UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CAMPUS DE JABOTICABAL

COMPORTAMENTO INDIVIDUAL DE BOVINOS NELORE E RELAÇÕES COM DESEMPENHO EM REGIME DE CONFINAMENTO E REPRODUÇÃO

Désirée Ribeiro Soares
Zootecnista

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TÍTULO: COMPORTAMENTO INDIVIDUAL DE BOVINOS NELORE E RELAÇÕES COM DESEMPENHO EM REGIME DE CONFINAMENTO E REPRODUÇÃO

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DADOS CURRICULARES DA AUTORA

DÉSIRÉE RIBEIRO SOARES - Nascida em 17 de janeiro de 1979, na cidade de Londrina-PR. Formada em Zootecnia pela Universidade Federal do Paraná – UFPR, Campus de Curitiba-PR no ano de 2009. Durante a graduação foi monitora em Etologia Aplicada a Animais Domésticos e aluna bolsista da Fundação Araucária desenvolvendo projetos de iniciação científica voltados a avaliação e desenvolvimento de estratégias para melhorias de bem-estar de equinos. No ano de 2010 ingressou no curso de mestrado pela Universidade Estadual Paulista – UNESP, Campus de Jaboticabal-SP desenvolvendo projetos de avaliação de temperamento e sua relação com o comportamentos social e alimentar de bovinos confinados, concluindo o seu mestrado em 2011. Neste mesmo ano iniciou seus estudos no curso de doutorado, aprimorando a pesquisa realizada durante o mestrado, com o intuito de avaliar o comportamento individual de bovinos Nelore.

« Eu sou o que posso, na medida que me permitem. Quando posso, ultrapasso as fronteiras. Quando não posso, do meu limite, faço arte. Sou semelhante ao rio. Se me barram, eu aprofundo. »

Padre Fábio de Melo

Dedico,

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RESUMO – Na presente tese, uma das nossas hipóteses era que o temperamento possa ser usado como indicador de indivíduos capazes de se adaptar ao regime de confinamento, bem como que novilhas prenhas com melhor temperamento possam mostrar melhores resultados de desempenho produtivo e reprodutivo, uma vez que estas possuem maior motivação para competir pelo alimento no cocho. Sendo assim, o objetivo do estudo do segundo capítulo foi avaliar a utilização de velocidade de saída do tronco de contenção (**FS**, $m.s^{-1}$), como indicador de temperamento, para prever a capacidade individual de bovinos para se adaptarem ao ambiente de confinamento. Foram avaliados 549 bovinos da raça Nelore durante dois anos consecutivos; ano 1 (2012, N = 268) e ano 2 (2013, N = 281). Os animais foram provenientes de dois grupos: Grupo 1, com média idade de 237,29 ± 22,30 dias e 280,09 ± 29,05 kg de PV (n = 141) no primeiro ano e com média de idade de 276,33 ± 12,24 dias e 299,26 ± 30,09 kg de PV (n = 143) no segundo ano; Grupo 2 com média de idade de 324,57 ± 32,20 dias e 318,68 ± 36,07 kg de PV (n = 127) no primeiro ano e 318,16 ± 47.99 dias e 318,16 ± 47,99 kg de PV (n = 138) no segundo ano. Durante o período experimental, os bezerros de cada grupo de idade, foram distribuídos em dois currais de confinamento (com aproximadamente 40 m^2/animal), sendo que cada possuía oito cochos eletrônicos (GrowSafe®) para avaliar o comportamento alimentar e consumo individual. As variáveis comportamentais incluíram a frequência de visitas ao cocho (**BAf,** visitas/dia), duração da visita ao cocho (**BAd**, min/dia), e taxa de alimentação (**ER,** g/min; o consumo alimentar foi avaliado pela ingestão de matéria-seca (**DMI**, kg/d). As medidas de FS ($m.s^{-1}$) foram realizadas quatro vezes durante o estudo, no primeiro dia (**FS1**, imediatamente antes da entrada dos animais em confinamento), e aproximadamente com intervalo de 28 dias (**FS2, FS3 e FS4**). Com base nestas medições foram calculados, para cada animal: as médias aritméticas de FS, denominada de FSmean; e as diferenças entre FS1 - FS4 (**FSdiff**). FSdiff foi utilizado para definir o FScategory, como se segue: **'Aclimatados'**- animais que reduziram FS em mais de um desvio padrão; **'Consistente'**- animais que não alteraram seu FS ao longo do tempo; e **'Sensibilizado'** - os animais que aumentaram FS em mais de um desvio-padrão. Os animais foram pesados em intervalos de 14 dias para se obter as características de desempenho, incluindo: o peso corporal no final do período de confinamento (**BWf**, $kg.animal^{-1}$), ganho de peso (**ADG**, $kg.animal^{-1}.dia^{-1}$) e conversão alimentar (**FCR**, kg de matéria-seca.kg de $ganho^{-1}$). Foram obtidos registros da área de olho de lombo (**AOL,** cm^2) por meio de ultrassonografia. Nossos resultados mostraram efeito significativo linear de FS1 somente sobre ER durante o período de adaptação (*P* = 0.0314), uma tendência de efeito linear sobre BAd (*P* = 0.0617) e BAf (*P* = 0.0550) durante os períodos de adaptação e total, respectivamente. Também verificamos um efeito quadrático de FS1 sobre BAd (*P* = 0.0192) e DMI (P = 0.0031) durante todo período

experimental. Nenhuma das características de desempenho foram afetadas por FS1. Por outro lado, FSmean mostrou um efeito linear (*P* = 0.0289) durante o período de adaptação, uma tendência linear sobre DMI (*P* = 0.0822) e BWf (*P* = 0.0831) durante todo período avaliado, efeito quadrático sobre ADG (*P* = 0.0194) e REA (*P* = 0.0411). A variável FSdiff mostrou um efeito linear somente para BAf (*P* = 0.0038) durante todo período. Com base nos resultados apresentados e sobre as condições do presente estudo, FSmean foi a única medida de FS com potencial para indicar animais da raça Nelore com melhores habilidades de adaptação ao regime de confinamento. Por outro lado, o terceiro capítulo foi desenvolvido com o objetivo de avaliar os efeitos do temperamento sobre o desempenho produtivo e reprodutivo de novilhas Nelore, usando diferentes abordagens na utilização dos dados de FS, e também verificar as relações de interações agonísticas com temperamento, desempenho produtivo e reprodutivo. Dados de 229 novilhas foram registrados durante dois períodos experimentais em dois anos consecutivos: 2012 (N = 105) e 2013 (N = 124). Durante o primeiro período, as novilhas apresentaram 525,33 ± 36,70 e 526,78 ± 33,26 dias de idade e pesaram 423,68 ± 39,45 e 427,76 ± 33,41 kg de peso vivo, para os anos 1 e 2, respectivamente, e foram alojadas em currais de confinamento (40 m^2/animal), sendo que cada possuía oito cochos eletrônicos (GrowSafe®) para avaliar a ingestão de matéria-seca (DMI, $kg.d^{-1}$). Foram utilizadas as mesmas medidas de FS e o mesmo protocolo de obtenção dos dados, bem como os registros das características produtivas em regime de confinamento (BWf, ADG e REA), previamente descrito para o capítulo 2. Além disso, as novilhas (N = 58) foram avaliadas por meio de dois índices comportamentais, 'Índice de agressividade' (**AI,** $frequência.d^{-1}$) e 'Índice de deslocamento' (**DI,** $frequência.d^{-1}$), sendo registradas a frequência de cada novilha sendo 'agressora' e 'receptora' da interação agonística durante as competições de acesso ao cocho. Durante o segundo período experimental, as novilhas foram alojadas a pasto e após o desmame, novilhas e seus bezerros foram avaliados para obter suas características de desempenho produtivo e reprodutivo a pasto: peso corporal dos bezerros ao desmame (**WW,** $kg.animal^{-1}$); peso corporal da novilha após o desmame do bezerro (**Wac,** $kg.animal^{-1}$); idade ao primeiro parto (**AFC,** d); dias para o parto (**DC,** d) e intervalo entre partos (**CI,** d). Durante o primeiro período experimental, nenhuma das características de desempenho foram afetadas pelas de temperamento (FS1, FSmean ou FSdiff). Durante o segundo período, FS1 somente mostrou quadrático efeito sobre Wac (*P* = 0.0437), enquanto que FSmean teve uma tendência linear sobre AFC (*P* = 0.0719) e DC (*P* = 0.0466) e FSdiff mostrou uma tendência linear sobre WW (*P* = 0.0735). Além do mais, as progênies das novilhas categorizadas como "Aclimatado" tiveram maior WW ($P < 0.05$) comparado aos grupos "Consistente" e "Agitado". Concluiu-se que nas condições do presente estudo, FSmean tem potencial para predizer precocidade sexual, enquanto que FSdiff, parece predizer desempenho da cria na desmama. FS1 não foi um bom indicador de desempenho produtivo e reprodutivo, e apesar de interações agonísticas (AI e DI) estarem associadas a temperamento, estas não afetaram o desempenho produtivo e reprodutivo.
Palavras-chave: comportamento, interação social, Nelore, reatividade

INDIVIDUAL BEHAVIOR OF NELLORE BEEF CATTLE AND ITS RELATIONSHIP WITH PERFORMANCE IN FEEDLOT SYSTEM AND REPRODUCTION TRAITS

SUMMARY – In the present thesis we hypothesized that temperament traits can be used as indicators of the individual capacity of cattle to adapt to the feedlot environment, and that pregnant heifers with better temperament can show better results of growth and reproductive performance, since they have greater motivations to compete to access the feed bunk. Thus, the aim of the study on the second chapter was to assess the use of flight speed method (**FS**, $m.s^{-1}$) to predict the cattle's ability to adapt to the feedlot environment. Five hundred and forty-nine Nellore bull calves were used in this study, which was carried out over two consecutive years; Year 1 (2012, N = 268) and Year 2 (2013, N = 281). The animals consisted of two groups: i) Group 1 averaging 237.29 ± 22.30 d of age and 280.09 ± 29.05 kg of BW (n = 141) in yr 1, and 276.33 ± 12.24 d of age and 299.26 ± 30.09 kg of BW (n = 143) in yr 2 and; ii) Group 2 averaging 324.57 ± 32.20 d of age and 318.68 ± 36.07 kg of BW (n = 127) in yr 1, and 332.83 ± 24.86d of age and 318.16 ± 47.99 kg of BW (n = 138) in yr 2. During the experimental period, calves from each group were allocated equally into one of two feedlot pens (~ 40 m^{2}·animal), and each pen contained eight adjacent electronic troughs (GrowSafe® System Ltd.) to document individual feeding behavior and intake. Feeding behavior variables assessed included bunk attendance frequency **(BAf, visits/d**), bunk attendance duration **(BAd, min/d)**, and eating rate **(ER, g/min^{-1}**; feed intake was assessed by dry matter intake (**DMI, kg/d**). FS ($m.s^{-1}$) was measured four times during the trial, on the first day (**FS1**, immediately before the animals entry in the feedlot facilities), and approximately every 28 days (**FS2, FS3, and FS4**) after. Based on these measurements, two additional FS variables were calculated for each animal including; the arithmetical means of FS (**FSmean**) and the differences between FS1 and FS4 (**FSdiff**). Consequently, FSdiff was used to define the **FScategory**, based on animals that reduced FS more than one SD (**Acclimated)**, animals that did not change FS (**Consistent)** and those that increased FS (**Sensitized**). The animals were weighed at 14-d intervals to obtain performance traits, including: body weight at the end of the feedlot period (**BWf**), **ADG** and feed conversion ratio (**FCR**). Carcass trait data also was collected at the end of the feedlot period using real-time ultrasound measurements of the ribeye area **(REA)**. Our results showed a linear effect of FS1 only for ER during the adaptation period (*P* = 0.0314), a trend of linear effect on BAd (*P* = 0.0617) and BAf (*P* = 0.0550) during the adaptation and entire period, respectively. Also, a quadratic effect of FS1 on BAd (*P* = 0.0192) and DMI (*P* = 0.0031) over the entire period. None of the productivity traits were affected by FS1. On the other hand, FSmean showed linear effects on BAd (*P* = 0.0029), ER (*P* = 0.0342), and DMI (*P* = 0.0289) during the adaptation period, a trend towards a linear effect on DMI (*P* = 0.0822) and BWf (*P* = 0.0831) over the entire period, and quadratic effects on ADG (*P* = 0.0194) and REA (*P* = 0.0411). The variable FSdiff showed only a linear effect on BAf (*P* = 0.0038) over the entire period. Based on these results and under the conditions of this study, FSmean was the only FS measurement with potential to indicate adaptability to the feedlot environment for Nellore bull calves. On the other hand, the third chapter was developed aiming to assess the effects of temperament on growth and reproductive performance of Nellore heifers, using different approaches for **FS** data-analysis, and also to verify the relationships of agonistic interactions with temperament, growth and reproductive traits. Data from two hundred and twenty-nine heifers were recorded during two experimental periods and over two consecutive years: 2012 (N = 105) and 2013 (N = 124). During the first period, heifers were 525.33 ± 36.70 and 526.78 ± 33.26 d of age and weighed 423.68 ± 39.45 and 427.76 ± 33.41kg of BW, for years 1 and 2, respectively, and they were allocated in the feedlot pens (~ 40 m^2.$animal^{-1}$). We used the same FS variables and the same protocol to assess temperament data as previously showed for chapter 2. The animals were weighed at 14-d intervals to obtain performance traits, including: body weight at the end of the feedlot period (**BWf,** kg.$animal^{-1}$), **ADG** (kg.$animal^{-1}$.d^{-1}) and feed conversion ratio (**FCR,** kg DMI. kg $gain^{-1}$). The aggressiveness of each heifer within the herd (**AI,** counts.d^{-1}) and how many individuals the aggressor was able to displace from the feed bunk (**DI,** counts.d^{-1}) were recorded in fifty-four heifers over 13 non-consecutive days. During the second period, heifers were housed on pasture, and after weaning, heifers and offspring were assessed to obtain their growth and reproductive traits, as follows: calves' body weight at weaning (WW, kg.$animal^{-1}$); heifer' weight 210 days after the first calving (Wac, kg.$animal^{-1}$); age at the first calving (AFC, d); days to calving (DC, d) and calving interval (CI, d). During the first period, none of the growth performance traits were affected by any FS measurement (FS1, FSmean or FSdiff). During the second period, FS1 only showed significant quadratic effects on Wac (*P* = 0.0437), while FSmean had a tendency of linear effects on AFC (*P* = 0.0719) and DC (*P* = 0.0466), and FSdiff showed a tendency of linear effects on WW (*P* = 0.0735). Therefore, the offspring of 'Acclimated' heifers had greater WW (*P* < 0.05) than 'Consistent' and 'Sensitized' heifers, which did not differ from each other. In contrast to FS1 that was not associated to AI or DI, FSmean showed a significant and positive correlation (*P* < 0.05) with AI and DI. We concluded that under the conditions of the present study, FSmean showed potential to predict sexual precocity, while FSdiff has potential to predict growth performance of the heifer's offspring at weaning. FS1 was not a good indicator of growth or reproductive performance, and although AI and AD were associated with FSmean, they were not associated to growth and reproductive performance.

Key-words: behavior, confinement, Nellore, reactivity

CHAPTER 1 – General considerations

1. INTRODUCTION

Historically, the beef production in Brazil has been predominantly extensive, occurring on vast fields of cultivated grass pastures. However, in the last decades, intensive production systems have been increasing, in particular, feedlot cattle production. This change is largely driven by the economic benefits associated with intensive production, by: preventing weight loss during the dry season, when there is low supply of poor quality forage; increasing the availability of arable lands for agriculture, and reducing the production cycle and (by this) anticipating return from the investment.

Generally, homogeneity in the performance of feedlot cattle from the same genetic origin and kept under the same environmental conditions is expected (and desired) from a production standpoint. However, significant individual variation in performance is still common in intensive production systems, mainly with *Bos indicus* cattle, which have not been systematically selected for performance in feedlot conditions. In addition, other factors such as diet and other environmental changes, e.g. space restrictions and feeding from troughs, are potential stressors for extensively raised animals (Fell et al., 1999).

Brazilian feedlot producers typically use weight gain as the main indicator of animal's ability to adapt to confinement. However, weight gain is useful only when the animal is over an extended period in the feedlot, and it is not a trait particularly useful to assess the capacity of newly placed animals to adapt to the feedlot. Therefore, it is extremely important to seek other indicators that are related to performance, providing an early assessment of the capacity of animals to adapt to the confinement.

Temperament seems to be one candidate for this, since the results of many studies agreed that the most temperamental individuals have higher basal levels of stress hormones, inhibiting the growth rate, immune functions, and reproductive performance (Fordyce et al., 1985, 1988; Fell et al., 1999; Curley Jr. et al., 2006; Barrozo et al., 2012), besides affecting also intake (Cafe et al., 2011) and feeding and social behaviors (Soares, 2011).

Based on these findings we present the hypotheses that temperament traits can be used as indicators of the individual capacity of cattle to adapt to the feedlot environment, and that pregnant heifers with better temperament can show better results of growth and reproductive performance, since they have greater motivations to compete to access the feed bunk. Thus, the aims of the present study were to assess the use of different measurements of a temperament trait, flight speed (FS) to: 1) predict the Nellore cattle ability to adapt to the feedlot environment, 2) assess the effects of FS measurements on growth and reproductive performance of Nellore heifers, and 3) study the relationship between agonistic interactions, temperament, growth and reproductive performance in Nellore heifers.

2. LITERATURE REVIEW

2.1. Temperament: Definition and methods of assessment

Although temperament has been considered a complex concept, defined by Réale et al. (2007) as “individual behavioral differences that are repeatable over time and across situations”, and that this individuality involves the expression of many behavioral traits, such as: “aggressiveness, avoidance of novelty, willingness to take risks, exploration, and sociality”, most of the studies assessing cattle temperament usually simplify the concept, assuming, for practical reasons, that cattle temperament can be measured by evaluating the individual responses of the animals during handling (Fordyce et al., 1985; Burrow, 1997). Due to this, most of the studies assessing cattle temperament has focused in only one or few traits, such as docility (Le Neindre et al., 1995; Grignard et al., 2000;), reactivity (van Reenen et al., 2004), fear (Forkman et al., 2007), reaction to humans (Windschnurer et al., 2009), and agitation (Kilgour et al., 2006), despite of many physiological and behavioral traits that can be used as temperament indicators.

Regarding the physiological traits, previous studies have suggested that animals with poor temperament (more excitable) tend to show a upper heart and respiratory rate (Le Neindre, 1989; Visser et al., 2002; Bachmann et al., 2003; Kilgour et al., 2006; von Borell et al., 2007), as well as higher concentrations of serum cortisol levels during routine handling in corral (Boissy and Bouissou, 1988;

Fell et al., 1999; Oliphint, 2003; Curley Jr. et al., 2006; Sanchez-Rodríguez et al., 2013). Then, individual variation in cattle temperament can be assessed using such indicators, in a combined way or not.

On the other hand, the behavioral traits can be used in a less invasive way, by recording the cattle's reactions when kept under specific situations with or without space restriction (Burrow, 1997). When using space restriction, the assessments are generally conducted with cattle contained in the squeeze chute or scale, where the observed variables have scores according to the animals' behavioral reactions to this restrictive situations. On the other hand, when not using the space restriction approach the measurements are carried out in open areas (e.g. in a corral pen), by assessing the animal's movements and the expression of certain behaviors.

While choosing the method (with or without restriction), the researcher must take into consideration a number of factors: the time taken to evaluate it, the necessity of equipment purchasing, and the necessity of training the observers. Summarizing, it is basically necessary to understand which of the behavioral expressions the temperament test can inform on a particular animal (e.g. fear of handler or social isolation) and, moreover, if the test has an easy application and consistency regarding its results, allowing a routine assessment in beef cattle herds (Piovesan, 1998).

2.2. Using flight speed to assess beef cattle temperament

One measurement that uses behavioral reaction to assess cattle temperament is flight speed (**FS**), which measures the exit velocity of cattle immediately after escaping from the squeeze chute (Burrow et al., 1988).Therefore, this chapter will present some studies that used 'Flight speed' test to assess cattle temperament.

Beginning with the fact that less reactive animals (calm) leave the squeeze chute with lower speed when compared to the more reactive ones (excitable), Burrow et al. (1988) developed the FS test that measures the speed of the animals after the release of the scale. According to the authors, the method was developed as an alternative method to another, highly regarded in the literature: the flight zone (Fordyce et al., 1982), which can be considered difficult to use in commercial situations, since it spends a lot of time for evaluation (Burrow et al., 1988). The measurements of FS can be done by using an electronic device composed of a pair of photoelectric cells, a stopwatch, and a processor programmed to register the time taken by each animal to cover a known distance, usually around 2.0 m (depending on the facilities), and later on the time data is converted into speed ($m.s^{-1}$) Faster animals are considered to have worse temperament, and vice versa (Burrow et al., 1988).

This is one of the most respected and validated methods to evaluate cattle temperament in international scientific literature (Burrow, 1997; Curley Jr. et al., 2006; Müller and von Keyserlingk, 2006; Cafe et al., 2011; Sant'Anna et al., 2012), perhaps because of a number of benefits of the method in question. For example, the objectivity of the methodology; since it involves a quantitative continuous assessment (using numeric ratio scale) and due to the fact that the method is not open to the observer’s interpretation, as well as having an easy use in commercial herds.

However, despite the good acceptance in the FS literature, researchers believe that given the complexity of behavioral characteristics expressed during the handling, the use of this measure by itself may be insufficient in the assessment of temperament (Müller and von Keyserlingk, 2006), thus requiring the combination of other methods for a better understanding of cattle temperament.

2.3. Factors related to cattle temperament

It has been found that temperament in farm animals may vary depending on a number of factors, such as genetics, sex, age, and prior experiences during the handling routines (Burrow, 1997; Grandin, 1997). According to Ramos and Mormède (1998), temperament in animals differs between and within species as well as between genotypes. *Bos indicus* cattle genotype has been considered in the literature as the most reactive (excitable) when compared to *Bos taurus* genotype (Hearnshaw and Morris, 1984; Fordyce et al., 1982; Voisinet et al., 1997). Moreover, several researches reported differences between breeds of cattle, as well as between sexes, (Fordyce et al., 1985; Burrow et al., 1988; Morris et al., 1994) with females being considered more reactive compared to males (Voisinet et al., 1997). Likewise, temperament differences between *Bos indicus* genotype cattle were reported by Piovezan et al. (2013), with Nellore considered less reactive (assessed by the flight speed test), compared to the Guzerat breed.

Regarding the effect of age on the expression of the temperament, researchers have shown that the reactivity of cattle is higher in young animals (Fordyce et al., 1988), though this response tends to reduce with the passage of age (Sato, 1981; Burrow, 1997; Tőzsér et al., 2003). In fact, Burrow et al. (1988) found estimates of heritability (h^2 = 0.54) during weaning, but lower heritability (h^2 = 0.26) on 18 months of age, and suggested that the inter-individual variation reduces over time. Studies have shown that this fact is due to the habituation process resulting from greater contact with humans, as well as familiarization with the management routines to which they are subjected, regardless of the breed (Andrade et al., 2001; Krohn et al. 2001; Curley Jr. et al., 2006). Habituation, defined by Harris (1943) as "... decrement of response that result from a repetitive stimulation ..." was considered one of the most elementary forms that prove the behavioral plasticity (Groves and Thompson, 1970). This phenomenon can be favored by a positive effect: the use of successive managements to perform the acclimatization of animals to management in the corral (Cooke et al., 2009).

Nevertheless, the opposite of habituation can also occur, regardless of the type of management used (aversive or not). This fact is referred to as sensitization, and has been reported in several studies (Petherick et al., 2002; Müeller and von Keyserlingk, 2006). According to Burdick et al. (2011), some animals considered to have worse temperament cannot adapt to farming systems that require intensive management, showing no sign of reduction in reactivity. Moreover, in some cases, animals may express a sensitization early in the feedlot, followed by habituation over time. In fact, in a study of feedlot with Nellore animals, Titto et al. (2010) reported an increase in the reactivity of animals only between the first two assessments (28 day interval), followed by a reduction throughout the confined period (between the 56 and 112 day interval). This suggests the occurrence of sensitization and habituation in the same evaluated animals.

Another factor reported by researchers, which can also influence the process of habituation of the animals, is the frequency and the duration between successive handlings (Sebastian et al., 2011), because the process involves the memory capacity of the animals (Siegel, 1989). In fact, there are reports that early life experiences can influence animal attitudes later (Grandin, 1993; Boivin et al., 1994; Curley Jr. et al., 2006; Hoppe et al., 2010). Australian researchers conducted a study in calves raised in extensive systems and reported that managers walking (quietly) between animals, and handling the cattle calmly and quietly during the managements in the corral, had a positive affect the reactivity of the same animals in later life (Fordyce et al., 1988). Similarly, zebu calves (between 30 and 60 days of age) managed successively in the corral became less reactive in later life when compared to the group not managed (Becker and Lobato, 1997).

Thus, it is clear that good management practices, while the animals are still young, can improve the human-animal interaction, by reducing their fear of humans (Boissy and Boissou, 1988).

2.4. Implications of individual behavioral variations

Researchers have stated that individuals are different, not only by morphological and physiological changes, but also in the expression of their behaviours (Searle et al., 2010), and the author further complemented that it is possible to manipulate certain individual behavior, first through breeding programs, in cases of inheritable characteristics, and second by providing favorable environment for the animals to demonstrate the desired behaviour by the producer, through lenience when driving and restraining during handling routines in the corral.

The estimate of flight speed heritability range from low to moderate, for instance Piovezan et al. (2013) estimated a FS heritability of 0.35 for zebu breeds, while other authors found lower estimates, e.g. 0.17 for Brahman (Prayaga et al., 2009), and between 0.11 and 0.36 for Angus, Charolais, Hereford, Limousin and Simmental cattle (Hoppe et al., 2010).

Several studies have shown that animals that express the worse temperament had their productive and reproductive traits negatively affected, including body weight, weight gain, conception rate, milk quality, carcass weight, meat tenderness, and immune function (Fordyce et al., 1985, 1988; Burrow and Dillon, 1997; Fell et al., 1999; Petherick et al., 2002; Prayaga and Henshall, 2005; King et al., 2006; Müeller and von Keyserlingk, 2006; Hoppe et al., 2010). But it is important to notice that the temperament is also associated to agonistic behavior, what can affect the traits cited above, as shown by Phocas et al. (2006), who estimated coefficients of correlation between aggressiveness score and docility (0.21) and among docility score and fertility (0.55), age at puberty (0.20), and ease calving (0.13).

However, we verified that several studies reported lack of, or weak genetic or phenotypic association between FS and performance traits (Fell et al., 1999; Burrow, 2001; Nkrumah et al., 2007; Prayaga et al., 2009; Sant'Anna et al., 2012), suggesting that temperament explains little of the individual variation in performance between cattle. Indeed, in a study conducted by Cafe et al. (2011) with Brahman cattle, the authors found that animals with higher FS spent less time in the trough and had lower dry matter intake, as well as lower body weight and average daily gain. The authors reported that for each meter per second added to FS there was a decrease of 370g per animal.day^{-1} in dry matter intake, and that the largest decline in weight gain occurred in animals with speeds exceeding 2.5 m/s (10g per animal.day^{-1}). It is evident that the association may be limited to some temperament traits, (Turner et al., 2011) due to large individual variations.

The importance of evaluations of individual variations in behavioral responses was largely reported in the literature (Reinhardt, 1983; Atwood et al., 2001; Lewis, 2002; Kilgour et al., 2006; Searle et al., 2010). However, some researchers suggest that the scientific community has emphasized only studies evaluating nutrition and behavior according to the average data of the group, as well as the managements recommendations prepared for the "average" as a whole, masking important individual variations (Atwood et al., 2001).

One way to assess individual differences in food intake, for example, would be keeping cattle in individual pen. However, this condition is very artificial because it excludes the social effects related to feeding behavior. Among them, we can mention the social facilitation, which is defined by the activity of certain individuals that in general is quickly followed by the majority of animals (Broom and Fraser, 2010). When a particular animal initiates ingestion, it stimulates others to the same conduct. In fact, Phillips (2004), evaluating the effects of isolation stress on the performance of calves, has found that animals kept in groups spend more time at the trough, which contributes to a higher food intake and ruminations frequency, compared to isolated animals in individual stalls.

Thereby, another evaluation possibility would be to use the electronic troughs that allow quantifying individual consumption even with the enclosed animals in a group, and this can be done by using the GrowSafe System® (GrowSafe Systems Ltd.). This equipment permits measuring, continuously and individually, the time spent in the trough, and the amount of food ingested in real time. Several studies were performed using this equipment in order to assess, for example, feed efficiency and its relation with weight gain (Schwarzkopf-Genswein et al., 2002; Durunna et al., 2011; Kelly et al., 2010), as well as the impact of social interactions on the feeding behavior (Zobel et al., 2011).

Groups of cattle kept together establish a dominance hierarchy. Based on this hierarchical order the priority of access to resources is set, including food (established through agonistic behavior, such as headers and displacement), especially in confinement. Certain individuals have the ability to select the food in the trough, and that the expression of this behavior is influenced by genetic and environmental factors (Snowder et al., 2001). If the dominant animal has the ability to select the food and has easy access to resources, there is evidence that the more submissive, those that have greater difficulty accessing the trough (Manteca and Deag, 1993) end up being affected. Therefore, under conditions where access to food is limited, as in commercial feedlots, some animals will probably eat less, both in quality and quantity of food (Zobel et al., 2011), affecting their welfare and weight gain. Thus, it is emphasized to the producers the need for reflection about individual variations, because when only the average results of the animal is considered, the opportunity to identify important factors that cause a reduction in profitability in confinement is lost.

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CHAPTER 2 - A comparison of different flight speed measurements to assess feedlot adaptability of Nellore bull calves

ABSTRACT - The aim of this study was to assess the use of FS method to predict the cattle's ability to adapt to the feedlot environment. Five hundred and forty-nine Nellore bull calves were used in this study, which was carried out over two consecutive years; Year 1 (2012, N = 268) and Year 2 (2013, N = 281). The animals consisted of two groups: i) Group 1 averaging 237.29 ± 22.30 d of age and 280.09 ± 29.05 kg of BW (n = 141) in yr 1, and 276.33 ± 12.24 d of age and 299.26 ± 30.09 kg of BW (n = 143) in yr 2 and; ii) Group 2 averaging 324.57 ± 32.20 d of age and 318.68 ± 36.07 kg of BW (n = 127) in yr 1, and 332.83 ± 24.86d of age and 318.16 ± 47.99 kg of BW (n = 138) in yr 2. During the experimental period, calves from each group were allocated equally into one of two feedlot pens (~ 40 m^{2}·animal), and each pen contained eight adjacent electronic troughs (GrowSafe® System Ltd.) to document individual feeding behavior and intake. Feeding behavior variables assessed included bunk attendance frequency **(BAf, visits/d**), bunk attendance duration **(BAd, min/d)**, and eating rate **(ER, g/min^{-1}**; feed intake was assessed by dry matter intake (**DMI, kg/d**). FS ($m.s^{-1}$) was measured four times during the trial, on the first day (**FS1**, immediately before the animals entry in the feedlot facilities), and approximately every 28 days (**FS2, FS3, and FS4**) after. Based on these measurements, two additional FS variables were calculated for each animal including; the arithmetical means of FS (**FSmean**) and the differences between FS1 and FS4 (**FSdiff**). Consequently, FSdiff was used to define the **FScategory**, based on animals that reduced FS more than one SD (**Acclimated)**, animals that did not change FS (**Consistent)** and those that increased FS (**Sensitized**). The animals were weighed at 14-d intervals to obtain performance traits, including: body weight at the end of the feedlot period (**BWf**), **ADG** and feed conversion ratio (**FCR**). Carcass trait data also was collected at the end of the feedlot period using real-time ultrasound measurements of the ribeye area **(REA)**. Our results showed a linear effect of FS1 only for ER during the adaptation period (*P* = 0.0314), a trend of linear effect on BAd (*P* = 0.0617) and BAf (*P* = 0.0550) during the adaptation and entire period, respectively. Also, a quadratic effect of FS1 on BAd (*P* = 0.0192) and DMI (*P* = 0.0031) over the entire period. None of the productivity traits were affected by FS1. On the other hand, FSmean showed linear effects on BAd (*P* = 0.0029), ER (*P* = 0.0342), and DMI (*P* = 0.0289) during the adaptation period, a trend towards a linear effect on DMI (*P* = 0.0822) and BWf (*P* = 0.0831) over the entire period, and quadratic effects on ADG (*P* = 0.0194) and REA (*P* = 0.0411). The variable FSdiff showed only a linear effect on BAf (*P* = 0.0038) over the entire period. Based on these results and under the conditions of this study, FSmean was the only FS measurement with potential to indicate adaptability to the feedlot environment for Nellore bull calves.

Key words: adaptation, beef cattle, behavior, confinement, reactivity, temperament

1. INTRODUCTION

Animals vary in their ability to cope with changes in their environment and efficiently use their habitat (Hohenboken et al., 2005). Studies assessing the impact of animal production systems reported that confinement production such as feedlot settings can be stressful for cattle and therefore certain individuals may have a reduced capacity to adapt to their environment, as evidenced by increased health problems (Fell et al., 1998) and reduced growth performance (Fell et al., 1999).

Brazilian feedlot producers typically use weight gain as the main indicator of an animal's ability to adapt to the feedlot environment. However, weight gain is most useful as an indicator over an extended period in the feedlot, and not particularly useful to assess newly placed animals. Therefore, it is extremely important to seek other indicators that are related to performance, but will enable the producer to make an early assessment of the capacity of animals to adapt to the feedlot environment.

One of the first responses to stressful events in animals is a change in behavior (Moberg, 2000) and as described by Burdick et al. (2011), the level of stress manifested by animals is modulated by their temperament. According to Réale et al. (2007) "temperament represents individual behavioral differences that are repeatable over time and across situations", and this individuality involves the expression of many behavioral traits such as: "aggressiveness, avoidance of novelty, willingness to take risks, exploration, and sociality". Studies interested in assessing cattle temperament usually simplify this definition, assuming, for practical reasons, that cattle temperament can be measured by evaluating the individual responses of the animals during handling (Fordyce et al., 1985; Burrow, 1997). One measurement that uses behavioral reaction to assess cattle temperament is flight speed (**FS**), which measures the exit velocity of cattle immediately after escaping from the squeeze chute (Burrow et al., 1988). The authors developed this method as they noted that some animals remain calm while being weighed and also exit the weigh scale more slowly than when they were excitable inside the squeeze chute. Since then, FS has been largely reported in the scientific literature as a cattle temperament trait (Burrow, 1997; Curley Jr. et al., 2006; Müeller and Von Keyserlingk, 2006; Behrends et al., 2009; Del Campo et al., 2010; Hoppe et al., 2010; Burdick et al., 2011; Cafe et al., 2011; Sant'Anna et al., 2013). One of the possible reasons for this is because the method is easy to incorporate into routine management procedures (Burrow et al., 1988) and that cattle with greater FS have been shown to have slower growth rates than calmer ones, particularly under more intensive conditions (Cafe et al., 2011). However, this relationship could also be attributed to other factors related to the assessment of temperament such as the objectivity of the assessment and the repeatability and heritability of temperament (Prayaga et al., 2009; Hoppe et al., 2010; Piovezan et al., 2013; Sant'Anna et al. 2012). Therefore, one could hypothesize that assessing temperament using FS would be a useful tool to rank cattle with greater and lesser ability to adapt to the feedlot environment. On the other hand, some studies did not confirm this association and suggested that cattle temperament traits explain only a small part of the individual variation in growth performance (Nkrumah et al., 2007; Prayaga et al., 2009).

Researchers have used FS in different ways to assess the relationship between cattle temperament and performance traits, as follows: by assessing FS once, before the animals were allocated in the feedlot environment (Curley Jr. et al., 2006; Behrends et al., 2009; Hall et al., 2011), and by measuring FS on repeated days, then calculating the mean values of this trait (Voisinet et al., 1997; Olmos and Turner, 2008; Cafe et al., 2011). Thus, we hypothesized that depending on the way that FS data is summarized (e.g., using means of all assessment days or a specific FS measurement), different results can be reached regarding the animals ability to adapt to the feedlot environment. The aim of the present study was to assess the use of FS method to predict the cattle's ability to adapt to the feedlot environment, using two age groups of Nellore bull calves aiming to determine: i) among four different FS measurements, which of them would be the best predictor of the feedlot cattle´s ability to adapt to the feedlot environment; ii) the consistency of FS over the feedlot period and; iii the use of FS changes over time as a predictor of cattle ability to adapt to the feedlot.

2. MATERIAL AND METHODS

This study was approved by the Committee of Ethical Use of Animals (CEUA process number: 005178/13) from Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal-SP, Brazil. It was conducted at a commercial farm located in Uberaba County, Minas Gerais State, Brazil.

2.1. Animals and feeding management

Five hundred and forty-nine Nellore bull calves were used in this study, which was carried out over two consecutive years; Year 1 (2012, N = 268) and Year 2 (2013, N = 281). The animals consisted of two groups: i) Group 1 averaging 237.29 ± 22.30 d of age and 280.09 ± 29.05 kg of BW (n = 141) in yr 1, and 276.33 ± 12.24 d of age and 299.26 ± 30.09 kg of BW (n = 143) in yr 2 and; ii) Group 2 averaging 324.57 ± 32.20 d of age and 318.68 ± 36.07 kg of BW (n = 127) in yr 1, and 332.83 ± 24.86d of age and 318.16 ± 47.99 kg of BW (n = 138) in yr 2. Data for Group 1 was collected between May, 21 and August, 22 of 2012 for yr 1, and between May, 25 and August, 20 of 2013 for yr 2, for a total experimental period of 93 and 87 d, respectively. For Group 2, data was collected between August, 23 and November, 19 of 2012 for yr 1 and between August, 26 and December, 05 of 2013 for yr 2, for a total experimental period of 89 and 102 d, respectively.

During the pre-confinement period (prior to the experimental period), animals from Group 1 and 2 were kept on pasture (*Brachiaria spp)*, with free access to natural water sources. Throughout this period all cattle were supplemented with a total mixed ration (TMR) provided in troughs (offered once per day) to adapt the animals to the feedlot diet. This adaptation diet consisted of corn silage (80%), soybean meal (11%), ground corn (6%), and additives and mineral supplement (3%). This resulted in a diet consisting of 12% CP and 68% TDN on DM basis, with minor adjustments to accommodate the nutritional requirements for group 1 and 2 used in this study, which were kept in separate paddocks.

During the experimental period, each group (1 and 2) was allocated equally into one of two feedlot pens (40 m × 70 m). Pen space available was ~ 40 $m^{2}\cdot$animal, and each pen contained eight adjacent electronic troughs (GrowSafe® System Ltd.). Concrete floors approximately 3 m x 10 m adjacent to the feeding troughs ensured a solid surface for the cattle to stand on while feeding, fresh water and feed were available *ad libitum* during the entire experimental period. All animals were fed daily at 0730, 1230 and 1530 h, receiving the same TMR offered during the pre-confinement period, as described above.

2.2. Temperament trait

Temperament was measured on four occasions (~28-d intervals) over the experimental period using flight speed measurement (**FS**; adapted from Burrow et al., 1988) by measuring the speed ($m \cdot s^{-1}$) at which each animal exited the cattle squeeze chute after being weighed. The measurements were performed with an electronic device that included 2 pairs of photoelectric cells, a chronometer and a small processor programmed to record the time taken by each animal to cover a 2.58 m distance, (measured as a diagonal line from the squeeze chute door to the end of the alley, since the exit corridor design required the cattle to turn left at 90º during the exit). Faster animals were considered to have more excitable temperament (Burrow et al., 1988).

FS was measured four times during the feedlot period, as follows: on the first day (**FS1**, on entry to the feedlot facilities), after ~28-d (**FS2**) and ~56-d (**FS3**), and at the end of the feedlot period (**FS4**, ~84-d). Assessment days were approximate according to the schedule of the farm for each group of age and year, but did not differ by less than 24 d and more than 37d. Based on these measurements 2 other FS variables were calculated for each animal 1) the arithmetic means of all FS recorded ((FS1 + FS2 + FS3 + FS4)/4) (**FSmean**) and; 2) the difference between FS1 and FS4 (**FSdiff**).

Flight speed difference (FSdiff) was used to define a new variable, categorizing flight speed (**FScategory)**, as follows: 1) 'Acclimated': animals that reduced FS more than one SD (FSdiff > 1SD) were assumed to represent the cattle that were best adapted to the feedlot; 2) 'Consistent': animals that did not change FS (FSdiff ± 1SD) were assumed to represent calves with a moderate ability to adapt to the feedlot and; 3) 'Sensitized': animals that increased FS more than one SD (FSdiff < -1SD) were assumed to represent calves with the lowest ability to adapt to the feedlot.

2.3. Feeding behavior and intake

The feeding behavior and intake of each animal was evaluated using the records obtained from the GrowSafe® system. All calves were fitted with an electronic identification (Allflex® Half duplex) transponder in their left ear which could be detected by an antenna within the rim of each feed trough. The files were recorded onto a computer every second over a 24 hour period throughout the confinement period.

The variable used to assess the feed intake was dry-matter intake (**DMI**, $kg.d^{-1}$), and the variables used to assess feeding behavior included: bunk attendance duration (**BAd**, total recorded time that the animal was present with their head in the trough, $min \cdot d^{-1}$), bunk attendance frequency (**BAf**, number of times the trough electronically registered the presence of the animal feeding, $visit \cdot d^{-1}$), and eating rate (**ER**, calculated as the ratio of DMI by BAd, $g \cdot min^{-1} \cdot d^{-1}$).

Two periods were considered to assess feeding behavior and intake: the adaptation period, first month in the feedlot (range from the first 14 to 24 d); and the entire experimental period, from the beginning until the end to the feedlot period (range 87 to 102 d).

2.4. Performance and carcass traits

Animals were weighed on entry (**BWi**, $kg.animal^{-1}$) and every ~14 d until the end of the feedlot period, according the schedule of the farm. Performance traits for each animal included: final body weight (**BWf**, BW assessed at the end of feedlot period; $kg.animal^{-1}$), ADG ($kg.\ kg.animal^{-1}.d^{-1}$), and also feed conversion ratio **(FCR,** $kg\ DMI.kg\ gain^{-1}$), which was calculated using DMI (obtained from GrowSafe) and ADG. Carcass trait data was collected using real-time ultrasound measurements of the ribeye area (**REA**, cm^2; measured between the 12th and 13th ribs). The device used to obtain ultrasound measurements for REA was an Aloka 500V, with a linear probe measuring 17 cm and 3.5 MHz, and an acoustic coupler with an image capture system (UltraInsights 1.0, LinXcel Video - Grabber USB 2.0). The images were obtained from all cattle within each group (1 and 2), in yr 1 and yr 2, on the same day, and by the same accredited technician, who was also responsible for interpreting the images, using UltrasInsights Processing Lab, Beef Lab Software - Maryville, MO.

2.5. Statistical analyses

2.5.1. Data cleaning and summary

Data was removed from the dataset used in the statistical analysis when the Growsafe® system failed (mainly due to power outage and equipment malfunction), or when the system was under maintenance, resulting in missing information, as described in Table 1.

Table 1. Number of days that Nellore bull calves were housed in a feedlot and the number of days with missing data from GrowSafe® system, per group of calves (1 and 2), year (2012 and 2013), and pen (1 and 2).

Group	*Year*	Period in feedlot, days	Missing data, days	
			Pen 1	Pen 2
1	2012	93	6	13
	2013	87	17	4
2	2012	89	10	9
	2013	102	19	17

Contrary to other studies which used a ‘meal criterion’ to summarize feeding behavior variables defined as: a return to the bunk within 300s of the previous departure (considered to be a continuation of the same visit) (Gibb et al., 1998; Schwartzkopf-Genswein et al., 2002), in the present study the raw GrowSafe data was not summarized and analyzed using a ‘meal criterion’. All statistical analysis was carried out separately for each group (1 and 2).

To identify outliers within group and year, the UNIVARIATE procedure of SAS, with analysis of residuals, was used. The data considered as outlier were removed from the dataset.

In addition, FS data from group 1 (N = 15) and group 2 (N = 15) that fell down at the time of exit from the squeeze chute were assigned a FS value of 3.00 $m \cdot s^{-1}$.

Multicollinearity with the VIF option (variance inflation factors) between the independent variables, age (on d 1 of experimental period) and BWi was tested before the final statistical models on the dependent variables. If the VIF value was greater than 10 among the factors they were removed, as it was assumed that the regression coefficients would be poorly estimated due to multicollineatity. Age was included as covariate instead of BWi, due to collinearity between these two factors.

2.5.2. Effect of FS on indicators of cattle ability to adapt to the feedlot

To identify which FS measurement (FS1, FSmean, and FSdiff) would be the best indicator of ability to adapt to the feedlot, the effects of each FS measurement on feeding behavior (BAf, BAd, and ER), intake (DMI), performance (BWf, ADG, and FCR), and carcass traits (REA), were analyzed fitting the linear mixed model and using the MIXED procedure of SAS. The statistical model included: the fixed effect of group (1 and 2, only for control purpose), year (yr 1 and yr 2, only for control purpose) and the interaction between group and year; the effect of age of animal on d 1 of the experimental period and one FS measurement (FS1, FSmean, or FSdiff) as covariate and; pen within the group nested within year as a random effect. A post-hoc (Tukey) test was used to compare the adjusted means. Main effects and interactions were considered significant at $P < 0.05$ and $0.05 < P < 0.10$ as tendency.

2.5.3. Consistency of FS over the feedlot period

The consistencies of FS over the feedlot period (FS 1-4) were evaluated using a one-way ANOVA with the use of the GLM procedure of SAS. Means among the assessment days were compared using a post-hoc (Tukey) test. In addition, the same procedure described above (GLM) was applied, using Partial correlation coefficients (MANOVA option) to estimate the associations among all FS assessment measurements (FS 1-4), FSmean and FSdiff. The statistical model included: the fixed effect of age of animal on d 1 of the experimental period, group (1 and 2), year (yr 1 and yr 2) and the interaction between group and year. The effects of FScategory were assessed on feeding behavior (BAf, BAd, and ER), intake (DMI), performance (BWf, ADG, and FCR), and carcass traits (REA), fitting the linear mixed model and using the MIXED procedure of SAS. The fixed effect of group (1 and 2, for control purpose), year (yr 1 and yr 2, for control purpose), interaction between group and year, and FScategory were included in the models; and age of animal on the d 1 of the experimental period as a covariate. The interactions of FScategory within the group nested within year were also included in the model; however, the interaction was maintained only if it was significant. In addition, the sampling distributions for FScategory were compared using χ^2 test. Main effects and interactions were considered significant at $P < 0.05$ and $0.05 < P < 0.10$ as tendency.

3. RESULTS AND DISCUSSION

Several studies have assessed the relationship between FS, feeding behavior, intake, and productive traits (Nkrumah et al., 2007; Cafe et al., 2011). However, the ideal FS measurements which have better relationships with those cited traits, which are related to the individual's ability to adapt to the feedlot environment, have yet to be determined.

3.1. Descriptive statistics

The descriptive statistics of FS measurements are shown in Table 2.

Table 2. Mean (±SD), minimum, and maximum observations of flight speed measurements[1] of Nellore bull calves

FS measurements (m/s)	N	Mean (±SD)	Min	Max
FS1	533	1.7 ± 0.51	0.4	3.9
FS2	511	1.6 ± 0.57	0.2	3.5
FS3	532	1.6 ± 0.56	0.3	4.1
FS4	520	1.3 ± 0.45	0.5	2.9
FSmean	549	1.5 ± 0.45	0.5	3.2
FSdiff	505	0.3 ± 0.50	-1.5	1.9

[1]FS1=flight speed assessed on the first day of the feedlot period; FS2: ~28d; FS3: ~56d; FS4:~84d; FSmean=((FS1 + FS2 + FS3 + FS4)/4); FSdiff=FS1 – FS4.

The descriptive statistics of feeding behavior, intake, performance and carcass traits are presented in Table 3.

Table 3. Mean (±SD), minimum, and maximum observations of feeding behavior[1], intake[2], performance[3], and carcass trait[4] of Nellore bull calves (N = 549)

Trait[1]	Adaptation period[5]			Entire experimental period[5]		
	Mean (±SD)	Min	Max	Mean (±SD)	Min	Max
BAf, visit/d	94.1 ± 28.71	31.9	243.1	113.1 ± 25.88	60.1	238.5
BAd, min/d	108.7 ± 34.10	16.6	203.2	111.4 ± 27.09	48.3	200.41
ER, g/min	62.9 ± 23.15	30.0	190.0	67.8 ± 21.78	30.0	170.0
DMI, kg/d	6.2 ± 1.17	1.7	10.1	7.2 ± 1.19	4.3	11.2
BWi, kg	---	---	---	303.6 ± 39.73	216.0	451.0
BWf, kg	---	---	---	376.1 ± 47.27	245.0	555.0
ADG, kg/d	---	---	---	1.0 ± 0.26	0.2	1.6
FCR, kg DM/kg gain	---	---	---	7.3 ± 2.25	4.4	26.6
REA, cm^2	---	---	---	63.0 ± 7.64	38.6	94.9

[1]BAf=Bunk attendance frequency, BAd=Bunk attendance duration, ER=eating rate. [2]DMI=dry matter intake. [3]BWi=Initial body weight, BWf=Final body weight, ADG=average daily gain, FCR=Feed conversion ratio. [4]REA=Ribeye area. [5]Adaptation period=first~15 days, Entire experimental period=~84 days.

3.2. Effect of FS1 as predictor of cattle ability to adapt to the feedlot

Assuming that FS assessment is a good predictor of cattle´s ability to adapt to the feedlot environment, we hypothesized that FS assessed before the cattle entered the feedlot environment could be an early predictor to this situation. Thus, the following results are described separately for adaptation and entire periods with the purpose to assess the effects of FS variables (FS1 FSmean, and FSdiff) over those two different periods.

Adaptation period. Even though it is known that excitable animals are more stressed than calmer ones facing novel situations (Grandin, 1993), our results showed that BAf was not affected by FS1 during the adaptation period (Table 4). This result suggests that all animals, independent of the temperament seem to have the same motivation to seek food at the trough. Motivation was defined as “a reversible brain state induced by internal and external signals, which results in an increased tendency to perform a specific behavior” (Forbes, 2007). Hunger is one example of the internal signals, and under the conditions of our study, the external signals could be the presence of the food in the trough, combined with social facilitation, which is characterized for example, when one animal seeks the feed bunk, independent of whether this animal is hungry or not, just because the other cattle have engaged in that activity.

However, despite all animals showing motivation to seek food during the first ~15 days of the feeding period, the decision to remain at the feed bunk was modulated by temperament, since excitable animals (those showing higher FS1) spent less time at the feed bunk (reduction of 4.1 $min.d^{-1}$ in BAd for each 1 $m.s^{-1}$ increased in FS1; P = 0.0617) than calmer ones (Table 4). Indeed, Amdam and Hovland (2011) reported that motivational behavior may vary between individuals, and generally is influenced by a multitude of intrinsic (e.g., genetic or physiological) or extrinsic (i.e., in the animal's environment) factors. Furthermore, it is known that some animals can exhibit positive motivation (approach-behavior) while others, negative motivation (avoidance-behavior) to the same circumstance (Kirkden and Pajor, 2006). Thus, the fact that our results showed that calm animals had greater BAd suggested that temperament could be an intrinsic factor for cattle's motivation to remain at the feed bunk eating, with excitable animals exhibiting negative motivation facing competitions among their conspecifics.

Despite the fact that excitable animals spent less time at the trough (Table 4) as previously discussed, they showed low DMI, such as calm animals due to the quadratic effect (P = 0.0007), as shown in Table 4. Therefore, excitable animals ate faster (increased of 2.2 $g.min^{-1}.d^{-1}$ in ER for each 1 $m.s^{-1}$ increased in FS1; P = 0.0314) than calmer ones (Table 4). These results suggest that excitable animals may be more stressed when competing for access to the trough, choosing not to spend an extended time at the feed bunk to avoid threatening situations (Soares, 2011), and consequently eating faster, while exhibiting the same intake as calm cattle. This result is opposite to findings of Nkrumah et al. (2007) which did not find any relationship between BAd and FS in young *Bos taurus* steers (252 ± 42 d of age).

Entire period. Given that competition at the feed bunk is highly prevalent during the first few days in the feedlot environment (Paranhos da Costa, 2000), it is possible that excitable calves may change their strategy to access the trough at this time. This hypothesis is based on the fact that several studies have reported cattle use different strategies to access feed resources (Zobel et al., 2011), such as changing the time of the day they access food to a time when there are fewer cattle eating are at the feed bunk. In contrast to the adaptation period, cattle categorized as excitable over the entire feeding period had a tendency to visit the feed bunk less frequently than calmer cattle (reduction of 3.5 visits.d^{-1} in BAf for each 1 m.s^{-1} increased in FS1; $P = 0.0550$) (Table 4). However, both excitable and calm cattle spent less time at the feed bunk ($P = 0.0192$), and consumed less feed ($P = 0.0031$) than animals that showed intermediary values of FS1 (Table 4). One explanation for calm animals having greater BAf, but reduced BAd, and DMI (similar to that observed in excitable animals) is that they are less affected by stressful situations (Grandin, 1993).

The stress response is one of the body's major coping mechanisms for environmental disturbance, which is comprised of the sympathetic-adrenal-medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axis. The HPA releases corticosteroid hormones (cortisol and corticosterone), as a result of a series of physiological events during stressful situations; and several researchers had reported that the high concentration of those hormones were associated with high FS and low growth performance in beef cattle (King et al., 2006; Curley Jr. et al., 2006).

One possible reason that FS1 did not affect any of the production traits (BWf, ADG, FCR, or REA) is that both excitable and calm animals had low DMI (as previously discussed) (Table 4). These results are opposite to those reported by Petherick et al. (2002) who suggested that FS could be a predictor of performance, since their results showed that calm animals (assessed using FS before animals enter into the feedlot environment) had greater ADG and FCR than excitable ones. Possible explanations for the difference between both studies include : 1) differences in BWi between the animals of those study (448 and 446 kg for calm and excitable cattle, respectively) and the animals of our study (303 kg); 2) contrary to our study where animals were fed with 12% CP and 68% TDN on DM basis during the entire feeding period, the cited study started the feeding period at the same level as ours however, they increased the level of grain to 26%, 21 days after entry to the feedlot and decreased the levels of roughage at weekly intervals until the end of the feedlot period (102 d), which could lead to increased growth performance.

In summary, based on the results described above we conclude that FS1 is not useful for the early prediction of individual adaptability to the feedlot environment, especially due to the lack of linear effects of FS1 on feed intake (during adaptation and entire periods) and productivity traits.

3.2. Effect of FSmean as indicator of cattle ability to adapt to the feedlot

Adaptation period. The effects of FSmean on feeding behavior variables (BAf, BAd, and ER) were very similar to those described for FS1. The variable FSmean did not affect BAf during the adaptation period (Table 4), suggesting that all animals had the same motivation to seek food at the trough, as previously discussed. Excitable cattle spent less time at the feed bunk (reduction of 7.4 $min.d^{-1}$ in BAd for each 1 $m.s^{-1}$ increase in FSmean; *P* = 0.0029) and ate faster than the calmer cattle (increase of 2.5 $g.min^{-1}.d^{-1}$ in ER for each 1 $m.s^{-1}$ increase in FSmean; *P* = 0.0342), as shown in Table 4. However, in contrast to FS1, a linear effect was observed between FSmean and DMI (reduction of 0.2 $kg.animal^{-1}.d^{-1}$ in DMI for each 1 $m.s^{-1}$ increase in FSmean; *P* = 0.0289) (Table 4), suggesting that excitable animals ate less than the calmer ones. This result was also reported by Cafe et al. (2011) with a tendency towards a linear effect of FSmean on DMI (0.26 $kg.animal^{-1}.d^{-1}$) in Brahman cattle approximately 7 mo. of age.

Entire period. During the entire feeding period BAf was not affected by FSmean (Table 4), however, both calm and excitable animals spent less time at the trough. On the other hand, a linear tendency was observed between FSmean and DMI (reduction of 0.1 $kg.animal^{-1}.d^{-1}$ in DMI for each 1 $m.s^{-1}$ increase in FSmean; *P* = 0.0822) (Table 4), suggesting that FSmean was a more sensitive predictor of calm calves with greater DMI. Significant linear effects of FSmean (assessed during the feedlot period) were reported by Cafe et al. (2011) on BAd (4.68 $min.d^{-1}$) and a tendency of the effect on DMI (0.26 $kg.animal^{-1}.d^{-1}$) as previously discussed.

Given our intake results (reduction of 0.1 kg.animal^{-1}.d^{-1} in DMI for each 1 m.s^{1} increase in FSmean; *P* = 0.0822, Table 4), it was expected that the performance and carcass traits of excitable calves would have been negatively affected. However, our results support this expectation only partially, with a tendency for FSmean to affect live weight (reduction of 0.26 kg.animal^{-1} in BWf for each 1 m.s^{-1} increase in FSmean; *P* = 0.0831) (Table 4). An unexpected quadratic effect was observed between FSmean, ADG (*P* = 0.0194) and REA (*P* = 0.0411), indicating that both, excitable and calm calves had low ADG and REA (Table 4). These findings were supported by other studies which reported a linear relationship between FSmean and BWf (Burrow and Dillon, 1997), a quadratic effect on ADG (Müeller and Von Keyserlingk, 2006), and no effect on FCR (Nkrumah et al., 2007).

However, others studies reported opposing results, for example, Hall et al. (2011) reported no correlation between FSmean and REA, using crosses of *Bos taurus* steers between 5 to 8 months of age. One possible explanation for the discrepancy between the studies is due to the fact that in our study we had a group of animals (Group 2) older (~ 12 months of age) than those used in the other study, which could lead to better carcass traits.

Overall, most relationships described in the literature between the FSmean and growth performance were negative and linear, suggesting that temperament can improve productivity in cattle kept in the feedlot environment (Nkrumah et al., 2007; Hall et al., 2011; Cafe et al., 2011). However, Cafe et al. (2011) also observed quadratic effects of FSmean on ADG, and suggested that this quadratic relationship was dependent upon which extremes in temperament exist within the herd, and the fact of the presence of extreme excitable animals could lead to a nonlinear relationship.

In summary, we verified that FSmean was a better predictor, in comparison to FS1, of an animal’s ability to adapt to the feedlot, in particular its consistent linear effects on DMI (during the adaptation and entire periods) and BWf, as shown in Table 3. However, due to the lack of linear effects on the other productivity variables (ADG, FCR, and REA), more studies will be necessary to clarify this relationship.

3.3. Effect of difference between the first to the fourth FS measurements (FSdiff) as indicator of cattle ability to adapt to the feedlot

In contrast to the other FS variables (FS1 and FSmean), FSdiff did not reflect to what extent an individual calf was calm or excitable, but rather how they changed their reaction to handling over time, indicating the degree of habituation or behavioral sensitization of the calves to handling. Habituation was defined by Harris (1943) as "... decrement of response that result from a repetitive stimulation ...", whereas sensitization, reported in several studies (Petherick et al., 2002; Müeller and von Keyserlingk, 2006), is the opposite of habituation, which can be characterized as a process of behavioral sensitization, defined as a transient increase in excitability resulting from repeated presentation of a stimulus (Sato, 1995).

Adaptation period. None of the feeding behavior variables (BAf, BAd, and ER), including intake (DMI), assessed during the adaptation period were affected by FSdiff (Table 4), suggesting that FSdiff was not able to identify animals with different feeding behaviors or intake during the first 15 days of the feeding period.

Entire period. Significant linear effect between FSdiff and BAf were observed during the entire experimental period (reduction of 5.5 visits.d^{-1} in BAf for each 1 m.s^{-1} increase in FSmean; P = 0.0038, Table 4). This result lead us to hypothesize that animals which were sensitized over time (increased FS) had more motivation to visit the trough or they needed to make more visits to attempt to eat the same amount of food as animals that reduced their FS; since the other feeding behaviors (BAd and ER) and intake (DMI) variables were not different among the different temperament types (Table 4). However, it is important to take into consideration that FCR and the other productivity traits (BWf, ADG, and REA) were not affected by FSdiff, suggesting that all animals had the same performance potential.

At this time no other published research studies have used FSdiff to assess the effect of temperament of beef calves on their adaptability to the feedlot. Thus, based on our results we conclude that FSdiff was not a good predictor of cattle adaptability to the feedlot environment.

Table 4. Significance (*P*-value) of effects of several flight speed measurements[1] on (±SE) feeding behavior[2], intake[3], performance[4], and carcass traits[5], determined during adaptation and entire experimental periods[6], recorded in Nellore bull calves (N = 549)

Trait	FS1			FSmean			FSdiff		
	Slope	SE	*P*-value	Slope	SE	*P*-value	Slope	SE	*P*-value
Adaptation period									
BAf, visit/d	- 1.2	1.72	0.4979	1.4	1.96	0.4637	- 2.8	1.82	0.1316
BAd, min/d	- 4.1	2.17	**0.0617**	- 7.4	2.46	**0.0029**	- 2.6	2.29	0.2507
ER, g/min/d	2.2	1.03	**0.0314**	2.5	1.20	**0.0342**	- 0.8	1.09	0.4509
DMI, kg/d	- 0.3	0.10	**0.0007**†	- 0.2	0.10	**0.0289**	0.1	0.09	0.3624
Entire experimental period									
BAf, visit/d	- 3.5	1.82	**0.0550**	0.3	2.07	0.8683	- 5.5	1.90	**0.0038**
BAd, min/d	- 5.7	2.50	**0.0192**†	- 8.7	3.66	**0.0178**†	1.6	2.10	0.4519
ER, g/min/d	1.0	1.12	0.3698	0.6	1.29	0.6007	- 0.2	1.18	0.8693
DMI, kg/d	- 0.2	0.08	**0.0031**†	- 0.1	0.07	**0.0822**	0.1	0.09	0.8458
BWf, kg	- 3.1	2.92	0.2908	- 5.8	3.36	**0.0831**	3.5	3.05	0.2477
ADG, kg/d	- 0.1	0.01	0.6387	- 0.1	0.03	**0.0194**†	- 0.1	0.02	0.2334
FCR, kg DM/kg gain	0.2	0.18	0.2939	- 0.2	0.21	0.3892	0.1	0.19	0.9509
REA, cm^2	- 0.3	0.58	0.5853	- 2.2	1.10	**0.0411**†	0.5	0.61	0.4252

[1]FS1=flight speed assessed on day 1 of the feedlot period; FSmean=[(FS1 + FS2 + FS3 + FS4)/4]; FSdiff=FS1 – FS4.[2]BAf=Bunk attendance frequency; BAd= Bunk attendance duration; ER= eating rate. [3]DMI=dry matter intake. [4]BWf=Final body weight; ADG=average daily gain; FCR=Feed conversion ratio.[5]REA=Ribeye area. [6]Adaptation=first ~15 days; Entire experimental periods=~84 days. Bold *P-values* represents significant effect ($P < 0.05$) and tendency ($0.05 < P < 0.10$); †Significant quadratic effect ($P < 0.05$) and tendency ($0.05 < P < 0.10$)

3.4. Consistency of FS over the feedlot period

Significant effects of days in the feedlot on FS were observed for bull calves ($P < 0.05$), as shown in Table 5. The variable FS was stable during the first two assessments, and showing a tendency of reduction between FS2 and FS3 ($P = 0.0620$) and a significant reduction between FS3 and FS4 ($P < 0.001$) and between FS1 and FS4 ($P < 0.001$), as shown in Table 5. These results suggest that cattle can be ‘trained’ or habituated to humans and become less fearful of them (Boissy and Boissou, 1988; Hemsworth et al., 1996).

These results are opposite to previous findings, where Cafe et al. (2011) showed a decline in the response of the cattle to handling over an 8 month period, with much of the decline occurring in the first 3 assessments. Also, the reduction in FS observed among all assessment days in the present study was much lower, ranging from 0.1 to 0.4 $m.s^{-1}$ (Table 5), when compared with the results of a previous study, reporting a FS reduction of 0.9 $m.s^{-1}$ between the first and the last measurements (Hall et al., 2011).

Table 5. Means (± SD) of flight speed measurements[1] taken during four assessments of Nellore feedlot bull calves (N = 549)

Type	FS1	FS2	FS3	FS4
FS, m/s	1.6 ± 0.03[a]	1.6 ± 0.03[ab]	1.5 ± 0.03[b]	1.2 ± 0.03[c]
Day[2]	0	~ 28	~ 56	~ 84

[1]FS1=flight speed assessed on the first day of the feedlot period; FS2= ~28d; FS3= ~56d; FS4= ~84d; [2]Approximate days from first FS measurement (range: 24 – 37d). [a - b]Means followed by the same letters in rows do not differ (P > 0.05) by Tukey test.

Given this we suggest that the reduction in FS in our study may have limited biological significance. One possible reason for this difference between the studies is the handling frequency, since in our study the cattle were handled every 14 d for weighing (totaling eight handling events), and FS data was obtained over a 28-d interval (over ~84 days of feedlot period), while Hall et al. (2011) assessed FS on eight occasions every 28-d over but for a longer period, 188 d. The authors also found that cattle exhibited a consistency in FS over time, (first four FS measurements 28-d intervals). This suggests that the feedlot period used in the present study (~84 days) may not have been enough to change temperament to the same degree as in Hall's study.

Indeed, we verified a significant partial correlation among the FS measurements (FS1, FS2, FS3 and FS4), ranging from r = 0.15 to 0.84 as shown in Table 6. In addition, FSmean had the highest correlation with the second assessment day (P < 0.001) and the lowest with FS1 (P < 0.001), while FSdiff had a positive correlation with FS1 (P < 0.001) and negative with FS3 (P < 0.001) and FS4 (P < 0.001), as indicated in Table 6.

These results indicated that FS seems to be consistent over the feedlot period, suggesting that the animals did not change their temperament, which is also in agreement with previous studies using *B. taurus* (Schrader, 2002; Van Reenen et al., 2004) and *B. indicus* cattle (Boissy and Boissou, 1995; Petherick et al., 2002). Indeed, for some researchers, individual temperament consistency is important because it can be useful in predicting future behaviors (Svartberg et al., 2002), such as high reactivity.

Table 6. Partial correlations coefficient between FS measurements[1] for the Nellore bull calves (N=549)

Item	FS1	FS2	FS3	FS4	FSmean	FSdiff
FS1		0.55**	0.49**	0.52**	0.78**	0.46**
FS2			0.58**	0.60**	0.84**	- 0.07
FS3				0.61**	0.83**	- 0.15**
FS4					0.83**	- 0.52**
FSmean						- 0.08
FSdiff						

[1]FS1=flight speed assessed on the first day of the feedlot period; FS2= ~28d; FS3= ~56d; FS4= ~84d; FSmean=[(FS1 + FS2 + FS3 + FS4)/4]; FSdiff=FS1 – FS4. *P < 0.05; **P < 0.001.

This lead us to conclude that, contrary to some studies that reported that quantity and quality of handling imposed on cattle has an impact on fear of humans (Petherick et al., 2002; Sebastian et al., 2011), the results in the present study indicated that, the duration between repeated exposures appears to affect habituation to human handling (Sebastian et al. 2011). Some cattle may need more time to habituate to handling to exhibit this change in FS. In fact, Soares (2011) reported reductions in FS over time with Nellore bull calves kept in a feedlot environment over 173 days , while no differences were observed in Guzerat cattle within the same study suggesting that they were more reactive and needed more time to habituate to handling.

Regarding individual differences in FS, our results show that bull calves slightly increased their variations in FS4 compared to FS1, as previously shown in Table 5. In a previous study, Behrends et al. (2009) reported similar results and concluded that the increase in FS variations over time provide some evidence that individual animals varied in their ability to habituate to handling.

Indeed, regarding FScategory, the percentage of animals within each category (‘Acclimated’, ‘Consistent’ and ‘Sensitized’) was not homogeneous ($\chi 2 = 292.56$; P < 0.001). Most of the bull calves did not change FS (62.48%; ‘Consistent’) between the first and the fourth assessments, followed by those which reduced FS (34.61%; ‘Acclimated’) and those which increased FS (2.91%; ‘Sensitized’), as described in Table 7.

The ability to habituate to humans and handling procedures, as indicated by ‘Acclimated’ category has been documented in the scientific literature (Curley Jr. et al. 2006; Hoppe et al. 2010). This habituation to handling may be evident in decreased reactivity over time, whereby animals become less reactive, or more docile in their behavioral responses. However, according to Burdick et al. (2011), some animals cannot adapt to farming systems that require intensive management, showing no sign of reduction in reactivity.

Table 7. Mean (± SD) observations of flight speed[1] by FScategory[2] of Nellore feedlot bull calves

FS, m/s	Acclimated	Consistent	Agitated
FS1	1.9 ± 0.05[aA]	1.5 ± 0.04[bA]	1.2 ± 0.12[cB]
FS4	1.0 ± 0.06[cB]	1.4 ± 0.05[aA]	2.1 ± 0.13[aA]
N	190	343	16

[a - b]Means followed by the same letters in rows (lowercase) and columns (uppercase) do not differ (P > 0.05) by Tukey test. [1]FS1=flight speed assessed on the first day of the feedlot period; FS4=flight speed assessed on the last day (~84 days) of the feedlot period. [2]Acclimated=animals that reduced FS in more than one SD; Consistent=animals that did not change FS; Sensitized=animals that increased FS in more than one SD.

Others studies have also reported an increase in FS over time in feedlot cattle (Petherick et al., 2002, Müeller and Von Keyserlingk, 2006). According to Kilgour et al. (2006) measuring individual differences in reactivity to handling is important in order to understand the level of threat to handlers. Despite the fact that FScategory represents an important practical variable in understanding the relationship between production and cattle temperament it was not useful to predict individual variation in feeding behavior, intake, performance, and carcass composition.

First, it is important to note that the farm studied herein had performed selective breeding of their Nellore cattle over the past 20 years, culling animals that were extremely excitable and aggressive during handling. This in combination with the fact that animals were weighed every 14 days, may help to explain the low FS variability observed in the present study, compared with other studies that had assessed Nellore cattle (Barrozo et al., 2013; Sant'Anna et al., 2013; Valente et al., 2014). According to Turner et al. (2011), frequent handling is one factor that can lead to low FS variability and to a weak relationship with performance.

Previous studies had reported that when herds with more docile animals had low FS variability they also had less pronounced effects on ADG (Graham et al., 2001; Elzo et al., 2009). Thus, these conditions could be responsible for the inconsistent relationship between FS and the indicators of adaptability to the feedlot environment used in the present study.

4. CONCLUSIONS

In conclusion, under the conditions of this study neither FS measured on entry of cattle into the feedlot or FSdiff can be used by commercial producers as a predictor of feedlot adaptability and performance. On the other hand, FSmean has potential to indicate adaptability to the feedlot environment, but future research in this area should continue to develop a practical way of using cattle temperament to assess their adaptability to confinement. This can be done by considering other behavioral indicators to evaluate the individual capacity of beef cattle to adapt to the feedlot, such as social behavior and strategies to access feed resources.

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CHAPTER 3 – Flight speed as indicator to growth and reproductive performance of Nellore heifers

Abstract - The success of the beef cattle industry is dependent on many factors, such as the stressors associated with the environment. Regarding that excitable cattle are more susceptible to stress, the aims of the present study were to assess the effects of temperament on growth and reproductive performance of Nellore heifers, using different approaches for **FS** data-analysis, and also to verify the relationships of agonistic interactions with temperament, growth and reproductive traits. Data from two hundred and twenty-nine heifers were recorded during two experimental periods and over two consecutive years: 2012 (N = 105) and 2013 (N = 124). During the first period, heifers were 525.33 ± 36.70 and 526.78 ± 33.26 d of age and weighed 423.68 ± 39.45 and 427.76 ± 33.41kg of BW, for years 1 and 2, respectively, and they were allocated in the feedlot pens (~ 40 m^2.$animal^{-1}$). FS ($m.s^{-1}$) was measured four times (~28 d-interval) and based on this, two FS measurements were calculated for each animal including; the arithmetical means of FS (**FSmean**) and the differences between the first and the last FS measurement day (**FSdiff**). FSdiff was used to define the **FScategory**, as follows: 1) **'Acclimated'**: FSdiff > 1SD; 2) **'Consistent'**: FSdiff ± 1SD; 3) **'Sensitized'** FSdiff < -1SD. The animals were weighed at 14-d intervals to obtain performance traits, including: body weight at the end of the feedlot period (**BWf,** kg.$animal^{-1}$), **ADG** (kg.$animal^{-1}$.d^{-1}) and feed conversion ratio (**FCR,** kg DMI. kg $gain^{-1}$). The aggressiveness of each heifer within the herd (**AI,** counts.d^{-1}) and how many individuals the aggressor was able to displace from the feed bunk (**DI,** counts.d^{-1}) were recorded in fifty-four heifers over 13 non-consecutive days. During the second period, heifers were housed on pasture, and after weaning, heifers and offspring were assessed to obtain their growth and reproductive traits, as follows: calves' body weight at weaning (WW, kg.$animal^{-1}$); heifer' weight 210 days after the first calving (Wac, kg.$animal^{-1}$); age at the first calving (AFC, d); days to calving (DC, d) and calving interval (CI, d). During the first period, none of the growth performance traits were affected by any FS measurement (FS1, FSmean or FSdiff). During the second period, FS1 only showed significant quadratic effects on Wac (P = 0.0437), while FSmean had significant linear effects on AFC (P = 0.0719) and DC (P = 0.0466), and FSdiff showed a tendency of linear effects on WW (P = 0.0735). Therefore, the offspring of 'Acclimated' heifers had greater WW (P < 0.05) than 'Consistent' and 'Sensitized' heifers, which did not differ from each other. In contrast to FS1 that was not associated to AI or DI, FSmean showed a significant and positive correlation (P < 0.05) with AI and DI. We concluded that under the conditions of the present study, FSmean showed potential to predict sexual precocity, while FSdiff has potential to predict growth performance of the heifer's offspring at weaning. FS1 was not a good indicator of growth or reproductive performance, and although AI and AD were associated with FSmean, they were not associated to growth and reproductive performance.
Key-words: behavior, confinement, Nellore, reactivity

1. INTRODUCTION

The main goal of a beef cattle breeding program is to produce at least one calf per cow every year; and based upon this, it is possible to evaluate the efficiency of the beef production system (Cooke et al., 2011). However, the success of the beef cattle industry is dependent on many factors, such as genetics, nutrition, health, and the stressors associated with the environment. It is well known that under stressful conditions, cattle trigger adaptive mechanisms involved in metabolic, physiological, and behavioral changes (Broom and Johnson, 1993), to eliminate or minimize their effects. However, there is scientific evidence that a considerable number of animals are not able to adapt to stressful situations (Gupta et al., 2007), which can directly affect herd productivity.

Several studies have reported that cattle with excitable temperament, assessed using flight speed method (**FS**), are more susceptible to stress during routine management (Grandin, 1997; King et al., 2006); and that those animals displayed lower growth (Behrends et al., 2009; Cafe et al., 2011) and reproductive rates (Burrow et al., 1988; Cooke et al., 2011) than calmer ones. However, most of the research to date have used a variety of different methods of collecting and summarizing FS, including as a single measurement (Behrends et al., 2009; Hall et al., 2011; Cooke et al., 2011), or the means of repeatable assessments (Olmos and Turner, 2008; Cafe et al., 2011; Cooke et al., 2009b and 2011). More recently, in a study with Nellore beef cattle (Cooke et al., 2011), the authors suggested that animals that habituated to handling (showing a reduction in FS over the assessment days) had better reproductive efficiency. Thus, we hypothesized that FS is a good indicator of growth and reproductive performance in beef cattle, but the results are dependent on the time this assessment is carried out.

Furthermore, agonistic interactions occurring in a group of cattle, (to establish social hierarchy) have been mostly studied in dairy cows (de Vries and Von Keyserlingk, 2005 and 2006; Val-Laillet et al., 2008); and these interactions are commonly verified particularly under situations where the resources are limited (e.g. feedlot environment) (de Vries and von Keyserlingk, 2006). Based on previous investigation, using crossbreed feedlot cattle (Nellore x Angus) we verified that animals characterized as having calm temperament were more motivated when competing for access to the feed bunk, than excitable ones, but growth performances were not different between groups (Soares, 2011). Thus, the aims of the present study were to assess the effects of temperament on growth and reproductive performance of Nellore heifers, using different approaches for FS data-analysis, and also to verify the relationships of agonistic interactions with temperament, growth and reproductive performance in Nellore cattle.

2. MATERIAL AND METHODS

This study was approved by the Committee of Ethical Use of Animals (Certified number: 005178/13) from Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal-SP, Brazil.

2.1. Farm, animals, and general management procedures

This study was conducted on a farm located in Uberaba County, Minas Gerais State, Brazil, where a pure bred Nellore herd was selected taking into account multiple traits, as follows: growth, temperament, carcass quality, maternal ability and reproductive traits. Sexual precocity and reproductive efficiency were the two main factors considered in the farms breeding program.

Data from two hundred and twenty-nine heifers were recorded over two consecutive years, 2012 (year 1: N = 105) and 2013 (year 2: N = 124), and during two experimental periods, as described later in the sections 2.2 and 2.3, respectively.

Prior to the experimental periods, general management procedures involved housing the heifers on pasture (*Brachiaria sp.)* with free access to natural water sources. Once daily, heifers were fed a TMR consisting of corn silage (80%), soybean meal (11%), ground corn (6%), and additives and mineral supplement (3%) resulting in a diet with 12% CP and 68% TDN on DM basis.

The heifers were weighed between 12 and 14 mo. of age and those that were > 300 kg of BW were assigned to an estrus synchronization + FTAI protocol during the breeding season (90 d, from December to February), then exposed to the bulls for 30 d. Only heifers receiving the first service of the breeding season were assigned to the present study. All heifers were inseminated by the same technician.

Following bull exposure, a pregnancy diagnosis was carried out using transrectal ultrasonography (Aloka SSD 500 with 5 MHz linear-array transrectal transducer, Tokyo, Japan). The images were obtained from all heifers on the same day, and by the same veterinarian, who was also responsible for interpreting the images.

2.2. First experimental period

The experimental period was 83 (year 1) and 88 (year 2) days in length, respectively. Heifers were 525.33 ± 36.70 and 526.78 ± 33.26 d of age and weighed 423.68 ± 39.45 and 427.76 ± 33.41kg of BW, for years 1 and 2, respectively, and were tested for feed efficiency by allocating them in feedlot pens (~ 40 m^2.$animal^{-1}$). Each pen contained eight adjacent electronic troughs (GrowSafe® System Ltd.), and concrete floors approximately 3 m x 10 m adjacent to the feeding troughs to ensure a solid surface for the heifers to stand on while feeding. All heifers were fed daily at 0730, 1230 and 1530 h, receiving the same TMR described previously in Chapter 2. Fresh water and feed were available *ad libitum* during the entire experimental period.

2.2.1. Temperament assessment

Temperament assessments were carried out by measuring flight speed (FS; Burrow et al., 1988) on four occasions, as follows: on the first day (FS1, on entry to the feedlot facilities), after ~28-d (FS2) and ~56-d (FS3), and at the end of the experimental period (FS4, ~84-d). Assessment days were approximate according to the schedule of the farm for each year, but did not differ by more than 32 d and less than 1 d. The measurements were performed with an electronic device that included 2 pairs of photoelectric cells, a chronometer and a small processor programmed to record the time taken by each heifer to cover a 2.58 m distance (measured as a diagonal line from the squeeze chute door to the end of the alley, since the exit corridor design required the cattle to turn left at 90º during the exit). The speed ($m.s^{-1}$) that each heifer exited the squeeze chute after being weighed was calculated, and cattle having faster FS are indicative of more excitable temperament (Burrow et al., 1988).

Based on the FS assessments FSmean ((FS1 + FS2 + FS3 + FS4)/4) and FSdiff (the difference between the first and the last FS assessed (FS1 – FS4) were calculated.

Furthermore, FSdiff was used to define the three FS categories **(FScategory)**, as follows: i) ‘Acclimated’: animals that reduced FS more than one SD (FSdiff > 1SD) over the experimental period, assumed to represent the heifers that were best adapted to the feedlot; ii) ‘Consistent’: animals that did not change FS over the experimental period (FSdiff ± 1SD), which we assumed to represent the heifers with a moderate ability to adapt to the feedlot and; iii) ‘Sensitized’: animals that increased FS more than one SD (FSdiff < -1SD) over the experimental period, which were assumed to represent heifers with the lowest ability to adapt to the feedlot.

2.2.2. Feed Intake

The feed intake of each animal was evaluated using the records obtained from the GrowSafe® system to obtain dry matter intake (DMI, $kg.d^{-1}$). All heifers were fitted with an electronic identification (Allflex® Half duplex) transponder in their left ear which could be detected by an antenna within the rim of each feed trough. The files were recorded onto a computer every second over a 24 hour period over the entire experimental period.

2.2.3. Agonistic interactions

Agonistic interactions were only assessed for heifers in year 1 and pen 1 (N = 58). All heifers were individually identified with numbers on their backs (approximately 10 cm behind the rump) made using black semi-permanent hair color paint without ammonia (SOFTCOLOR®).

Direct observations with focal animal sampling and continuous recording (Martin and Bateson, 2000) were performed by one trained observer, who recorded the ID of heifers competing for access to the feed bunk, within the feeding area (about 3 m x 10 m). An agonistic interaction was recorded when a heifer displaced another one from the feed bunk using physical contact with their head and/or body;

the first was considered as aggressor, and the second receptor of the displacement (adapted from Welfare Quality®, 2009). All observations were recorded from 0730 (after the first feed delivery of the day) to 1830 h over 13 non-consecutive days (3, 5, 7, 12, 14, 16, 18, 20, 22, 24, 26, 82 and 83 days in the feedlot), totaling 143 h of observations.

From this, agonistic interactions were analysed in two different ways: firstly, evaluating an ‘Aggressive index’ (AI) to evaluate the aggressiveness of each heifer within the herd (adapted from Gibbons et al., 2009), suggesting that the higher the index value, the greater the motivation and heifers’ ability to access the feed bunk; secondly, evaluating a ‘Displacement index’ (DI), defined as the number of individuals the aggressor was able to displace from the feed bunk (Mendl et al., 1992), suggesting that the higher the number of individual index, the greater the social status of the aggressor within the herd.

In the first case, the AI was calculated as follows: AI = frequency aggressor / total frequency of interactions; the AI values ranged between 0 and 1, corresponding to whether an individual was always a recipient or an aggressor (Gibbons et al., 2009). The DI was documented to evaluate a heifer’s ability to displace another heifer at the feed bunk and was calculated as follows: DI = number of heifers able to displace / (number of heifers able to displace + number of heifers able to displace her) (Mendl et al., 1992). The DI values ranged between 0 and 1 corresponding to always being displaced or always successfully displaying others.

2.2.4. Growth performance traits

Pregnant heifers were weighed on entry (**BWi**, kg.animal^{-1}) and then every 14 d until the end of the experimental period. Growth performance traits included final body weight (**BWf**, BW assessed at the end of feedlot period; kg.animal^{-1}), ADG (kg.animal^{-1}.d^{-1}), and feed conversion ratio **(FCR,** kg DMI·kg gain^{-1}), which was calculated using DMI (obtained from GrowSafe) and ADG.

2.3. Second experimental period

During the second experimental period, all the pregnant heifers were housed on pasture (*Brachiaria spp.),* where they remained, with their offspring, until weaning that occurred when the calves were around 210 d of age.

2.3.1. Reproductive performance traits

After weaning, both heifers and calves were assessed to obtain their growth and reproductive performance traits, which are defined in Table 1.

Table 1. Definition of growth and reproductive performance traits obtained in Nellore heifers and their calves (N=229)

Trait	Definition
Growth performance traits	
Weaning weight[1]	Calves' body weight at weaning (**WW**, kg/animal).
Body weight after calving[1]	Heifer' weight 210 days after the first calving (**Wac**, kg/animal)
Reproductive performance traits	
Age at first calving	Difference in days between the date of the first calving and the date of the dam's birth. This trait is related to the onset of a females' reproductive activity (**AFC**, d).
Days to calving	Time interval in days, between the first breeding season day and the subsequent calving. This trait identifies a females' fertility through the heifer's ability to conceive early in the breeding season and calve earlier in the calving season (**DC**, d).
Calving interval	Number of days between the date of birth of the first calf and the date of birth of subsequent calf, both from the same heifer (**CI**, d).

[1]Heifers and calves were handled in the corral, in the same day, to obtain WW and Wac.

2.4. Statistical analysis

2.4.1. Data cleaning and summary

Data was removed from the dataset used in the statistical analysis when the Growsafe® system failed or was under maintenance, resulting in 21 and 13 days of missing information from pens 1 and 2, respectively for year 1; and in 25 and 26 days from pen 1 and 2, respectively for year 2.

Contrary to the methods of Gibb et al. (1998) and Schwartzkopf-Genswein et al. (2002), (who used a ‘meal criterion’ to summarize feeding behavior variables), the GrowSafe® data used in the present study was not summarized according to any meal criterion but instead documented each heifer visit to the feeders. The outliers were identified using the UNIVARIATE procedure of SAS, with analyses of residuals, and removed from the dataset.

Multicollinearity tests between the independent variables were carried out by using the variance inflation factors (VIF) option, and only two variables, heifers age on d 1 of experimental period and BWi, were collinear, and due to this the former one was excluded from the statistical models.

2.4.2. Effect of FS variables on growth and reproductive performance

To identify which of the FS variables (FS1, FSmean, or FSdiff) would be the best indicator of cattle growth (BWf, ADG, FCR, WW, and Wac) and reproductive performance (AFC, DC, and CI), these data were analyzed fitting linear mixed models using the MIXED procedure of SAS. The statistical model included: the fixed effect of year (yr 1 and yr 2), the random effect of pen within year, the effect of BW on d 1 of the experimental period (BWi), and one FS measurement (FS1, FSmean, or FSdiff) as a covariate. A post-hoc (Tukey) test was used to compare the adjusted means. Main effects and interactions were considered significant at $P < 0.05$ and $0.05 < P < 0.10$ as tendency.

2.4.3. Consistency of FS over the feedlot period

The effects of FS changes over the experimental period were assessed by including the FScategory variables on the statistical models of growth (BWf, ADG, FCR, WW, and Wac) and reproductive performance (AFC, DC, and CI). This was done fitting a linear mixed model and using the MIXED procedure of SAS. The fixed effect of year (yr 1 and yr 2) and FScategory were included in the models, as well as the random effect of pen within year, and BWi as a covariate. The interactions of FScategory with year were also included in the model and maintained only if they were significant. In addition, the sampling distributions for FScategory were compared using χ2 test. Main effects and interactions were considered significant at *P* < 0.05 and 0.05 < *P* < 0.10 as tendency.

2.4.4. Agonistic interaction traits

The estimation of Pearson coefficients of correlation was used to evaluate the association between AI and DI, and FS variables (FS1, FSmean, and FSdiff), growth (BWf, ADG, FCR, WW, and Wac) and reproductive performance traits (AFC, DC, and CI). Only data from pen 1 and year 1, was considered in this analysis (N = 58). Associations were considerate significant at *P* < 0.05 and 0.05 < *P* < 0.10 as tendency.

3. RESULTS AND DISCUSSION

3.1. Descriptive statistics

The descriptive statistics of all FS variables are shown in Table 1.

Table 1. Mean (± SD), minimum, and maximum observations of flight speed measurements[1] of pregnant Nellore heifers housed in a feedlot environment

FS measurement, m/s	N	Mean (± SD)	Min	Max
FS1	216	1.1 ± 0.60	0.10	2.86
FS2	212	1.4 ± 0.51	0.27	2.69
FS3	220	1.3 ± 0.46	0.34	2.43
FS4	198	1.3 ± 0.53	0.09	2.74
FSmean	229	1.3 ± 0.42	0.27	2.63
FSdiff	186	- 0.3 ± 0.65	- 2.18	1.49

[1]FS1: flight speed assessed on the first day of the feedlot period; FS2: ~28 d; FS3: ~56d; FS4: ~84d; FSmean: ((FS1 + FS2 + FS3 + FS4)/4); FSdiff: FS1 – FS4.

The descriptive statistics of feed intake (DMI), agonistic interactions (AI and DI), and growth performance (BWi, BWf, ADG, and FCR) variables of pregnant Nellore heifers housed in a feedlot environment are shown in Table 2; and the descriptive statistics of growth performance (WW and Wac) of Nellore heifers and their offspring and reproductive performance (AFC, DC, and CI) of pregnant Nellore heifers housed on pasture are shown in Table 3.

Table 2. Mean (± SD), minimum, and maximum observations of feed intake[1], agonistic interactions[2], and growth performance[3] traits of pregnant Nellore heifers housed in feedlot environment

Trait	N	Mean (±SD)	Min	Max
Feed intake[1]				
DMI, kg/d	229	7.9 ± 1.48	4.6	12.1
Agonistic interactions[2]				
AI, frequency/d	58	0.5 ± 0.06	0.4	0.7
DI, frequency/d	58	0.5 ± 0.04	0.4	0.6
Growth performance[3]				
BWi, kg	229	425.0 ± 36.42	340.0	516.0
BWf, kg	229	500.6 ± 41.83	398.0	621.0
ADG, kg/d	229	1.04 ± 0.24	0.2	1.6
FCR, kg DM/kg gain	229	7.9 ± 1.80	4.0	20.8

[1]DMI: dry matter intake. [2]AI: frequency aggressor / total frequency of interactions; DI: number of individuals able to displace / (number of heifers able to displace + number of heifers able to displace her).[3]BWi: Initial body weight; BWf: Final body weight; ADG: average daily gain; FCR: Feed conversion ratio.

Table 3. Mean (± SD), minimum, and maximum observations of growth[1] and reproductive performance[2] traits of Nellore heifers and their offspring (N = 229)

Trait	Mean (±SD)	Min	Max
Growth performance traits[1]			
WW, kg	203.1 ± 33.2	115.0	283.0
Wac, kg	451.8 ± 44.3	350.0	575.0
Reproductive performance traits[2]			
AFC, d	724.7 ± 72.27	633.1	1150.5
DC, d	305.4 ± 66.84	242.5	713.2
CI, d	388.6 ± 75.69	330.0	820.0

WW: calves' body weight at weaning; Wac: heifer' weight 210 days after the first calving; AFC: difference in days between the date of the first calving and the date of the dam's birth; DC: time interval in days, between the first breeding season day and the subsequent calving; CI: number of days between the date of birth of the first calf and the date of birth of subsequent calf, both from the same heifer.

3.2. Effect of FS variables on growth and reproductive performance

Several research studies reported that excitable animals (assessed using FS method) have reduced growth (Behrends et al., 2009; Nkrumah et al., 2007; Cafe et al., 2011) and reproductive performance (Burrow et al., 1988; Cooke et al., 2011), indicating that temperament has important effects on profitability of the herd. However, we also verified that there are a considerable number of studies that did not find any relationship between those cited traits (Sant'Anna et al., 2012; Valente et al., 2014), and the reason this are not clearly understood at this time. Based on this, we hypothesized that the lack of agreement between these finding and ours could be a result of the FS variable used in the data analysis.

3.2.1. Effect of FS variables on growth performance

Considering the practical feasibility of using FS as a single measurement, and assuming that it is a good indicator of growth and reproductive performance in beef cattle, we hypothesized that FS assessed as early in the animal's life as possible could be a good predictor for determining female herd replacements.

During the first experimental period, none of the growth performance traits (BWf, ADG, and FCR) were affected by any FS variable (FS1, FSmean or FSdiff), suggesting that individual differences in growth performance were independent of the heifers' temperament. It is important to note that during the first trimester of gestation (as verified in the present study) the development of foetus and placenta represent only 4 kg of dam' body weight (NRC, 2000), indicating that observed differences in ADG in the present study were not related to that.

One possible reason for these results is that heifers were fed a diet that supported growth rates of 1 $kg.day^{-1}$. Thus, this diet (with only 20% concentrate) was not formulated for rapid weight gain in the heifers, but with the purpose of facilitating the precocity of reproductive efficiency (based on maintenance of pregnancy), as well as feed efficiency. Given this, we expected to find a significant relationship between FS and FCR, but our findings were consistent with another study that reported no relationship between these traits (Nkrumah et al., 2007). Similarly, Black et al. (2013) did not find any association between FSmean (measured in growing heifers, every 14 d, over a 70 d experimental period) growth performance or feed efficiency (measured during the heifers growth phase and subsequent lactating phase).

Regarding the growth performance of heifers, while they were housed on pasture (second experimental period) we did not find any effect of FS1 and FSmean on WW, as shown in Table 5. This result corroborates Cooke et al. (2012) observations, since they also did not observe any effect of FS, assessed once and immediately after fixed-time artificial insemination (FTAI) on WW of *Bos taurus* cows. These findings combined, indicate that the growth performance of the offspring is independent of dam' temperament assessed as a single measurement or using the mean values of FS obtained on four repeatable occasions.

Contrary to FS1 and FSmean which characterized animals as having excitable or calm temperament, FSdiff reflected if the animals changed their temperament over time, as an indicator of which cattle became habituated to or sensitized to handling, as described in our previous study (Chapter 2). A tendency towards of linear effects of FSdiff were found on WW (P = 0.0735) (Table 5). If we take into account that one of the main purposes of a beef cattle breeding program is to predict which heifers will wean heavier offspring; changes in temperament over time could be a good indicator of this reproductive performance trait.

To date, few studies have examined the relationship between changes in FS over time on economically relevant traits to beef production (Behrends et al., 2009; Cooke et al., 2009a, 2009b, 2011). In one of those studies (Cooke et al., 2009b) crossbreed heifers (Angus x Hereford) that were exposed to repeated handling without restraint in the squeeze chute, and categorized as 'acclimated' (heifers that reduced FS over time), had greater ADG than heifers that were 'not acclimated'. This was attributed to altered grazing behavior and additional exercise that the 'acclimated' group was exposed to, during the acclimation process. On the other hand, the same research group (Cooke et al., 2009b) tested the effect of acclimation on the reproductive performance of Brahman-crossbreed heifers, controlling those environmental effects cited above, and their findings are in agreement with our results, showing no significant effect of FS on ADG. It is relevant to note that these authors did not test the effect of this acclimation process on WW or Wac, but they reported that 'acclimated' heifers had reduced plasma concentrations of cortisol (Cooke et al., 2012). Several studies also reported that cattle with excitable temperament exhibited greater basal concentrations of cortisol, and that those animals had lower growth performance (Curley Jr. et al., 2006 and 2008; Cooke et al., 2011; Burdick et al., 2011).

Thus, animals that improve their temperament are less susceptible to stress. Based on the findings of a human study in which they concluded that the concentration of the cortisol secreted in breast milk may influence infant temperament (Sørensen et al., 2005); we hypothesized that the low body weight observed in the offspring of heifers that increased their FS over time could be explained by a similar mechanism. Another possibility based in previous research showing that more excitable cattle have reduced milk production (Breuer et al., 2000), is that reduced milk production could negatively affect the growth performance of the offspring. In addition, excitable animals may have reduced nutrient availability to support body functions when facing stressful situations (Cooke et al., 2009a; 2009b). Heifers in our study that improved their FS over time had offspring with greater WW; consequently we expected that excitable heifers assessed on entry to the feedlot could have greater WW, as well. However, our results showed only significant quadratic effects of FS1 on Wac (P = 0.0437), suggesting that both calm and excitable heifers had greater body weight at weaning (Table 5).

In summary, our results suggested that independent of the FS variable (FS1, FSmean, or FSdiff) under the conditions of our study and using pregnant Nellore heifers, FS was not able to predict heifers with better growth performance in a short period of time (~84 d of the feedlot period). However, FSdiff showed potential to identify heifers that will wean heavier offspring.

3.2.2. Effect of FS variables on reproductive performance

The variable FS assessed once early in a heifers' life (approximately 12 - 14 mo. of age) was not a good predictor of sexual precocity, since FS1 did not affect AFC, DC, and CI, the same for FSdiff (Table 5). However, we found a tendency of linear effects of FSmean on AFC (P = 0.0719) and DC (P = 0.0466), but not on CI, as shown in Table 5.

To explain differences in the results between FS variables we should consider the findings of previous studies. For example, Valente et al. (2014) found a weak genetic correlation between FS and AFC (0.14 ± 0.11) in Nellore beef cattle (~550 d of age) kept on tropical pastures, suggesting that excitable animals showed higher AFC, and consequently reduced sexual precocity. Similar to our study, the authors assessed FS as a single measurement, and reported a 0.27 heritability estimate for FS and a lower value for AFC (h^2 = 0.09), indicating that FS (as a single assessment) and AFC are greatly affected by environmental factors. Similar heritability estimates for AFC (ranged from 0.08 to 0.19) were also reported by other researchers working with Nellore cattle (Pereira et al., 2002; Dias et al.; 2004). One example was Burrow and Corbet (2000) who reported a 0.33 heritability for FS, as a single measurement assessed in calves at 12 mo. of age, but higher heritability was found for FSmean (h^2 = 0.50). Thus, Burrow and Corbet (2000) suggested that using 2 or 3 repeated FS measurements could provide most reliable selection criterion to improve herd temperament. Based on those findings, we hypothesized that we did not find a significant relationship between AFC and FS1, but with FSmean, due to the fact that using more than one assessment we could limit the effects of environmental factors. This could also explain the lack of relationship between FSdiff and AFC (Table 5); hence this measurement is based on only two FS measurements, while FSmean was based on four.

The significant linear effect between FSmean and DC previously discussed in the present study, indicate that DC followed the same trend as AFC, since they are highly genetically correlated in Nellore cattle (r_g = 0.76), as reported by Forni and Albuquerque (2005). On the other hand, CI was not related with any FS variable (FS1, FSmean and FSdiff; Table 5).

Table 5. Summary of the effects (Slope ±SE and *P*-value) of FS variables[1] on growth[2] and reproductive performance traits[3] recorded in Nellore heifers housed on pasture (N = 229)

Trait	FS1			FSmean			FSdiff		
	Slope	SE	*P*-value	Slope	SE	*P*-value	Slope	SE	*P*-value
Growth performance traits[2]									
WW, kg	6.8	4.38	0.1250	4.0	5.48	0.4653	8.8	4.89	**0.0735**
Wac, kg	14.1	6.90	**0.0437**†	5.4	6.74	0.4264	1.5	5.41	0.7785
Reproductive performance traits[3]									
AFC, d	8.1	9.70	0.4046	21.4	11.86	**0.0719**	- 5.8	10.03	0.5595
DC, d	9.3	9.95	0.3539	24.0	12.0	**0.0466**	- 9.9	10.70	0.3554
CI, d	- 18.1	13.93	0.1971	- 16.3	18.02	0.3672	- 10.5	14.50	0.4705

[1]FS1: Flight speed assessed on the first day of the feedlot period; FSmean: ((FS1 + FS2 + FS3 + FS4)/4); FSdiff: FS1 – FS4. WW: calves' body weight at weaning; Wac: heifer' weight 210 days after the first calving; AFC: difference in days between the date of the first calving and the date of the dam's birth; DC: time interval in days, between the first breeding season day and the subsequent calving; CI: number of days between the date birth of the first calf and the date birth of subsequent calf, both from the same heifer. Bold *P-values* represents significant effects (10% of probability) †Significant quadratic effect (10% of probability).

Some researchers had questioned the use of CI as an indicator of reproductive efficiency in beef cattle (Bourdon and Brinks, 1983), since it is commonly used (FTAI) during breeding season, which could also lead to the lack of association with FS measurement, contrary to the other traits (AFC and DC). Thus, DC has been suggested as a better indicator for this assessment under this situation (Pereira et al., 2002).

In summary, based on data from Nellore cattle participating in the PAINT®, commercial breeding program of the Brazilian company CRV Lagoa Ltda. (Sertãozinho, São Paulo, Brazil) recorded from 173 herds (from 1994 to 2007) (Barrozo et al., 2012), we verified that average AFC (1053.96 ± 131.44 d; N = 1402) was greater than the one observed in this study (724.7 ± 72.72; N = 229), leading us to conclude that the farm's breeding program was efficient in improving the sexual precocity of their herd. Also, considering that our results showed that AFC increased 21.4 days and DC 24 days for each 1 $m.s^{-1}$ on FSmean, we suggest that more studies are needed to estimate the genetic parameters of FSmean, in order to evaluate the potential use of this trait in genetic selection for sexual precocity.

3.3. Consistency of FS over the experimental period

Significant effects of days were observed on FS over the experimental period ($P < 0.05$) (Table 6). Results showed a significant increase in FS between the first and second assessment days ($P < 0.05$), returning to the initial value between the second and third day ($P < 0.05$), and remaining stable from the third to fourth assessment day (Table 6).

We observed a tendency towards increasing FS between the first and last assessment day ($P < 0.05$) (Table 6), suggesting that heifers became sensitized to handling (contrary to findings reported in Chapter 2 showing a reduction from FS1 to FS4). Sensitization is defined as a transient increase in excitability resulting from repeated presentation of a stimulus (Sato, 1995), the opposite of habituation, which was defined by Harris (1943) as "…a response decrement to repetitive stimulation…". This may be due to the effect of previous handling, when the heifers were submitted to the FTAI protocol (before the first experimental period), which is a known stressor for heifers, as reported by Rueda et al. (2015), and the animals were weighed every 14 days during the first experimental period in the same facilities where FTAI was carried out. Then, we assume that this previous negative experience could be the cause of increased FS between FS1 and FS4. This hypothesis was based on findings reported by Burdick et al. (2011) indicating that some animals with excitable temperament may not show any sign of habituation, especially in farming systems that require intensive management.

Even though the difference (0.2 $m.s^{-1}$) in FS between the first and the last day (FS1-FS4) in the present study was statistically significant ($P < 0.05$) (Table 6), it was smaller than the difference reported by Hall et al., (2011) (0.9 $m.s^{-1}$) assessed on eight occasions every 28-d over, but for a longer period, 188 d.

Table 6. Means (± SD) of flight speed measurements[1] taken during four assessments of pregnant Nellore heifers housed in a feedlot environment (N = 229)

Type	FS1	FS2	FS3	FS4
Pregnant heifers	1.1 ± 0.60[b]	1.4 ± 0.51[a]	1.3 ± 0.46[ab]	1.3 ± 0.53[ac]
Day[2]	0	~28	~56	~84

[a - b]Means followed by the same letters in rows (lowercase) and columns (uppercase) do not differ (P > 0.05) by Tukey test. [1]FS1: Flight speed assessed on the first day of the feedlot period; FS2= ~28 d; FS3= ~56 d; FS4: ~84 d; FSmean: ((FS1 + FS2 + FS3 + FS4)/4); FSdiff: FS1 – FS4. [2]Days from first FS measurement.

This suggests that the changes in FS in the present study (ranging between 0.1 to 0.3 $m.s^{-1}$) may not be biologically relevant. The dissimilarity of the experimental assessment intervals (~84 d and 188 d for our study and Hall et al., (2011), respectively), combined with the consistency in FS (from the first four FS measurements) reported by those authors, suggests that if an extended assessment period would have been used in our study greater changes in FS between the first and the last assessment day may have occurred.

Indeed, significant partial correlations were found between FS1 and FS4 (r = 0.33), as shown in Table 7, but this value was lower compared with the previous study using bull calves (r = 0.50) (Chapter 2). Despite the weak correlation between FS1 and FS4, the results described previously (Table 6) suggest that heifer temperament was consistent over time. It is relevant to note that the strongest correlation was found between FSmean, second and third FS measurements, followed by FS4 and then FS1 (Table 7).

Table 7. Partial correlations coefficient between FS measurements[1] and variables for pregnant Nellore heifers housed in a feedlot environment (N = 229)

Item	FS1	FS2	FS3	FS4	FSmean	FSdiff
FS 1	---	0.42**	0.40**	0.33**	0.73**	0.63**
FS 2	---	---	0.67**	0.61**	0.84**	- 0.13
FS 3	---	---	---	0.69**	0.84**	- 0.23
FS 4	---	---	---	---	0.83**	- 0.52**
FSmean	---	---	---	---	---	- 0.03
FSdiff	---	---	---	---	---	---

[1]FS1: Flight speed assessed on the first day of the experimental period; FS2: ~28-d; FS3: ~56-d; FS4: ~84d; FSmean: ((FS1 + FS2 + FS3 + FS4)/4); FSdiff: FS1 – FS4. ***P* < 0.001.

Regarding individual differences, the percentage of animals within each FScategory were not homogeneous ($\chi^2 = 83.23$; $P < 0.01$) (Table 8). Most of the heifers did not change FS (59.39%; Consistent) between the first and the last assessments, followed by heifers that increased (30.13%; Sensitized) and those that reduced FS (10.48%; Acclimated), as described in Table 8.

Table 8. Means (± SD) of observed initial and final flight speed[1] by FScategory[2] (Acclimated, Consistent and Sensitized) of pregnant Nellore heifers housed in feedlot environment

FS, m/s	Acclimated	Consistent	Sensitized
FS1	1.7 ± 0.38^{aA}	1.1 ± 0.51^{bA}	0.7 ± 0.57^{cB}
FS4	0.9 ± 0.43^{cB}	1.2 ± 0.46^{bA}	1.7 ± 0.49^{aA}
N	24	136	69

[1]FS: FS1: flight speed assessed on the first day of the feedlot period; FS4: flight speed assessed on the last day (84 days) of the feedlot period. [2]Group of FScategory: Acclimated: animals that reduced FS in more than one SD; Consistent: animals that did not change FS; Sensitized: animals that increased FS in more than one SD. [a - b]Means followed by the same letters in rows (lowercase) and columns (uppercase) do not differ (P > 0.05) by Tukey test.

However, this individual variation did not affect the variables of growth performance (BWf, ADG, and FCR) assessed during the experimental period, nor did they affect reproductive performance traits (AFC, DC, CI, and Wac), however, the offspring of heifers categorized as ‘Acclimated’ had greater WW (221.8 ± 7.71 kg.animal^{-1}; $P < 0.05$) than ‘Consistent’ and ‘Sensitized’ heifers (202.7 ± 3.04 and 198.6 ± 4.46 kg.animal^{-1}, respectively), which did not differ from each other. These results support those discussed previously (Table 5).

In summary, most heifers exhibited consistent temperament during handling over the experimental period. However, a considerable number of animals exhibited behavioral differences over time, which where predictive of heifers with better reproductive performance.

3.4. Agonistic interactions traits

Significant positive correlation was found between AI and DI (r = 0.66; $P < 0.10$), suggesting that more aggressive heifers also had a higher social ranking within the herd. However, none of the variables related to growth (BWf, ADG, FCR, WW, Wac), or reproductive performance (AFC, DC, and CI) were associated with AI or DI.

One of the first hypotheses of the present study was that FS assessed once, early in a heifers' reproductive life could be a predictor of those animals with greater growth and reproductive performance. However, the results of the present study did not support this hypothesis. Given this, we could assume that agonistic behavior could be one key factor influencing these results since, as indicated by Miranda-de La Lama et al. (2013), showed that social interactions affected the growth performance of goats. However, our results indicated that FS1 was not associated with AI or DI, suggesting that temperament assessed as a single measurement was also independent of aggressiveness and social status within the herd. However, in contrast to FS1, FSmean showed a significant and positive correlation ($P < 0.05$) with AI (r = 0.33) and DI (r = 0.36), suggesting that heifers characterized as excitable (considering four repeatable measurement days) were more aggressive when competing for access to the feed bunk, and were ranked higher in the herd social hierarchy. This is not in agreement with previous findings from our research group, where crossbreed female cattle (Angus x Nellore) with calm temperament were more competitive at the feed bunk than moderate and excitable cattle (Soares et al., 2011). One explanation for the discrepancy between studies is differences in the methodology used. In the present study we measured the frequencies of being an actor or recipient of agonistic interactions, while in this previous study we only measured the frequencies of aggression per heifer.

Given this, one would expect that the more aggressive heifers would have higher AFC, but this was not the case, since no association was found between AFC and AI. To our knowledge, there is no other published study assessing the relationship between agonistic interactions, temperament and growth and reproductive performance traits in Nellore cattle, making the interpretation of ours findings difficult.

Regarding the association between temperament and agonistic interactions, (for similar reasons as previously discussed for FS1), we hypothesized that FSdiff would not be associated with agonistic interactions traits (AI and DI), because this variable only considers two FS measurements (FS1 and FS4). These results suggest that some heifers expressed an improvement in their temperament over time, which was associated with improved offspring WW. It was also not predictive of animals that were more or less competitive at the feed bunk as AD and AI did not affect WW, therefore those agonistic interaction traits were not relevant for predicting animals with better reproductive performance.

4. CONCLUSIONS

Our results indicate that growth performance traits were not associated with any FS variable and that FS1 was also not associated with either growth or reproductive performance. Based on this, we conclude that none of the FS variables tested were a good predictor of a heifers' growth performance in feedlot system, and that FS1 is also not a useful predictor of a heifer's reproductive performance.

On the other hand, we found a relevant association between FSmean and a heifers' sexual precocity, suggesting that calmer heifers had a higher probability of conceiving earlier in life. Based on this, one could conclude that FSmean would be a good predictor of sexual precocity. However, further research on this subject is needed to ensure the consistency of this association.

Our results also indicate that it is necessary to consider not only which animal is more or less excitable, but also how cattle temperament changes over a period of time. This conclusion is based on the results that the heifers that reduced their FS over time had calves with higher weights at weaning. In addition, FSmean was associated with agonistic interactions; we concluded that under conditions of this study, agonistic interactions were not related with growth and reproductive performance traits.

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CHAPTER 4 - Final considerations

The use of temperament as a genetic selection criterion in Nellore beef cattle has recently been adopted by some Brazilian breeding programs, where individuals with excitable or aggressive temperaments are selected against. Currently, the majority of producers assess cattle temperament by scoring the reaction of each animals during handling in a corral just after being weighed (Sant'Anna et al., 2012), while others use a flight distance test within a corral pen, just after weighing. At this time none of the Brazilian breeding programs for Nellore cattle use flight speed (FS) to assess temperament, despite the fact that FS seems to be the best proxy indicator given its greater heritability estimates compared to the other measures of temperament cited above. In addition, it is practical, low cost, and most importantly an objective measure that does not require training of observers to do the measurements.

There are several studies assessing the use of FS as a proxy for temperament to predict productive traits in beef cattle. In fact, when we decided to conduct the study (described in the second chapter), we hypothesized that animals categorized as calm (assessed using FS prior to entering to the feedlot) would be more motivated to seek food at the bunk, and also have better weight gain and feed efficiency. In addition, behavioral variations among individuals (calm and excitable animals) could be used as an early predictor of how well an individual adapts to a feedlot environment. The relationship between FS (assessed as single measure or assessed as the average of repeated measurements; FSmean) and temperament has not been studied to our knowledge. Given this, we formulated another question with the expectation of predicting relevant production traits and adaptability to the feedlot, as follows: When is the best time to assess FS and what is the best way to use this measurement? Based on the results of this thesis we concluded that different temperament outcomes are associated with the way FS is summarized and when it is assessed.

Overall, the most important results in the second chapter, firstly was that FS assessed prior to entering the feedlot was not a good predictor of cattle adaptability to confinement and secondly that calm animals (assessed by FSmean) had greater live weights at the end of the experimental period. However, those animals also had greater feed intake and visited the feed troughs more frequently than excitable ones. Considering that food accounted for over 70% of the variable cost of beef cattle production, we suggest that further studies are necessary to ensure that selecting calm animals will not increase the cost of feedlot production.

We must highlight that the farm where the present study was conducted does not represent the typical Brazilian feedlot, but it was the first in Brazil to use electronic feed trough monitoring technology to assess feed efficiency in a feedlot setting, which could provide data of important economic traits (feeding behavior, intake and feed efficiency) for each animal. Thus, we think that to recommend FS as an indicator of feed efficiency, the replication of the present study is necessary, but under commercial feedlot conditions, in order to compare the effects of different methods of FS on feed efficiency; and also on the adaptability to confinement, including physiological measurements as confirmatory of stress. We did not assess physiological indicators of stress in this thesis, since was not the goal of this study, but a recent research (Macitelli Benez, 2015) assessing stress in Brazilian feedlots found that the levels of stress experienced by the calves under feedlot conditions are associated with mud, dust and high pen density.

In the third chapter we hypothesized that calm heifers (assessed as a single measure of FS) would have better performance and reproductive traits as they would be more motivated to compete by the food at the feed bunk. However, this hypothesis was not confirmed, suggesting that the relationship between FS assessed prior to entering the feedlot was not clear (significant quadratic effect) to the subsequent feedlot performance. Therefore, contrary to our expectations, excitable heifers (assessed using FSmean) were found to be more motivated to compete for food (significant positive correlation with AI and DI). However; the motivation to compete for food was not related to greater feed intake, growth (BWf, ADG, FCR, WW, and Wac) or reproductive (AFC, DC, and CI) performance traits. This suggests that cattle of differing temperaments develop different strategies to access feed within a feedlot environment where feed bunk space is limited. We found only one other study (Zobel et al., 2011) indicating that feedlot cattle use different feeding strategies to gain access to feed, and no studies have explored the relationship between these strategies and performance and therefore more studies in this area are required to increase understanding.

The other hypothesis formulated when we started this study was that it is possible to improve temperament of Nellore feedlot cattle, due to the fact that they would have greater contact with humans during the handling routines. The cattle on the farm where our research was conducted had significant exposure to humans as they were weighed frequently, were fed and checked daily for health by one person walking inside the feedlot pens and therefore should have been less fearful of humans. However, based on the results presented in both, the second and the third chapter, we found that mean FS did not change over time indicating that their temperament remained constant over the course of the study. But, when we refine our assessments, highlighting the differences between individuals, we found that the vast majority of animals truly were not affected by environmental factors imposed by confinement (which explains the apparent consistency in average data FS), but we verified that some animals can improve or deteriorate their temperament over time.

Based on the results of the third chapter, heifers that habituated to handling (those that decreased their FS over time), showed a tendency to wean heavier offspring, compared to those that did not habituate or their FS increased over time. Although the other growth and reproductive performance traits were not affected by the environmental factors of the feedlot, these results lead us to highlight the importance of individual animal variation. This was already pointed put by Larson et al. (1992), who cautioned the use of only group averaged data may mask important individual variations. This observation is very relevant to producers particularly for growth performance as when only the average results of weight gain may not identify problems that reduce profitability during production.

Furthermore, regarding the results of the third chapter, differences in outcomes using different FS summary techniques were clearly verified. For this study we had a similar hypothesis to that described in the second chapter, that FS1 has potential to predict of heifers with better growth and reproductive performance. However, our results only partially supported our expectations. One example of this was that with the use of FSmean it was possible to identify that calm heifers had earlier puberty onset than excitable heifers. However, if the purpose is to predict offspring with greater live weight immediately after weaning, FS1 and FSmean measured in their dams were not able to predict it. Regarding the increase of Brazilian beef producers adopting the intensive production system for cow-calf operations, the results of the present thesis provides support to producers of how to evaluate the temperament of the animals in order to reduce operating costs of production.

Based on the results of this thesis we recommend using FSmean as indicator of productive and reproductive traits, due to the fact that this measure considers the environmental effects of all assessment days (and not just a single point in time). However we would like to draw attention to the importance of other measures such as FSdiff that was found to be a useful measure for assessing the temperament of heavier calves, but contrary to what was expected FS1 was not associated with important economic traits (BWf, ADG, FCR, or REA) and consequently could not predict which animals will adapt better to confinement.

In the last decade, social pressure from both consumers and the media regarding animal welfare practices on farm is increasing. Initially it was to improve food quality and safety however, it is well known and accepted that animals are sentient beings, able to experience fear, cold, and hunger, which increased the social pressure. We highlight the importance continued studies using the same approaches in this thesis. If producers can identify animals that are not coping well within the feedlot environment this will help to reduce poor welfare outcomes of individuals, as well as the herd.

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