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Metabolic Adaptations Due to the Inclusion of Pasture in the Diet of Dairy Cows Fed Total Mixed Ration during Early Lactation

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Abstract

The aim of this study was to evaluate the metabolic adaptations due to the inclusion of pasture in the diet of dairy cows fed on a total mixed ration (TMR) *ad libitum* during early lactation. Multiparous cows (n = 18) were used in a randomized complete block design and were randomly assigned according to parity, BW and BCS to one of two feeding strategies from calving to 60 DIM: 1) cows fed TMR *ad libitum* (without access to pasture; 100% TMR) and 2) cows fed on a mixed system with pasture grazing (6 h of access to paddock in one grazing session, 8:00 to 14:00 h) and supplemented with 50% of *ad libitum* TMR (Pasture Group, PG). At 61DIM, TMR fed cows were assigned without an adjustment period to a similar feeding and management routine than PG group (Post-TMR), while PG cows remained in their original routine throughout the experiment. Thus, at 61DIM and thereafter, both, PG and Post-TMR cows grazed a second-year pasture and were supplemented with 50% TMR (DM basis). Milk production was determined daily until 80 DIM, and cow BCS and BW were registered and blood samples and liver biopsies were obtained one week before and one week after dietary change (−1 to +1 wk; +55 and +69 DIM). Milk yield, BW and BCS did not differ between treatments but decreased or tended to decreased from −1 to +1 wk only in Post-TMR cows. Serum IGF-1 tended to increase in Post-TMR cows. Hepatic expression of *IGFBP5* and *IGFBP6* mRNA, were greater while *IGF1* and *IGFBP3* mRNA tended to be greater for Post-TMR than PG cows. Hepatic expression of *IGF1*, *IGFBP5* and *IGFBP6* mRNA increased from −1 to + 1 wk only in Post-TMR cows. Expression of *ACADVL* and *PDH1A* mRNA had a 2-fold increase in both groups from wks −1 to +1. The results confirm that changes in feeding strategy without an adaptation period modified animal metabolism. The inclusion of grazing to cows that were fed TMR during early

lactation, increased IGF-1 concentrations and modified hepatic expression of genes related with IGF system and fatty acid metabolism indicating redistribution of nutrients and energy towards maintenance requirements (increased due to walking and grazing activity) in detriment of milk production.

Keywords

Turnout to Pasture, Hepatic Expression, Dairy Cattle

1. Introduction

Pasture-based systems allow low-cost and high-nutritional value feeding in dairy cows, and offer benefits for animal welfare and environmental care [1]. However, the quantity and quality of the pasture offered is variable throughout the year, determining an imbalance between nutrient supply and demand at various stages of the production cycle of the modern dairy cow [2]. Thus, the inclusion of concentrates and silages in the dairy cow diet has increased to meet the requirement of the milking herd [3] [4]. In this regard, the use of a total mixed ration (TMR) in early lactation allows dairy cows achieve maximum performance in times of reduced pasture dry matter (DM) production, determining an increase in milk yield, cow body condition score (BCS), metabolic status and reproductive performance [5] [6] [7].

The transition from a TMR to a pasture based systems involved significant changes in feed intake [8], cow behavior and activity [3], and rumen microbiota [9] causing changes in milk production and composition [10] [11]. However, little is known about dairy cow metabolic adaptations to changes in feeding regimes, from a TMR diet in early lactation (0 - 60 days in milk; DIM) to a pasture-based diet in a later stage of lactation. Metabolic adaptations during the transition from a TMR to a pasture-based diet (+1.75 or 3.6 kg DM of concentrate) in mid-lactation cows (160 to 200 DIM) included decreased insulin and increased non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB), indicating an increased lipomobilization during the first days or weeks on pasture, associated to decreased dry matter intake (DMI), milk yield and BCS [11] [8]. Nevertheless, liver changes on the somatotrophic axis and energy - glucose and fatty acid - metabolism in response to this dietary change has been scarcely reported and understood. Better understanding of the effects of different feeding strategies on animal metabolism and performance will promote improvements in actual feeding practices. To our knowledge, there are no studies that evaluated energy metabolism when TMR-fed dairy cows are changed to a feeding regime that included pasture grazing and an appropriate supplementation.

We hypothesized that when cows in early lactation change from a 100% TMR to a pasture + 50% TMR feeding regime, metabolism would rapidly adapt in order to cope with changes in amount and type of nutrients absorbed as well as in energy balance. Therefore, the aim of this study was to examine alterations in

metabolic and endocrine profiles as well as hepatic expression of genes related with nutrient partitioning and energy metabolism when dairy cows fed TMR *ad libitum* during the first 60 DIM (100% TMR) changed to a feeding regime that included pasture grazing (6 h/d) and supplementation with a reduced allowance of TMR (50%) without an adaptation period.

2. Materials and Methods

The experiment was performed according to the protocol approved by the Animal Experimentation Committee (CHEA) of the Universidad de la República (UdelaR, Uruguay). It was carried out the Experimental Station "Dr. Mario A. Cassinoni" of the School of Agronomy (Paysandú, Uruguay).

2.1. Animals and Experimental Design

Eighteen multiparous Holstein cows (fall calving; body weight (BW) = 709 ± 52.5 kg and BCS = 3.25 ± 0.25 units and parity = 4.5 ± 1.7) were used in a randomized block design with two replications (groups). Cows were grouped according to their due calving date (beginning and end of fall) and were randomly assigned within group according to their parity, BW and BCS to one of two feeding strategies from calving (day 0) to 60 DIM: 1) cows fed a TMR *ad libitum* (without access to pasture) and 2) cows fed on a mixed system with grazing (6 h of access to paddock in one grazing session, 8:00 to 14:00 h) and supplemented with 50% of *ad libitum* TMR (PG). At61 ± 2 DIM, TMR fed cows were assigned without an adjustment period to a similar feeding and management routine than PG group (Post-TMR), while PG cows remained in their original routine throughout the experiment. Therefore, in the present study we evaluated two treatments: Post-TMR (0 to 60 DIM fed TMR and 61 to 80 DIM fed pasture grazing + 50% TMR) and PG as a control group (0 to 80 DIM pasture grazing + 50% TMR). Details on cow feeding and management during the prepartum and early postpartum as well as productive, metabolic, endocrine and reproductive responses during the first 60 DIM have been previously reported [6] [7].

At 61 DIM, all cows (PG and Post-TMR) grazed a second-year pasture of *Festuca arundinacea*, *Trifoliumrepens* and *Lotus corniculatus* (located 1.7 km from the milking parlour) in a 7-d rotation system with a mean herbage allowance at 4 cm above the ground level of 15 kg DM/cow/d (279 ± 14DM g/kg, and 184 ± 14 g/kg DM of crude protein (CP), and 201 ± 2.5 g/kg DM acid detergent fiber (FDA)). The TMR was offered once a day in the afternoon (after pm milking), had a forage/concentrate ratio of 45/55 (dry basis) and was composed of sorghum silage (0.45) and a concentrate that included ground corn (0.19), wheat (0.12), soybean expeller (0.09), sunflower expeller (0.11), urea (0.003) and minerals and vitamins (0.009) (597 ± 43 DM g/kg, 167 ± 18 g/kg DM PC, and 141 ± 16.0 g/kg DM FDA).

Cows were milked twice a day (5:00 and 15:00 h) and milk production was determined daily from 61 until 80 DIM of lactation. Milk samples to determine fat, protein and lactose composition and BCS (score 1 a 5) [12], BW, blood sam-

ples and liver biopsies were obtained one week before and one week after dietary change (−1 and 1 wk relative to dietary change or +55 and +69 DIM). Blood samples were obtained in heparinized tubes by venipuncture of the coccygeal vein and were centrifuged (2000 x g for 15 min at 4˚C) within 2 h after collection and serum was stored at −20˚C until assayed. After blood collection, liver biopsies (500 mg) were obtained using a 14-gauge biopsy needle (Tru-Core®-II Automatic Biopsy Instrument; Angiotech, Lausanne, Suitzerland) as described by Carriquiry *et al.* [13]. Liver samples were immediately frozen in liquid nitrogen and stored at −80˚C until total RNA was isolated.

2.2. Metabolite and Hormone Analyses

Concentrations of serum NEFA, BHB, glucose and urea were determined by colorimetric assays using commercial kits according to [7]. Concentrations of insulin, IGF-1 and leptin were measured using commercial kits by immunoradiometric assays (IRMA, for insulin and IGF-1) or by a liquid-phase radioimmunoassay (RIA, for leptin). Assay details and detection limits as well as intra-assay CV were described in Astessiano *et al.* [7].

2.3. Isolation and Purification of RNA

Isolation of total RNA from hepatic tissue and synthesis of cDNA by reverse transcription was performed according with Astessiano *et al.* [7]. Primers (**Supplementary Table S1**) to specifically amplify cDNA of target genes: *IGF*1, *IGF*2, *IGF binding proteins*-1 *to* 6 (*IGFBP*1, *IGFBP*2, *IGFBP*3, *IGFBP*4, *IGFBP*5, *IGFBP*6), insulin receptor (*INSR*), and long isoform of leptin receptor (*LEPRB*), glucose 6-phosphatase (*G6PC*), pyruvate carboxylase (*PC*), pyruvate dehydrogenase (*PDH*1*A*), acyl-CoA oxidase (*ACOX*), carnitine palmitoyltransferase (*CPT*1*A*), acyl-CoA dehydrogenase very long chain (*ACADVL*), hydroxymethylglutaryl-CoA synthase (*HMGCoA*), citrate cintase (*CS*), and from endogenous controls: *β-actin* (*ACTB*), *hypoxanthine phosphoribosyl transferase* (*HPRT*), and *ribosomal protein S*9 (*RPS*9) were used [7].

Real time PCR reactions were performed in a total volume of 15 µL using KAPA SYBR® FAST Universal 2X qPCR Master Mix (KapaBiosystems, inc. Woburn, MA, USA) according with [14]. Gene expression one week after dietary change (+1 wk) was measured by relative quantification [15] to the expression of one week before dietary change (−1 wk) and normalized to the geometric mean expression of the endogenous control genes (*HPRT*, *ACTB* and *RPS*9). Expression stability of 3 selected housekeeping genes was evaluated using MS-Excel add-in Normfinder (MDL, Aarhus, Denmark). The stability values obtained with Normfinder they were 0.144, 0.121, and 0.178 for *HPRT*, *ACTB*, and *RPS*9, respectively. Amplification efficiencies or target and endogenous control genes were estimated by linear regression of a dilution cDNA curve (n = 5 dilutions, from 100 to 6.25 ng/tube). Intra and inter-assay CV values were 1.9% and 4.2%, respectively.

2.4. Calculation and Statistical Analyses

Net energy (NE) calculations was based on [16]. Maintenance NE requirements were calculated as NEM = 0.08 × BW^0.75 + NEmact, where NEmact = (((Distance/1000) × Trips) × (0.00045 × BW)) + (0.0012 × (BW)). Lactation NE requirements were calculated as NEL = milk yield × [(0.0929 × fat %) + (0.0563 × true protein %) + (0.0395 × lactose %)], using composition data derived from analysis of samples collected weekly.

Data were analyzed in a randomized block design using the SAS System program (SAS Institute Inc., Cary, NC, USA). Univariate analyses were performed on all variables to identify outliers and inconsistencies and to verify normality of residuals. Data of milk production, BW, BCS, and serum metabolite and hormone concentrations were analyzed by repeated measures using the MIXED procedure with DIM as the repeated effect, and the unrestricted (UN) covariance structure. The model included the treatment (PG vs. Post-TMR), week (before and after dietary change), and the interaction between treatment and week as fixed effects, replication as a random effect and calving date as a covariate. The Kenward-Rogers procedure was used to adjust the denominator degree of freedom. Tukey-Kramer tests were conducted to analyze differences between treatments and weeks ($\alpha = 0.05$). Data of hepatic gene expression were analyzed using the MIXED procedure with a model that included treatment as fixed effect, replication as a random effect and calving date as a covariate. One-way t-test were conducted to assess week effect within treatment. Correlation coefficients were estimated using the CORR procedure. Data are presented as least square means ± pooled standard errors.

3. Results

Milk yield did not differ between treatments (P = 0.16) but decreased (P < 0.05) from −1 to +1 wk only in Post-TMR (**Figure 1(A)**) whereas milk NEL output and milk composition was not affected (P > 0.10) by treatment or week. Cow BW and BCS tended to be affected by treatment and week (P = 0.12) as they decreased (P = 0.03) from −1 to +1 wk only in Post-TMR cows (**Figure 1(B)** and **Figure 1(C)**). Maintenance NEL requirement was affected by the interaction between treatment and week (P = 0.01) as while it did not change from −1 to +1 wk in PG cows (12.81 to 12.66 ± 0.3 Mcal/d), an increase (P < 0.05) was observed in Post-TMR cows (10.73 to 11.91 ± 0.3 Mcal/d).

Serum concentrations of NEFA, BHB, urea and glucose were not affected by treatment or week (**Table 1**). Serum IGF-1 concentrations were not affected by treatment or week but tended to be affected by the interaction between treatment and week ($P = 0.06$) as they tended to increase in Post-TMR cows with the change of diet (129.5 vs. 167.5 ± 13.5 ng/mL from −1 to +1 wk, respectively). Concentrations of insulin and leptin were not affected by treatment, week or their interaction (**Table 1**).

Hepatic expression of *IGFBP5* and *IGFBP6* mRNA (P < 0.05), were greater while IGF1 and IGFBP3 mRNA tended to be greater (P = 0.14) for Post-TMR

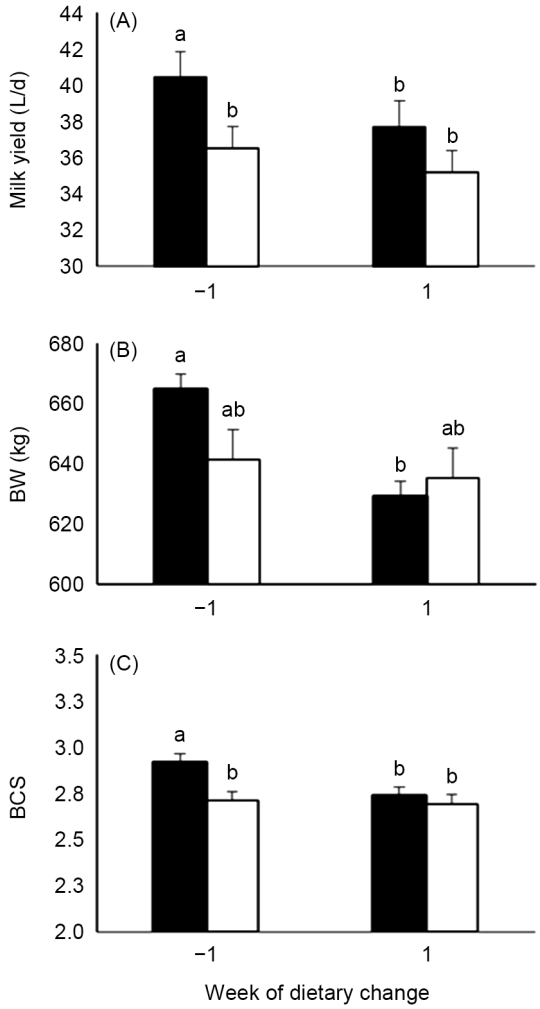

Figure 1. Changes in milk yield, BW and BCS one week before and one week after dietary change (+55 and +69 DIM, respectively) in multiparous dairy cows (Post-TMR; ■ and PG; □). Within treatments: a vs b indicates significant differences ($p < 0.05$).

Table 1. Effect of dietary change on serum metabolite and hormone concentrations in multiparous dairy cows.

	Treatments[1]			P*value*		
	Post-TMR	PG	SE	T	WK	T x WK
NEFA (mmol/L)	0.6	0.5	0.06	0.66	0.56	0.20
BHB (mmol/L)	0.4	0.5	0.04	0.65	0.14	0.25
Urea (mmol/L)	5.3	5.3	0.50	0.96	0.88	0.56
Glucosa (mmol/L)	4.2	4.0	0.04	0.23	0.57	0.58
IGF-I (ng/mL)	148.5	143.5	13.40	0.83	0.91	0.06
Insulina (μIU/mL)	17.5	18.4	1.50	0.69	0.47	0.62
Leptina(ng/mL)	4.1	3.6	0.50	0.53	0.45	0.47

[1]**Post-TMR**: Cows changed at 61 DIM to a mixed feeding regime that included pasture grazing (one am grazing session of 6 h) + 50% TMR (DM basis) after being fed 100% TMR (*ad libitum*) during the first 60 DIM. **PG**: control group, cows fed a mixed feeding regime of pasture grazing (one am grazing session of 6 h) + 50% TMR (DM basis) from calving. T: treatment; WK: week relative to dietary change.

than PG cows (Table 2). In addition, expression of *IGF*1, *IGFBP*5 and *IGFBP*6 mRNA had a 2.3 to 3.4-fold increase ($P < 0.025$) from −1 to +1 wk only in Post-TMR cows. Expression of *IGF*2, *IGFBP*1, *IGFBP*2, *IGFBP*4, *INSR*, and *LEPRB* mRNA were not affected by treatments ($P > 0.22$; Table 2). Expression of the genes related to glucose and fatty acid metabolism in the liver did not differ ($P > 0.50$) between treatments (Table 2), but *ACADVL* and *PDH*1*A* mRNA had a 2-fold increase ($P < 0.01$) in both groups from −1 to +1 wk. In addition, the ratio *PDH*1*A* to *PC* mRNA tended ($P = 0.07$) to have a 1.5-fold increase from −1 to +1 wk only in Post-TMR cows. Independently of treatment, hepatic *ACADVL* mRNA was positively linearly correlated with serum NEFA concentrations ($r = 0.71$, $P = 0.07$).

Table 2. Effect of dietary change on relative hepatic mRNA expression of genes related to IGF system, glucose and fatty acid metabolismin multiparous dairy cows.

	Treatments			P *value*
	Post-TMR	PG	SE	
*IGF*1	2.41	1.00	0.25	0.14
*IGF*2	1.00	0.97	0.20	0.96
*IGFBP*1	0.60	1.22	0.13	0.22
*IGFBP*2	0.95	1.00	0.12	0.93
*IGFBP*3	1.33	0.76	0.20	0.14
*IGFBP*4	0.84	1.05	0.16	0.66
*IGFBP*5	3.43	0.75	0.35	0.01
*IGFBP*6	2.32	0.66	0.12	0.04
INSR	1.03	0.83	0.11	0.65
LEPRB	1.46	1.46	0.12	0.99
ACADVL	2.01	1.85	0.23	0.80
ACOX	1.20	1.47	0.12	0.46
CS	1.18	1.49	0.24	0.65
PC	1.32	1.47	0.24	0.80
G6PC	1.16	1.48	0.11	0.63
HMGCOAS	1.92	1.56	0.12	0.79
*PDH*1*A*	2.09	1.78	0.14	0.62
*CPT*1*A*	1.05	1.05	0.12	0.99

[1]**Post-TMR**: Cows changed at 61 DIM to a mixed feeding regime that included pasture grazing (one am grazing session of 6 h) + 50% TMR (DM basis) after being fed 100% TMR (*ad libitum*) during the first 60 DIM. **PG**: control group, cows fed a mixed feeding regime of pasture grazing (one am grazing session of 6 h) + 50% TMR (DM basis) from calving. [2]Genes: *IGF*1, *IGF*2 = insulin like growth factor 1 and 2; *IGFBP*1, *IGFBP*2, *IGFBP*3, *IGFBP*4, *IGFBP*5, *IGFBP*6 = IGF binding proteins-1, 2, 3, 4, 5 and 6; *INSR* = insulin receptor; *LEPRB* = full-lengthleptin receptor; *ACADVL* = Acyl-CoA dehydrogenase very long chain; *ACOX* = Acyl-CoA oxidase; *CS* = citrate cintase; *PC* = pyruvate carboxylase; *G6PC* = glucose 6-phosphatase; *HMGCoA* = hydroxymethylglutaryl-CoA synthase; *PDH*1*A* = pyruvate dehydrogenase; *CPT*1*A* = carnitinepalmitoyltransferase.

4. Discussion

The inclusion of grazing, without an adaptation period, in the diet of dairy cows that were fed only TMR during early lactation, modified hepatic IGF system associated with changes in productive performance. Milk production decreased only in Post-TMR group after dietary change. Although this decrease could accompany the normal decline in the lactation curve around the peak of milk production [17], as it was only observed in Post-TMR cows, it was probably associated to a restriction in DMI and/or increased in energy requirements after the change of the diet [6]. It has been reported that not only characteristics of the pasture, but also its management can limit DMI of grazing animals [4] [18]. Bargo *et al.* [19] reported decreased milk production and DMI for cows grazing pasture and supplemented with TMR (as 17 kg DM/d per cow) when compared to cows fed only TMR. In addition, grazing as such produces an increase in energy expenditure (for walking and grazing) compared to animals with food easily available as a TMR [20]. This idea is supported by the fact that cows in the Post-TMR group increased estimated energy requirements maintenance [16] by 10% from −1 to +1 wk (+55 to +69 DIM).

The observed decreases in milk yield, BW and BCS from −1 to +1 wk only in Post-TMR cows suggested that the reduction in TMR intake and the greater energy maintenance demands were not compensated by an adequate intake from pasture in these cows. Indeed, DMI and grazing behavior of the same experimental treatments were reported by [6] that despite not finding differences in average DMI and grazing behavior between Post-TMR and PG cows after the change of diet, they reported lower grazing activity on Post-TMR during the last hours in the pasture. In addition, it is also known the transition from one diet type to another causes changes in the rumen microbiota and rumen stratification [21] [22]. These changes could lead to alterations in fermentation patterns [23] and physiological and structural adaptations of the rumen epithelium [24] that could affect nutrient utilization in the gastrointestinal tract and also explain the decline in performance (milk yield, BW and BCS) in Post-TMR cows.

Dietary change in this study was done after peak milk yield and nadir of BCS [7], thus, no changes in BCS would have been expected according to the lactation stage. Nevertheless, as reported before, both, BW and BCS decreased for Post-TMR cows after the change of diet, indicating mobilization of body reserves, probably to cope with the increased maintenance requirements. Indeed, milk fatty acid profile of these cows showed an increase of preformed fatty acids, particularly of stearic acid (Barca *et al. unpublished results*), which may reflect lipomobilization [25]. However, NEFA concentrations did not increase in Post-TMR cows, which suggested that lipid mobilization was not extensive and/or there was a differential tissue NEFA utilization due to the increased walking and grazing activities in these cows [7]. The oxidation of mitochondrial fatty acids produces energy in muscle for muscle contraction or exercise [26].

In agreement with the inverse association between serum IGF-1 and milk production in dairy cows [27], we found that the concentrations of IGF-1 tended

to increase after the dietary change in Post-TMR cows. The role of IGF-1 around calving, when its concentrations decreased consistent with an uncoupled somatotropic axis, mediating nutrient partitioning towards milk production has been extensively reviewed [27] [28]. Therefore, considering that in the present study the change of diet occurred after the peak of milk production, results suggested that the increase in circulating IGF-1 would modify nutrient redistribution in detriment of milk production. The increased serum IGF-1 concentrations were also consistent with an increased hepatic mRNA expression of *IGF*1 and three of its binding proteins, *IGFBP*3, *IGFBP*5 and *IGFBP*6, after the change of diet only in Post-TMR cows. The association between hepatic *IGF*1 mRNA and circulating concentrations of IGF-1 has been previously reported [29] [30]. It is known that, once released from the cell, the majority of IGF-1 found in circulation is bound with high affinity to one or more of the 6 known IGF binding proteins. However, binary complexes that IGF-1 form with IGFBP-3 or IGFBP-5 (including the binding with the acid-labile) extend IGF-1 half-life from 10 min to 16 hours or longer increasing availability of IGF to cellular functions in the body [31] [32] [33]. Thus, increased serum IGF-1 could have been responded not only to a greater hepatic IGF-1 secretion, but also to an increased circulation of this hormone in blood as a ternary complex due to greater availably of IGFBP3 and IGFBP5. Consistent with our results, Fenwick *et al.* [30] reported increased *IGFBP*3, *IGFBP*5 and *IGFBP*6 mRNA expression in the liver of early lactation dairy cows with reduced milk production and moderate negative energy balance when compared with cows with greater milk production and severe negative energy balance. In addition, Coyne *et al.* [34] reported increased hepatic *IGFBP*5 mRNA in heifers fed a high n-3 polyunsaturated diet. Indeed, in the present study n-3 PUFA intake increased after the change of diet (by the inclusion of pasture) for Post-TMR cows (Barca *et al. unpublished results*).

The increase in *ACADVL* mRNA in the liver at +1 wk in both treatments indicated a metabolic adaptation to promote β-oxidation of fatty acids [35]. Indeed, we found a positive correlation between serum NEFA concentrations and hepatic *ACADVL* mRNA. The increase in liver *ACADVL* mRNA as lactation progresses could reflect a better liver capacity to use NEFA as fuel as has been shown previously [35]. Interestingly, although no changes in hepatic *PC* mRNA were observed, *PDH*1*A* mRNA increased its expression in both treatments suggesting that the shift in the metabolism later in lactation was not directed to improve gluconeogenesis but to the complete oxidation of the carbons (Krebs Cycle). Moreover, the *PDH*1*A* to *PC* mRNA ratio tended to increase only in Post-TMR cows after the change of diet suggesting a greater flux of pyruvate to acetyl-coA than to oxaloacetate, probably due to a reduced demand for the generation of oxaloacetate via PC [36] as milk production, thus lactose production and the need for glucose, was reduced in this group.

The results confirm that metabolic regulation in dairy cows is a dynamic system, and changes in feeding strategy without an adaptation period modified animal metabolism. In the present study, the inclusion of grazing to cows that

were fed TMR during early lactation, increased IGF-1 concentrations and modified hepatic expression of genes related with IGF system and fatty acid metabolism indicating redistribution of nutrients and energy towards maintenance requirements (increased due to walking and grazing activity) in detriment of milk production.

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Supplementary

Table S1. Primers used for the quantification of target and endogenous control gene cDNA.

Gene[1]	Accession no.[2]		Primer sequence	Length (bp)	Efficiency
IGF1	XM_612412	Sense	CCAGACAGGAATCGTGGATG	89	1.27
		Antisense	ACTTGGCGGGCTTGAGAG		
IGF2	NM_174087	Sense	TTGCAGGTAGGCTTGTCCTT	98	1.09
		Antisense	CAGGTTTGGGTCTTTGGTGT		
IGFBP1	NM_174554	Sense	TACAGAAGTGGAAGGAGCCCT	127	1.21
		Antisense	AATCCATTCTTGTTGCAGTTT		
IGFBP2	NM_174555	Sense	ATGCGCCTTCCGGATGA	83	1.15
		Antisense	GTTGTACAGGCCATGCTTGTCA		
IGFBP3	NM_174556	Sense	AGCACAGACACCCAGAACTTCT	86	1.19
		Antisense	TTCAGCGTGTCTTCCATTTCC		
IGFBP4	NM_174557	Sense	ARGTGCCTGATGGAGAAAGG	145	0.98
		Antisense	AAGGCAGAGCCACAGACAGT		
IGFBP5	NM_1105327	Sense	CAAGCCAAGATCGAAAGAGACT	86	1.01
		Antisense	AAGATCTTGGGCGAGTAGGTCT		
IGFBP6	NM_1040495	Sense	GGAGAGAATCCCAAGGAGAGTAA	100	0.97
		Antisense	GAGTGGTAGAGGTCCCCGAGT		
INSR	XM_590552.4	Sense	CTGAAGCCAAGGCAGATGATATT	77	0.90
		Antisense	GCCACATCAAGTGAACAACGTT		
LEPRB	AB199589	Sense	ACCACACCTTCCGTTCTCAG	164	1.08
		Antisense	GGGACAACACTCTTGACTC		
ACADVL	NM_174494.2	Sense	CCAGC-CCCTG-TGGAA-AATAC-TA	62	1.04
		Antisense	GCCCC-CGTTA-CTGAT-CCAA		
CS	NM_001044721.1	Sense	AGCCAAGATACCTGTTCCTC	217	1.10
		Antisense	TGTGCTGGAAGAAACGATTGC		
PC	NM_001040716	Sense	AGGGAAGCTCCTATTTGCTCC	234	1.15
		Antisense	CGGTGGATGTGGTCCTTCTCT		
G6PC	NM_00151	Sense	TGAGGATGGAGAAGGGAATG	203	1.02
		Antisense	TGAACCAATCCTGGGAGTTC		
PDH1A	NM_000284	Sense	ATCCTCTGTCGTCCCCTTCT	89	0.98
		Antisense	CACCTCATGCGAAGAGTTGA		
CPT1A	NM_001876	Sense	CAAAACCATGTTGTACAGCTTCCA	140	1.01
		Antisense	GCTTCCTTCATCAGAGGCTTCA		
ACTB	BT030480	Sense	CGTGGCTACAGCTTCACC	53	1.15
		Antisense	GAAATCGTCCGTGACATCAA		
HPRT	XM_580802	Sense	TGGAGAAGGTGTTTATTCCTCATG	105	1.05
		Antisense	CACAGAGGGCCACAATGTGA		
RPS9	NM_1101152	Sense	CCTCGACCAAGAGCTGAAG	63	1.03
		Antisense	CCTCCAGACCTCACGTTTGTTC		

[1]*GHR* = growth hormone receptor, *GHR1A* = growth hormone receptor 1A, *IGF1* = insulin-like growth factor-I, *IGF2* = insulin-like growth factor-II, *IGF1R* = insulin-like growth factor-I *receptor, IGF binding proteins*-1 *to* 6 (*IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, IGFBP6*), *INSR* = insulin receptor, ***LEPRB*** **=** full-length leptin receptor; *ACADVL* = Acyl-CoA dehydrogenase very long chain; *CS*= citrate cintase; *PC*= pyruvate carboxylase; *G6PC*= glucose 6-phosphatase; *PDH1A*= pyruvate dehydrogenase; *CPT1A* = carnitine palmitoyl transferase, *and ACTB* = ß-actin, *HPRT* = hypoxanthine phosphoribosyl-transferase and *RPS9* = *ribosomal protein S9* as an endogenous control gene. [2]Gene Bank bovine sequences.