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Original

Fiber (NDF) consumption and its effect on the response to FTAI in cows of the tropics of Mexico

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ABSTRACT

Objective. To evaluate the reproductive performance of crossbred cows in the tropics of Mexico fed two levels of neutral detergent fiber (NDF), eliciting them to ovulate with a hormonal protocol and fixed-time artificial insemination (FTAI). **Material and methods.** Twenty-seven multiparous cows were used. Twenty-one days before calving, cows were assigned to two treatments: T1= 4.5 kg of concentrate/cow/d and T2= 3.0 kg of concentrate/cow/d, and forage *ad libitum* in both treatments. **Results.** Cows assigned to T2 consumed more NDF (p<0.05) than cows assigned to T1 (38 *vs* 44% of the diet). The increment in forage consumption, up to the NDF allowed, compensated the provision of metabolizable energy (ME) and protein (MP), which were similar in cows of both treatments during the 15 days before calving. Cows fed with T2 tried to compensate the nutrients deficit consuming more forage (6.25 *vs* 7.37 kg of DM/d). The MP was always deficient during lactation for T1 and T2 cows. In T2 cows, the MP deficiency resulted in less milk nitrogen content. Cows assigned to T1 lost less body condition, which resulted in higher (p<0.05) pregnancy rate at first service (75.2 *vs* 42.8%), although they did not produce more milk than T2 cows. **Conclusions.** NDF reduction in the ration from 44 to 38% and the application of the FTAI hormonal protocol reduce the open period to 105 d in lactating cows in the tropics.

Keywords: Dual-purpose system; feed intake; fertility; crossbred cows; hormonal protocol; transition period (*Source: CAB*).

RESUMEN

Objetivo. Evaluar el comportamiento reproductivo de vacas cruzadas del trópico de México alimentadas con dos niveles de fibra detergente neutro (FDN), induciéndolas a ovular con un protocolo hormonal e IATF. **Materiales y métodos**. Se utilizaron 27 vacas multíparas. Veintiún días antes del parto, las vacas se asignaron a dos tratamientos: T1=4.5 kg de concentrado/vaca/d y T2=3.0 kg de concentrado/vaca/d, y forraje *ad libitum* en ambos tratamientos. **Resultados.** Las vacas del

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T2 consumieron más FDN (p<0.05) que las vacas del T1 (38 *vs* 44% de la dieta). El incremento en el consumo de forraje, hasta donde la FDN permitió, logró compensar el aporte de EM y PM, siendo similares para ambos tratamientos durante los primeros 15 días antes del parto. Las vacas del T2 intentaron compensar el déficit de nutrientes consumiendo más forraje (6.25 *vs* 7.37 kg de MS/d). La PM siempre fue deficiente durante la lactación para T1 y T2. En las vacas del T2, la deficiente PM repercutió en menor contenido de N en leche. Las vacas del T1 perdieron menos condición corporal; esto se reflejó en una mayor (p<0.05) tasa de gestación a primer servicio (75.2 *vs* 42.8%), aunque no produjeron más leche que las del T2. **Conclusiones.** La disminución de la FDN en la dieta de 44 a 38% y la aplicación del protocolo hormonal de IATF disminuye el período abierto a 105 d en vacas lactando en el trópico.

Palabras clave: Consumo voluntario; fertilidad; periodo de transición; protocolo hormonal; sistema doble propósito; vacas cruzadas (*Fuente: CAB*).

INTRODUCTION

Dual-purpose cattle farming in the humid tropics of Mexico bases its feeding on diets composed almost exclusively of forages, in the prepartum and postpartum stages. In some cases, forages are supplemented with concentrate feed after calving. It is well known that three weeks pre- until three weeks post-calving, which is called "transition" period, cows demand special attention. However, in most dual-purpose systems this period is completely ignored.

This period is characterized by a decrease in feed intake, which is recovered from one to two weeks after calving. This decrease in feed intake causes a nutritional unbalance between nutritional requirements and nutrients intake, mainly causing a negative energetic unbalance at the beginning of lactation.

To reduce the risk factors associated with the transition period, it is important to achieve a gradual ruminal adaptation (particularly with those diets that imply challenges associated with quantity and quality of feed) and implement strategies for vitamin-mineral or antioxidant-nutrients supplementation to mitigate the immunosuppression state and the metabolic and oxidative stress during pregnancy and lactation, to prevent these problems and, consequently, to achieve a favorable impact on the productive and reproductive performance (1).

Cattle milk production in the tropics is based on grazing, therefore, forage quality has a direct effect on production and reproduction, because it is the main source of energy and protein. Forage dry matter intake (DMI) is the most important factor that regulates grazing cattle production (2). The chemical composition of a forage determines its nutritional quality and, consequently, its consumption and value in animal production (3), therefore, fiber voluntary intake should be known or predicted to determine the proportion of nutrients that can be provided by tropical forages so the supplementary concentrate quantity needed per day can be calculated.

It is necessary to have a clear knowledge of the factors that regulate, in the mid (consumed quantity) and long term (body fat), the appetite and voluntary DMI, which depend on the physiological state of the animal. The objective of this work was to evaluate the reproductive performance of crossbred cows in the tropics of Mexico fed two levels of neutral detergent fiber (NDF), eliciting them to ovulate with a hormonal protocol and fixed-time artificial insemination (FTAI).

MATERIALS AND METHODS

Location. The study was carried out from August 2017 to February 2019 at 'La Posta' Research Station (INIFAP), located in Paso del Toro, Veracruz, Mexico, at kilometer 22.5 of the Veracruz-Córdoba federal highway, at 19º02' North Latitude and 96º08' West Longitude. The altitude above the sea level is 16 m.

Environment. The climate of the region is classified as Aw with average annual temperature, pluvial precipitation and relative humidity of 25°C, 1461 mm and 75%, respectively (4).

Animals. From a dairy herd of 100 Holstein x Zebu cows under a grazing dual-purpose system in tropical climate, the multiparous, pregnant cows programmed to calve in the rainy season (from June to December) of 2017 were used.

Twenty-seven cows enter the experiment 30 days before calving and were allocated in individual paddocks until day 70 postpartum.

Adjustment period. To adapt the cows to pens and diets, from day 30 to 15 of the prepartum period, all cows were offered a fixed quantity of concentrate (2 kg) with 16% of CP and 70% of TDN, and grass (*Digitaria decumbens*) hay *ad libitum*.

Treatments. Fifteen days before the expected calving date, cows were randomly assigned to treatment 1 (T1) and 2 (T2): T1= 4.5 kg of concentrate/cow/d, and T2= 3.0 kg of concentrate/cow/d, until completing 14 and 13 replicates, respectively. The concentrate feed formulation is presented in Table 1. Grass hay was offered *ad libitum*. Cow's daily forage intake was calculated as the difference between the amount of forage offered to the cow and the amount of forage rejected by the cow, in accordance with cow's nutritional requirements, which were estimated with the Cornell Net Carbohydrate and Protein System (CNCPS) (5) based on cow's milk yield.

Table 1. Ingredients used to formulate the concentrate feed for lactating crossbred cows in both treatments.

Ingredient	Percentage
Corn grain (ground)	43.5
Sorghum grain (ground)	21.0
Wheat bran	12.0
Soybean meal	10.0
Molasses	10.0
Vitamins and minerals	2.0
Soybean oil	1.0
Urea (feed grade)	0.5

Animal management. After calving, cows were milked once a day, in the morning, with the suckle of their calves to stimulate milk ejection. Eight hours after milking, calves were allowed to nurse their dams for 1 h. Cows' and calves' body weight (BW) as well as cows' body condition score (BCS; 1=thin to 9=fat) was measured every 14 days. Milk yield was recorded daily, while milk composition (fat, protein and lactose) was estimated every 28 days with an ultrasonic milk analyzer (LACTOSCAN S, Milkotronic Ltd, Nova Zagora, Bulgaria).

Feed analysis. The content of nutrients in the diet was calculated every three months through chemical composition analyses of the forage and concentrate feed throughout the experiment. Two 0.5-kg samples were taken from each type of feed. To determine dry mater (DM) content, one sample was dried at 100°C for 24 h and discarded; the other sample was dried at 55°C until constant weight was achieved. This last sample was ground in a Wiley mill (Model 4, Arthur H. Thomas Co., Philadelphia, PA, USA) with a 1-mm mesh. The content of DM, ash, crude fat, crude protein and the fractions of NDF, ADF and lignin (6), as well as the nitrogen fractions (7), were calculated in accordance with the CNCPS recommendations (8).

Estimation of nutrients input and nutritional requirements. The CNCPS program (v 6.5.5.1) was used to estimate the nutrients input and nutritional requirements of cows in both treatments. Environmental (temperature, relative humidity), management (confinement), genetic (Holstein x Zebu crossbred cows), physiological (BW, BCS, calving age, calving number, yield and composition of milk), and feeding variables (intake and nutritional content of the concentrate and forage) were considered to model the daily nutritional status of the cows. This information was divided into five 15-day periods: 15 days before calving, and 15, 30, 45 and 60 days postpartum.

The variables evaluated in these periods were: changes in BW and BCS; concentrate, forage and NDF intake, and metabolized energy and protein requirements for maintenance, body reserves, pregnancy and lactation. For each period, treatments (T1 and T2) were compared with the Student's t-test. The nutritional implications in these periods were associated with the beginning of the postpartum ovarian activity and the subsequent reproductive performance of cows in both treatments.

Resumption of ovarian activity and reproductive management. Cows' follicular dynamics was evaluated by a bug BCF ultrasound Innovative Imaging with a 7.5 MgHz scanner, every three days, from day 30 postpartum until the appearance of a follicle with a diameter greater than 10 mm. From the appearance of such a follicle or from day 70 postpartum, which was considered day 0, cows were induced to estrous and ovulation with a hormonal protocol that consisted of the administration of a CIDR with 1.9 g of progesterone plus 2 mg of estradiol benzoate. The CIDR was removed at day 8, then 25 mg of $PGF_{2\alpha}$, 0.5 mg of estradiol cypionate and 400 IU of eCG were injected intramuscularly. Cows were tail painted at CIDR removal. At day 10 (58 h after CIDR removal) painted and non-painted cows were artificially inseminated (60 h after CIDR removal) and injected with 2 µg of GnRH. Calves were weaned for 72 h (temporary weaning) from day 8, when the CIDR was removed, to 10, when timed AI was performed.

Reproductive variables. Reproductive traits were analyzed under a completely randomized design. The evaluated variables were: days open, calving interval, number of services per conception, and first service conception rate. First service conception rate was considered as a binary variable; therefore, when a cow resulted pregnant at the first AI service this variable was recorded as 1, on the contrary (non-pregnant cow), it was recorded as 0.

Statistical analysis of reproductive variables. Number of AI services per conception and first service conception rate were analyzed with the GENMOD procedure of SAS (9). For number of services per conception, a Poisson distribution was specified in the model statement; in the analysis of first service conception rate, a binomial distribution was specified, and a logit link function was used. Days open and calving interval were analyzed with the GLM procedure of SAS (9). In all cases, the statistical model included the effects of treatment, calving year, calving season, and age of the cow at calving, except for days open and calving interval, because this continuous variable was not significant ($p>0.05$) in preliminary analyses.

RESULTS

Table 2 shows cows' nutritional status in the period corresponding to 15 days before calving, per treatment. Cows had very similar physiological conditions at the beginning of the study because assignment of cows to treatments was standardized based on expected calving date, age, lactation number, BW and BCS. Similarity in physiological conditions allowed similar DMI of cows assigned to both treatments, except for concentrate intake that was experimentally controlled, since T1 cows were offered more concentrate than T2 cows (4.5 *vs* 3.0 kg/cow/d). Due to the lower concentrate intake of T2 cows, they tried to compensate the nutrients deficit by consuming more forage (6.25 *vs* 7.37 kg of DM/d). Statistically, this trend was not manifested ($p=0.27$), because NDF intake has a limit due to the limited ruminal capacity during late pregnancy. Even so, T2 cows consumed more NDF (38 *vs* 44% of the diet; $p<0.05$) than T1 cows. The increase in forage consumption, as far as the NDF allowed it, compensated the contribution of metabolizable energy (ME) and protein (MP), which were similar in T1 and T2 cows 15 days before calving, although the nitrogen balance indicated deficit of NH_3 in the rumen of T1 and T2 cows, being more accentuated in T2 cows (89 *vs* 86% of the required; $p=0.02$).

The first physiological variable that changes at the beginning of lactation is body condition. Treatment 2 cows presented lower BCS than T1 cows because of greater use of body energy reserves to support lactation, due in part to a limited DMI of T2 cows compared to that of T1 cows (Table 3). Even though T2 cows attempted to compensate it by consuming more forage ($p=0.08$), this was not enough, affecting their milk to feed ratio, producing only 0.56 kg of milk per kilogram of feed consumed, making milk more expensive (3.34 *vs* 4.89 pesos/kg; $p=0.03$).

With almost 50% of NDF in the diet, T2 cows did not have the capacity to consume more than 3.2 kg of NDF. The impact of this was not only on energy consumption (33.1 *vs* 28.2 Mcal; $p=0.03$), but also on energy distribution, since T2 cows used less ME for lactation and tended to prevent a more drastic fall in body condition by using almost three times more energy for reserve (Mcal/d) in relation to T1 cows (1.0 *vs* 2.77; $p=0.14$), even though the amount of ME for maintenance in both treatments was the same. In consequence, T2 cows produced 4 kg of milk/d less than T1 cows (11.8 *vs* 15.7 kg; $p=0.03$) during the first 15 days after calving. Cows on T1 and T2 had ruminal ammonium deficiency (negative numbers). No effects were observed on protein and lactose content of milk between treatments.

Table 2. Response of dry cows 15 days before calving to two levels of concentrate supplementation.

Description	Treatment 1			Treatment 2			*P*-value
	Average	SD	SEM	Average	SD	SEM	
Physiological condition							
Age, months	113	40	23	90	48.9	28	0.18
Lactation number	5	1.7	1	3.3	2.3	1.3	0.15
Calf's birth weight, kg	34.4	3.61	2.1	36.1	11	6.4	0.43
Body weight, kg	489	46.1	27	506	19.3	11	0.24
Condition score (1 to 9)	4.5	1.26	0.73	4.0	1.63	0.94	0.32
Dry matter intake (DMI)							
Observed total DMI, kg	10.7	2.27	1.3	10.5	2.51	1.4	0.45
Concentrate intake, kg	4.46	0.02	0.01	3.12	0.02	0.01	0.00
Forage intake, kg	6.25	2.25	1.3	7.37	2.53	1.5	0.27
Feeding cost, $/d	51	10.3	5.9	50	11.1	6.4	0.44
Effective fiber intake (eNDF)							
eNDF intake, kg	1.63	0.95	0.55	2.27	1.05	0.61	0.19
eNDF intake, % diet	38	5.8	3.3	44	5.6	3.2	0.05
eNDF, % body weight	0.81	0.26	0.15	0.93	0.34	0.20	0.27
Metabolizable energy (ME) requirements							
ME available, Mcal/d	24.4	4.53	2.6	24.5	4.69	2.7	0.52
ME maintenance, Mcal/d	13.6	1.16	0.67	14.2	0.42	0.24	0.22
ME pregnancy, Mcal/d	7.2	1.6	0.93	8.13	3.74	2.20	0.38
ME reserves, Mcal/d	3.7	4.11	2.4	2.2	3.81	2.2	0.36
Average daily gain, kg/d	0.27	0.25	0.15	0.15	0.25	0.15	0.32
Metabolizable protein (MP) requirements							
MP available, g	862	110	64	794	129	75	0.39
MP maintenance, g	466	95.3	55	491	109	63	0.38
MP pregnancy, g	246	58.3	34	283	137	79	0.37
MP reserves, g	128	146	84	129	196	113	0.50
MP balance, g	22	120	69	-48	193	112	0.26
MP, % of required	106	17.2	9.9	100	22.7	13	0.26
Nitrogen balance							
NH_3, % of required	89	12.1	7.0	86	11.2	6.4	0.02
Urea, cost Mcal/d	0.17	0.07	0.04	0.09	0.08	0.05	0.21

Treatment 1= 4.5 kg of concentrate/cow/d; Treatment 2= 3.0 kg of concentrate/cow/d; SD= standard deviation; SEM= standard error of the mean; Body condition score: 1= thin to 9= fat.

Table 3. Response of lactating cows during the first 15 days in milk to two levels of concentrate supplementation.

Description	Treatment 1			Treatment 2			P-value
	Average	SD	SEM	Average	SD	SEM	
				Physiological condition			
Body weight, kg	477	46.9	27.1	500	25.4	14.6	0.29
Condition score (1 to 9)	5.4	0.81	0.47	4.5	0.46	0.26	0.05
				Dry matter intake (DMI)			
Observed total DMI, kg	13.1	2.32	1.34	12.34	2.82	1.63	0.05
Concentrate intake, kg	4.5	0.04	0.02	3.1	0.01	0.01	0.00
Forage intake, kg	8.6	2.35	1.36	9.24	2.83	1.64	0.08
Feeding cost, $/d	62.3	10.7	6.2	55.8	17.5	10.1	0.13
Ratio milk/feed	1.01	0.35	0.20	0.56	0.41	0.24	0.00
Cost, $/kg of milk	3.34	1.62	0.93	4.89	2.09	1.21	0.03
				Effective fiber intake (eNDF)			
eNDF intake, kg	2.5	1.06	0.61	3.2	0.85	0.49	0.01
eNDF intake, % diet	41.7	5.17	2.98	48.6	1.1	0.63	0.05
eNDF, % body weight	1.17	0.44	0.25	1.21	0.27	0.16	0.37
				Metabolizable energy (ME) requirements			
ME allowable milk, kg	15.7	2.06	1.19	11.8	2.07	1.20	0.03
ME available, Mcal/d	33.1	2.45	1.41	28.2	2.83	1.64	0.03
ME maintenance, Mcal/d	13.2	0.85	0.49	14.0	0.63	0.37	0.17
ME lactation, Mcal/d	18.9	2.44	1.41	11.4	1.98	1.14	0.01
ME reserves, Mcal/d	1.0	1.73	1.00	2.77	3.72	2.15	0.14
				Metabolizable protein (MP) requirements			
MP allowable milk, kg	12.8	2.44	1.41	6.1	3.23	1.86	0.00
MP available, g	1140	43.4	25.1	958	78.3	45.2	0.01
MP maintenance, g	580	92.7	53.5	590	115.9	66.9	0.28
MP lactation, g	655	86.4	49.9	538	94.1	54.3	0.05
MP reserves, g	127	109.8	63.4	260	103.8	59.9	0.00
MP balance, g	-120	121	70	-260	104	60	0.00
MP required, %	91	9.24	5.33	79	5.86	3.38	0.01
				Nitrogen balance			
N-urea in milk, mg/dL	0.93	1.55	0.90	-0.90	1.06	0.61	0.17
NH_3 ruminal balance, g	-21	20.7	11.9	-31	5.6	3.2	0.21
NH_3 ruminal, % of required	89	11.14	6.43	82	1.53	0.88	0.21

Treatment 1= 4.5 kg of concentrate/cow/d; Treatment 2= 3.0 kg of concentrate/cow/d; SD= standard deviation; SEM= standard error of the mean; Body condition score: 1= thin to 9= fat.

From day 15 to 30 after calving (Table 4), body condition stabilized. Cows on T2 tended to consume less DM than cows on T1 (12.6 *vs* 14.4 kg/d; p=0.09) due to lower concentrate intake, since forage and NDF intake was similar in T1 and T2 cows.

Table 4. Response of lactating cows 15 to 30 days in milk to two levels of concentrate supplementation.

Description	Treatment 1			Treatment 2			*P*-value
	Average	SD	SEM	Average	SD	SEM	
Physiological condition							
Body weight, kg	477	46.9	27.1	500	25.4	14.6	0.29
Condition score (1 to 9)	5.13	0.92	0.53	4.64	1.83	1.06	0.25
Dry matter intake (DMI)							
Observed total DMI, kg	14.4	1.52	0.879	12.6	1.04	0.598	0.09
Concentrate intake, kg	4.4	0.01	0.00	3.13	0.07	0.04	0.00
Forage intake, kg	10	1.53	0.88	9.46	1.09	0.63	0.31
Feeding cost, $/d	65.2	13.1	7.56	62.5	14.3	8.27	0.31
Ratio milk/feed	0.82	0.116	0.067	0.48	0.234	0.135	0.11
Cost, $/kg of milk	4.17	0.821	0.474	5.07	1.532	0.884	0.08
Effective fiber intake (eNDF)							
eNDF intake, kg	3.37	0.31	0.176	3.23	0.74	0.426	0.60
eNDF intake, % diet	46.1	1.0	0.58	48.5	4.64	2.68	0.19
eNDF, % body weight	1.4	0.20	0.115	1.25	0.232	0.134	0.29
Metabolizable energy (ME) requirements							
ME allowable milk, kg	15.8	3.04	1.76	12.5	1.99	1.15	0.02
ME available, Mcal/d	32.5	4.75	2.74	26.9	2.0	1.15	0.04
ME maintenance, Mcal/d	13.4	1.10	0.633	14.1	0.361	0.208	0.28
ME lactation, Mcal/d	19	3.65	2.11	12.1	1.93	1.11	0.01
ME reserves, Mcal/d	1.33	2.31	1.33	0.80	0.92	0.53	0.29
Metabolizable protein (MP) requirements							
MP allowable milk, kg	11.8	1.82	1.05	6.0	2.72	1.57	0.05
MP available, g	1170	99.2	57.2	896	103.5	59.7	0.01
MP maintenance, g	652	48.6	28.1	604	70.9	41.0	0.21
MP lactation, g	658	126.8	73.2	570	90	52	0.03
MP reserves, g	164	57.3	33.1	297	137.8	79.5	0.13
MP balance, g	-164	57.3	33.1	-297	137.8	79.5	0.13
MP required, %	87.7	2.52	1.45	75.7	10.6	6.12	0.11
Nitrogen balance							
N-urea in milk, mg/dL	0.73	1.19	0.69	-0.27	1.66	0.96	0.04
NH_3 ruminal balance, g	-28.7	4.2	2.4	-33.0	17.5	10.1	0.32
NH_3 ruminal, % of req.	85	3.61	2.08	81	9.07	5.24	0.15

Treatment 1= 4.5 kg of concentrate/cow/d; Treatment 2= 3.0 kg of concentrate/cow/d; SD= standard deviation; SEM= standard error of the mean; Body condition score: 1 = thin to 9= fat.

After 30 days postpartum (Table 5), T2 cows began to recover body condition, but lost body weight; in contrast, T1 cows maintained body weight, but lost body condition.

Table 5. Response of lactating cows 30 to 45 days in milk to two levels of concentrate supplementation.

Description	Treatment 1			Treatment 2			*P*-value
	Average	SD	SEM	Average	SD	SEM	
Physiological condition							
Body weight, kg	479	42.3	24.4	491	41.4	23.9	0.39
Condition score (1 to 9)	4.74	1.96	1.13	4.85	2.07	1.2	0.42
Dry matter intake (DMI)							
Observed total DMI, kg	15.2	2.4	1.38	12.2	1.31	0.76	0.12
Concentrate intake, kg	4.5	0.11	0.06	3.2	0.08	0.04	0.01
Forage intake, kg	10.7	2.48	1.43	9.0	1.38	0.80	0.23
Feeding cost, $/d	74.87	15.85	9.15	66.99	14.58	8.42	0.03
Ratio milk/feed	0.61	0.29	0.17	0.70	0.47	0.27	0.38
Cost, $/kg of milk	5.41	1.32	0.76	4.6	1.18	0.68	0.01
Effective fiber intake (eNDF)							
eNDF intake, kg	3.5	1.15	0.67	3.0	1.08	0.62	0.29
eNDF intake, % diet	45.8	5.11	2.95	46.9	6.01	3.47	0.37
eNDF, % body weight	1.46	0.255	0.147	1.21	0.416	0.24	0.25
Metabolizable energy (ME) requirements							
ME allowable milk, kg	14.0	2.84	1.64	14.7	2.02	1.17	0.15
ME available, Mcal/d	33.2	4.43	2.56	28.15	2.04	1.18	0.03
ME maintenance, Mcal/d	13.5	1.24	0.72	13.8	0.26	0.15	0.38
ME lactation, Mcal/d	16.9	3.43	1.98	14.2	1.97	1.13	0.04
ME reserves, Mcal/d	2.83	1.58	0.91	0.17	0.21	0.12	0.04
Metabolizable protein (MP) requirements							
MP allowable milk, kg	9.3	3.69	2.13	8.1	4.95	2.86	0.29
MP available, g	1140	167.9	96.9	951	125	72.2	0.01
MP maintenance, g	687	124.6	71.9	580	106.2	61.3	0.19
MP lactation, g	585	118.7	68.5	669	91.5	52.8	0.02
MP reserves, g	219	150	87	300	179	104	0.23
MP balance, g	-200	183	106	-300	179	104	0.21
MP required, %	86	13.1	7.54	76	12.4	7.17	0.12
Nitrogen balance							
N-urea in milk, mg/dL	1.2	2.05	1.18	-0.63	1.92	1.11	0.01
NH_3 ruminal balance, g	-31.7	18.3	10.6	-30.0	24.3	14.0	0.44
NH_3 ruminal, % of req.	84.7	8.02	4.63	82.7	10.97	6.33	0.31

Treatment 1= 4.5 kg of concentrate/cow/d; Treatment 2= 3.0 kg of concentrate/cow/d; SD= standard deviation; SEM= standard error of the mean; Body condition score: 1= thin to 9= fat.

Dry matter intake maintained the same trend as in the previous period, causing efficiency of milk production per kilogram of feed in T2 cows to recover in such a way that the feed cost per kilogram of milk produced was lower (5.4 vs 4.6 pesos/kg of milk; p<0.01). This trend was the result of a match in milk production (14.0 vs 14.7 kg/d; p=0.15). In the last stage of the study, 45 to 60 days postpartum, prior to the reproductive evaluation of cows, the physiological status of the cows changed (Table 6). At calving, cows on T1 weighed 489 kg and body scored 4.5 units, and reached day 60 postpartum weighing 481 kg and body scoring 5.35 units; that is, they lost 8 kg and gained 0.85 BCS units. However, T2 cows calved with 506 kg of BW and 4.0 BCS units, and at day 60 postpartum they weighed 488 kg with a BCS of 4.33 units.

Table 6. Response of lactating cows 45 to 60 days in milk to two levels of concentrate supplementation.

Description	Treatment 1			Treatment 2			P-value
	Average	SD	SEM	Average	SD	SEM	
Physiological condition							
Body weight, kg	481	38.3	22.1	488	46.0	26.6	0.438
Condition score (1 to 9)	5.35	1.86	1.07	4.33	2.12	1.22	0.096
Dry matter intake (DMI)							
Observed total DMI, kg	14.5	1.41	0.81	13.1	1.94	1.12	0.080
Concentrate intake, kg	4.5	0.08	0.049	3.1	0.08	0.048	0.001
Forage intake, kg	9.97	1.49	0.86	9.98	2.00	1.15	0.493
Feeding cost, $/d	79.2	15.61	9.01	61.1	10.59	6.11	0.073
Ratio milk/feed	0.71	0.514	0.297	0.60	0.52	0.30	0.206
Cost, $/kg of milk	5.77	1.098	0.634	4.19	0.506	0.292	0.070
Effective fiber intake (eNDF)							
eNDF intake, kg	3.13	1.185	0.684	3.37	1.29	0.745	0.211
eNDF intake, % diet	44.4	5.89	3.4	48.0	6.29	3.63	0.026
eNDF, % body weight	1.34	0.291	0.168	1.36	0.53	0.307	0.449
Metabolizable energy (ME) requirements							
ME allowable milk, kg	13.9	2.89	1.67	14.6	1.47	0.85	0.326
ME available, Mcal/d	32.9	1.42	0.820	28.4	1.03	0.597	0.003
ME maintenance, Mcal/d	13.5	1.17	0.674	14.1	0.611	0.353	0.291
ME lactation, Mcal/d	16.7	3.5	2.02	14.1	1.47	0.85	0.128
ME reserves, Mcal/d	2.77	2.42	1.4	0.17	0.21	0.12	0.095
Metabolizable protein (MP) requirements							
MP allowable milk, kg	9.9	6.94	4.00	7.2	5.71	3.30	0.080
MP available, g	1136	91.9	53	978	112.4	64.9	0.003
MP maintenance, g	651	117.8	68.0	644	136.5	78.8	0.444
MP lactation, g	578	120.9	69.8	663	66.9	38.6	0.132
MP reserves, g	184	201	116	335	258	149	0.048
MP balance, g	-171	214	124	-335	258	149	0.051
MP required, %	85	12.4	7.17	76	15.9	9.21	0.038
Nitrogen balance							
N-urea in milk, mg/dL	1.27	2.69	1.55	-0.57	2.35	1.35	0.008
NH_3 ruminal balance, g	-28	24	14	-212	360	208	0.222
NH_3 ruminal, % of req.	86	9.5	5.5	84	24.3	14.1	0.435

Treatment 1= 4.5 kg of concentrate/cow/d; Treatment 2= 3.0 kg of concentrate/cow/d; SD= standard deviation; SEM= standard error of the mean; Body condition score: 1= thin to 9= fat.

This resulted in a loss of 18 kg of BW and a poor increase of 0.33 BCS units. Considering that T2 cows lost 10 kg of BW more than T1 cows, it is assumed that T1 cows had better energy balance to restart reproductive activity than T2 cows. The amount of concentrate offered to T1 cows always favored greater availability of ME and MP, since T1 cows had higher ($p<0.05$) pregnancy rate at first service than T2 cows (Table 7).

Table 7. Least-squares means and their standard errors for number of services per conception (NSC), pregnancy rate at first service (PR1), days open (DO) and calving interval (CI).

Treatment	NSC	PR1	DO	CI
1	1.4 ±0.4[a]	75.2 ±16.4[a]	105.4 ±26.5[a]	392.6 ±27.2[a]
2	1.6 ±0.5[a]	42.8 ±21.1[b]	117.8 ±28.3[a]	382.6 ±31.8[a]

[a,b]Means with different letter within column are different (p<0.05).

DISCUSSION

At the beginning of lactation, glucose requirements of cows increase to produce lactose, but in the absence of glucose, the animal mobilizes glucogenic substrates (10). Part of the incompetence of T2 cows to compensate with forage the energy required by the homeorhesis of lactation is due to the forage's NDF content. The NDF intake is a function of the size of the animal's digestive tract. The size of the digestive tract in cattle is a function of their BW. A bovine can consume 1% of its BW as NDF (11). In the last month of pregnancy, cows' intake capacity is limited due to the presence of the fetus, placenta, and associated fluids. Before calving (Table 2), it was observed that NDF intake was less than 1% in cows on both treatments (0.81 and 0.93% of BW). However, at the beginning of lactation, the energy demand of T1 cows showed that NDF intake was greater than 1% due to the ruminal distension factor, consuming 1.17, 1.40, 1.46 and 1.34 of NDF as a percentage of BW at days 15, 30, 45 and 60 of lactation (Tables 3, 4, 5 and 6, respectively). In T2 cows, which had lower milk production at the beginning of lactation, the energy requirement for lactation followed a different trend of NDF consumption (1.21, 1.25, 1.21 and 1.36% of BW) for the same periods. These results show that in dual-purpose systems with forage-based feeding, providing 4.5 and 3.0 kg of energetic concentrate (T1 and T2, respectively) at the beginning of lactation, provides more ME for milk production (15.8 *vs* 12.5 kg/d; Table 4), which improves NDF consumption (1.4 *vs* 1.25% of BW), maximizing forage use.

At the beginning of lactation, cows experience negative energy balance (NEB) due to a decrease of DMI and higher energy demand for milk production, which cause high mobilization of lipids from adipose tissue to the liver, compensating glucose levels needed to equilibrate energy balance (12). Cows on T1 started with 489 kg of BW at calving and 4.5 BCS units, and reached day 60 postpartum with 481 kg of BW and 5.35 BCS units. That is, they lost 8 kg of BW and gained 0.85 units of BCS. However, T2 cows started with 506 kg of BW and 4.0 units of BCS, and at day 60 postpartum they weighed 488 kg with BCS of 4.33 units. This resulted in a loss of 18 kg of BW and a poor increase of 0.33 BCS units. With this information and using previously reported equations (13), the energy retained at day 60 of lactation was calculated for each treatment, resulting that T1 cows retained 132.6 Mcal of net energy for lactation (NEL) from the diet, but T2 cows only retained 9.2 Mcal of NEL. Treatment 1 cows had better energy balance to restart reproductive activity than T2 cows.

Several studies have shown that glucose synthesis in cows can be stimulated with the use of gluconeogenic precursors such as propionate (14) or propylene glycol (15), so this may be an option to correct NEB without reducing NDF consumption. The MP allowable milk was even more critical for both groups. In T1 cows, of the 15.7 kg of milk produced, 12.8 kg were covered by dietary protein; in T2 cows, of the 11.8 kg of milk produced, only 6.1 kg were provided by dietary protein.

A trade-off between MP for lactation and reserve was observed between treatments, being 117 g greater the MP for lactation in T1 cows, and the MP for reserve in T2 being 133 g greater, perhaps because protein stress was more accentuated in T2 cows, since only 79% of their MP requirements were covered. Dietary protein represents 42 to 50% of the total cost of dairy cattle concentrates (16) and plays an important role in the profitability of the production system, as it affects the performance of dairy cows and the environment (17).

A direct metabolic strategy to save N is to tend to secrete less milk-urea N (mg/dL), which was 0.93 and -0.90 (p=0.17) for T1 and T2 cows, respectively. In an investigation with medium-production cows, the increase of MP supplies did not modify DMI, however, milk protein, fat and lactose increased linearly. It was also found that the efficiency of MP decreased from 0.70 to 0.60 in high to low MP supplies, respectively (18).

Different models of nutrient requirements use different MP efficiencies to predict protein requirements in lactating dairy cows; for example, the NRC (19) model proposes a MP efficiency of 0.67, which is higher than that of INRA (20) and CNCPS (13), which propose MP efficiencies of 0.64 and 0.65, respectively.

Given the experimental conditions, T1 cows managed ($p<0.05$) to consume more ME/d (32.5 *vs* 26.9 Mcal), derive more ME for lactation (19 *vs* 12.1 Mcal) and produce more milk (15.8 *vs* 12.5 kg/d). However, in our study the first limiting nutrient was always MP; cows on T1 and T2 ($p<0.05$) used 1170 and 896 g/d, respectively, of which 658 and 570 g/d were destined for lactation, enough to produce just 11.8 and 6.0 kg of milk/d. Both treatments had a negative MP balance, covering only 88 and 76% of the requirement. The consequence for T2 cows was that they had to reduce the N-urea in milk (0.73 *vs* -0.27; p= 0.04) to save N.

It has been shown that increasing dietary protein supplies below the requirement increases protein in milk; however, NEL decreases (14,21). Part of the MP deficiency was due to a reduction in microbial protein synthesis because only about 80% of the NH_3 required for the fermentable organic matter available in the rumen was provided.

Even though T1 cows consumed more ME (33.2 *vs* 28.1 Mcal/d), the difference was not derived entirely towards milk production, but in equal parts towards reserve. There must be some signaling (perhaps hormonal) that tells the cow how to distribute the nutrients for lactation and reserve, depending on the physiological and nutritional state in which she is. Some authors (22) explained that more glucogenic diets (as is the case of T1) decrease energy expenditure for milk, and tend to stimulate the partition of energy towards body reserves, and improve energy balance and reproductive performance in comparison with lipogenic diets (lower energy density in the diet) due to a lower plasma concentration of BHBA, NEFA and FFA in the liver. This signaling could also explain why T2 cows derived more MP allowable milk than T1 cows (585 *vs* 669 g; p= 0.02) during this period, still maintaining negative urea N in milk (1.2 vs -0.63 mg/dL) and NH_3 excretion low (30 *vs* 12 g/d).

Cows on T1 lost less BCS; this advantage was reflected in a higher ($p<0.05$) pregnancy rate at first service. This result indicates that T1 cows, although they did not derive enough energy from gluconeogenesis for greater lactogenesis, did provide the necessary glucose that the ovarian follicles use for their development and maturity, which together with the FTAI protocol, culminated in that more than half of the cows became pregnant at first service, unlike T2 cows, which less than half responded to the ovulation synchronization treatment at day 70 postpartum.

Dairy cow's fertility problems in the humid tropics are conditioned by several factors: environmental conditions, management, lack of energy in the diet, health, infrastructure and the breed groups used; all these factors influence cow's reproduction (23). It is difficult to determine a causal basis for the decline in fertility, as genetics and the environment have markedly changed over the past few decades. Part of the observed decrease in fertility is caused by genetic selection for higher milk production; in addition, the heritability of reproduction traits is low (24).

Nutritional influences during the transition period (± 4 weeks peripartum) may be of importance (25), but the effect of diet on fertility during this period is complex and multifactorial. Therefore, the use of FTAI allowed to serve the cows shortly after the voluntary waiting period, regardless of the cyclical state in which they were, increasing the service rate (26). In this sense, the hormonal techniques used to accelerate the resumption of the ovarian activity during the postpartum period have a great impact on cattle production (26,27).

It is recommended that in the period of 45 to 60 days postpartum the ME concentration in the ration be increased. At the same time, the NDF concentration in the ration must be reduced, because it was shown that cows had a limit to consume fiber (1% of their BW). Above this percentage, the consumption of DM and nutrients is compromised. Since the increase of ME in the ration of crossbred cows in the tropics is limited by a high consumption of NDF, the administration of glucogenic precursors during the transition period could result in a positive effect.

Another observation from this study is that MP availability was always low during lactation. Under tropical conditions, MP will always be the first limiting nutrient, since tropical grasses are low in protein (28). Under the conditions of the present study, to produce 14 kg of milk/d at least 30 Mcal of ME and 1,350 g of MP are required. Another observation in T2 cows is that MP deficiency resulted in lower N content in milk, deficiency that directly impacts the cheese industry, which is the main destination of milk in the region. On the other hand, supporting with restricted suckling (8 h after milking) and temporary weaning of 72 h after CIDR removal when applying the FTAI hormonal protocol, the fertility of cows in the tropics was also maximized by implementing a program of synchronization of ovulation at day 70 postpartum. Up to 75% first service pregnancy rate was obtained with open periods from 105 to 117 days and calving interval from 13 to 14 months, results that would also impact cow's reproductive efficiency in the tropics of Mexico.

It is clear that achieving a balance between energy and protein in early lactation will improve energy balance and fertility, and suggests that starch and sugars (glucogenic diets) may have different effects on the proportion of cows that conceive with artificial insemination (22).

In conclusion, at the beginning of lactation the energy demand of T1 cows requires maximum consumption, taking advantage of the ruminal distension factor. It is recommended that 45 to 60 days postpartum the concentration of ME be increased. The concentration of NDF in the ration must be reduced, because it has been shown that cows have a limit of 1% of their BW to consume fiber. The increase of ME in the ration of crossbred cows in the tropics is limited by a high consumption of NDF, so that the administration of glucogenic precursors during the transition period could be favorable. Since the MP balance was always negative, it is necessary to focus on the contribution of specific amino acids during the first 15 days of lactation. The decrease in NDF in the diet from 44 to 38% and the application of the FTAI hormonal protocol reduce the open period to 105 d in lactating cows in the tropics.

Conflict of interest

The authors have no conflicts of interest.

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