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Short communication: Administration of an appeasing substance to *Bos indicus*-influenced beef cattle improves performance after weaning and carcass pH

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ABSTRACT

Stressful situations, such as weaning and transport, are known to impact beef cattle health and performance. Hence, alternatives to minimize these stress-related losses are warranted and the bovine appeasing substance (**BAS**) is expected to have calming effects in cattle going through stressful events. Therefore, 2 studies were designed to investigate the impacts of BAS administration at weaning (Experiment 01) and immediately prior to transport to slaughter in beef animals (Experiment 02). In Experiment 01, 120 *Bos indicus*-influenced calves were weaned at 7 mo of age (day 0). At weaning, calves were ranked by BW and assigned to receive BAS (Nutricorp; Araras, SP, Brazil; n = 60) or water (**CON**; n = 60). Treatments (5-mL) were topically applied to the nuchal skin area of each animal. Calf body weight (**BW**) was recorded twice on days 0, 14, and 45 of the study. Calves that received BAS were heavier than CON cohorts at the end of the experimental period ($P < 0.01$). Additionally, starting on day 14, calves that received BAS had a greater average daily gain (**ADG**) when compared to CON cohorts ($P \leq 0.01$). In Experiment 02, a total of 835 Nelore-influenced animals, originated from 4 farms were used herein. Immediately prior to transport, animals were ranked by BW and assigned to receive BAS (n = 422) or water (CON; n = 413). Treatments (5 mL) were applied as previously described for Experiment 01. For all animals transported, meat pH was determined from the 12th rib 48 hours post-mortem. Animals having a meat pH > 5.80 were classified as higher-risks for having dark, firm, and dry (**DFD**) and was further evaluated herein. Additionally, for animals originated from farm 1 (n = 59), a portion of the *Longissimus dorsi* muscle was collected for colorimetric analysis. A treatment effect was detected for meat pH ($P < 0.0001$), so that animals receiving BAS had a reduced pH vs. CON cohorts (5.75 vs. 5.82, respectively). Additionally, the risk of DFD% was greater ($P < 0.0001$) for CON vs. BAS when pH > 5.80 (42.2 vs. 26.2%, respectively) was evaluated. In summary, BAS administration to animals prior to stressful events (weaning and transport to slaughter) improved performance, decreased the risk if occurrence of DFD cuts, and maintained the pH at levels below the threshold considered as critical for DFD occurrence.

1. Introduction

Stress is recognized as an important factor affecting beef cattle productive and reproductive performance, as well as health of the herd (Cooke, 2017). Among the stressful situations cattle are exposed to, transportation, feed and water restriction, and weaning are recognized as the main ones eliciting adrenocortical and acute-phase protein responses in cattle (Carroll & Forsberg, 2007; Marques et al., 2012). Weaning has been shown to impact health and performance of beef animals (Arthington et al., 2005), whereas transportation to slaughter might increase the occurrence of dark, firm, and dry (**DFD**) cuts and reduce acceptability of edible products by consumers. Hence, strategies

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to mitigate the stress-related responses elicited by weaning and transportation to slaughter are warranted.

Appeasing pheromones have been initially discovered in swine, and shown to reduce agonistic behavior in piglets (Archunan et al., 2014). In cattle, the synthetic analogue of the appeasing pheromone is based on a mixture of fatty acids, reproducing the composition of the original substance (Osella et al., 2018) Recently, Cooke et al. (2019) demonstrated that bovine appeasing substance (**BAS**) administration to *Bos indicus* beef steers at weaning alleviated the acute-phase response and improved performance over a 45-day period. In dairy cows, Osella et al. (2018) observed that cows administered BAS had greater milk yield and less somatic cell count, suggesting reduced stress caused by changes in the environment. Research investigating BAS administration to cattle is still limited, particularly in beef cattle managed under commercial settings (Cooke et al., 2019). Moreover, DFD occurrence is greater in *B. indicus*-influenced cattle and also in non-castrated animals, as usually observed in Brazil. Therefore, we hypothesized that BAS administration immediately before weaning would improve performance of beef animals (Exp. 01) and would improve carcass characteristics of *B. indicus*-influenced cattle when administered prior to transport to the slaughter facility (Exp. 02). Hence, this study investigated performance, carcass characteristics, and DFD occurrence of *Bos indicus*-influenced animals receiving or not BAS prior to stressful events, such as weaning and transport to slaughter.

2. Materials and methods

For both experiments reported herein, animals were managed according to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

2.1. Experiment 01

2.1.1. Animals and treatments

This experiment was conducted in a commercial dairy farm (Fazenda Barra, located in Aporé, Goiás state, Brazil) and 120 Nelore calves were used. 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 BW (initial BW = 191.1 ± 26.03 kg) and alternately assigned to receive BAS (Nutricorp; Araras, SP, Brazil; n = 60) or water (CON; n = 60) in a manner that treatments had equivalent BW. Calves were immediately segregated by treatment into 1 of 2 groups, processed for treatment application, and a second BW collection. Upon segregation, treatment groups had no physical contact, and were maintained ≥300 m from each other throughout the experimental period (day 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 as described by Cooke et al. (2019). Both treatment groups were maintained in a continuous grazing system of *Panicum maximum* cv. Zuri (2 similar pastures; 20 ha/pasture) with abundant availability of standing forage and stocking density adjustment, as observed by a trained personnel, and in order to avoid any confounding results between treatments and forage availability/quality. Moreover, animals were dewormed with moxydectin at weaning (Ônix™; Zoetis Animal Health, São Paulo, SP, Brazil).

Following weaning and allocation into pastures, all animals were group-fed on a daily basis a 0.3% of BW energy-protein supplement and water for ad libitum consumption. The supplement contained (as-fed basis) 74.0% corn, 18.5% soybean meal, 2.5% calcium salts of soybean oil (Nutri Gordura™; Nutricorp, Araras, SP, Brazil), 1.5% urea, and 3.5% of a mineral-vitamin mix (Tecnobeef, Altair, SP, Brazil).

Moreover, BW measurements were performed twice on days 14 and 45, within 30 min interval and averaged as described on day 0, whereas average daily gain (**ADG**) was calculated from days 0–14, 14–45, and 0–45.

2.1.2. Statistical analysis

All data were analyzed using animal as experimental unit. Performance data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, USA). The model statement used for these data contained the effects of treatment. Random variable included was animal(treatment). All results are reported as least square means. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and $P \leq 0.10$.

2.2. Experiment 02

2.2.1. Animals and treatments

A total of 835 Nelore-influenced animals, originated from 4 farms (farm 1 = 59 animals; farm 2 = 507 animals; farm 3 = 178 animals; farm 4 = 97 animals), were used in this experiment. In all farms, animals were fed a high-concentrate diet for the finishing phase (≥80% concentrate in total diet). It is important to mention that dry matter intake (**DMI**) and subsequent performance of the animals between farms was not the objective of the study; hence, these data were not reported herein and BW measurement at end of the finishing phase was taken mostly for randomization to treatments and not for comparison purposes among farms.

At arrival to the cattle working facility, animals were weighed and randomly assigned to receive BAS (Nutricorp; Araras, SP, Brazil; n = 422) or water (**CON**; n = 413). As reported by Brandão et al. (2019), treatments (5 mL) were topically applied to the nuchal skin area of each animal. Immediately after treatment administration, animals were segregated into 2 pens (100 m distance) and loaded into different trucks, so that animals had no physical contact and were maintained away from each other. This management procedure was adopted in order to avoid any confounding effect that animals from different treatments could have on the response of specific treatment, if in contact with counterparts receiving different treatments. Nonetheless, trucks left the farms at the same time, followed the same route and upon arrival at the slaughter facility, animals within each respective treatment were also maintained in segregated pens for 16 hours (without access to feed and water) before slaughter.

For all animals transported to the slaughter facility, meat pH was determined from the 12th rib of the left carcass at 48 hours post-mortem (model HI99163; Hanna Instruments; Barueri, São Paulo, Brazil). According to these data, the proportion of animals having a meat pH > 5.80 were classified as high-riskers for DFD occurrence and the proportion of this classification was further evaluated herein. Additionally, for animals originated only from farm 1 (n = 59), a portion of the *Longissimus dorsi* muscle (±100 gr) was collected for further colorimetric analysis. The reason why only animals originated Farm 01 were sampled was due to align sampling between personnel from the study and the slaughter facility. The determination of the components L*, a*, and b* and performed according to procedures described by Abularach et al. (1998). Color was evaluated on the surface of the samples using the CIE L*, a*, and b* system with a D65 illuminating and 10° as the standard evaluation point. A Minolta Cr-200b device (Konica Minolta, Osaka, Japan) will be used for color determination and will be calibrated with a blank standard sample. The following color parameters were used: L* is a lightness index (0 = black and 100 = white), a* is the intensity of red color, an index that ranges from green (–) to red (+), as well as b* is the intensity of yellow color, an index ranging from blue (–) to yellow (+; Houben et al., 2000).

2.2.2. Statistical analysis

All data were analyzed using animal as experimental unit. The pH and colorimetric parameters data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, USA). The model statement

Table 1
Performance data of beef calves receiving (BAS; n = 60) or not (CON; n = 60) a bovine appeasing substance at weaning (day 0; Experiment 01)[1]

Item	CON	BAS	SEM	*P*-value
Body weight, kg				
Initial	191.9	191.1	3.40	0.86
Day 14	212.0	219.6	3.16	0.09
Day 45	240.3	256.5	3.33	< 0.01
Average daily gain, kg				
Day 0–14	1.43	2.04	0.151	< 0.01
Day 14–45	0.91	1.18	0.031	< 0.0001
Day 0–45	1.08	1.45	0.052	< 0.0001

[1] Treatments (5 mL) were topically applied to the nuchal skin area of each animal.

used for these data contained the effects of treatment, farm, and treatment × farm interaction. Random variable included was animal (farm × treatment). The proportion of animals with meat pH > 5.80 were analyzed with the GLIMMIX procedure of SAS (SAS Inst., Inc.) and the model statement contained the effects of treatment only, whereas random variable included was animal(treatment). All results are reported as least square means. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and $P \leq 0.10$.

3. Results

3.1. Experiment 01

No treatment effects were observed on initial BW ($P = 0.86$), indicating that animals were under the same management prior to weaning on day of the study and that the randomization process was performed correctly (Table 1). Nonetheless, animals that received BAS tended to be heavier on day 14 of the study ($P = 0.09$) when compared with CON cohorts. Moreover, this parameter was significant on day 45 of the study, in a manner that BAS-administered animals were heavier at the end of the study vs. CON cohorts ($P < 0.01$; Table 1). Similarly, ADG from days 0–14, 14–45, and 0–45 were also greater for BAS vs. CON cohorts ($P \leq 0.01$; Table 1).

3.2. Experiment 02

No treatment × farm interaction was observed for any of the parameteres evaluated herein (pH and% of DFD animals; $P \geq 0.12$); hence, only the main effects will be reported throughout the article. A farm effect was observed for both pH and% of DFD animals ($P < 0.0001$; Table 2). Moreover, a treatment effect was detected for meat pH ($P < 0.0001$; Figure 1-A), so that animals receiving BAS immediately prior to transport had a reduced meat pH when compared to CON cohorts (5.75 vs. 5.82, respectively). Additionally, the proportion of animals with DFD was greater ($P < 0.0001$) for CON vs. BAS (42.2 vs. 26.2%, respectively; Figure 1-B). Conversely, no treatment effects were observed for the colorimetric parameters evaluated on animals originated from farm 1 ($P \geq 0.28$; Table 3).

Table 2
Measurements of pH and proportion (%) of animals with pH > 5.80 receiving (BAS) or not (CON) a bovine appeasing substance in the different farms (n = 4) immediately prior to transport to slaughter[1]

Farm	n	Item Meat pH	% pH > 5.80
1			
CON	30	5.75	30.0
BAS	29	5.58	3.4
2			
CON	248	5.78	42.3
BAS	259	5.75	34.7
3			
CON	411	5.74	34.3
BAS	418	5.68	10.8
4			
CON	45	5.93	60.0
BAS	45	5.83	42.2
SEM	–	0.017	3.932
P-value	–	< 0.0001	< 0.0001

[1] Treatments (5 mL) were topically applied to the nuchal skin area of each animal.

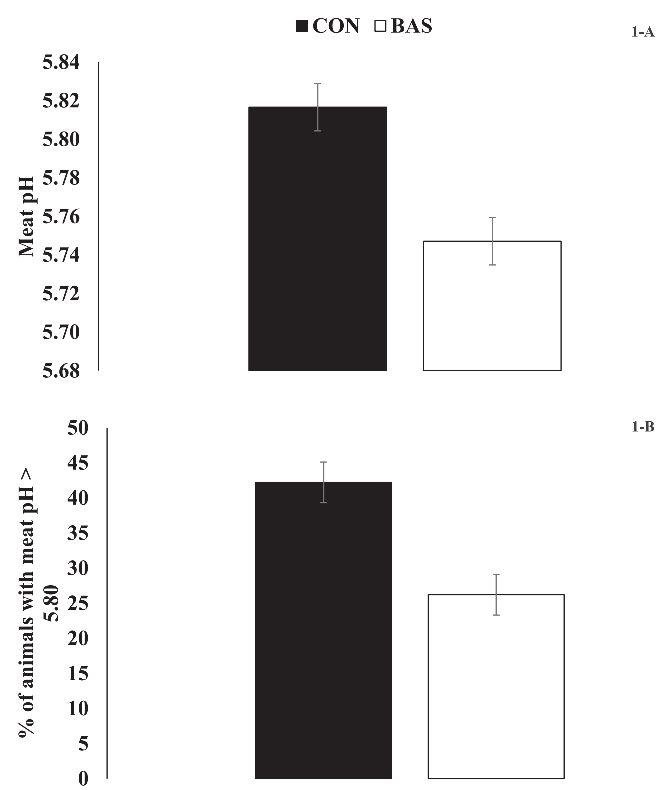

Figure 1. Meat pH (1-A) and proportion (%) of animals with meat pH > 5.80 (1-B) following administration (BAS; n = 422) or not (CON; n = 413) of a bovine appeasing substance immediately prior to transport to slaughter. A treatment effect ($P < 0.0001$) was observed for both parameters.

4. Discussion

Other researchers have reported that stress elicits a neuroendocrine response that, in turn, impairs performance and health of beef animals (Carroll and Forsberg, 2007; Cooke et al., 2012). Hence, alternatives that mitigate these neuroendocrine responses and improve performance and health of the herd are warranted. In the present study, BAS administration at weaning improved performance of the herd, starting 14 days post-BAS-administration. In agreement with our data, 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. The benefits reported by these authors included a 1.65 kg/d improvement on milk production and a reduction in somatic cell count. In beef cattle, Cooke et al. (2019) reported that beef animals administered BAS at weaning had greater ADG and BW at the end of a 45-day post-weaning evaluation period. One might speculate the differences observed between both weaning studies evaluating BAS administration to beef animals. In the study of Cooke et al. (2019), all animals had access to a low-intake protein supplement, whereas in the present study, a medium-intake supplement was offered to the herd, which likely optimized growth performance of the animals. Additionally, it is important to mention that even under a greater plane of

Table 3
Colorimetric parameteres of animals receiving (BAS; n = 29) or not (CON; n = 30) a bovine appeasing substance immediately prior to transport to slaughter.[1]

Item[2]	CON	BAS	SEM	*P-value*
L*	38.29	38.47	0.554	0.81
a*	19.86	19.67	0.511	0.79
b*	15.00	15.87	0.566	0.28

[1] Treatments (5 mL) were topically applied to the nuchal skin area of each animal.
[2] L* = lightness index (0 = black and 100 = white); a* = intensity of red color, an index that ranges from green (-) to red (+); b* = intensity of yellow color, an index ranging from blue (-) to yellow (+; Houben et al., 2000).

nutrition as reported herein, stress resulting from weaning significantly limited the performance of the animals throughout the 45-day period.

In cattle, a high pH will result in a darker piece of meat, whereas a low pH will result in a lighter piece of meat (Grandin, 1980). The pH is affected in part by the breakdown of glycogen to lactic acid and the rate of glycogen metabolism is affected by immediate and long-term stress (Grandin, 1980). Therefore, it is clear that stress situations that are lengthened prior to slaughter have a primary effect on meat pH, given that physical and psychological stressors lead to a depletion in muscle glycogen reserves, impairing the rate by which meat pH drops (Apple et al., 2005). These prolonged stressful situations are often observed as animals are shipped to slaughter considering that this management involves human handling procedures, transport to the slaughter facility, loading and unloading, arrival in a novel environment (i.e., slaughter facility), as well as feed and water deprivation. In fact, Marques et al. (2012) reported that feed and water deprivation resulted in a greater stress response when compared to transported and non-transported cohorts. Supporting our hypothesis, BAS administration immediately prior to transport to slaughter resulted in a maintenance of meat pH at adequate values, decreasing the% of animals likely presenting DFD cuts (pH > 5.80). Conversely, no positive effects were observed on colorimetric parameters due to BAS administration and the reason why BAS did not impact colorimetric parameters, even though a positive effect was observed on the reduction of DFD risk cuts is unknown, given that a reduction in DFD occurrence is related to changes in colorimetric parameters on beef meat (Grandin, 1980). One might speculate that the small number of animals sampled and analyzed for meat quality might be one of the reasons for these responses, given that the negative effects of stress on meat quality were observed when a greater number of animals were evaluated (Sant'Anna et al., 2019). Moreover, Cooke et al. (2019) failed to detect a positive effect of BAS on plasma and tail-hair cortisol concentrations, indicating that the mechanisms by which this technology improved productive parameters herein (Experiment 01), in the previous studies (Osella et al., 2018; Cooke et al., 2019) and meat quality (Experiment 02) might involve other stress-related responses and warrant further investigation.

5. Conclusions

The utilization of BAS appears to be a promising alternative to improve performance of newly-weaned beef calves, as well as improve meat quality and reduce the risk of incidence of DFD cuts. Nevertheless, additional research is still warranted to evaluate the mechanisms by which BAS improves productivity of beef animals.

Declaration of Competing Interest

None.

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