

Research Article

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Effect of bovine appeasing substance on multiparous Holstein cows during the transition period

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Abstract

The aim of the experiment reported in this research paper was to determine the influence of the bovine appeasing substance (BAS) on milk yield, energy metabolism, inflammation, and stress in cows during the transition period. Twenty-four multiparous Holstein cows (day 28 pre-partum to 21 postpartum) were distributed randomly into two groups: control (n = 12) and BAS (Secure Cattle[®]; n = 12). Each animal was administered 5 mL of the product on days 28 and 14 pre-partum and on the day of calving. The feed intake was assessed using automated, individual feeders and the milk yield was determined electronically. Six milk samples were obtained from each animal, which were analyzed for chemical composition and somatic cell count. Six blood samples were obtained per animal for future biochemical analyses (free fatty acids, beta hydroxybutyrate, cortisol, myeloperoxidase and paraoxonase 1). The statistical analyses were conducted with the JMP Pro 14 software, with $P \leq 0.05$ being considered as statistical significance. BAS-treated cows showed higher milk yield than controls. Dry matter intake (DMI) during the pre- and postpartum periods was greater for the control than treated group. The BAS group exhibited reduced plasma cortisol postpartum. In conclusion, cows treated with BAS showed higher milk yield, lower DMI, and reduced plasma cortisol concentrations than controls.

The term ‘well-being’ pertains to physical and psychological status relative to an individual’s efforts to adapt to the environment (Broom and Johnson, 2019). According to John Webster, a fitting breeding environment must favor five freedoms: (1) freedom from thirst, hunger, and malnutrition; (2) freedom from pain, injury, and disease; (3) freedom from discomfort; (4) freedom to express their natural behavior; and (5) freedom from fear and stress (Pacheco *et al.*, 2012). These guidelines facilitate the qualitative assessment of physical, mental, and natural factors of well-being and of the likely discomforts in each of these aspects.

Today, technologies that encourage animal welfare include the use of pheromones, which are the semiochemicals that can influence the behavior of conspecifics (Falewee *et al.*, 2006; Taylor and Mills, 2007; Temple *et al.*, 2016). These pheromones can be recognized by the vomeronasal (or Jacobson’s) organ (Crowell-Davis and Houpt, 1985; Kekan *et al.*, 2017). The porcine appeasing pheromone was first isolated from sow mammary glands (Pageat, 2001). Thereafter, these appeasing pheromones were investigated in other mammalian species, such as dogs, cats, and cattle (Pageat and Gaultier, 2003; Gunn-Moore and Cameron, 2004; Tod *et al.*, 2005; Hargrave, 2014; Osella *et al.*, 2018).

Bovine appeasing substance (BAS) is a synthetic pheromone similar to the natural hormone that cows secrete just after calving. It is composed of a blend of fatty acids that reproduces the original substance (Osella *et al.*, 2018; Cappellozza and Cooke, 2022). When BAS was used in cattle, it increased daily weight gain and the final weight of calves (Cooke *et al.*, 2020). Additionally, it lowered hair cortisol and haptoglobin levels in beef steers (Schubach *et al.*, 2020). Furthermore, it improved the immune response of animals with respiratory diseases (Hervet *et al.*, 2021) and supported increased milk production while decreasing somatic cell count (SCC) in dairy cows (Osella *et al.*, 2018).

The greatest challenge for dairy cattle welfare occurs during the peripartum period, known as the transition period, which begins 3 weeks before calving and lasts approximately 3 weeks after.

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During this time, cows undergo homeorhetic adaptations to support the rise in colostrum and milk production (Marett *et al.*, 2015). However, these metabolic adjustments can increase susceptibility to illnesses and immunological dysfunctions, with over 70% of illnesses occurring during this stage (Ortolani, 2009; Daros *et al.*, 2022). Recent research has examined the relationship between energy balance and immune function during this critical period. Understanding these interactions is vital for developing strategies to minimize the negative impacts associated with the transition period (Daros *et al.*, 2022). Therefore, implementing technologies that enhance animal welfare and support cow health during this phase is essential (Falewee *et al.*, 2006; Taylor and Mills, 2007; Carroll and Forsberg, 2007; Temple *et al.*, 2016; Hervet *et al.*, 2021; Cappelozza and Cooke, 2022).

In this context, the use of bovine appeasing substance (BAS) has shown promise. Studies suggest that BAS can improve milk production and quality, optimize energy metabolism, and strengthen the immune system of cattle (Osella *et al.*, 2018; Angeli *et al.*, 2020; Hervet *et al.*, 2021; Cappelozza and Cooke, 2022). Additionally, BAS may reduce the adverse effects of the transition period on the metabolism and performance of highly productive dairy cows.

Thus, this study aims to evaluate the impact of BAS on feed intake, milk production, blood parameters, energy metabolism, stress, and inflammation in dairy cows during the transition period.

Materials and methods

This study was conducted on a commercial property in the southern region of the state of Rio Grande do Sul (32° 16'S, 52 67° 32' E), with 400 housed lactating cows producing a total of 12,000 L/d. The cows were kept in a compost barn-type confinement system and were fed a total mixed ration (TMR) using automatic, individual feeders (AF 1000 electronic trough, Intergado®, Betim, Minas Gerais, Brazil) twice daily. The animals had access to water *ad libitum*. They were milked twice daily: at 8:00 and 20:00 h. All animal procedures were approved by the Animal Ethics and Experimentation Committee of the Federal University of Pelotas (project number 044683).

To determine sample size, blood cortisol levels were evaluated from three weeks prior to calving until to two weeks post-calving. The calculation was performed using the OpenEpi epidemiological calculator, following the formula available at (<https://www.openepi.com/SampleSize/SSMean.htm>). Therefore, for the sample size calculation of each cycle, a total sample size of 24 animals was determined.

Separation of animals and experimental design

Twenty-four multiparous cows were selected and monitored from day-28 pre-partum to day-21 postpartum. A completely randomized experimental design was adopted, and the cows were separated into two experimental groups: the control group (n = 12) and BAS group (n = 12) (SecureCattle®, Nutricorp®, Araras, São Paulo, Brazil; n = 12). The inclusion criterion was cows in their 2nd to 5th lactation, with an average lactation number of 3 for both groups. All animals were housed in a single compost barn-type shed, which was subdivided by electric fencing to maintain a minimum distance of 50 m between groups due to the volatile nature of the product. A dose of 5 mL/animal was administered topically at the nape on day-28 and day-14 relative to the expected calving date, and on the calving day (D0). No management procedures were performed

to simulate the application in the Control group (placebo), as the topical application did not induce stress. Throughout the experiment, a minimum distance of 50 m was maintained between the groups. The experiment took place from January to August 2022.

Feed samples and bromatological analyses

Corn silage and pre-dried ryegrass samples were obtained twice weekly to form a weekly pool. TMR samples from the experimental batches were also collected daily for dry matter analysis and a weekly pool was created for other analyses. The samples were frozen for analysis of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), non-fibrous carbohydrates (NFC), and mineral residue (MR).

The feed samples were analyzed at the Animal Nutrition Laboratory, in the NUPEEC Hub, at the Federal University of Pelotas. First, the samples were subjected to a 72-h pre-drying process in a forced circulation oven, set to 55 °C. Next, the samples were ground in a knife mill. For the definitive DM analysis, the ground samples were placed in an oven set to 105° C for at least 8 h (Easley *et al.*, 1965). For mineral matter analysis, 1 g of ground sample was weighed in triplicate and added to the muffle furnace at 600 °C for 3 h to burn the organic residue (Association of Official Analytical Chemists – International [AOAC], 1995). The value obtained was then subtracted to define the gross mineral matter.

To determine crude protein, the method of Kjeldhal (Association of Official Analytical Chemists – International [AOAC], 1995) for nitrogen determination was used, which included 4% (w/v) boric acid solution as a free ammonia receptor during distillation, a 0.2% (w/v) bromocresol green solution, and 0.1% (w/v) methyl red as the indicator, along with a standard sulfuric acid solution for titration (Kozloski *et al.*, 2003). Following this, the NDF and ADF analyses were performed as previously described (Van Soest and Robertson, 1985). NFC were estimated using the formula $NFC = 100 - CP - MR - EE - NDF$ (Sniffen *et al.*, 1992). The average chemical composition of the diets provided to the animals during the experimental phase is shown in Table 1. The formulated diets followed the recommendations of National Academies of Sciences, Engineering, and Medicine [NASSEM] (2021).

Feed consumption

Feed intake was recorded continuously, using automatic, individual intelligent feeders (Electronic Trough AF 1000, Intergado®, Betim, Minas Gerais, Brazil). The control group was housed on one side of the barn and had access to eight feeders, while the BAS group was restricted to the other side, with access to eight different feeders. According to the manufacturer's recommendations, there was a maximum 1.6 animals per feeder.

Animals were given a space in the compost barn that allowed sole access to the feeders via a radio-frequency identification tag. One TMR sample was obtained daily from the output and dried in an air fryer to ascertain the concentration of DM (Erker and Brus, 2023). The TMR was made available twice a day at 11:00 a.m. and 4:00 p.m.

Milk production and analysis of milk constituents

Milk production was assessed electronically using DelPro™ software (DeLaval®, Tumba, Botkyrka, Sweden), and in-person

Table 1. Average chemical composition and ingredients of pre- and postpartum diets during the experimental period

	Pre-partum	Postpartum
Nutrients		
Dry matter (%)	43.14	42.29
Non-fibrous carbohydrates (% DM)	22.71	26.79
Ether Extract (% DM)	3.09	4.90
Acid Detergent Fiber (% DM)	37.70	31.33
Neutral Detergent Fiber (% DM)	51.64	46.13
Crude Protein (% DM)	12.29	14.67
Mineral Residue (% DM)	9.47	8.20
Chlorine (g/kg DM)	6.20	*
Boron (mg/kg DM)	10.71	*
Calcium (% DM)	0.60	*
Copper (mg/kg DM)	22.42	*
Sulfur (% DM)	0.27	*
Iron (mg/kg DM)	506.02	*
Phosphorous (% DM)	0.43	*
Ingredients		
Concentrate pre-partum (kg)	5,00	-
Concentrate lactation (kg)	-	11,90
Corn silage (kg)	12,00	35,80
Oat hay (kg)	2,50	-
Pre-drying of ryegrass and clover (kg)	-	6,00

*Nutrient was not analyzed in the diet.

monitoring was performed during the two daily milkings. The milk from two sequential milkings was collected twice a week for each animal, totaling six samples per cow. The samples were preserved with bronopol and kept at room temperature, analyzed within 7 days, and sent to the Centralized Milk Quality Analysis Laboratory (LCAQL) (APCBRH, Curitiba, Paraná, Brazil) to analyze the milk constituents (fat, lactose, protein and total solids) via spectrophotometry, as well as the somatic cell count (SCC) via flow cytometry (NexGen, Bentley Instruments®, Chaska, United States).

Blood analyses

Six blood samples (4 mL) were retrieved per cow by puncturing the coccygeal arteriovenous complex on day-28 and day-14 before expected parturition, on the parturition day (D0), and on days 7, 14, and 21 postpartum. These collections were performed prior to the provision of the morning diet. The samples were kept at room temperature for a maximum of 30 min before processing and centrifuged at $1,800 \times g$ for 15 min. Serum samples were drawn and analyzed for free fatty acids (FFA), beta-hydroxybutyrate (BHB), myeloperoxidase (MPO), paraoxonase (PON-1) and blood cortisol. Plasma (KF-EDTA) was used for glucose analysis. The FFA, BHB and glucose were analyzed using an automatic biochemical analyzer (Labmax Pleno®, LabTest, Lagoa Santa, Minas Gerais, Brazil) with commercial kits (DiaSys Diagnostic Systems kits). Myeloperoxidase

was investigated using the enzyme-linked immunosorbent assay (ELISA; ELK Biotechnology CO., LTD, Wuhan, Hubei, China) with an ELISA plate reader at a wavelength of 450 nm (Thermoplate, Palm City, FL, USA). PON-1 activity was assessed using a commercial kit (ZeptoMetrix Corporation®, USA) via kinetic spectrophotometry (T80 UV/VIS, PG Instruments, England) in duplicate. Blood cortisol was analyzed using an immunoassay analyzer (Access 2, Beckman Coulter®, Brea, California, United States) with a commercial kit (Access Cortisol kit). The Metabolism Laboratory, NUPEEC Hub (Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil) supplied all the equipment utilized for these tests. Intra- and inter-assay precision was determined by analyzing six samples obtained on the same or different days, and the coefficient of variation remained below 5%.

Clinical and zootechnical evaluations

Clinical examinations of all the animals were performed weekly, and their weight and body condition score (BCS) were recorded. A bovine weighing tape was used to record the weights by positioning it posterior to the scapulohumeral joint to determine the circumference of the thoracic perimeter. Heart rate, respiratory rate, rumen movements, rectal temperature, color of the ocular and vaginal mucous membranes, and capillary refill time were evaluated (Feitosa, 2020).

Feed efficiency was calculated as total milk production divided by DMI (Blake and Custodio, 1984). A trained evaluator assessed BCS on a scale of 1 to 5, using 0.25 subdivision points (Edmonson *et al.*, 1989). Energy balance (EB) was calculated for each cow using previously described equations. Net energy intake (NEI) was determined by multiplying daily DMI by the laboratory-calculated net energy for lactation (NEL) density of the diet. Net energy for maintenance was calculated as $NEM = 0.10 \times BW \text{ kg}^{0.75}$.

Requirements for NEL for milk production were calculated as $NEMILK = 9.29 \times \text{kg fat/kg milk} + 5.5 \times \text{kg crude protein/kg milk} + 3.95 \times \text{kg lactose/kg milk}$. NEL available (Mcal/kg loss) was calculated as $NEL \text{ available} = 5.6 \text{ Mcal NEL/kg BW loss}$. The equation used to calculate postpartum EB was $EBPOST \text{ (Mcal/d)} = NEI - (NEM + NEMILK + NEL \text{ available})$.

For the equations, milk production and DMI data were obtained daily during the 21 d postpartum; weight data were obtained from the two postpartum weights and averaged over the 21 d. The milk component data were from six postpartum samples and were averaged over the 21 d. Using daily intake and milk production data, milk efficiency (ME) was calculated with the formula $ME = \text{Milk Quantity (L)}/\text{Dry Matter Intake (kg)}$ (Nasrollahi *et al.*, 2017).

Statistical analysis

Statistical analyses were conducted using the JMP Pro 14 software. Analysis of the dependent variables was performed using a mixed analysis of variance by repeated measures (ANOVA) model, adopting the PROC MIXED procedure. The model was constructed with the treatment and time (days) variables, and interactions between variables were treated as fixed effects, while animals were regarded as a random effect. In the randomization process, both the number of lactations and the production in the previous lactation were considered. The Tukey test was used to compare the means between the groups. The covariance structure used was Variance Components, selected for having the lowest value in the Bayesian information criterion (BIC). Data were analyzed for normality and homogeneity of variance through histogram analysis and the

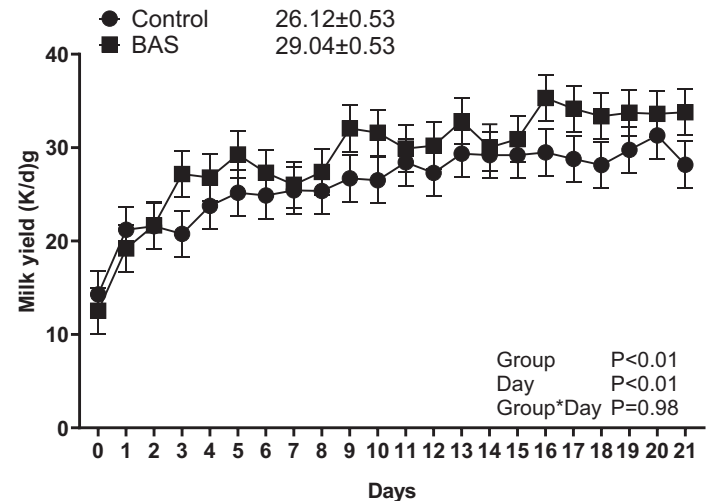


Figure 1. Milk yield of multiparous cows in the control and BAS groups over 21 d postpartum (data are shown as mean \pm standard error).

Shapiro-Wilk method, with a value above 0.80 considered acceptable. The accepted level of significance was $P \leq 0.05$, and trends were recognized if $P > 0.05$ and $P \leq 0.10$.

Results

The BAS group produced 29.04 ± 0.53 kg of milk/day, while the control group produced 26.12 ± 0.53 kg milk/day, a difference of 2.92 kg milk/day ($P < 0.05$) as shown in Fig. 1. Cows in the control group exhibited a DMI of 14.31 ± 0.40 kg/day pre-partum, compared to 10.34 ± 0.34 kg/day for the cows in the BAS group ($P < 0.05$) (Fig. 2A). Moreover, the control group exhibited a DMI of 16.24 ± 0.44 kg/day postpartum, while the BAS group consumed 12.91 ± 0.41 kg/day ($P < 0.05$) (Fig. 2B). This indicates that cows in the BAS group produced milk more efficiently despite lower feed consumption (feed efficiency: BAS = 2.21; Control = 1.38; $P < 0.01$).

The results of the blood analyses for the control and BAS groups are shown in Table 2. Pre-partum, all parameters for both groups were within the physiological range. Postpartum, the plasma cortisol level was lower in the BAS group ($P < 0.05$). Cows in the BAS group had an energy balance of -10.69 ± 0.92 Mcal/day, whereas cows in the control group had 3.01 ± 0.92 Mcal/day ($P < 0.01$). No differences were found in the parameters of FFA, BHB, MPO, glucose and PON-1 between the groups both prepartum and postpartum ($P > 0.05$).

The results for body weight are presented in Table 3 and values did not differ between groups ($P > 0.05$), with the control and BAS groups presenting mean prepartum body weights of 734 ± 11 kg and 735 ± 11 kg, respectively. However, there was a trend towards lower BCS in the BAS group compared to the control group in the prepartum period ($P = 0.07$). In the postpartum period, no differences were found in body weight and BCS parameters between the groups, with a mean weight of 705 ± 11 kg observed in the control group and 728 ± 11 kg in the BAS group, and BCS values of 3.56 ± 0.12 and 3.45 ± 0.12 , respectively ($P > 0.05$), as shown in Table 3.

Milk composition results for multiparous cows in both groups are shown in Table 4. The BAS group had slightly higher lactose levels and total solids than the control group, ($P = 0.10$ and $P = 0.08$, respectively). There were also no significant differences between

the groups for fat, protein, and SCC parameters ($P = 0.13$; $P = 0.42$ and $P = 0.99$, respectively; Table 4).

Discussion

In the present study, the milk yield was 2.92 kg/day ($P < 0.05$) higher in cows that received BAS than in controls. This is consistent with previous findings showing a benefit of 1.65 kg milk/day in cows receiving BAS during the transition between a confined and a semi-confined system (Osella *et al.*, 2018). The authors attributed this result to the reduced levels of environmental change-related stress. Cortisol can inhibit the production of oxytocin, which controls milk ejection. High output of cortisol can negatively affect prolactin levels which control milk production. Therefore, animals that experience stressful periods show decreased milk production caused by the negative effect that stress exerts on oxytocin and prolactin (Edmonson *et al.*, 1989; Bobić *et al.*, 2011).

BAS activity can reduce reactivity to neuroendocrine stress (Cappelozza and Cooke, 2022). This is consistent with our results, which demonstrated a clear BAS-induced reduction in plasma cortisol levels. A drop in serum cortisol following BAS treatment was previously described in Angus steers in a feedlot system for Colombo *et al.* (2020) 7 days after BAS application, as well as by Fonseca *et al.* (2021) when using BAS before transport and upon arrival at the feedlot in Nellore bulls. The pheromone used in this study likely decreased the response of the hypothalamic-pituitary-adrenal axis (Cappelozza and Cooke, 2022). In contrast, BAS did not reduce cortisol concentrations when administered to *Bos indicus* calves at weaning, as reported by Cooke *et al.* (2020), nor in Nelore calves at weaning (Schubach *et al.*, 2020). Cortisol concentrations have been extensively used to assess the adrenocortical responses in cattle (Carroll and Forsberg, 2007); however, the outcomes may be affected by stress during sampling (Cooke, 2017; Colombo *et al.*, 2020). Therefore, hair retrieval is suggested to determine hair cortisol, which provides a long-term response (Cooke, 2017). Schubach *et al.* (2020) observed a reduction in hair cortisol in calves at weaning after 14 days of BAS application. More recently, Pickett *et al.* (2024) also found lower hair cortisol in Angus calves using a reliever pheromone than in control animals. However, BAS had no effect on hair cortisol in studies by Cooke *et al.* (2020) and Schubach *et al.* (2020), suggesting that this

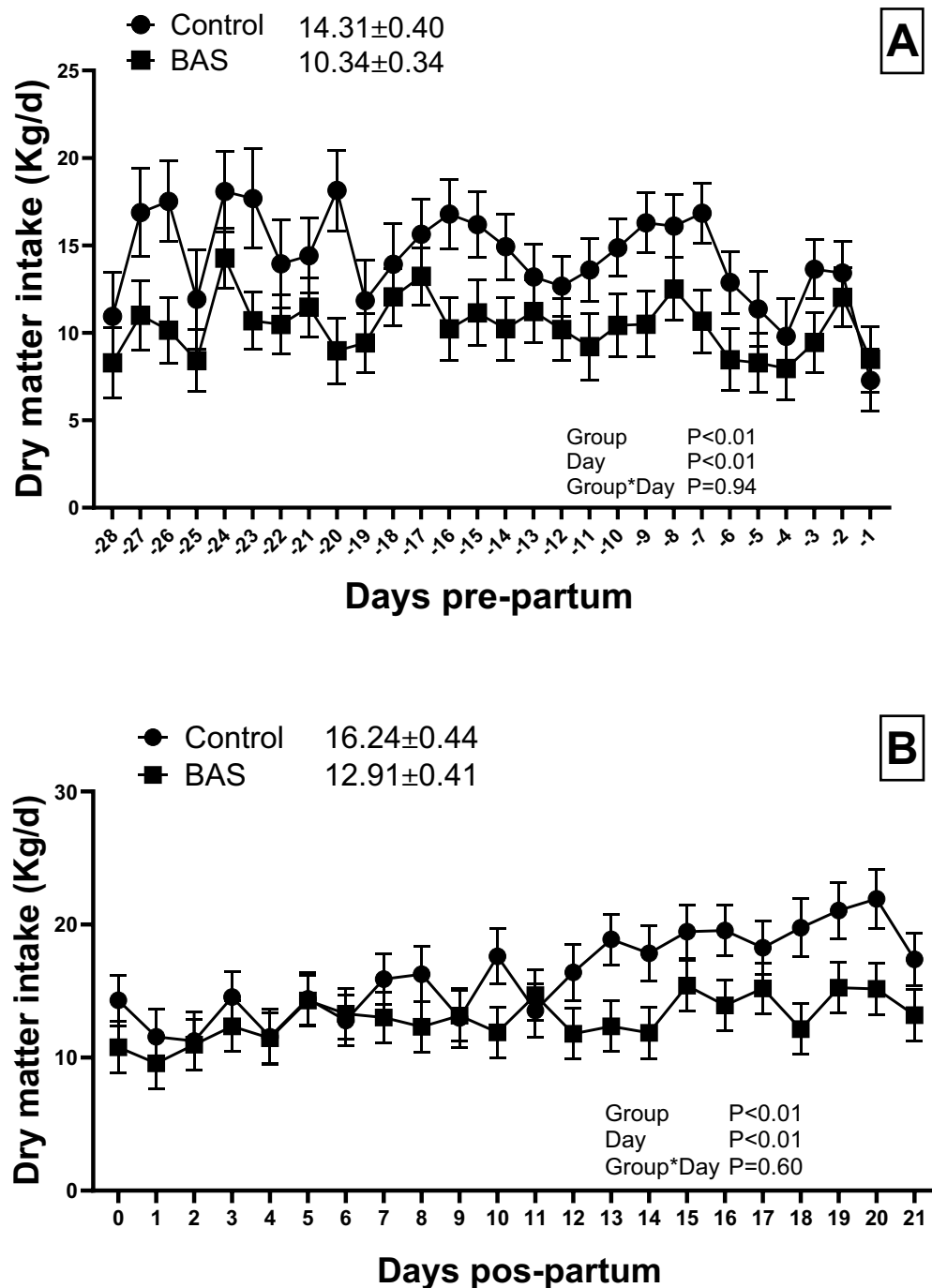


Figure 2. (A) DMI of multiparous cows in the control and BAS groups over 28 d pre-partum. (B) DMI of multiparous cows in the control and BAS groups over 21 d postpartum excluding calving day. All data are shown as mean \pm standard error.

substance likely affects metabolism through alternative metabolic pathways.

In this study, the influence exerted by the pheromone on the hypothalamic-pituitary-adrenal pathway partially explains the increased milk production in treated cows. However, the galactopoietic hormones, serotonin and prolactin, can also affect milk production. BAS is a well-known promoter of comfort and well-being with the ability to lower serum cortisol concentrations. Furthermore, serotonin plays fundamental roles in animal metabolism and is also an important hormone produced in the

mammary gland to assist in coordinating lactation (Forslund *et al.*, 2010). Recently, studies have demonstrated the influence of serotonin on calcium homeostasis during lactation in rodents and dairy cows (Schubach *et al.*, 2017; Connelly *et al.*, 2021), and several studies have demonstrated the relationship between serotonin and prolactin in humans and rodents (Noel *et al.*, 1974; Panerai *et al.*, 1985; Laporta *et al.*, 2015; Slater *et al.*, 2018). Therefore, we hypothesize that BAS increases serotonin concentrations by reducing cortisol levels, ultimately increasing prolactin secretion and milk production. Nonetheless, prolactin has been scarcely evaluated

Table 2. Energy balance and blood metabolites analyses of multiparous cows in the control and BAS groups

Variable	Groups		P values		
	Control	BAS1	Group	Day	Group*Day
Pre-partum					
Free Fatty Acids (mmol/L)	0.27 ± 0.03	0.29 ± 0.03	0.70	0.68	0.60
Beta-hydroxybutyrate (mmol/L)	0.46 ± 0.04	0.42 ± 0.03	0.36	0.22	0.76
Glucose (mg/dL)	59.13 ± 1.63	62.57 ± 1.46	0.12	<0.01	0.98
Cortisol (ng/mL)	20.94 ± 2.86	17.98 ± 3.30	0.51	0.37	0.72
Myeloperoxidase (ng/mL)	03.11 ± 0.14	3.37 ± 0.16	0.26		
Postpartum					
Free Fatty Acids (mmol/L)	0.52 ± 0.04	0.42 ± 0.03	0.46	<0.01	0.90
Betahydroxybutyrate (mmol/L)	0.57 ± 0.05	0.57 ± 0.04	0.92	0.11	0.90
Glucose (mg/dL)	54.49 ± 2.42	54.12 ± 2.34	0.91	<0.01	0.96
EB2 (Mcal/d)	-0.09 ± 0.92	-13.87 ± 0.92	<0.01	0.76	0.29
Cortisol (ng/mL)	15.70 ± 1.07	11.58 ± 0.92	<0.01	<0.01	0.98
Paraoxonase (U/L)	56.01 ± 3.09	59.48 ± 3.25	0.44	0.19	0.99
Myeloperoxidase (ng/mL)	3.05 ± 0.08	3.02 ± 0.08	0.75	0.92	0.42

1BAS: Bovine Appeasing Substance; 2EB: energy balance; The metabolites that have * do not present the P values on the day and group*day because there was only one analysis of this parameter in the pre-partum period. Data are shown as mean ± standard error.

Table 3. Body weight and body condition score of multiparous cows from the control and BAS groups

	Groups		P values		
	Control	BAS1	Group	Week	Group*Week
Pre-partum					
Body weight	734 ± 11	735 ± 11	0.92	0.33	0.77
2BCS	4.06 ± 0.12	3.75 ± 0.12	0.07	0.40	0.40
Postpartum					
Body weight	705 ± 11	728 ± 11	0.15	0.41	0.92
2BCS	3.56 ± 0.12	3.45 ± 0.12	0.52	0.17	0.74

1BAS: Bovine Appeasing Substance; 2BCS: Body Condition Score
Data are shown as mean ± standard error.

Table 4. Milk composition and somatic cell count (SCC) of multiparous cows in the control and BAS groups

Component	Groups		P values		
	Control	BAS1	Group	Days	Group*Day
Fat	3.95 ± 0.22	4.43 ± 0.23	0.13	0.77	0.58
Lactose	4.08 ± 0.06	4.24 ± 0.07	0.10	0.10	0.92
Protein	3.34 ± 0.05	3.41 ± 0.07	0.42	<0.01	0.98
Total solids	11.79 ± 0.29	12.63 ± 0.38	0.08	<0.01	0.98
2SCC	2.87 ± 0.10	2.87 ± 0.13	0.99	0.54	0.96

1BAS: Bovine Appeasing Substance; 2Somatic Cell Count (SCC): log₁₀/mL; Fat, lactose, protein and total solids: g/100 g.
Data are shown as mean ± standard error.

in studies with cattle. Greater clarity regarding the relationship between prolactin, serotonin, cortisol and peripartum conditions in dairy cows will significantly improve understanding on how well-being enhances milk production.

Cows receiving BAS produced milk more efficiently, with an intake of 3.33 kg of DM/day, which is less than that of cows in

the control group. These data are consistent with the mechanism of BAS which includes improved nutrient utilization resulting in greater feed efficiency (Apfelbaum, 1987; Cappelozza and Cooke, 2022). Reduced DM intake is a common feature of peripartum cows (Coiro *et al.*, 1987; Marett *et al.*, 2015) generally exerts harmful effects on animal metabolism. The negative energy balance

(NEB) during early lactation results from the gap between the energy needed for maintenance and milk secretion and the energy supply provided by feed intake (Drackley *et al.*, 2005). To sustain increasing milk yields during the first weeks of lactation, dairy cows must mobilize body reserves, mainly fat. The release of fatty acids (FA) from adipose depots is reflected in increased circulating FA concentrations (Schoenberg *et al.*, 2012). In the present study, reduced feed intake and higher milk production in cows treated with BAS resulted in a NEB. Despite this, no difference was found between the groups in terms of FFA levels. Additionally, the body weight of the animals did not differ pre- and postpartum, and the BCS tended to be higher in the control than in the BAS group pre-partum, which did not occur postpartum.

It was expected that, with reduced intake and higher milk production in the BAS group, the levels of BHB and FFA would increase, indicating mobilization of body reserves to meet energy demand (Grummer, 1995), which was not observed. Furthermore, it was expected that, as the control group had a higher DMI, glucose levels would be higher in these animals compared to the BAS group, which was not demonstrated (Grummer, 1995; Jermann *et al.*, 2022). However, this may have occurred due to the stability of glucose concentrations in cattle resulting from the action of insulin (Grummer, 1995; Fonseca *et al.*, 2021). These evaluations aimed to clarify the effect of BAS on the energy metabolism of dairy cows in the transition period, considering its already proven effects on health and welfare parameters. However, as there were no differences between the groups for these parameters, the route of action of this pheromone remains unclear, necessitating further studies focused on this aspect.

In addition, regarding milk composition and quality, it was expected to find effects of BAS mainly in the reduction of SCC in animals, associated with an improvement in the immune response resulting from the reduction of stress levels (Osella *et al.*, 2018). However, this was not demonstrated in our study. It is known that SCC, in addition to being indicative of mammary gland health (Santos and Fonseca, 2019), reflects litter and environmental quality in confined animals. Thus, considering that both groups had SCC greater than 200,000, it may be related to the management of bedding and milking (Magalhães *et al.*, 2006). Regarding the parameters of fat, protein, lactose and total solids, a difference was expected in the BAS group because it had a higher volume of milk produced, which can change compounds such as fat and total solids (Huhtanen and Rinne, 2007; Costa *et al.*, 2019), but it was not evidenced. There was a tendency to increase lactose and total solids in animals treated with BAS, indicating possible effects of the soothing pheromone on milk composition that can be better explored in future studies, as indicated by Osella *et al.* (2018).

In the parameters related to immunity, we did not find differences between the groups in the serum levels of myeloperoxidase, which is an inflammatory marker with a microbiocidal role, present in immune cells such as neutrophils (Prata *et al.*, 2016). Additionally, both groups showed similar levels of PON-1, indicating that the animals in both groups did not display signs of inflammatory processes. PON-1 is an acute-phase negative protein, meaning its values are reduced when there is inflammation (Pezzulo *et al.*, 2012). In the peripartum period, Bionaz *et al.* (2007) state that it is common to have a drop in PON-1 levels close to delivery, considering that the event itself involves inflammation to assist in the expulsion of the fetus and placenta (Sheldon *et al.*, 2014), however, we did not find a difference between the groups. It was expected that there would be differences in inflammatory markers in the BAS group, as reported by Hervet *et al.* (2021), but it is worth

noting that the evaluated period may have been too short to verify the effects of BAS on inflammatory responses.

Conclusions

In conclusion, the treatment with BAS during the transition phase significantly reduced plasma cortisol levels, leading to increased milk production while simultaneously decreasing dry matter intake (DMI). This outcome reflects an improvement in feed efficiency among multiparous Holstein cows. These findings suggest that BAS not only promotes animal welfare by mitigating stress but also enhances productivity, indicating its potential as an effective management tool in dairy farming.

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