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Reactivity of Nellore steers in two feedlot housing systems and its relationship with plasmatic cortisol

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ABSTRACT

To evaluate reactivity to assess the temperament of Nellore steers in two feedlot housing systems (group pen or individual pen) and its relationship with plasmatic cortisol, 36 experimental units were observed five times at 28-day intervals of weight management during a 112-day feedlot confinement. A reactivity score scale ranging from 1 to 5 was applied when an animal was in the chute system. To the calmest animal, a reactivity score of 1 was ascribed and to the most agitated, 5. Blood samples were collected for cortisol analysis. No differences were found in reactivity and feedlot system. There was a relationship noted between reactivity and feedlot time in both housing systems ($P<0.01$). There was a relation between reactivity and cortisol levels for group animals ($P=0.0616$) and for individual ones ($P<0.01$). Cortisol levels varied among housing systems ($P<0.01$). Feedlot time influenced the cortisol levels ($P<0.09$ individual; $P<0.01$ group) and when variable time was included, these levels changed, decreasing in the group pen and increasing in individual pens. The continuous handling reduces reactivity and plasmatic cortisol, and group pen system seems to be less stressfully than individual pens.

1. Introduction

Despite the extensive grazing system for cattle in Brazil, the intensive feedlot system has been a widely used option in the final stage of the marketing process, with the aim of shortening the productive cycle. Nellore is one of the Zebu cattle breeds most used by Brazilian farmers because of its adaptability to tropical conditions. Due to the animals' nervousness, feedlot management can be more prone to accidents involving people and animals. Thus, this system demands more labor and is also more time consuming.

Among the most important behavioral traits, temperament reflects the ease with which animals respond to handling, treatment, and routine management. Animals with disposition problems are a safety risk to handlers, themselves, and other animals in the herd (Dolezal et al., 2002).

In cattle, there is a significant relationship between temperament and productivity. Cattle that became agitated during restraint in a squeeze chute had lower weight gains and harder meat (Burrow, 2003; Voisinet et al., 1997). Burrow and Dillon (1997) found that cattle that exited slowly from a squeeze chute had greater weight gains than those that exited the squeeze chute quickly.

Reactivity is one aspect of temperament and is defined by the quality or state of the one who protests or fights. Its expression depends on a series of components—intensity of

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stimuli, motivation, and response intensity (see review by Burrow, 1997).

Besides behavioral evaluation, some physiological responses can be directly related to the measure of reactivity, as plasmatic concentrations of cortisol. The hormones produced by the endocrine system play a critical role in the development and expression of a series of behaviors (changes in position, movements, and flight speed). Of all the endocrine axes, the HPA axis has been studied the most (Chackleu, 1996; Nemeroff, 1996) and was found to play a key role in handling internal and external stress stimuli. The behavior is the first stress response during stressful conditions, than the physiological activation of the HPA axis and the consequent variation of plasmatic cortisol (Moberg, 2000).

The objectives in this study were to compare temperament appraisals using chute score (a subjective technique) with two housing systems (individual pen vs. group pen) over repeated observations, as well as the relationship of the temperament assessments with concentrations of plasmatic cortisol.

2. Materials and methods

The experiment was conducted at the Laboratory of Biometeorology and Ethology—FZEA-USP, in Pirassununga, SP, Brazil (21° 80′ 00″ S, 47°25′42″ W, 634 m altitude) and all procedures have been approved by the USP Ethics Commission under the University of São Paulo, Brazil.

2.1. Animals and housing

Thirty-six Nellore steers with initial weight of 322.5 kg (±24.3) and 20 (±3) months that were under extensive management systems were used in the study. The animals were placed in two types of experimental housing systems: the first one consisted of two pens with 230 m^2 each (19 m^2/animal) with a capacity load of 12 animals/pen. These pens had an automatic gate system in the feed bunk that avoids dominance and aggressive interactions among animals. The second system was composed of 12 individual pens, with 20 m^2 each, partially covered with cement roofing, and concrete feed bunks. The animals had an 8-day adaptation phase in the two corrals; they were then allocated according to the management system of the research, 12 in each pen (total of 24 animals) and 12 in individual pens. Each animal was defined as one replicate in its housing group for statistical analysis (Tapki and Sahin, 2006).

2.2. Collection of data

All animals were evaluated five times every 28 days until the end of the feedlot period in 112 days, during weighing management between 6:00 h and 9:00 h. To evaluate how the temperament reflects the ease with which animals respond to handling, housing type, and weighing routine management, cattle walk into a chute system where they were weighed and a score was assigned. The observation for posterior attribution to the score was made by one observer by focal method, for 30 s, during the stay of the steer on the scale (contention method). The reactivity score for each animal was defined by a scale (Table 1) adapted from posterior research such as those of Hearnshaw and Morris (1984), Voisinet et al. (1997) and Dolezal et al. (2002).

After weighing, steers were subsequently processed through a next chute and restrained with a squeeze to allow the collection of blood samples through jugular puncture using heparinized tubes. The blood samples were then centrifuged at 3000 rpm for 15 min at 4 °C to separate the plasma. Each sample was placed in 1.5 ml *eppendorf* tubes and frozen at −25 °C until the analyses were made.

The analysis of cortisol levels was done at the Laboratory of Animal Physiology of FZEA-USP, according to manufacturer's instructions. The readings of the samples were made by ELISA (Labsystem Multiskan Version 8.0). The plasmatic cortisol measures were calculated through the absorbance of the samples and the standard curve, determined by the Labsystem Gênesis V3.03 software.

The same group of people and the same facilities were always used to handle the animals during the experimental period. Handling was done to avoid fear-inducing situations, as humans shouting or waving sticks with plastic or cloth attached.

2.3. Statistical analysis

For statistical analysis, data collected from the reactivity evaluation in the scale (chute) and the values of plasmatic cortisol were considered. Behavioral data were tested for normality by a histogram and parametric statistics were used. Traditionally, researchers such as Fordyce et al. (1988) and Mourão et al. (1998) have used this system, which is possible due to the approximation of a normal distribution that these scales present. As such, the measured characteristics were analyzed statistically considering the composite symmetric covariance, which applies the maximum restricted likelihood, with the use of the MIXED procedure statistical program SAS 9.1 (2004). This covariance structure was used due to the presence of repeated measurements. The same covariance method was used because it allows the evaluation of homogeneity in the variances and it adjusts to the covariance of the samples. Type "cs" was used in the analysis to include each animal as an experimental unit ($n=36$; $df=1$ for treatment; $df=34$ for residual). The mathematical models were established according to each variable measured in the study.

Cortisol: the effect of the treatment (individual pen or group), time as covariable (in relation to days) of treatment, effect on the animal and residual as random.

Table 1
Scoring system to subjectively evaluate reactivity by temperament.

Score	Description of temperament
1	Non-reactive, calm, still and inattentive
2	With little reaction still but showing eye movement
3	Reactive, continuous but not vigorous movements
4	Reactive, with continuous movements, vigorous and somewhat abrupt
5	Extremely reactive: continuous movements and extreme fright, quivers, vocalizations, and attempts to climb out of the scale

Scores assigned when cattle are contained in a chute system, observation during 30 s.

Reactivity 1: the effect of the treatment (individual pen or group), cortisol as covariable in the treatment, effect on the animal and residual as random.

Reactivity 2: treatment effect, time as covariable (linear and quadratic), effect on the animal and residual as random.

The results are presented as least square means through the *F* test. The effects of the possible interactions that were not significant ($P>0.10$) in the study and/or could not be tested due to the distribution of the information were withdrawn from the study's final analysis. Statistical comparisons were made on least square means with an approximation using chi-square test.

3. Results

Although there was no significant difference in the reactivity parameter considering the kind of housing, group pen or individual pen, the reactivity tends to reduce ($P<0.01$) considering the long stay of the steers in the feedlot until their transfer to the slaughterhouse (Fig. 1; Table 2).

There was a relation between reactivity and cortisol levels for animals in group pen ($P=0.0616$) and in individual pens ($P<0.01$). However, this relationship was positive for grouped animals and negative for individual ones (Table 3).

There was a positive effect of time spent in the feedlot in relation to cortisol sampled from the animals in the individual pens ($P=0.09$) and a negative relation was found for animals in the group pens ($P<0.01$; Table 3; Fig. 2). The cortisol levels sampled from the grouped animals tend to decrease along the time. There was interaction between day of sampling and cortisol values of the animals.

The cortisol levels differed between housing systems, individual or group ($P<0.01$; Table 3). The higher mean values were found for the group (28.36 ng/mg) as against the individual pens' 18.79 ng/mg (Fig. 3).

4. Discussion

The subsequent experiences with weighing resulted in a decrease of reactivity scores independent of setting (individual or group). These results are similar to the findings of Crookshank et al. (1979) and Curley et al. (2006)—the more acquainted to the procedure the animal is, the less reaction they present. On the other hand, using flight speed (FS) as another tool to measure temperament, some authors found that FS increased slightly over time in both *B. indicus* and *B. taurus* cattle (Müller and von Keyserlingk, 2006; Petherick et al., 2002). Authors may attribute this increase to repeated handling of the test animals, which may led to an increase in their fearfulness (Petherick et al., 2002). Because an animal's behavior can be influenced by past experiences, scoring should be conducted at weaning. This will reduce the extent to which current behavior has been influenced by prior handling experiences (Dolezal et al., 2002). Nevertheless, in the present study, there was no stressful handling; only positive experiences were looked for, avoiding fear memories that can never be erased.

Temperament and stress have been closely associated, and the behavior of cattle has become a method for indicating or selecting cattle that could be more stress responsive cattle or less adaptive (Curley et al., 2006).

The animals that lived alone (in individual pens) did not learn to express a normal submissive behavior, on the other hand, animals in group pens learned how and when to limit their aggressiveness through interaction with peers (Price and Wallach, 1990). This explanation matches the findings of this research—the steers that were kept alone presented visible aggressive traits during handling, especially toward the end of the experiment. Many tests to assess fear incorporate social isolation (Mason, 2000).

The results found for steers placed in pens confirmed the observations of Andrighetto et al. (1999), who studied social interactions among young cattle; they detected certain social behaviors within a group, like visual, vocal and olfactory communications. It helped to minimize the habitual discomfort of a feedlot environment, which is limited in space and comfort, thus making the animals less reactive and less physiological stressed.

Becker and Lobato (1997) reported that the smallest reactivity scores presented at the end of certain processes such as raising and handling, happened because of adaptation to human handling and to facilities used, which is also responsible for the lower cortisol levels of these animals. Relationship found between high levels of cortisol and greater hesitation when entering a stressful situation (a holding

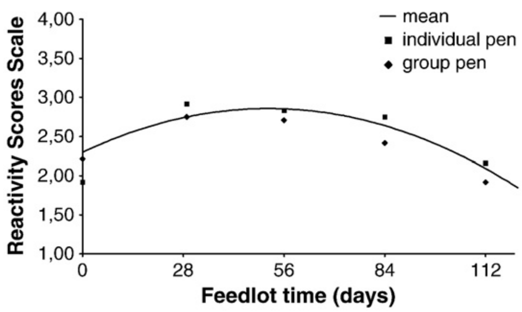

Fig. 1. Variation in reactivity in steers housed in group or 1 individual pens throughout the feedlot time. During the feedlot period, reactivity was observed every time the animals were weighed ($n=36$). The points show the observed reactivity for the two housing systems and the line shows the mean predicted values for both systems.

Table 2
Least square means of reactivity scores for steers housed in group pen or individual pen, and relation with feedlot time (112 days).

	β	SEM	*P*-value
Cortisol (individual pen)	0.08369	0.02565	<0.0026
Cortisol (group pen)	0.01412	0.007297	<0.0616
Feedlot time (112 days)	+0.02152	0.006068	<0.0012

Table 3
Least square means of plasmatic cortisol for steers housed in group pen or individual pen, and relation with feedlot time (112 days).

Housing system	Plasmatic cortisol		β	Feedlot time	
	Mean	SEM		SEM	*P*-value
Individual pen	13.6[b]	3.32	+0.07708	0.0448	<0.09
Group pen	22.9[a]	2.35	−0.1494	0.0368	<0.0001

Different superscript letters differ ($P<0.01$).

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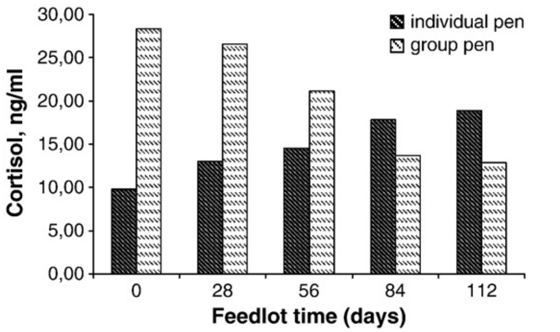

Fig. 2. Mean concentrations of plasma cortisol of steers housed in the individual pens or group pen during feedlot period. Animals blood was drawn every 28 days after the reactivity score was assigned ($n = 12$ for individual; $n = 24$ for group).

corral) and more vocalizations in the stressful situation provide strong and consistent evidence that cortisol levels are related to anxiety-related behaviors in cattle in non-stressful and stressful situations (Bristow and Holmes, 2007).

Grandin (1993) also found that repeated weighing resulted in a lower mean value of cortisol concentration. The decrease of cortisol could suggest physiological down-regulation or adaptation to the new environment (Higashiyama et al., 2007).

Cattle tend to be less agitated during handling if they are grouped rather than single (Grandin, 1987), probably because they are less fearful (Petherick et al., 2002). Therefore, as reactivity seems to reduce along the feedlot period, this allowed us to suppose that housing animals as a group is a better feedlot system as plasmatic cortisol levels decreased along confinement period.

There is a possibility that the higher mean value of detected cortisol in the animals in group pens was due to a higher stress level during adaptation to learn how to use the electronic gates at the feeding bunks. These hormonal changes presumably could underlie adaptive changes.

The chronic stress could be another explanation of the reducing of cortisol levels in grouped animals. A decrease in plasma cortisol levels has been observed previously in chronically heat-stressed cows (Bell et al., 1989; Christison and Johnson, 1972). Although reactivity behavior seems to have the same decrease tendency between the two housing types,

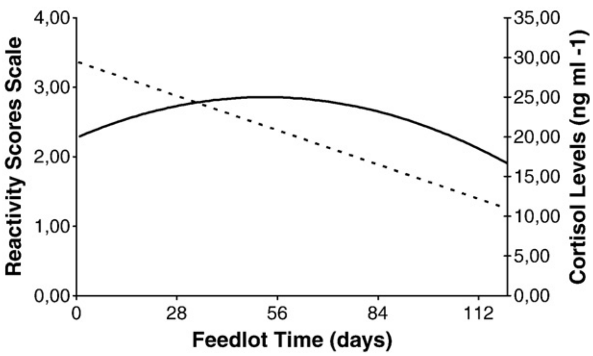

Fig. 3. Evolution of cortisol levels (dotted line) and reactivity scores (full line) observed in group pen animals ($n = 24$) weighed every 28 days. Blood samples were drawn after the reactivity score was assigned.

the differences of cortisol levels could be the physiological answer in stress situation. The handling system was the same in the two housing systems, so living as a group can be attributed to the decreased of plasmatic cortisol concentrations as cattle are gregarious animals (Bouissou et al., 2001).

5. Conclusion

The results of this study show that housing cattle in group or individual in feedlots did not alter reactivity of weighing, which reduced along the confinement period. However, the cortisol levels tend to increase in individual housed animals and decreased in grouped animals. This relationship between cortisol and housing system should be considered with caution and warrants further investigation to avoid the hypothesis of chronic stress in animals in group.

References

Andrighetto, I., Gottardo, F., Andreolli, D., Cozzi, G., 1999. Effect of type of housing on veal calf growth performance, behaviour and meat quality. Livestock Prod. Sci. 57, 137–145.

Becker, A., Lobato, J.F.P., 1997. Effects of gentle handling on the reactivity of zebu crossed calves to human. Appl. Anim. Behav. Sci. 53, 219–224.

Bell, A.W., McBride, B.W., Slepetis, R., Early, R.J., Currie, W.B., 1989. Chronic heat stress and prenatal development in sheep: I. Conceptus growth and maternal plasma hormones and metabolites. J. Anim. Sci. 67, 3289–3299.

Bouissou, M.F., Boissy, A., Neindre, P.L., Veissier, I., 2001. The social behaviour of cattle. In: Keeling, L.J., Gonyou, H.W. (Eds.), Social Behavior in Farm Animals. CABI Publishing, Wallingford, pp. 113–135.

Bristow, D.J., Holmes, D.S., 2007. Cortisol levels and anxiety-related behaviors in cattle. Physiol. Behav. 90, 626–628.

Burrow, H.M., 1997. Measurements of temperament and their relationships with performance traits of beef cattle. Anim. Breeding Abstr. 65, 477–495.

Burrow, H.M. 2003. Improving cattle performance and meat quality by measuring temperament. On homepage of Cooperative Research Centre for cattle and Beef Quality. (2003), http://www.beef.crc.org.au.

Burrow, H.M., Dillon, R.D., 1997. Relationship between temperament and growth in a feedlot and commercial carcass traits of *Bos indicus* crossbreeds. Aust. J. Exp. Agric. 37, 407–411.

Chackleu, S., 1996. The neuroendocrinology of depression and chronic stress. Brit. Med. Bul. 52 (3), 597–617.

Christison, G.I., Johnson, H.D., 1972. Cortisol turnover in heat-stressed cows. J. Anim. Sci. 35, 1005–1010.

Crookshank, H., Elissaude, N., White, R., Canton, D., Smolley, H., 1979. Effect of handling and transportation of calves upon blood serum composition. J. Anim. Sci. 48, 430.

Curley Jr., K.O., Paschal, J.C., Welsh Jr., T.H., Randel, R.D., 2006. Technical note: exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J. Anim. Sci. 84, 3100–3103.

Dolezal, S. L., D. H. Crews, Jr., M. E. Dikeman, T. W. Marston, L. W. Olson, J. C. Paschal, G. H. Rouse, R. L. Weaber, R. E. Williams and D. E. Wilson. 2002. Animal evaluation. In: Guidelines for Uniform Beef Improvement Programs, 8th edition. Beef Improvement Federation. Available: www.beefimprovement.org.

Fordyce, G., Whites, R.N., Dodt, J.R., 1988. Cattle temperament in extensive beef herds in northern Queensland. 1. Factor affecting temperament. Aust. J. Exp. Agric. 28, 683–687.

Grandin, T., 1987. Animal handling. Vet. Clin. North Am.: Food Anim. Pract. 3, 323–338.

Grandin, T., 1993. Behavioral agitation during handling of cattle is persistent over time. Appl. Anim. Behav. Sci. 36, 1–9.

Hearnshaw, H., Morris, C.A., 1984. Genetic and environmental effects on a temperament score in beef cattle. Aus. J. Agric. Res. 35, 723–733.

Higashiyama, Y., Nashiki, M., Narita, H., Kawasaki, M., 2007. A brief report on effects of transfer from outdoor grazing to indoor tethering and back on urinary cortisol and behaviour in dairy cattle. Appl. Anim. Behav. Sci. 102, 119–123.

Mason, W.A., 2000. Early developmental influences of experience on behavior, temperament and stress. In: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress. CABI Publishing, Wallingford, pp. 269–290.

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Moberg, G.P., 2000. Biological response to stress: implications for animal welfare. In: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress. CABI Publishing, Wallingford, pp. 1–21.

Mourão, G.B., Bergmann, J.A.G., Ferreira, M.B.D., 1998. Genetic differences and heritability estimates of temperament in Zebus and F1 Holstein × -Zebu females. R. Bras. Zootec. 27 (4), 722–729.

Müller, R., von Keyserlingk, M.A.G., 2006. Consistency of flight speed and its correlation to productivity and to personality in *Bos taurus* beef cattle. Appl. Anim. Behav. Sci. 99, 193–204.

Nemeroff, C.B., 1996. The corticotropin-realising factor (CRF) hypothesis of depression: new findings and new directions. Mol. Psychiatry 1 (4), 336.

Petherick, J.C., Holroyd, R.G., Doogan, V.J., Venus, B.K., 2002. Productivity, carcass and meat quality of lot-fed *Bos indicus* cross steers grouped according to temperament. Aust. J. Exp. Agric. 42, 389–398.

Price, E.O., Wallach, S.J.R., 1990. Physical isolation on hand-reared Hereford bulls increases their aggressiveness towards humans. Appl. Anim. Behav. Sci. 27, 263–267.

SAS Institute, Inc., 2004. AS/STAT User's Guide, 12th ed. SAS Inst., Inc, Cary, NC.

Tapki, I., Sahin, A., 2006. Comparison of the thermoregulatory behaviours of low and high producing dairy cows in a hot environment. Appl. Anim. Behav. Sci. 99, 1–11.

Voisinet, B.T., Grandin, T., Patum, S.F., O'Connor, S.F., Struthers, J.J., 1997. Feedlot cattle with calm temperament have higher average daily gain than cattle with excitable temperament. J. Anim. Sci. 75, 892–896.