior, welfare

1 | INTRODUCTION

Different dairy production systems influence milk composition and production, physiology, behavior, and animal welfare. Pasture-based dairy systems have productive advantages compared with confinement systems, such as low production costs and improvements in milk quality, e.g.: fatty acid profile (Barca et al., 2018; Bargo, Delahoy, Schroeder, Baumgard, & Muller, 2006; White, Benson, Washburn, & Green, 2002; White et al., 2001) and industrialization products, such as cheeses (Martin, Verdier-Metz, Buchin, Hurtaud, & Coulon, 2005). From the animal welfare point of view, pasture-based dairy systems are considered a natural environment for dairy cattle (Clutton-Brock, 1987) since it allows the expression of the normal behavior of the species, such as grazing (Olmos et al., 2009). Overall, the cows spend between 90% and 95% of the day grazing, ruminating, and resting in pastoral systems (Kilgour, 2012) and all these behaviors are considered as positive indicators of animal welfare (Krawczel & Grant, 2009; Olmos et al., 2009; Roca-Fernández, Ferris, & González-Rodríguez, 2013). In this sense and in comparison with confinement systems, pasture-based system has advantages on the animal welfare.

On the other hand, with regard to productive disadvantages, cows in pasture-based systems consume less dry matter and have less milk yield than in confinement systems (Fontaneli, Sollenberger, Littell, & Staples, 2005; Kolver & Muller, 1998). Besides, cows in pasture conditions are more exposed to extreme environmental variations, which can affect their physiology, production and welfare (Schütz, Rogers, Poulouin, Cox, & Tucker, 2010). For example, in summer, high environmental temperatures and humidity (heat stress) affects the production and milk composition, health and reproduction (Bernabucci et al., 2010, 2015; Cowley, Barber, Houlihan, & Poppi, 2015; Jordan, 2003). Thus, due to the fluctuation in the availability of forage that occurs at different times of the year (Bargo, Muller, Delahoy, & Cassidy, 2002; Chilibroste, Soca, Mattiauda, Bentancur, & Robinson, 2007; Wales et al., 2013) and adverse weather conditions (heat stress), confinement system with access to shade is used as an alternative management in dairy cattle to reduce the negative effects on milk production and improve animal welfare (Charlton & Rutter, 2017; Schütz et al., 2010;). In this sense, total mixed ration (TMR) has been strategically incorporated to increase total dry matter (DMI) and energy intake to ensure an adequate balanced supply of nutrients in quality and quantity throughout the year. In this way, it is easier to meet the nutritional requirements of dairy cows and therefore achieve a better productive level (Bargo, Muller, Kolver, & Delahoy, 2003; Cajarville, Mendoza, Santana, & Repetto, 2012; Charlton, Rutter, East, & Sinclair, 2011; Fajardo et al., 2015; Soriano, Polan, & Miller, 2001). Then, the use of confinement system as strategies of management could be an alternative to mitigate the climatic conditions in summer and minimize the negative effects on milk yield. Therefore, achieving the best use of the different production systems according to environmental variations and respecting the welfare is a challenge.

Due to the fact that the “welfare of an individual is its state as regards its attempts to cope with its environment” (Broom, 1986) changes in systems and environments can affect animal welfare. However, there is little information, about how cows adapt to these changes of management and feeding. In addition, the available information was focused on a change of diet from TMR to pasture. In these studies, the way cows responded to these changes (from TMR to pasture system) was evidenced through the concentrations of several blood parameters (insulin concentration decrease, nonesterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) concentration increase), lower milk production, and by decreases in DMI and body conditional score (BCS; Agenäs, Holtenius, Griinari, & Burstedt, 2002; Astessiano et al., 2017; Schären et al., 2016). If the change is made gradually (per 10 weeks), the cows show a negative effect on blood parameters and milk during the first week; but after, they adapted to the new system (recovery of body weight, lower concentration of NEFA, greater DMI and decrease in energy deficit), although they maintained negative energy balance and high levels of BHB (Schären et al., 2016). They evaluated how the gradual inclusion of pasture in cows maintained in TMR systems affects blood parameters and milk. However, according to our knowledge, there is no information on how the cows adapt to the abrupt change from a mixed system (grazing plus TMR) to a single system in confinement (TMR) with an integrated perspective that includes/covers blood, milk and behavioral parameters. We hypothesize that the abrupt change from a mixed to a confinement system improves milk production and composition and metabolic profile, but negatively affects some behavioral indicators of welfare in dairy cows. Therefore, the objective of this study was to determine if a diet change, from a mixed system (grazing plus TMR) to confinement system (100% TMR) affects milk production and composition, behavior, and blood biochemistry of dairy cows.

2 | MATERIALS AND METHODS

2.1 | Location, animals, and treatments

Experimental protocol was evaluated and approved by the Comisión Honoraria de Experimentación Animal (CHEA), Universidad de la República, Montevideo, Uruguay. The study was conducted at the commercial farm located in the department of Paysandú, Uruguay.

Thirty multiparous Holstein dairy cows with mean date of calving on September 15th± 13 days, number of lactations of 3.1 ± 1.2 and an average live weight of 660 ± 82.1 kg were used. All cows were under the same management and feeding conditions throughout the 21days before expected calving date (prepartum diet). Cows were blocked by calving date, number of lactation, pre-calving body condition and live weight and randomly assigned to one of the two following treatments immediately after calving: cows confined and fed with TMR ad libitum (GTMR, n = 15) throughout the period and cows that changed their diet (GCHD, n = 15) from a mixed system (pasture plus TMR) to a single TMR system, in the same way as GTMR.

Diet change in GCHD was carried out from November 16th, according to historical records of Temperature-humidity index (THI) values in the region. This change of management from mixed systems to systems in confinement, which is usually done at the beginning of summer, is called in some farms of the region as "summer management". This date (November 16th) corresponds to the second month of calving (2M), 70 ± 14 DIM (week 9).

The TMR was offered from 11:00 to 15:00 in both groups (ad libitum), in open stalls with and access to water. Feeders covered (70 × 3 m) with concrete floor and metal roof and an area with dirt floor without roof but with shade in each group (50 × 35 m). Both groups were in the same environment and confinement system but in different and adjacent pens. The drinking troughs were plastic-made (3.76 × 0.76 × 0.44 m; Figure 1). GCHD group cows were grazing in two sessions (08:00–11:00 and 19:00–06:00 hr) after each milking and fed with TMR in one session (11:00–15:00 hr, until milking in the afternoon) equivalent to 25% of that received by the GTMR. The pasture used was composed of *Festuca arundinacea* and *Dactylis perseo*. The herbage allowance was ample over 40 kg/cow of dry matter (4 cm above ground level).

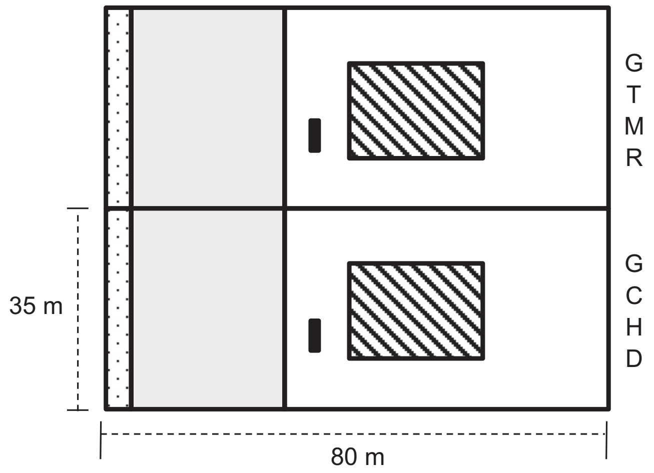

FIGURE 1 Barn diagram. GTMR: barn where cows were in confinement system during all experiment. GCHD: barn where cows were fed with TMR before change (mixed system) and after diet change with only TMR. : Feeders covers, : concrete floor, : drinking troughs, : shade. Feeders covers and concrete floor were covers with metal roof. The others places were "open" with dirt floor and without roof. Both barns were separate with wire fence

2.2 | Feed chemical composition

Ration components were analyzed by near-infrared spectro- scopy (methods 167.03, 42.05, and 984.13; AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured sequentially (Van Soest, Robertson, & Lewis, 1991) without sodium sulphite in the neutral detergent solution) using an ANKOM200 Fibre Analyzer (ANKOM Technology Corp.). NDF was assayed without a heat stable amylase. Both fiber contents were expressed inclusive of residual ash.

Total Mixed Ration (TMR) consisted of silage of whole sorghum plant (33%), dry grain of sorghum (12.5%), citrus pulp (10%), canola expeller (16.5%), sorghum burlanda (10%), and soybean husk (16%). In addition, a premix of minerals and vitamins formulated to measure (1.3%) and urea (0.2%) was added (Table 1).

Prepartum diet consisted of whole sorghum plant silage (51%), Corn grain (26%), Canola meal (21%), CaCo3 (0.4%), Insalmix® (0.6%), Prepartum Bovigold® (1.0%). The chemical composition of prepartum diet was: crude protein (CP): 19.9%; ether extract (EE): 2.4%; neutral detergent fiber (NDF): 36%: acid detergent fiber (ADF): 19%; dry matter (DM): 88.5%; Calcium (Ca):0.5%; Phosphorus (P): 0.5%.

2.3 | Consumption of TMR

The TMR consumption was estimated by difference between the feed offered and rejected (each groups) in two consecutive days each week. Consumption of TMR (kgDM/cows) was in week 8 (before change): GTMR: 27.6kgDM, GCHD: 8.4kgDM; in week 10 (after change) GTMR: 26.4kgDM, GCHD: 27.1kgDM and in week 14 (one month after change) GTMR: 25.2kgDM, GCHD: 25.2kgDM.

2.4 | Temperature-humidity index

Mean and maximum THI value, were recorded during study period. The mean THI values recorded in each month were in October: 59.8 ± 3.2, November: 64.2 ± 2.4, and December: 67.8 ± 2.5. The maximum THI values registered for each month (October, November and December; Figure 2).

2.5 | Body condition score, sampling procedures, and laboratory analysis

The body condition score (BCS) was registered every 15 days (scale 1 = emaciated, 5 = fat) by one trained observed using the scale of Edmonson, Lean, Weaver, Farver, and Webster (1989).

All cows were milked twice a day at 06:30 and 15:30 hr. Milk production was individually recorded with Waikato® meters. Milk samples were taken at one month of lactation (week 4 and 5), at two month of lactation (before changing: week 8 and after changing: week 9 and 10) and three month of lactation (week 12 and 14; Figure 3). Analyzes for the determination of milk composition (fat, total protein, lactose) were performed using: LactoScope FT infrared (FTIR; Delta Instruments, Drachten the Netherlands). Milk yield and composition were analyzed as an average of the two daily milkings.

Blood samples were taken from coccygeal vein into 5-ml, after morning milking, at one month of calving (week 4), at two month of calving (before changing: week 8 and after changing: week 10), and three month of calving (week 12 and 14; Figure 3). The samples were centrifuged at 2,500 × *g*, 10 min to obtain the serum. The serum was aliquotted and stored at −20°C. Blood biochemistry was analyzed in the Laboratorio de Endocrinología y Metabolismo Animal, Facultad de Veterinaria, Universidad de la República according to the following colorimetric methodologies: total proteins (TP): Biuret reaction; albumin: Bromocresol green, creatine kinase (CK): immunoinhibition; urea: UV GIDH; cholesterol: CHOD-PAP,

TABLE 1 Mean chemical composition of samples of the total mixed diet (TMR) and herbage hand clipped in the months of the experiment (%DM)

Feed	Month	CP	EE	NDF	ADF	C
Festuca + Dactylis	October	13.56	2.29	48.94	24.01	10.05
Festuca + Dactylis	November	12.63	1.23	55.1	27.31	10.5
TMR	October	16.8	3.48	40.4	22.34	6.5
TMR	November	17.35	3.75	38.41	20.99	5.44
TMR	December	16.59	3.14	35.66	20.51	6.03

Abbreviations: ADF, acid detergent fiber; C, Ash; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber.

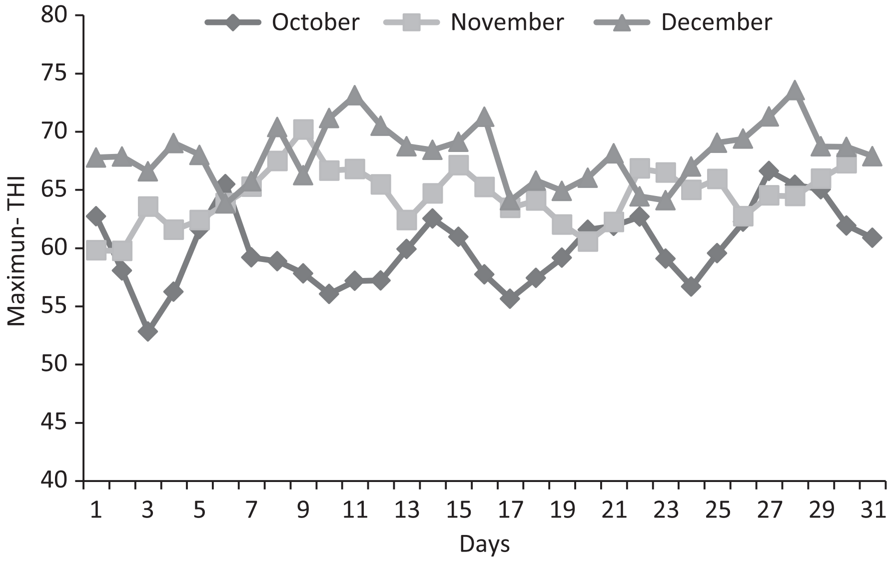

FIGURE 2 Maximum ITH value in each day of October, November, and December

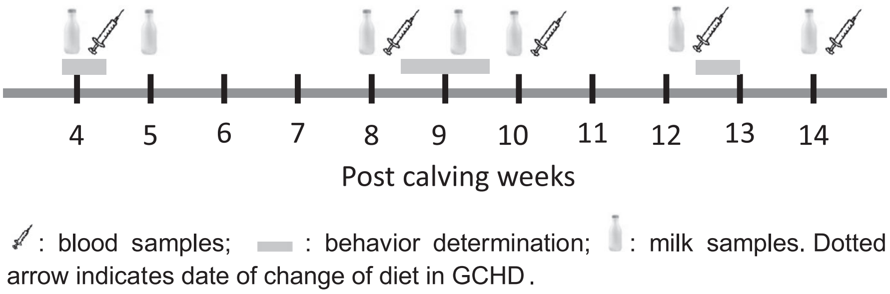

FIGURE 3 Experimental design. Moments (post calving weeks) in which the determinations of blood, behavior, and milk samples were made

calcium: o-cresolphtaleine; phosphorus: phosphomolybdate UV; aspartate aminotransferase (AST); and alanine aminotransferase (ALT): IFCC optimized (37°C). For all these determinations commercial kits were used from the Wiener laboratory (Rosario, Argentina). For CK, BioSystem commercial kits were used. The equipment used was the Vitalab Selectra 2 automatic autoanalyzer (Vital Scientific, Dieren, Netherlands). The concentration of NEFA was determined by the ACS-ACOD method (NEFA-C kit; Wako Chemicals) and BHB by the d-3-hydroxybutyrate kit (Randox Laboratories Ltd.). The controls used were those included in the kit and internal laboratory controls. The concentration of globulins was determined by the difference between the concentration of total proteins and albumin. The interassay and intraassay CV for all determinations was less than 10%

2.6 | Animal behavior

Cows of each treatment were identified with different color (red and blue) collars and numbers drawn on the body to individualize each animal, two days before starting the evaluation. Behavior of individual cows (ruminating, lying, walking, and eating (grazing or TMR; Table 2) were recorded with instantaneous scan sampling every 10 min, performed by two observers simultaneously one in each group (Schütz et al., 2010). Data were recorded during two observation periods daily (08:00–11:00 and 12:00–15:00 hr) for three consecutive days to first and third month after calving (1M and 3M respectively) and for six consecutive days to second month after calving (2M): 3 days before (2M-B) and 3 days after (2M-A) change diet made in GCHD (Figure 3).

2.7 | Statistical analyses

Data of BCS, behavior, milk production, milk composition, and blood biochemistry were analyzed by repeated measures using the PROC MIXED of SAS (SAS Institute Inc., 2004). The BCS, milk yield, milk composition (percentage and yield), and blood biochemistry were analyzed as fixed effects of treatment (GTMR or GCHD), time (weeks) and interaction between treatment and time. The cows in each treatment

TABLE 2 Behaviors observed and their respective descriptions

Behavior	Description
Lying	Lying down in any resting position
Walking	All four legs are moved with head raised or not
Eating: Grazing/ TMR	Picking or consuming pasture/TMR, with the head above ground, still or moving slowly
Ruminating	Chewing movements without feed in the mouth, regurgitation of feed, or both

were considered as a random effect. In BCS, milk and blood variables, post calving days was included as co-variable. Behavior variables were analyzed separately in each month (1M, 2M, and 3M) and the results are presented as average of 3 days in each period. In October (1M) and December (3M) the model considered the treatment (GTMR or GCHD) as fix effect and in November the treatment (GTMR or GCHD) and interaction between treatment and period were considered (2M-B and 2M-A) as fix effects. Post hoc comparisons were performed with least significant difference (LSD). Results were considered significant at α ≤0.05. Data are presented as mean ± *SEM*.

3 | RESULTS

3.1 | Milk production, milk composition, and body condition score

There was not effect of the treatment in milk, lactose, and protein yield ([kg/d] ($p > .30$). All these variables showed an interaction between treatment and time ($p < .0001$, Figure 4). Milk yield in week 9 was greater in GTMR than in GCHD ($p = .009$, Figure 4a). Milk yield increased from week 4 to 9 ($p < .0001$) and decreased from week 9 to 14 ($p < .0001$) in GTMR, while that in GCHD increased from week 9 to 10 ($p < .0001$) and then remained stable until week 14 (Figure 4a). The GTMR cows had greater lactose yield in week 8 and 9 than GCHD cows ($p = .01$ and $p = .002$, respectively, Figure 4b). In GCHD, lactose yield decreased from week 4 to 9 ($p = .01$) and increased from 9 to 10 ($p < .0001$) and then remained at high level until week 14. In GTMR, lactose yield increase from week 4 to 9 ($p = .004$) and decreased from week 10 to 14 ($p = .001$). Protein yield had similar patterns to the lactose yield (Figure 4c). Fat yield did not show effect of treatment ($p = .40$) or interaction between treatment and time ($p = .12$). The differences found between groups (GTMR vs. GCHD) in milk, lactose and protein yield one week before change disappeared after diet change ($p > .05$). The percentage of fat was greater in GCHD than in GTMR (3.2 ± 0.1% vs. 2.5 ± 0.1%, respectively, $p = .01$, Table 3). The GCHD cows had greater percentage of fat than GTMR cows on weeks 4 (3.8 ± 0.2% vs. 2.1 ± 0.2%, $p < .0001$), 5 (3.7 ± 0.2% vs. 2.5 ± 0.2%, $p = .0001$), 8 (3.0 ± 0.2% vs. 2.1 ± 0.2%, $p = .004$) and 9 (3.3 ± 0.2% vs. 2.4 ± 0.2%, $p = .004$). The percentage of fat in GTMR cows increased from week 8 to 10 (2.1 ± 0.2% and 2.8 ± 0.2%, $p = .004$) and remained with high levels and in GCHD decreased from week 4 to 14 (3.8 ± 0.2% and 2.8 ± 0.2%, $p < .0001$). The GTMR cows presented greater percentage of protein than GCHD cows on week 8 (GTMR: 3.1 ± 0.04% vs. GCHD: 2.9 ± 0.04%; $p = .0009$), and lower on week 12 (GTMR: 3.1 ± 0.04% vs. GCHD: 3.3 ± 0.04%; $p = .01$). In GTMR, the percentage of protein decreased from week 4 to 10 (3.2 ± 0.03% and 3.1 ± 0.03%, $p = .02$), while in GCHD decreased from week 4 to 8 (3.1 ± 0.04% and 2.9 ± 0.04%, $p < .0001$) and then increased from week 8 to 14 (2.9 ± 0.04% and 3.2 ± 0.04%, $p < .0001$). In GTMR cows the percentage of lactose increased from week 10 (4.7 ± 0.1%) to 12 (4.9 ± 0.1%, $p = .007$). In GCHD cows the percentage of lactose increased from week 8 to 12 (4.6 ± 0.1% and 5.1 ± 0.1%, respectively, $p < .0001$) and decreased from week 12 to 14 (5.1 ± 0.1% and 5.0 ± 0.1%, respectively, $p = .02$).

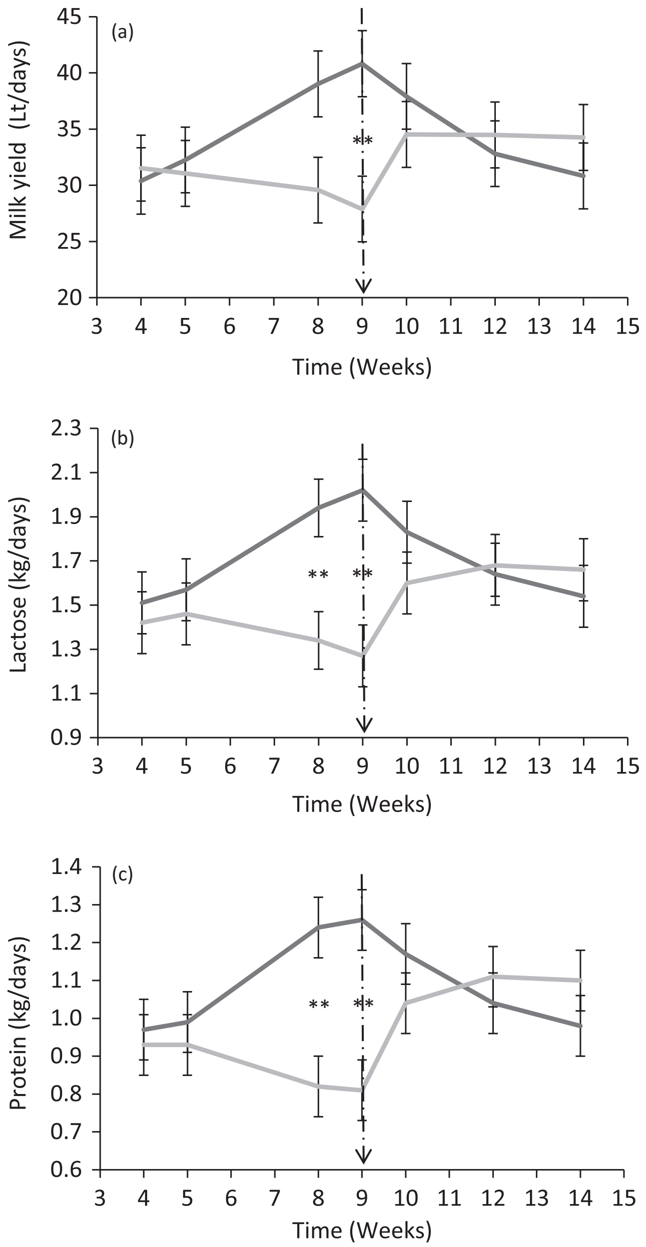

FIGURE 4 Milk yield (Lt/d) (a), Lactose (kg/d) (b), Protein (kg/d) (c) (mean ± *SEM*) in milk of GTMR (line black) and GCHD (line gray). The dotted arrow indicates date of change of diet in GCHD. Asterisks indicate differences between treatment in the same week: $^{**}p < .01$

The BCS was greater in GTMR cows than in GCHD cows ($p = .04$; Table 3).

3.2 | Animal behavior

3.2.1 | First month of lactation

Frequency in which cows were observed ruminating was greater in GCHD than in GTMR ($p < .001$, Figure 5a). There was not difference between groups in the frequency in which cows were observed eating (Figure 5d), lying (Figure 6a), and walking (Figure 6d).

TABLE 3 Effect of treatment (T: GTMR vs. GCHD), week (W) and interaction between treatment and week (T×W) on BCS; percentage of Fat; Protein and Lactose (mean ± *SEM*) during the experimental period

	Treatment			*p* value		
	GTMR	GCHD	*SEM*	T	W	T×W
Fat (%)	2.5	3.2	0.11	.01	.005	.0006
Protein (%)	3.1	3.1	0.13	.49	<.0001	<.0001
Lactose (%)	4.8	4.8	0.2	.96	<.0001	.003
BCS	3.0	2.5	0.11	.04	.0003	.11

Abbreviations: BCS, body condition score; GTMR, group fed TMR; GCHD, group diet change.

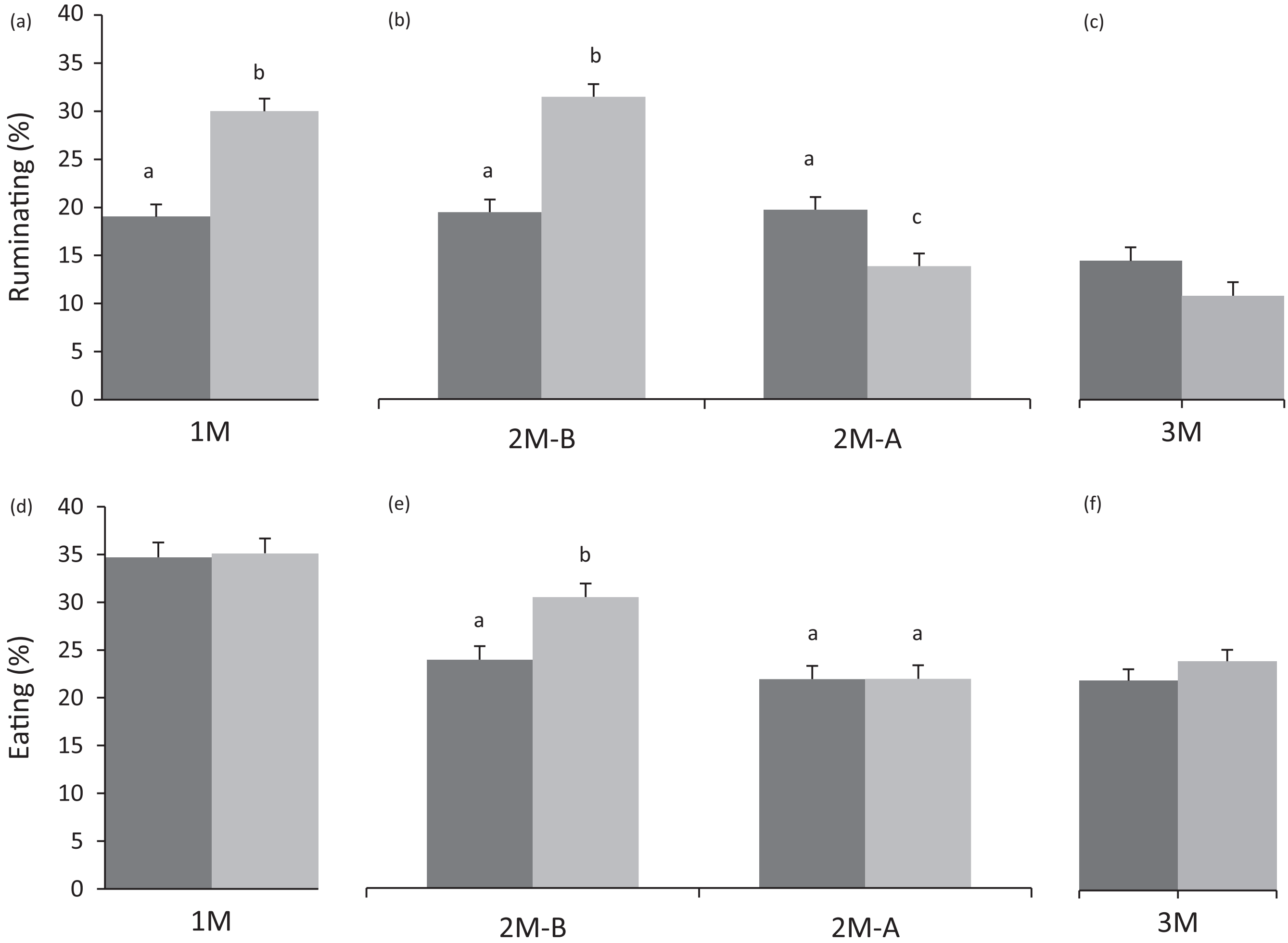

FIGURE 5 Frequency (mean ± *SEM*) in which cows were observed ruminating (%) (a, b, and c) and eating (%) (d, e, and f) in GTMR (black bar) and GCHD (grey bar) at first month from calving (1M), second month from calving: before change feeding (2M-B) and after change feeding (2M-A), and at third month from calving (3M). Different small letters show differ between treatment and between period (2M-B and 2M-A) in second month ($p < .05$)

3.2.2 | Second month of lactation

The frequency in which cows were observed ruminating was greater in GCHD than in GTMR (22.6 ± 0.9% vs.19.5 ± 0.9%; $p = .02$; respectively). There was interaction between treatment and periods ($p < .0001$) in this variable (Figure 5b). During period 2M-B, GCHD cows were ruminating with greater frequency than GTMR cows ($p < .0001$). After the diet change (2M-A) GCHD cows had lower frequency of rumination than GTMR cows ($p = .001$; Figure 5b). The frequency of rumination in GCHD cows decreased from 2M-B (31.4 ± 1.3%) to 2M-A (13. 8 ± 1.3%; $p < .0001$). In GTMR cows there was no difference in the frequency of rumination between periods (2M-B vs. 2M-A; Figure 5b).

The frequency in which cows were eating was greater in GCHD (25.8 ± 0.9%) than in GTMR (22.5 ± 0.9%; $p = .02$). There was an interaction between treatment and period ($p = .02$): before diet

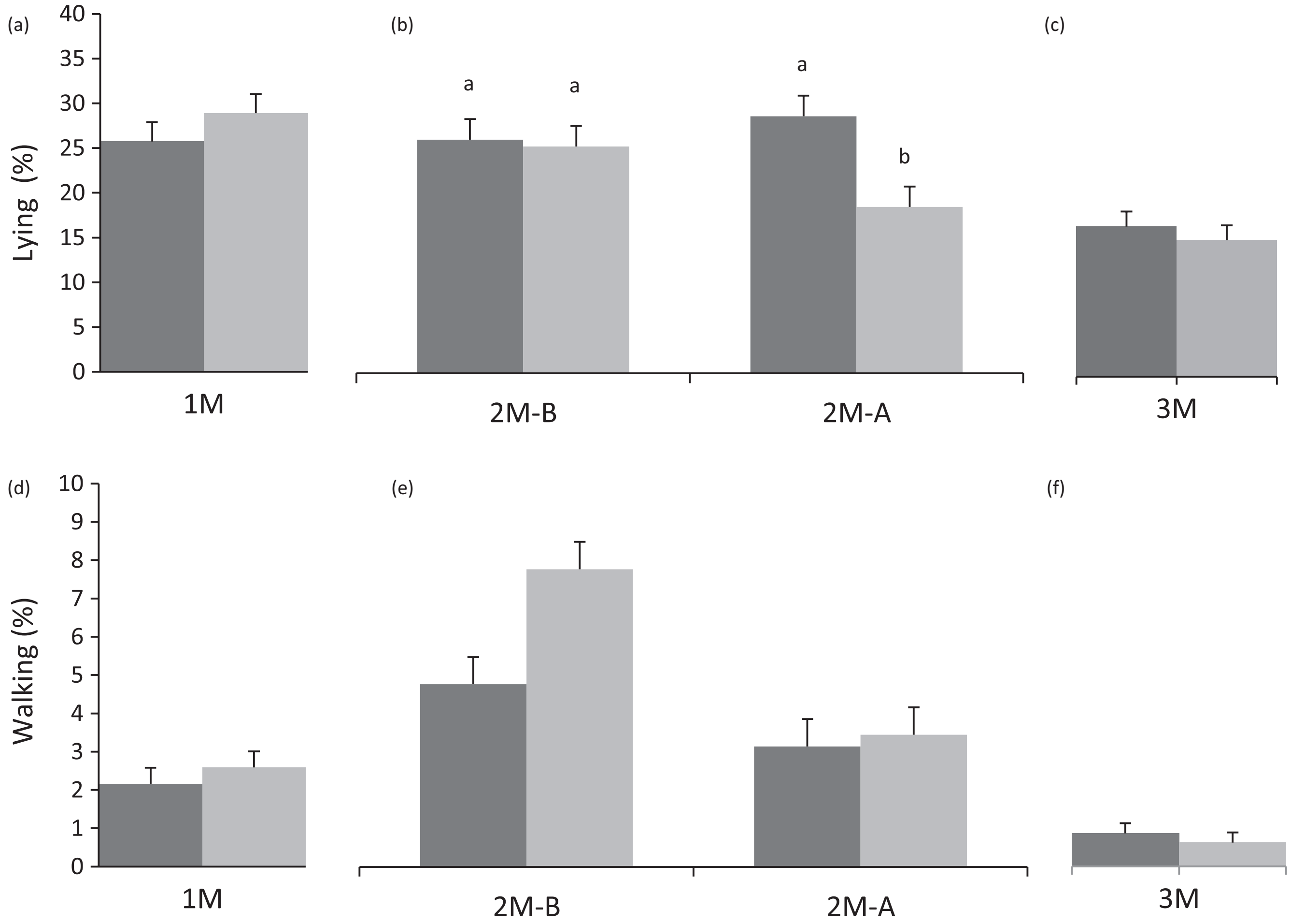

FIGURE 6 Frequency (mean ± *SEM*) in which cows were observed lying (%) (a,b,c) and walking (%) (d, e, f) at one month from calving (1M), two month from calving: before change feeding (2M-B), and after change feeding (2M-A) and at three month from calving (3M), in the groups GTMR (black bar) and GCHD (bar Gray). Different small letters show differ between treatment and period (2M-B and 2M-A) in second month ($p < .05$)

change, GCHD cows had greater frequency of eating than GTMR cows (p = .001). The GCHD cows had greater frequency of eating during 2M-B (39.0 ± 1.6%) than during 2M-A (21.6 ± 1.6%; $p < .0001$; Figure 5e).

The GTMR cows were lying with greater frequency than GCHD cows (25.4 ± 1.5% and 20.3 ± 1.5%, respectively; p = .02). There was an interaction between treatment and period (p = .04; Figure 6b): GCHD cows had greater frequency of lying before diet change (2M-B; 23.5 ± 2.1%) than after diet change (2M-A; 17.3 ± 2.1%; p = .03). After diet changing (2M-A) frequency of cows lying was lower in GCHD (17.2 ± 1.5%) than in GTMR (26.6 ± 1.5%; p = .02; Figure 6b).

The frequency in which cows were walking was greater in GCHD (5.5 ± 0.5%) than in GTMR (3.9 ± 0.5%; p = .02). There was a tendency of interaction between treatment and period in the frequency in which cows were walking (p = .06; Figure 6e).

3.2.3 | Third month of lactation

There was no difference in the frequency in which cows were observed eating lying and walking between treatments (Figures 5f, 6c and f) The GTMR cows tended to ruminate more than GCHD cows (14.6 ± 1.5% and 11.0 ± 1.4% respectively, p = .06; Figure 5c).

3.3 | Blood biochemistry

Nonesterified fatty acids concentration did not show effects of the treatment (p = .7). There was an interaction between treatment and time ($p < .001$) for NEFA concentration. In week 4, GCHD cows had greater NEFA concentrations (0.8 ± 0.1 mmol/L) than GTMR cows (0.4 ± 0.1 mmol/L; p = .002; Figure 7a). There was interaction between treatment and time (p = .03) for BHB concentration. In GCHD, BHB concentration decreased from week 4 to 14 ($p < .0001$) and in GTMR decreased from 4 to 8 (p = .0005; Figure 7b). Cholesterol concentration showed interaction between treatment and time ($p < .001$). In week 10, GTMR cows had greater concentration of cholesterol (5.4 ± 0.39 mmol/L) than GCHD cows (4.0 ± 0.4 mmol/L), (p = .049; Figure 7c). The cholesterol concentration increased from week 4 to 10 ($p < .0001$) and decreased from week 10 to 12 ($p < .0001$) in GTMR cows and increased from week 4 to 8 ($p < .0001$) and decreased from week 8 to 12 ($p < .001$) in GCHD cows (Figure 7c). The

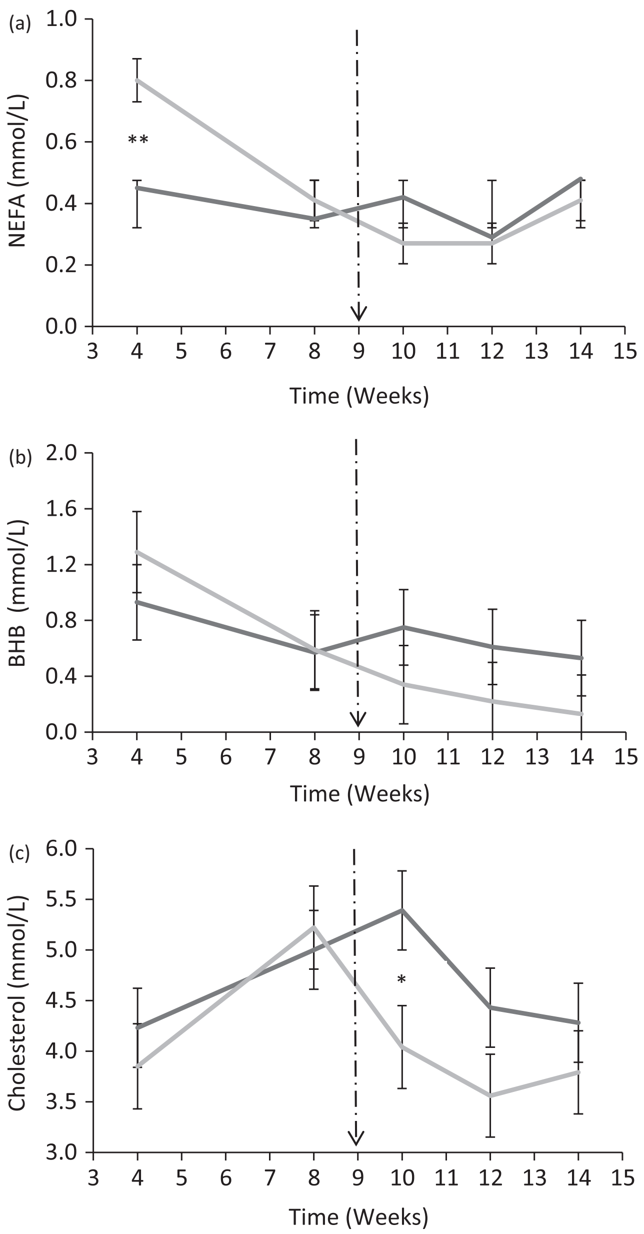

FIGURE 7 Non-esterified fatty acids (NEFA) (a); Beta-hydroxybutyrate (BHB) (b); Cholesterol (c) (mean ± *SEM*) in GTMR (black line) and GCHD (gray line). Dotted arrow indicates date of change of diet in GCHD. Asterisks indicate differences between treatments in the same week: *$p < .05$, **$p < .01$

AST concentration showed interaction between treatment and time ($p = .01$). The AST concentration was greater in GTMR cows than in GCHD cows (114.9 ± 9.1 U/L and 103.2 ± 9.5 U/L respectively; $p = .05$) in week 14. Their AST concentration decreased from week 4 to 14 ($p = .03$) in GCHD cows, in contrast to GTMR cows, whose level increased from week 4 to 8 ($p = .03$) and remained the same until the end of the experiment. There was no effect of the treatment in other blood variables, except for a tendency in ALT (Table 4). There was no interaction between treatment and time for the other blood variables (Table 4), except for a tendency in the concentration of globulins ($p = .08$).

4 | DISCUSSION

The diet change from a mixed system (pasture grazing plus TMR) to single confinement system (100% TMR) in dairy cows affected the milk production and composition, behavior, and some metabolic parameters in blood. On one hand, the change from a mixed system to a single system in confinement was positive in terms of milk production and composition and metabolism. However, the abrupt change from one system to the other negatively affected the behavior of cows, evidencing the difficulty to adapt quickly during the first days. After the change, cows were ruminating and lying with less frequency than before, and they even performed these behaviors less frequently than those cows kept in TMR. The fact that both behaviors (ruminating and lying) decreased after the change of system could be due to the lack of access to the pasture or the factors associated with it. Since these behaviors along with the grazing in the dairy cow are associated with good well-being and comfort (Arnott, Ferris, & O'Connell, 2017; Higashiyama, Nashiki, Narita, & Kawasaki, 2007; Kilgour, 2012; Tucker, Weary, & Fraser, 2004), it is possible that the change of system and the difficulty to adapt during the first days resulted in worse welfare. Ruminating and lying are associated behaviors and occur when cows are at rest (Schirmann, Chapinal, Weary, Heuwieser, & von Keyserlingk, 2012), and in more comfortable housing conditions cows lie more frequently (Charlton & Rutter, 2017), highlighting the importance of these behaviors as welfare indicators. In our study, before the change, cows were in a mixed system, and possibly preferred to perform behaviors of ruminating and lying more in pasture than in confinement, where they do not find a comfortable ground to lie on, as it has been reported in other works (Arnott et al., 2017; Charlton & Rutter, 2017). Nevertheless, after the change of system the cows lost access to the pasture, and possibly in an uncomfortable environment to rest during confinement, they reduced the frequency of lying. Rumination activity is influenced mainly by diet characteristics (Erina et al., 2013; Welch & Smith, 1970) therefore the decrease in rumination activity after the change of system may be due to changes in the composition of the diet. But also, rumination activity is negatively affected by different stressors, such as high stocking density, heat stress, and acute stress (Grant & Albright, 2001; Herskin, Munksgaard, & Ladewig, 2004; Kadzere, Murphy, Silanikove, & Maltz, 2002; Moallem, Altmark, Lehrer, & Arieli, 2010; Moretti, Biffani, Chessa, & Bozzi, 2017). In addition, more stressed cows (with greater serum cortisol concentration) spent less time ruminating (Bristow & Holmes, 2007; Lindström, Redbo, & Uvnäs-Moberg, 2001). Therefore, the reduction in rumination after the change of system may be an indicator of negative emotional response as was suggested by Herskin et al. (2004). In short, the cows expressed difficulties to adapt quickly in a short time window to the abrupt change from a mixed system to a single confinement system with TMR.

One month after the system change, cows of both groups displayed a pattern of similar behavior, which could suggest that cows were adapted to the system. However, even one month after the change, cows of GCHD tended to ruminate less than those of GTMR, suggesting that the process of adapting cows to change was gradual, and that animals need a longer window of time to fully adapt to a

TABLE 4 Effect of treatment (T: GTMR vs. GCHD), week (W) and interaction between treatment and week (T×W) on blood metabolites (mean ± *SEM*) during the experimental period

	Treatment			*p* value		
Blood metabolites	GTMR	GCHD	*SEM*	T	W	T×W
BHB (mmol/L)	0.7	0.5	0.3	.7	<.001	.03
NEFA (mmol/L)	0.4	0.4	0.1	.7	<.001	<.001
Cholesterol (mmol/L)	4.7	4.1	0.4	.8	<.001	<.001
TP (g/L)	79.0	80.6	1.2	.4	<.0001	.16
Albumin (g/L)	34.5	33.5	0.7	.4	<.0001	.4
Globulin (g/L)	44.1	47.1	1.0	.2	.0008	.08
CK (U/L)	225.6	331.1	89.6	.5	.8	.3
Urea (mmol/L)	6.3	5.9	0.1	.8	.02	.4
Calcium (mmol/L)	2.2	2.3	0.1	.6	.01	.3
Phosphorus (mmol/L)	2.2	2.3	0.1	.6	.01	.3
AST (U/L)	117.5	103.6	6.8	.3	.1	.01
ALT(U/L)	44.1	31.8	3.6	.08	.08	.8

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BHB, Beta-hydroxybutyrate; CK, creatine-kinase; GCHD, group diet change; GTMR, group fed TMR; NEFA, Non-esterified fatty acids; TP, Total protein.

system change. Our results match those of Enriquez-Hidalgo et al. (2018) who reported that cows needed more than 10 days to adapt their behavior to the change of management from a pasture system to one of confinement. Therefore, the cows need a window of time of some weeks to adapt their behavior to a new environment after abrupt change from a pasture-based system to a confinement system. Given that dairy cows do not adapt easily to abrupt changes of system, it would be interesting to evaluate how animals can adapt their behavior to gradual changes in these systems of production.

Around one week before the diet change, cows of GCHD produced less milk, and less protein and lactose yield than the cows of GTMR, but such differences disappeared after the change of system. These results are in agreement with the available information on the advantages of the system in confinement, where cows consuming TMR diet produce more milk than cows grazing or in mixed systems, which is associated with an increase in DMI and greater availability of energy (Bargo et al., 2002; Fajardo et al., 2015; Kay, Mackle, & Auldist, 2002; Kolver & Muller, 1998; Meikle, Adrien, Mattiauda, & Chilibroste, 2013; Vibart, Fellner, Burns, Huntington, & Green, 2008; Wales et al., 2013). In addition, the change from a mixed system to a single system in confinement with TMR improved the production and composition of milk in a short period of time, evidenced from one week before the change to one week after. These changes in milk production and composition were accompanied by some metabolic changes, such as a continuous decrease in the concentration of BHB and AST toward the end of the experimental period and an early fall in cholesterol concentration, suggesting less mobilization of body reserves and liver activity after the change of system (Garcia, Cardoso, Campos, Thedy, & Gonzalez, 2001; Noro, Cid, Wagemann, Arnés, & Wittwer, 2013). Although cows in a mixed system during the first month of lactation had greater negative energy balance compared to cows of GTMR (evidenced by greater values of NEFA and lower BCS), they failed to recover their BCS after the change. This could be due to the fact that during early lactation the energy generated by the dairy cow prioritizes the mammary gland for milk production rather than the body reserves (Gross, van Dorland, Bruckmaier, & Schwarz, 2011). Therefore, in terms of milk production, milk composition and metabolism, dairy cows respond favorably to change of diet and environment from mix system to TMR system.

It is interesting to note that according to our knowledge there are few studies that evaluated the adaptation of dairy cows to changes in diet and management considering behavioral variables. This study reinforces the concept of the need to evaluate behavior along with other variables (productive or physiological) for a comprehensive analysis of animal welfare in dairy production systems.

5 | CONCLUSION

Abrupt change from a mixed system to confinement system (100% TMR) was favorable in productive and physiological variables, such as milk production, milk composition, and metabolism of dairy cows. However, in relation to behavior, the cows expressed difficulties to adapt quickly in a short time window to the abrupt change of system.

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