Journal MVZ Cordoba
2021; January-April. 26(1):e1958.
https://doi.org/10.21897/rmvz.1958

Original

Effect of restricted suckling and feed complementation on weight and cortisol in Simbrah calves

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Received: February 2020; Accepted: August 2020; Published: November 2020.

ABSTRACT

Objective. Determine the effect of restricted suckling, early weaning and feed supplementation on weight gains and plasma cortisol concentration of Simbrah calves. **Materials and methods**. One hundred and twenty 15-day-old lactating calves were distributed in two treatments: T1) calves with restricted suckling, early weaning and feed supplementation (n=30 males/n=30 females); and T2) calves with continuous suckling without feed supplementation (n=30 males/n=30 females). Calves were weighed at the beginning of the experiment, and every 15 days to assess changes in body weight from weaning unto 0 months of age. Blood sampling was carried out at the start of study and for 10 days between 7:30 and 8:30 am. Response variables were evaluated using general linear models. **Results.** The weight (310.1 and 268.5 kg) and post-weaning weight gain (0.980 kg/day and 0.800 kg/day) was better in the T1 treatment than, for calves in T2 (168.3 and 159.6 kg; and -0.500 and -0.480 kg/day) for males and females, respectively. The cortisol concentration (ng/mL) was higher in T1 compared to T2 from day 0 to day 3 ($p<0.05$), but on day 4 no difference was found. T1 allowed weaning at 3.5 months of calf life and increased post-weaning weight gain. **Conclusions.** Live weight and post-weaning weight gain were better in T1 treatment animals; however, plasma cortisol levels were increased at the star of the study to subsequently decrease.

Keywords: Early weaning; nutrition; stress; suckling; animal welfare (*Source CAB*).

RESUMEN

Objetivo. Determinar el efecto del amamantamiento restringido, destete precoz y complementación alimenticia sobre las ganancias de peso y concentración plasmática de cortisol en becerros Simbrah. **Materiales y métodos.** Se utilizaron 120 becerros lactantes de 15 días de edad, distribuidos en dos tratamientos: T1) becerros con amamantamiento restringido, destete precoz y complementación

How to cite (Vancouver).
Severino-Lendechy VH, Montiel-Palacios F, Gómez-de-Lucio H, Peralta-Torres JA, Segura-Correa JC. Effect of restricted suckling and feed complementation on weight and cortisol in Simbrah calves. Rev MVZ Cordoba. 2021; 26(1):e1958. https://doi.org/10.21897/rmvz.1958

ISSNL: 0122-0268

alimenticia (n=30 machos/n=30 hembras) y T2) becerros con amamantamiento continuo sin complementación alimenticia (n=30 machos/n=30 hembras). Los becerros se pesaron al inicio del experimento, y cada 15 días para evaluar los cambios en peso corporal del destete hasta los 10 meses de edad. Los muestreos sanguíneos se realizaron al inicio del estudio y durante 10 días, entre las 7:30 y 8:30 am. Las variables de respuesta se evaluaron usando modelos lineales generales. **Resultados.** El peso (310.1 y 268.5 kg) y ganancia de peso postdestete (0.980 kg/día y 0.800 kg/día) fue mejor en el tratamiento T1 que, para los becerros en T2 (168.3 y 159.6 kg; y -0.500 y -0.480 kg/día) para machos y hembras, respectivamente. La concentración de cortisol (ng/mL) fue mayor en T1 comparado con T2 del día 0 al día 3 (p<0.05), pero el día 4 no se encontró diferencia. El T1 permitió el destete a los 3.5 meses de vida de los becerros e incrementó la ganancia de peso posdestete. **Conclusiones.** El peso vivo y ganancia de peso posdestete fue mejor en los animales del tratamiento T1; sin embargo, los niveles de cortisol plasmáticos se incrementaron al inicio del estudio para disminuir posteriormente.

Palabras clave: Destete precoz; nutrición; estrés; amamantamiento; bienestar animal (*Fuente CAB*).

INTRODUCTION

Controlled or restricted suckling, as well as temporary, early or early weaning, are breeding techniques applied in beef cattle (1,2), which seek to raise the calf in part, temporally or in full after birth. The use of food supplements (milk substitute, balanced feed and green or dry forage), and restricted suckling are intended to: 1) gradually decrease the dependence of the offspring on dam´s milk and develop ruminal capacity, as soon as possible, to be weaned at a young age. 2) improve the cow's reproductive behavior (postpartum anestrus) by decreasing the stress of lactation and the cow-calf bond that is generated by suckling (3,4,5).

Improvements have been reported in some reproductive characteristics of cows (early restart of cyclicity, improvement in body condition score and increase in gestation rate) (6,7). However, the calves, having a higher consumption of udder milk and low or no solid food consumption (balanced feed and/or forage) do not have an efficient rumen development. Therefore, at weaning, the offspring undergo abrupt changes at a physical, social and physiological level (increased bellowing, agitation, constant walking and reduced food consumption) that can affect their status quo, generating stress in animals and in some cases death (5,8,9). Under these circumstances, the use of restricted suckling or controlled early weaning methods are positive for the cows (2,10); but negative for the calves, due to the lack of animal welfare, by causing stress by separating them from the mother (1).

The use of these technologies in tropical regions is limited (11,12,13), due to various factors that discourage their implementation by producers, such as feed costs and constant animal management. Therefore, the management that the calves receives during the separation process from the mother at an early age must strategically be used. That is, the offspring must be feed supplemented with food according to their physiological stage, facilitating their adaptation to the change of diet (milk-solid food). At the same time, animal welfare indicators should be used, which allow knowing the stress level that the offspring experience during growth, to reduce the stress and make it more productive (10,14).

Few studies have determined the effect of feed supplementation and stress level, caused by weaning, on post-weaning performance in beef cattle, especially Simbrah cattle, in tropical regions (5,10,11,13). The objective of this study was to determine the effect of restricted suckling, early weaning and feed supplementation on ~~body~~ weight gain and serum cortisol concentration in Simbrah calves at weaning and after weaning.

MATERIALS AND METHODS

The procedures carried out during this experiment adhered to the Official Mexican Standard (15), published in the Official Gazette of the Federation on the Technical Specifications for the production, care and use of laboratory and experimental animals.

Geographical location of the production unit. The study was carried out in a livestock production unit dedicated to the production of calves for slaughter. The farm was located in Macuspana, Tabasco, Mexico (17° 45′ 17 ″ N and 92° 33′ 32″ W), at a height 10 meters above sea level, with tropical climate, temperature and annual average precipitation of 26.4°C and 3186 mm, respectively (16).

Characteristics of the study and of the calves. Prior to the experiment, a 90-day breeding was performed in 300 cows with 75±15 days post-partum and 3.5±1.5 calvings. The cows were synchronized with conventional protocol using natural progesterone, and mated with bulls previously evaluated andrologically, to give effective service. The pregnancy diagnosis was carried out 60 days at the end of breeding. After calving, 120 Simbrah (n=60 males and n=60 females) lactating calves (15±2 days of age, and 63.8±6.5 kg body weight) were selected, and identified according to the existing records of the production unit.

The internal deworming of the calves was carried out with Levamisol Hydrochloride 15%, (Lab. Genfar, dose 1 mL/20 kg intramuscularly (im)) and external deworming with Flumethrin 1% (Lab. Bayer, dose 10 mL/100 kg of weight topically). In addition, the animals received an iron ferric hydroxide complex (Lab. Bayer, dose 1000 mg via im) and fat-soluble vitamins such as Vitamin A 500,000 IU, Vitamin D3 75,000 IU, Vitamin E 50 IU (Lab. Bayer, dose 2 mL via im). Animals were vaccinated against rabies virus at 3 and 9 months of age using an inactivated vaccine (Pasteur RIV strain: ≥2 IU; Lab. MSD, dose 2 mL via im) and against *Bacillus anthracis* (Sterne strain with a concentration minimum of 2,000,000 spores not encapsulated per mL; Lab. Bayer, 1 mL dose subcutaneously). Calves were also vaccinated against *Pasteurella multocida* type A and D, *Mannheimia* (*Pasteurella*) *haemolytica* A-1, *Clostridium chauvoei* and *Clostridium septicum*, (Lab. MSD, dose 5 mL via im).

Treatments. The experiment lasted 303 days (10 months), the calves were identified with progressive numbers and were randomized to the following treatments. T1) 60 calves with restricted suckling and feed supplementation during weaning and post-weaning (n= 30 males/ n= 30 females), and T2) 60 calves with continuous suckling without feed supplementation during weaning and post-weaning (n=30 males /n=30 females). The calves from the T1 treatment were separated from the dam 15 days after birth and kept in a pen with firm-floor, semi-roofed and a vital space of 5 m^2/animal. The calves suckled once a day in the morning (7:00 a.m. to 8:00 a.m.) until weaning (3.5 months old) bringing the dam to the pen. In addition, the calves received feed supplementation with balanced feed *ad libitum* (21% CP and 75.26% TDN). After weaning, dietary supplementation was given at a rate of 2.5% live weight per animal/day. Throughout the process, dry forage was offered, arranged in bales of Humidicola grass (*Brachiaria humidicola*) and water *ad libitum*.

T2 calves remained with the dam since birth, which were kept in a rotational grazing system (every 5 days / paddock) in 30 paddocks of 5 ha. The paddocks were sown with MG5 grass (*Brachiaria brizanta*; 10% of CP) and Humidicola grass, (7.5% of CP), in an area of 150 ha with availability to drinking water in mobile drinkers and freely accessible mineral salts (8% phosphorous). Subsequently, upon reaching 7 months of age, the calves were weaned and managed under the same feeding system mentioned above.

Measurement of body weight change. At the beginning of the study (15 days of age), the calves of both treatments (T1 and T2) were weighed with a livestock scale with capacity to 2000 kg (Revuelta®, México), and then every 15 days until 303 days of age. With the information obtained, weaning weight, weaning weight gain, total weaning weight gain, postweaning weight, postweaning weight gain, total postweaning weight gain, and total weight gain were determined.

Blood sampling and serum cortisol determination. Blood samples were taken between 7:30 and 8:30 am, in order to determine the serum concentration of cortisol, due to the effect of separating the calf from the cow. Calves from treatments T1 and T2 were sampled from day 0 (start of treatment) to day 10 (30 min before and 30 min after separating them from the dam), by puncturing the coccygeal vein, with a 21G x 38 mm gauge needle and 6 mL Vacutainer® tubes, without anticoagulant.

The samples were centrifuged at 700 g/10 min, using a HETTICH Zentrifugen® brand centrifuge (model D-78532 Tuttlingen, Berlin, Germany) in a time not exceeding 4 hours after the samples were taken. The serum was separated and aliquots made that were frozen at -20°C until the determination of the cortisol concentration (ng/mL), by means of solid phase enzyme immunoassay (ELISA), in the Laboratory of Physiology of the Faculty of Veterinary Medicine and Animal Science of the Universidad Veracruzana.

Commercial cortisol kits (Cortisol, EIA-1887, DRG Diagnostics, Germany) were used, following the manufacturer's instructions, where the specificity of antibodies for cortisol and corticosterone cross-reaction was 100 and 45%, respectively, and the precision of the intra and inter-trial variation was 5.63 and 6.93%, respectively. The ELISA plates were read at 450 nanometers in an HLAB® brand ELISA reader (model HReader1, HLab supply LTD, Berlin, Germany).

Statistical analysis. Weights at the beginning of the study, at weaning, after weaning, total weight gain from weaning up to 303 days, and total weight gain at start of study up to 303 days were analyzed using a general model that included the fixed effects of treatment and sex of the calf. The variables daily gain from the beginning of the study up to 303 days and weaning at 303 days, as well as the data on serum cortisol concentration were evaluated using a general linear model where the animal was considered the experimental unit and the weighing of the animal or cortisol sampling were the repeated measurements.

The general model that described the variables with repeated measures is shown below:

$$Y_{ijkl} = m + t_i + s_j + a\,(t * s)_{ijk} + r_l + e_{ijkl}$$

Where:
Y_{ijkl} = any of the response variables with repeated measures.
m = the general mean of the data of the variable of interest.
t_i = fixed effect of the i-th treatment.
s_j = fixed effect of the j-th calf sex.
$a(t*s)_{ijk}$ = random effect of the k-th animal nested within the treatment-sex combinations (experimental error, NID $(0, \sigma^2)$).
r_l = fixed effect of the l-th measurement.
e_{ijkl} = random effect of NID residual error $(0, \sigma^2)$.

Treatment and sex means were compared using adjusted p-values for multiple comparisons by Tukey's range tests. All statistical analyzes were performed using the SPSS package (17).

RESULTS

Differences were found between treatments for pre and post-weaning characteristics, as well as sex and number of weighing or sampling. The weaning weight of male and female calves, the daily weight gain and total weight gain at weaning for animals in treatment T1 were lower ($p<0.05$) compared to treatment T2. However, after weaning, the calves (males and females) of the T1 treatment had higher post-weaning weight, daily weight gain and total weight gain with respect to T2 (Table 1, Figure 1).

Tabla 1. Means ± standard deviations by treatment and sex for pre-and post-weaning productive characteristics in Simbrah calves in the tropics of Mexico.

Treatment	T1			T2
Sex/Variable	**Male (n=30)**	**Female (n=30)**	**Male (n=30)**	**Female (n=30)**
Initial body weight (kg; 15 days of age)	63.5±7.0^a	61.0±5.0^a	62.1±6.1^a	60.1±5.5^a
Body weight at weaning (kg)*	104.3±6.4^a	98.5±5.4^a	213.3±35.3^b	202.9±25.1^c
Daily body weight gain unto weaning (kg/day)	0.450±0.2^a	0.400±0.5^b	0.720±0.6^c	0.680±0.7^c
Total body weight gain unto weaning (kg)	40.5±5.2^a	36.5±6.2^a	151.2±30.7^b	142.8±21.7^b
Post-weaning body weight unto 303 days (kg)	310.1±20.1^a	268.5±21.5^b	168.3±25.1^c	159.6±24.2^c
Daily post-weaning daily body weight gain (kg/day)	0.980±0.3^a	0.800±0.1^b	-0.500±0.2^c	-0.480±0.3^c
Total post-weaning body weight gain (kg)	205.8±19.3^a	168.9±19.3^b	-45.0±7.1^c	-43.3±7.1^c
Total body weight gain (kg)**	246.3±12.2^a	205.4±13.2^b	106.3±14.6^c	99.5±14.6^c

[a,b,c] Different letters by rows indicate statistically differences ($p<0.05$; Tukey test); T1= Restricted suckling, early weaning and feed supplementation; T2= Continuous suckling without feed supplementation. * Calves of T1 were weaned at 105 days and those of T2 unto 210 days; ** Total body weight from 15 to the 303 days of age.

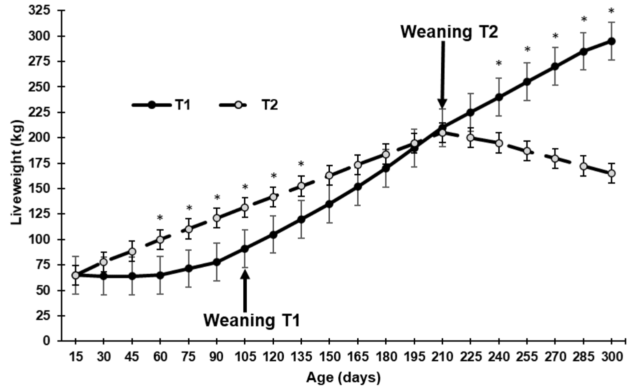

Figure 1. Body weight of Simbrah calves with restricted suckling, early weaning and feed supplementation (T1) and continuous suckling without feed supplementation (T2) under tropical conditions.

No difference was found between males and females for cortisol concentration, but treatment and sample number were. The average cortisol concentration (ng/mL) in the first blood sample, from days 0 to 10 (before the separation of the calf from the dam) showed no statistically significant difference between T1 treatment calves with respect to T2. However, in the second sampling on day 0 to 3, a difference ($p<0.05$) was found between treatments (Table 2). That is, the plasma cortisol concentration in male and female T1 and T2 calves was similar before separation from the dam (day 0 to 10), but not after separation (day 0 to 3). From day 4, the cortisol concentration was similar before and after the separation of the calves from the dam (Table 2).

Table 2. Mean ± standard deviation by treatment, sex and number of sample (30 min before and 30 min after separation of the calf from the dam) for cortisol concentration of Simbrah calves.

Treatment	T1				T2			
Sex/Days	Male (n=30)		Female (n=30)		Male (n=30)		Female (n=30)	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
0	10.5±2.5[a]	56.2±8.0[b]	11.5±2.6[a]	57.2±7.6[b]	9.9±2.7[a]	10.1±1.5[a]	10.9±2.4[a]	11.7±1.7[a]
1	11.7±1.2[a]	41.0±6.2[b]	11.5±1.2[a]	40.0±7.1[b]	10.5±2.3[a]	11.5±2.1[a]	10.1±2.3[a]	11.4±2.3[a]
2	9.7±1.4[a]	31.2±2.6[b]	9.9±1.5[a]	30.2±2.6[b]	9.9±1.9[a]	10.5±1.0[a]	9.5±2.9[a]	10.4±1.0[a]
3	10.5±2.1[a]	20.3±1.5[b]	10.3±2.2[a]	21.3±1.7[b]	10.9±1.8[a]	10.5±2.9[a]	10.7±1.8[a]	10.8±2.5[a]
4	9.7±1.5[a]	10.1±2.7[a]	9.5±1.9[a]	10.2±2.5[a]	9.5±1.3[a]	10.5±2.1[a]	9.3±1.3[a]	10.6±1.4[a]
5	9.7±1.3[a]	9.1±1.7[a]	9.4±1.5[a]	9.1±1.5[a]	9.5±1.3[a]	9.5±1.1[a]	9.6±1.3[a]	9.7±1.1[a]
6	10.0±1.0[a]	9.0±1.2[a]	10.0±1.5[a]	9.5±1.2[a]	9.8±1.5[a]	9.9±1.9[a]	9.9±1.7[a]	10.1±1.5[a]
7	9.9±1.0[a]	9.6±1.2[a]	9.8±1.0[a]	9.5±1.5[a]	9.5±1.6[a]	9.7±1.7[a]	9.3±1.6[a]	9.5±1.7[a]
8	9.6±1.6[a]	9.8±1.4[a]	9.5±1.5[a]	9.7±1.4[a]	9.7±1.8[a]	9.8±1.1[a]	9.5±1.7[a]	9.8±1.7[a]
9	9.8±1.7[a]	9.8±1.8[a]	9.7±1.7[a]	9.9±1.5[a]	10.2±1.8[a]	10.0±1.1[a]	10.0±1.2[a]	10.5±1.2[a]
10	9.5± 1.0[a]	9.8± 1.4[a]	9.9±2.0[a]	9.8±1.5[a]	9.7±1.5[a]	9.8±1.1[a]	9.8±1.9[a]	9.7±1.1[a]

[a,b,c] Different letters by rows indicate statistically differences ($p<0.05$; Tukey test); T1= Restricted suckling, early weaning and feed supplementation; T2= Continuous suckling without feed supplementation.

DISCUSSION

In this study, the productive performance, physical and physiological welfare of calves subjected to two rearing strategies were compared. The differences between treatments, found here, are attributable to the adaptation process in the consumption of solid food (quantity and time) and to the development at the gastrointestinal level, necessary for the digestibility and absorption of nutrients from solid foods (8,9) of T1 treatment animals compared to T2. The adaptation process in T1 began after the calves were separated from the dam, 15 days after birth, until being weaned at 3.5 months of age, unlike the T2 treatment calves that remained with the dam until 7 months old, when they were weaned. This adaptation process was also observed by Lambertz et al (5) in calves separated from their mothers 4 hours after birth, to whom milk (6 to 8 L /day) was supplied with a bottle until 36 days of age, and from that moment on, milk intake decreased to 4 L/day until weaning (6 or 8 weeks). It is worth mentioning that they were also offered pelletized food for calves (22% CP; ME = 2.63 Mcal/kg), oat straw (8% CP; ME = 1.55 Mcal/kg) and water *ad libitum*. In addition, in that study, it was reported that the calves that were weaned at 8 weeks compared to those at 6 weeks, had higher consumption of solid feed (1.36 and 0.40 kg/day) and weight gain at weaning (0.79 and 0.34 kg/day), respectively.

Those authors also mention that the same post-weaning performance (150 days of age) was observed in calves weaned at 8 weeks compared to 6 weeks for feed consumption (2.51 and 1.16 kg/day) and weight gain at weaning (1.05 and 0.35 kg/day). They attributed those differences to the animals' exposure time of 8 weeks to solid food intake and better gastrointestinal development compared to those of 6 weeks. In the present experiment, although the changes in body weight were better in the T2 treatment compared to T1 during the weaning stage, the trend in T1 was towards gaining weight, which indicate that there was a decrease in dependence on breast milk and a rapid adaptation to the consumption of solid food (5,9,18). de-Castro et al (19) report that daily weight gain (1.12 kg/day) was more efficient in feeding calves for longer periods (≥180 days) compared to short periods (≤90 days; 0.91 kg/day), in addition to being more costly. Hence, the benefit of given feed supplement to calves during the post-weaning stage.

Post-weaning body weight changes were better in calves of treatment T1 compared to those of treatment T2, observing a negative trend in daily weight gain and in total weight gain in T2. This was a consequence of the T2 calves being in the adaptation stage (weaning) that the T1 calves had already passed. What was relevant in the changes in body weight was the performance of the T1 animals with respect to the T2 animals, where the first ones did not present evident weight loss at the stage of separation (15 days of age), weaning or post-weaning, compared to those of T2, which lost weight after weaning (Figure 1). This was attributed to dependence on dam milk and limited ability to eat solid foods (20,21). This circumstance reduced the weight gained up to that moment under continuous suckling, and that finally put them at a disadvantage with the calves of the T1 treatment. This is explained because, from the weaning of the animals of the T2 group (7 months of age) and up to 10 months of age, the performance in the weight changes of the calves of the T1 treatment improved and that of the T2 calves decreased.

Pérez et al (13) reported that animals under temporary weaning (24, 48 or 72 hours) at 25 or 45 days after birth, and weaned at 150 days of age (130 kg) had greater weight gain at weaning (200 kg) and post-weaning (270 days of age), when they were isolated from the dam for 48 or 72 hours. This was regardless of age, compared to animals in the control group, and those weaned for 24 hours which weighed 175 kg. These differences were attributed to the previous separation process that the animals had experienced, which prepared the calves separated for 48 or 72 hours, to be better adapted to not having the dam present, and forced them to eat more solid food.

Pazoki (22) and Mageste et al (23) mention that food supplementation before and after weaning improves daily weight gain (≥0.800 kg/day) and the physical development of animals. However, in post-weaning, animals with previous exposure to the consumption of solid feed (concentrated feed and/or grass) developed better physical and chemical conditions that allowed the ruminant better stability of the ruminal pH, as well as spending more time on rumination of food (increased ruminal muscles). In addition, it is mentioned that the morphometric attributes of the rumen wall are stimulated and improved at the micro and macroscopic level, which increase and improve nutrient digestibility and metabolic status; characteristics that provide productive improvements to calves and economic improvements to producers.

From the economic and productive point of view, a calf that gains weight is better than one that loses weight (18). The decrease of milk dependency and the increase of solid food intake allows weaning male calves at a young age (≤ 5 months old) with good weight (≥209 kg), to be sent to fattening and finished at 10.5 months old and with 451 kg of body weight (18). Likewise, it is stimulated that females can start their reproductive life at ≤16 months of age (23), both economically profitable circumstances. Therefore, controlled suckling, early or temporary weaning or any other zootechnical technique that uses the temporary or permanent separation of the calf from the dam, coupled with the gradual stimulation of the consumption of solid food (balanced feed and/or forage), is an effective tool that reduces dependence on milk. In addition, inducing early weaning increases weight gain, and the physical and productive development of animals in the short term, in an economically profitable manner.

The temporal or permanent separation of the calf, for weaning purposes or productive improvement, affects it due to the loss or absence of the mother, access to the udder to consume milk, as well as, to changes in diet, in the social structure and physical environment, which alter its physical, physiological and welfare (14,24). To determine the effect of those changes, measures of the welfare of the animals, directly is reflected in the stress that the calf experiences during production and, which alter the plasma concentration of cortisol, heart rate, body temperature and locomotion have been proposed (2,10,24). However, changes in cortisol concentration seem to be particularly useful as an indicator of acute stress, as well as of the functioning of the hypothalamic-pituitary-adrenocortical system, in response to emotional and physical experiences (2,10).

Differences in plasma cortisol concentration, analyzed considering the average of the two cortisol samples, were evident on day 0 (T1; 32.8 and T2; 10.52; $p<0.05$). However, on day 1 (T1; 26.2 and T2; 11.4), 2 (T1; 19.9 and T2; 10.1) and 3 (T1; 15.3 and T2; 10.7), although significant difference was found, the concentration decreased as the days passed and, by day 4 (T1; 9.8 and T2; 9.9), the calves from treatment T1 and T2 had similar plasma concentration ($p>0.05$; Table 2). Therefore, calf separation by controlled suckling affects the stress levels of the animal, but that physiological state invariably stabilizes rapidly, indicating that the animals adapt to the separation within a few days.

Acevedo et al (10) report that plasma cortisol concentrations of calves with restricted suckling (RS; temporary separation, allowing suckling once a day for 30 min from 8:00 am to 8:30 am and forage consumption), and temporary weaning (TW; separation, without any type of contact for 72 hours, with forage consumption) were higher in TW (23.36 ng/mL), 24 hours after the starting of treatment. However, these values decreased significantly to levels similar to calves with restricted suckling on the second day (48 hours) (TW; 10.99 and RA; 11.44 ng/mL), and on the third day (72 hours), no differences found between treatments ($p>0.05$). This was attributed to the fact that animals with restricted suckling reduced stress through physical contact that is provided to them in a controlled and temporary way (when they are allowed to suckle) compared to calves subjected to temporary weaning that were totally separated from the dam. Although, of in the end the animals with temporary weaning on the second day of the experiment had already become accustomed to the isolation.

On the other hand, Pérez-Torres et al (2) determined the effect of stress caused by temporary weaning according to the age of the animals (25 and 45 days old), to which they permanently separated from the dam by a 24, 48 or 72 hours, providing them with commercial food (16% of CP), mineral salt and water *ad libitum*. It was observed that the 25-day-old calves had higher plasma cortisol concentration (ng/mL) (10.3, 5.3 and 2.0, at 24, 48 and 72 hours, respectively) compared to those temporary weaned 45 days (6.2, 2.1 and 0.8, at 24, 48 and 72 hours, respectively). These results were attributed to the cow-calf bond and a higher social dependence of young animals compared to older animals; although the plasma cortisol concentration decreased markedly in both groups at 72 hours after separation.

Therefore, considering the results of these studies and the present, it could be inferred that the separation of the calves in any of its modalities of temporary weaning or restricted suckling, generates stress in the animals. However, this physiological condition is a natural response and/or reaction of the animals to the change in their circumstances, which will decrease until normalizing in an average period of 48 to 96 hours after separation. Finally, the weaning process is better, stimulating the consumption of solid feed in calves, gradually and reducing the dependence on dam milk, so that they can adapt, calmly and without negative effects on weight gain, health and physical development.
In conclusion, restricted suckling, early weaning, and feed supplementation allowed weaning of male and female calves at 3.5 months of age and increased post-weaning weight gain. Live weight and post-weaning weight gain were better in T1 treatment animals. Plasma cortisol levels increased at the start of the study, but decreased later.

Conflict of interest

The authors of this paper declare no conflict of interest.

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