


## Short communication: administering an appeasing substance to *Bos indicus*-influenced beef cattle at weaning and feedlot entry

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(Received 17 May 2019; Accepted 17 September 2019; First published online 22 October 2019)

The bovine appeasing substance (BAS) is expected to have calming effects in cattle experiencing stressful situations. Therefore, this study investigated the impacts of BAS administration during two of the most stressful events within beef production systems: weaning and feedlot entry. In experiment 1, 186 *Bos indicus*-influenced calves (73 heifers, 113 bulls) were weaned at  $211 \pm 1$  days of age (day 0). At weaning, calves were ranked by sex and BW, and assigned to receive BAS (Nutricorp, Araras, SP, Brazil;  $n = 94$ ) or water (CON;  $n = 92$ ). Treatments (5 ml) were topically applied to the nuchal skin area of each animal. Calf BW was recorded and samples of blood and tail-switch hair were collected on days 0, 15 and 45. Calves that received BAS had greater ( $P < 0.01$ ) BW gain from day 0 to 15 compared with CON. Overall BW gain (days 0 to 45) and BW on days 15 and 45 were also greater ( $P \leq 0.03$ ) in BAS v. CON. Plasma haptoglobin concentration was less ( $P < 0.01$ ) in BAS v. CON on day 15, whereas cortisol concentrations in plasma and tail-switch hair did not differ between treatments ( $P \geq 0.13$ ). In experiment 2, 140 *B. indicus*-influenced bulls (~27 months of age) from 2 different pasture-based systems (70 bulls/origin) were transported to a commercial feedlot ( $\leq 200$ -km transport; day -1). On day 0, bulls were ranked by source and BW, and assigned to receive BAS ( $n = 70$ ) or CON ( $n = 70$ ) and the same sampling procedures as in experiment 1. Bulls receiving BAS had greater ( $P = 0.04$ ) BW gain from day 0 to 15, but less ( $P < 0.01$ ) BW gain from day 15 to 45 compared to CON. No other treatment effects were detected ( $P > 0.14$ ). Therefore, BAS administration to beef calves alleviated the haptoglobin response associated with weaning, and improved calf growth during the subsequent 45 days. Administration of BAS to beef bulls at feedlot entry improved BW gain during the initial 15 days, but these benefits were not sustained throughout the 45-day experiment.

**Keywords:** appeasing, bovine, growth, pheromone, stress

### Implications

The bovine appeasing substance is expected to have calming effects in cattle under stressful conditions. In this study, bovine appeasing substance administration to beef calves at weaning reduced circulating concentrations of haptoglobin, a biomarker of stress-induced inflammation, and improved calf growth during a 45-day post-weaning period. Administering BAS to beef bulls entering a feedlot had initial benefits to growth, which were not sustained throughout a 45-day experimental period. Additional research is warranted to understand the underlying reasons for these outcomes; however, bovine appeasing substance appears to be an alternative to improve welfare and productivity of cattle exposed to stressful management procedures.

### Introduction

Beef cattle are inevitably exposed to psychologic, physiologic and physical stressors resultant from routine management (Cooke, 2017). These include weaning and feedlot entry, which elicit adrenocortical and acute-phase protein responses known to impair cattle immunocompetence and growth (Carroll and Forsberg, 2007). Hence, strategies to mitigate the stress elicited by management procedures are warranted to promote beef cattle welfare and productivity.

Appeasing pheromones have been initially discovered in swine, and shown to reduce agonistic behavior in piglets (Archunan *et al.*, 2014). Accordingly, synthetic analogues of appeasing pheromones have been developed for swine and various other species. In cattle, the synthetic analogue

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of the appeasing pheromone is based on a mixture of fatty acids, reproducing the composition of the original substance (Osella *et al.*, 2018). These latter authors administered the synthetic analogue (bovine appeasing substance, **BAS**) to Valdostana dairy cows during turn-out from confinement to pastures, which is a stressful event due to changes in social, housing and dietary habits. Cows administered BAS had greater milk yield and less milk somatic cell count, suggesting reduced stress caused by the change in management system (Osella *et al.*, 2018).

Research investigating BAS administration to cattle is still limited, particularly beef cattle managed under commercial conditions. Moreover, BAS may be of greater relevance to cattle with *Bos indicus* influence, due to their predisposition to psychological stress (Cooke, 2014). Therefore, we hypothesized that BAS administration will alleviate the stress caused by weaning and feedlot entry, and improve subsequent performance of *B. indicus* cattle. To test this hypothesis, this study investigated BW gain, adrenocortical and acute-phase protein responses in Nelore-influenced cattle receiving or not BAS at weaning (experiment 1) or feedlot entry (experiment 2).

## Material and methods

### Animals and treatments

In experiment 1, 186 Nelore-influenced calves (73 heifers, 113 bulls) were weaned at (mean  $\pm$  SEM)  $211 \pm 1$  days of age (day 0). Calf BW was recorded upon separation from the dam, followed by anthelmintic administration (Dectomax; Zoetis Brasil, São Paulo, SP, Brazil). Calves were then ranked by sex and BW, and alternately assigned to receive BAS (Nutricorp;  $n = 94$ , 36 heifers and 58 bulls) or water (**CON**;  $n = 92$ , 37 heifers and 55 bulls) in a manner that treatments had equivalent BW and proportion calves from each sex. Calves were immediately segregated by treatment into one of two groups, processed for treatment application and a second BW collection. Upon segregation, treatment groups had no physical contact, and were maintained  $\geq 300$  m from each other throughout the experimental period (days 0 to 45). Treatments (5 ml) were topically applied to the nuchal skin area of each animal, and both BW collected on day 0 were averaged as weaning BW. The dose and route of application of BAS utilized herein were according to manufacturer's recommendation and Osella *et al.* (2018). Both treatment groups were maintained in a rotational system of *Brachiaria decumbens* pastures (three similar pastures; 25 ha/pasture) with abundant availability of standing forage, and received a supplement at 0.1% of BW and water for *ad libitum* consumption. Only two of the three pastures were utilized in this experiment, with an empty pasture between groups to maintain distance. Groups were rotated between pastures every 15 days.

In experiment 2, 140 Nelore-influenced bulls from 2 different pasture-based systems (70 bulls/origin) were road-transported to a commercial feedlot on day -1. Bulls were transported for

150 or 200 km in a single truck, according to origin. Upon arrival on day -1, bulls were maintained in a single *B. decumbens* pasture for 24 h. On day 0, bulls were processed for BW collection, vaccinated against respiratory pathogens (Biopoligen HS; Biogenesis Bago SA, Buenos Aires, Argentina) and *Clostridium* (Fortress 7; Zoetis Brasil), and anthelmintic administration (Dectomax; Zoetis Brasil). Bulls were ranked by origin and BW and alternately assigned to receive BAS ( $n = 70$ ) or CON ( $n = 70$ ), in a manner that treatments had equivalent BW and 35 bulls from each origin. As in experiment 1, bulls were immediately segregated by treatment into one of two groups, processed for treatment application and another BW collection. Each treatment group had no physical contact and was maintained  $\geq 200$  m from each other throughout the experimental period (days 0 to 45). Both BW collected on day 0 were averaged as initial BW. Both treatment groups were maintained in two identical drylot pens ( $75 \times 150$  m), receiving a total-mixed ration (**TMR**) and water for *ad libitum* consumption (diet A, days 0 to 18; diet B, days 19 to 45). Three empty feedlot pens were maintained between groups to maintain distance, whereas groups were rotated between pens every 15 days.

### Sampling and laboratorial analyses

In experiment 1, samples of forage and supplement were collected every 15 days, and pooled into a single sample. In experiment 2, samples of the TMR were collected on days 1 and 19. All samples were analyzed for nutrient content via wet chemistry procedures (3rlab, Belo Horizonte, Brazil). Nutritional profile of all feedstuffs, as well as composition of supplement and TMR are described in the Supplementary Material S1.

In both experiments, BW was recorded twice on days 15 and 45, within 1-h interval and averaged as described for day 0. Blood samples were collected from all animals as cattle were restrained in the squeeze chute for the first BW assessment on days 0, 15 and 45. All procedures for BW and blood collection were performed in the morning. Blood was collected from either the coccygeal vein or artery, and processed as in Schubach *et al.* (2017). Plasma samples were analyzed for haptoglobin (Cooke and Arthington, 2013) and cortisol (Burdick *et al.*, 2009). Concurrently with blood sampling, hair samples were collected from the tail switch of 30 animals randomly selected from each treatment (balanced for calf sex or bull origin in experiments 1 and 2, respectively), and analyzed for hair cortisol concentrations (Schubach *et al.*, 2017). Intra- and inter-CVs are reported in Supplementary Material S1.

### Statistical analysis

All data were analyzed using animal as experimental unit, with the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, USA). In both experiments, BW and physiological results from day 0 were included as independent covariate in each respective analysis. Significance was set at  $P \leq 0.05$  and tendencies were determined if  $P > 0.05$  and  $P \leq 0.10$ . Additional details about statistical analyses are provided in Supplementary Material S1.

**Table 1** Performance and physiological responses of beef calves receiving (BAS; n = 94) or not (CON; n = 92) a bovine appeasing substance at weaning (day 0)<sup>1</sup>

Item	CON	BAS	SEM	P-value
BW parameters				
Day 0 (kg)	235.4		2.1	–
Day 15 (kg)	246.1	249.2	0.7	<0.01
Day 45 (kg)	248.6	251.4	0.9	0.03
BW gain (days 0 to 15, kg/day)	0.74	0.95	0.05	<0.01
BW gain (days 15 to 45, kg/day)	0.07	0.07	0.02	0.97
BW gain (days 0 to 45, kg/day)	0.29	0.36	0.02	0.03
Plasma haptoglobin (mg/dl)				
Day 0	0.395		0.020	–
Day 15	0.530	0.279	0.026	<0.01
Day 45	0.246	0.236	0.027	0.77
Plasma cortisol (ng/ml)				
Day 0	21.7		1.1	–
Day 15	19.3	20.2	1.9	0.32
Day 45	24.8	22.1	1.9	0.13
Hair cortisol (pg/mg of hair)				
Day 0	2.48		0.14	–
Day 15	2.47	2.51	0.30	0.82
Day 45	2.55	2.59	0.30	0.85

BAS = bovine appeasing substance; CON = negative control (water placebo).

<sup>1</sup>Treatments (5 ml) were topically applied to the nuchal skin area of each animal. Values from day 0 were used as independent covariate; hence, results are reported as covariately adjusted least square means.

## Results

In experiment 1 (Table 1), BW gain was greater ( $P < 0.01$ ) in BAS v. CON from day 0 to 15, but similar ( $P = 0.97$ ) from day 15 to 45. Overall BW gain during the experimental period was greater ( $P = 0.02$ ) BAS v. CON. Calf BW was greater ( $P \leq 0.03$ ) in BAS v. CON on days 15 and 45. A treatment  $\times$  day interaction was detected for plasma haptoglobin ( $P < 0.01$ ), which was less ( $P < 0.01$ ) in BAS v. CON on day 15, but similar ( $P = 0.77$ ) on day 45. No treatment differences were detected ( $P \geq 0.13$ ) for plasma or hair cortisol concentrations.

In experiment 2 (Table 2), BW gain was greater ( $P = 0.04$ ) in BAS v. CON from day 0 to 15, but greater ( $P < 0.01$ ) in CON v. BAS from day 15 to 45. Overall BW gain and BW values during the experiment did not differ ( $P \geq 0.14$ ) between treatments. No treatment differences were detected ( $P \geq 0.38$ ) for plasma haptoglobin, plasma cortisol and hair cortisol concentrations.

## Discussion

Weaning and feedlot entry are two of the most stressful events within beef production systems, known to stimulate adrenocortical and inflammatory reactions that impair cattle immunocompetence and performance (Cooke, 2017). During

**Table 2** Performance and physiological responses of beef bulls receiving (BAS; n = 70) or not (CON; n = 70) a bovine appeasing substance at feedlot entry (day 0)<sup>1</sup>

Item	CON	BAS	SEM	P-value
BW parameters				
Day 0 (kg)	332.7		1.9	–
Day 15 (kg)	353.9	356.8	1.7	0.14
Day 45 (kg)	403.9	399.8	1.9	0.23
BW gain (days 0 to 15, kg/day)	1.45	1.65	0.08	0.04
BW gain (days 15 to 45, kg/day)	1.65	1.42	0.06	<0.01
BW gain (days 0 to 45, kg/day)	1.58	1.50	0.04	0.18
Plasma haptoglobin (mg/dl)				
Day 0	0.215		0.015	–
Day 15	0.188	0.217	0.055	0.55
Day 45	0.452	0.410	0.55	0.38
Plasma cortisol (ng/ml)				
Day 0	21.2		1.4	–
Day 15	32.8	30.9	2.3	0.48
Day 45	30.4	29.7	2.2	0.79
Hair cortisol (pg/mg of hair)				
Day 0	1.69		0.12	–
Day 15	1.94	1.80	0.15	0.48
Day 45	2.20	2.00	0.15	0.38

BAS = bovine appeasing substance; CON = negative control (water placebo).

<sup>1</sup>Treatments (5 ml) were topically applied to the nuchal skin area of each animal. Values from day 0 were used as independent covariate; hence, results are reported as covariately adjusted least square means.

these events, cattle are often handled for routine processing, creating the opportunity for application of novel technologies such as BAS. Circulating concentrations of cortisol have been widely used as biomarker to assess adrenocortical responses in cattle (Carroll and Forsberg, 2007); however, results may be influenced by the process of handling cattle for blood collection (Schubach *et al.*, 2017). Alternatively, circulating cortisol is gradually accumulated in the emerging tail hair, and its concentration represents long-term adrenocortical activity (Schubach *et al.*, 2017). Elevated cortisol stimulates pro-inflammatory and acute-phase reactions in cattle, which can be assessed via circulating concentrations of haptoglobin (Cooke, 2017). Therefore, concentrations of cortisol in plasma and tail-switch hair, as well as plasma haptoglobin concentrations were evaluated herein to determine the impacts of BAS administration to *B. indicus* cattle at weaning and feedlot entry.

Supporting our hypothesis, BAS administration at weaning improved BW gain and reduced plasma haptoglobin concentrations by day 15. No advantages in BW gain were noted after day 15, although overall average daily gain and final BW were improved by BAS administration. Research with BAS to beef cattle is limited, whereas the period in which BAS remains active upon a single administration is unknown. Results from experiment 1 suggest that BAS was active within 15 days upon administration, when the benefits of BAS on calf growth and


haptoglobin were noted. Accordingly, Osella *et al.* (2018) administered BAS to dairy cows weekly upon turn out to pasture, and reported productive benefits throughout their 28-day experimental period. Administration of BAS at weaning, however, did not impact cortisol responses to corroborate with plasma haptoglobin results. Specific reasons to why BAS improved initial BW gain are also unknown, including potential increases in feed intake and efficiency. Hence, the biological mechanisms by which BAS reduced plasma haptoglobin concentrations and increased BW gain upon weaning warrant investigation.

Results from experiment 2 partially support our hypothesis, given that BAS administration at feedlot entry improved BW gain during the initial 15 days. In turn, BW gain after day 15 was less in cattle administered BAS, perhaps associated with compensatory growth and DM intake of CON cattle (Ryan *et al.*, 1993). Overall BW gain and bull BW at the end of the experiment were not improved, whereas haptoglobin and cortisol responses were not impacted by BAS administration. As in experiment 1, the biological mechanisms by which BAS administration impacts BW gain of feedlot bulls, either positively or negatively, require investigation. Moreover, additional administrations of BAS were perhaps warranted to extend its benefits throughout the 45-day experimental period (Osella *et al.*, 2018).

In conclusion, additional research is warranted to examine the potential benefits of BAS in beef production systems, including biological mode of action and multiple BAS administrations. Nonetheless, use of BAS appears to be a promising alternative to improve cattle welfare and productivity upon stressful management procedures

## Acknowledgements

All authors acknowledge Nutricorp for their financial support (Araras, Brazil).

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## Declaration of interest

Nothing to report.

## Ethics statement

Animals were managed according to FASS (2010).

## Software and data repository resources

No data deposited in an official repository.

## Supplementary material

To view supplementary material for this article, please visit <https://doi.org/10.1017/S1751731119002490>

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