CAPÍTULO VII - Animal welfare related to temperament and different pre slaughter procedures in Uruguay[6]

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Abstract

Sixty Hereford (H) and Braford (B) steers were assigned to two diets: D1) native pasture plus corn grain (1 % of live weight) (H n=15, B n=15); and D2) high quality pasture (H n=15, B n=15) for finishing purposes. Temperament was individually assessed and monitored during the experiment. All animals were slaughtered the same day in two groups (50 % of animals from D1 and 50 % from D2 in each group) after staying 15 and 3 hours in lairage pens, respectively. Different physiological indicators were used to assess stress after transport, lairage and immediately preslaughter. Carcass quality was determined through the incidence of bruising and final pH. Calmer animals had higher average daily gains (ADG) with no differences between diets. Transport was not a psychological stressful stage but animals were physically affected. The group that remained 3 hours in lairage pens showed a higher frequency of negative behaviour. These stressed animals did not have enough time to cope with the environment, with the consequent deleterious effects on final pH. The long lairage group had a higher metabolic response but these animals could rest and recover, and reached adequate final pH values. Braford steers were more excitable during the finishing period and also during lairage. Regardless of breed, temperament appears to be a valid tool for increasing productivity and decreasing the physiological stress response during all preslaughter stages. Further research should be carried out to establish the proper intermediate lairage duration according to animal welfare, and carcass and meat quality criteria.

Keywords: lairage time, stress response, temperament, transport in cattle

1. Introduction

Transport and handling of slaughter animals are associated with a series of events which cause stressful and unfavourable conditions, compromise animal welfare, increase the

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chance of spreading disease (Gebresenbet and Eriksson, 1998; Gross and Siegel, 1993) and reduce meat quality (Honkavaara and Kortesniemi, 1994; Warriss et al., 1995).

Strict regulations and directives have been issued to promote animal welfare during transit and the significant relationship between pre-slaughter stress and meat quality has been widely documented (Ferguson et al., 2001). However, adequate scientific data are lacking on stress-inducing factors in cattle transport and lairage. In many European countries and North America it is common to slaughter animals on the day of arrival, whereas in Australia, New Zealand and Uruguay, animals are more typically slaughtered the day after arrival. Several authors sustain that time lairage potentially allows cattle to replenish muscle glycogen concentrations, reduce dehydration of body tissues and carcase weight loss and to rest and recover from the effects of transport. Other authors think that the lairage environment itself may inhibit the ability of cattle to rest or recover from the effects of feed and water restriction (Jarvis et al., 1996).

The magnitude and quality of the stress response will be greatly affected by individual differences (Moberg, 1985). Animals with the most excitable temperaments may be most susceptible to stress generated by routine handling practices, such as loading and unloading, transport or the new environment in the abattoir (Lensink et al., 2000) and these differences will be reflected in physiological indicators. Stress may activate the pituitary-adrenocortical system (Ryjnberk and Mol, 1989) and these hormonal changes may affect cellular metabolic processes (Terlouw et al., 2005). Previous research has indicated that cattle with more excitable temperaments had more extensive responses to a simulated stress challenge and higher basal concentrations of glucocorticoids (Curley, 2004). This suggests that stress response mechanisms are much more active in excitable animals than in their calmer counterparts. By choosing appropriate physiological measurements of psychological and physical stress in conjunction with behavioural observations of animals in slaughterhouses, it is possible to make an initial assessment of the effect of being restrained, the new environment, feed and water restrictions, as well as other kinds of stress associated with transport and pre-slaughter management, on the welfare of cattle (Moss, 1984).

The objective of this experiment was to evaluate the effect of animal temperament and different preslaughter procedures, on animal welfare in Braford and Hereford steers.

2. Materials and methods

2.1 Animals and diets

This study was run by the National Institute of Agricultural Research at INIA Tacuarembó Research Station, Uruguay (Latitude South 32º 02' 12.4''; Longitude West 57º 09' 15.2'') over a period of 7 months (December 2006 to June 2007). Sixty Hereford and Braford steers 2.5 years old were assigned to the following two feeding strategies with finishing purposes according to live weight, horn presence and breed: D1) rangeland plus corn grain with the grain supplied at 1 % of live weight (LW) (Hereford n=15, Braford n=15); and D2) high quality pasture composed mainly of lotus (*Lotus corniculatus)* with a small proportion of white clover *(Trifolium repens)* (Hereford n=15, Braford n=15). The area for each finishing strategy was divided into two plots by electric fencing and animals alternated plots every 14 days. The system was planned in order to avoid over-grazing.

2.2. Field determinations

Productivity measurements

Animals were weighed early in the morning without previous fasting, every 14 days. For D1, amounts of corn grain were adjusted at this time according to LW. The supplement was provided once a day early in the morning (6 AM). Animals from both finishing strategies had *ad libitum* access to water.

Tameness and temperament

Tameness and temperament were evaluated by non-restrained and restrained tests based on existing or adapted methodologies (Burrow, 1988; Fordyce et al., 1982; Grandin and Deesing, 1998; Hemsworth et al., 1989; Le Neindre et al., 1995). The Flight zone (FZ) is the animal´s personal space and its size depends on the tameness of livestock. It was registered in each finishing strategy 2 days before slaughter. The test was always performed by the same person, who slowly walked (2 steps/second) toward the group of animals, registering the distance (in meters) when half of them turned away. Hair whorl position (HWP) was recorded on the first day of the experiment, looking for a correlation with temperament. If the center of the hair whorl was above the top of the eyes, the animal was categorized as “excitable”; “medium” if the center was located at eye level and “calm” if the center was located below the bottom of the eyes (Grandin et al., 1996). Temperament ratings were assessed individually every 14 days by the following 3 tests: 1) *Crush score* (CS): is the behaviour of each animal when put into a crush using a 1 (calm) to 5 (combative) scale, 2) *Flight-time* (FT): the amount of time (in seconds) it takes an animal to cover a fixed distance (5 meters) after it leaves the restraining device and 3) *Exit speed* (ES): the speed on leaving the squeeze chute and was ranked as: 1 = walked, 2 = trotted, and 3, if the animal ran away from the chute. A multicriterial temperament index (Tindex) was built from FT, CS and ES.

Health status

Pathological event or trauma and the corresponding medical treatments were daily observed and registered throughout the entire experimental period.

2.3. Transport and slaughter plant

All animals were slaughtered on the same day in a commercial abattoir licensed to export meat, following standard animal welfare procedures. Each slaughter group was composed of 50 % of animals from D1 and 50 % from D2, remaining in pens for 3 and 15 hours pre-slaughter, respectively. Animals were transported for 4 hours in a commercial truck with two compartments, allowing 420 Kg/m^2 (1-1.2 m^2/head) according to the abattoir protocol (based on international recommendations). Steers from different diets within each slaughter group were not mixed either in the truck or in the abattoir. The same truck and driver were used for both journeys. Distance from the farm to the slaughter house was 140 km. Average driving time was 3 hours and a half, with 3 stops of 3-4 minutes for animal monitoring. No problems were registered during loading and unloading, this being fluid in both groups. After arriving at the abattoir, animals from each diet (n=15) within each slaughter group were taken to a 37.5 m^2 pen with 2 divisions (8 and 7 animals per division). The space allowance in lairage pens was 420 Kg/2.5 m^2, according to the protocol mentioned above.

Physiological indicators

Three blood samples were taken from all animals looking for basal values in welfare indicators and their respective changes, according to the following periods:

- before leaving the farm (basal values), *Time A*
- immediately after arriving at the slaughter house (transport effect), *Time B*
- after lairage (lairage effect), *Time C*
- during bleeding (effect of the last handling procedures), *Time D*

One of these samples was collected into anticoagulant, cooled and immediately sent for hematocrit determination. The other 2 samples were kept cool until they arrived at the laboratory. Serum was extracted following centrifugation at 3000 rpm for 15 minutes. The serum fractions were frozen and immediately sent for analysis:

Sample 1 *Hematocrit* was determined by the micro hematocrit technique in the Veterinary Faculty, Uruguay. Results are expressed in percentages.

Sample 2. *Cortisol and Creatine kinase (CPK).* Serum samples were asssayed in the Nuclear Techniques Laboratory, Veterinary Faculty, Uruguay.

- Cortisol was determined by a direct solid-phase radioimmunoassay (RIA) using DPC kits (Diagnostic Product Co., Los Angeles, CA, USA). All samples were determined in the same assay. The RIA had a sensitivity of 8.2 nmol l^{-1}. The intra-assay coefficients of variation for low (36 nmol l^{-1}), medium (224 nmol l^{-1}) and high (427 nmol l^{-1}) controls were 10%, 6.8% and 4.6%, respectively. Results are expressed in nmol/L.

- CPK. Method: CK NAC liqui UV. Liquid test for creatine kinase determination (EC 2.7.3.2.) activated by NAC and measured by spectrofotometry at 340 nm. Results are expressed in U/L.

Sample 3. *Non esterified fatty acids (NEFA) and ß-hidroxibutirate (βHB).* Serum samples were assayed in the Rubino Laboratory, Uruguay.

- NEFA. Method: ACS-ACOD (acil-CoA sintetasa-acil-CoA oxidasa). WAKO laboratory kits were used (refs. 999-34691, 995-34791, 991-34891 y 993-35191) - lots TK 365, TK 366, TK 367 y TK 368. This method was adapted for use in a VITALAB Selectra 2 Autoanalyzer. Results are expressed in nmol/L.
- βHB. Method: D-3-hidroxybutyrate oxidation into acetoacetate through the 3-hidroxibutirate dehydrogenase enzyme. As a consequence, NAD+ from the reactive is reduced to NADH and absorvance change to 340 nm. RANDOX laboratory kits were used (ref. RB 1008) - 094293 in a VITALAB Selectra 2 autoanalizer. Results are expressed in nmol/L.

Behaviour

Cattle behaviour was evaluated by 8 trained observers working in pairs, who rotated between divisions each hour. Direct observation was performed within each division (experimental unit) using a scan sampling technique (Martin and Bateson, 1993). Due to operative restrictions, animals were observed for 1.5 hours in the short lairage and 7 hours in the long lairage group. At each scan, the following states/events were recorded: walking, ruminating, lying, standing (without rumination), drinking, negative behaviour, social behaviour, and self grooming. Results are shown as the percentage of time spent performing the behaviour (related to the observation period). Fighting and mounting are considered significant behaviours, for which it is important to record each occurrence and this type of behaviour would tend to be missed by scan sampling (Martin and Bateson, 1993). In this experiment, conflicts (fighting and mounting) were also registered with the behaviour sampling technique at each pen division, between 2 scan periods. Each consecutive sample interval took 7.5 minutes. Animals were individually identified with a number painted on both sides of the body.

2.4. Carcass traits

Before carcasses were dressed they were visually inspected, recording the number and severity of bruises at the individual level. Severity was scored as major or minor, depending on whether or not they involved tissue remotion (minor: subcutaneous or no tissue remotion; major: affecting muscle). Carcass pH was measured at 24 hours *post mortem* (pm) at the *Longissimus dorsi* (LD) between 12-13th ribs, using a pHmeter (Orion 210A) with a gel device.

2.5. Statistical analysis

Exploratory analyses were performed for all variables using Statgraphics and SAS packages.

Productivity. A general linear model (PROC GLM; SAS, 2007) was used to determine the effects of diet, horns presence, hair whorl position, breed and temperament on ADG. Initial and final liveweight were included in the model as covariates. Interactions were considered and if not significant, were removed from the model.

Tameness and temperament. A multicriterial temperament index (Tindex) was built with FT, CS and ES. A standardised ranking was created for each variable in a 1-100 scale. Tindex was created by: Tindex=$\sum$ W.d, where “W” is the weight assigned to each variable estimated by the Analytic Hierarchy Process-AHP (Saaty, 1980); and “d” is each individual record, standardised (within each variable). Considering that FT is an objective test, it was assigned a relatively higher ranking in the index. The higher Tindex, the calmer the animal. For several analysis an *Average* Tindex was constructed, including variations in temperament during the whole period. A general linear model (PROC GLM; SAS, 2007) was used to evaluate the effect of diet, breed, horn presence and hair whorl position on Average Tindex. In order to study the effect of the diet and breed on Tindex through time (5 consecutive dates), an analysis of variance was carried out using a mixed model with repeated measures considering the animal as a random effect inside each diet. Initial and final liveweight were included in the model as covariates (PROC MIXED; SAS, 2007). When interactions between independent variables were not significant they were removed from the model.

Physiological data. Due to absence of normality, cortisol and CPK values were normalized by taking a natural logarithm. The effects of diet, breed and temperament on physiological indicators through time (4 consecutive occasions) were evaluated through analysis of variance using a mixed model with repeated measures considering the animal as a random effect inside each diet (PROC MIXED; SAS, 2007). Initial and final liveweight were included in the model as covariates. Each animal was bleeded on four consecutive occasions. To model the correlation between repeated measures for each animal, a general linear mixed model was used (PROC MIXED, SAS 2007). For each dependent variable analysed, several covariance structures were tested (variance components [VC], first-order autoregressive structure [AR (1)] and compound symmetry [CS]), in order to fit the best model. Goodness of fit was defined by the lower Akaike’s Information Criteria (AIC) value (Akaike, 1974). After each model had been adjusted, robustness was tested excluding from data standardised, residual values higher than 2 and lower than -2. The model was considered robust when explanatory variables stayed in the model after data filtering and model rerunning. A regression analyses was performed to evaluate Average Tindex, lairage duration and final liveweight effects, on cortisol concentration during slaughter.

Behavioural data: Binomial data from each activity was modeled assuming a binary distribution and a logit link function, using the pen division as the subject/experimental unit. A General linear mixed model was used to study the effect of lairage time, diet, breed and temperament, on the frequency of each behaviour (PROC GLIMMIX; SAS, 2007). Mounting&fighting data from the *Behaviour technique* was modeled and a log link function was set, assuming a Gamma distribution. A General linear mixed model was used to study the effect of diet, breed and temperament on fighting in the first hour in lairage (PROC GLIMMIX; SAS, 2007). Hypothesis tests (Binomial proportion) were performed to analyze differences in fighting frequency (number of events per hour) between consecutive and non consecutive hours in lairage.

Carcass quality. Bruising frequency was compared by the χ^2 test (PROC FREQ; SAS, 2007) and regression analysis were performed to study the effect of independent variables on bruising frequency (PROC LOGISTIC; SAS, 2007) and pH values (PROC REG; SAS, 2007).

3. Results and discussion

3.1. Field determinations

Productivity

ADG did not differ between diets (0.63 ± 0.02 in D1, 0.64 ± 0.02 in D2; p<0.05). The crude protein (CP) content of Uruguayan rangeland pastures seems not to be restrictive for animal production (Rovira, 1996) covering cattle maintenance requirements (Carámbula, 1996) but low ADG especially in autumn, are usually due to the unbalanced chemical composition of native pasture, with low energy availability for the digestive process (Andreo et al., 2001). In our experiment, grazing was not restricted in any diet, CP contents were above critical values (9.22% and 22% in D1 and D2, respectively) and energy restrictions in D1 were compensated by the energetic supplementation, providing the animals in this diet with adequate daily gains. Horn presence and HWP were not related to daily gains (p<0.05).

Tameness and temperament

The D1 Flight zone was lower at the end of the experimental period (0 vs 8 meters in D2). In more intensive systems the Flight Zone is necessarily reduced as compared with an open range system (Phillips, 1993; Uetakee al., 2002). However, the D2 Flight Zone (8 meters) also implied that all the animals got used to the presence of humans. Average Tindex was not affected by diet, but breed did have a significant effect on it ($p<0.05$). Braford animals were more excitable showing a lower Average Tindex than Hereford steers (62.10 ± 4.10 in Hereford and 50.90 ± 4.00 in Braford). Based on a review of temperament of beef cattle, Burrow (1997) reported that without exception, *Bos indicus* were more excitable, temperamental and difficult to handle under extensive management conditions than *Bos Taurus* breeds (Burrow and Corbet, 2000; Elder et al., 1980; Fordyce et al., 1988; Hearnshaw and Morris, 1984; Powell and Reid, 1982). Regarding *B.indicus*-cross cattle, Voisinet et al (1997) reported that they also had higher mean temperament scores than *B. taurus*. Although these differences could be partly due to environmental factors, genetic differences in tameness cannot be ruled out (Manteca and de la Torre, 1996).

Braford steers had higher ADG than Hereford (0.73 ± 0.05 and 0.53 ± 0.05, respectively; $p<0.05$) and calmer animals had higher ADG within both breeds ($p<0.05$). These results are consistent with those of Voisinet et al. (1997), who reported that calmer *Bos indicus*-cross *and Bos Taurus* cattle had higher ADG than steers with excitable temperaments. Barnett, et al. (1983) and Hemsworth et al. (1996) sustained that a fall in the rate of growth is the consequence of a series of acute or chronic responses to human presence, and is probably more accentuated in temperamental animals. Regardless of temperament, gentler animals are known to be less susceptible to stress generated by management practices in which human presence is involved (Boivin et al., 1994) and their productivity is therefore less affected. In this study, all animals had been subjected to good animal husbandry practices prior to and during the test, which probably contributed to the satisfactory ADG levels obtained.

Average Tindex was not related to horn presence ($p<0.05$). These results are not consistent with those of Kilgour and Scott (1959) who reported that dairy cows that had not been dehorned created more trouble while being milked. These cows may have been dominant by virtue of their horns. According to Grandin et al. (1996) animals from *B. Taurus* and *B. indicus* * *B. taurus* breeds could be classified as temperamental, medium or calm according to the HWP. This relation may be explained by the fact that hair patterns in the foetus form at the same time as the brain (Smith and Gong, 1974). However, in our experiment, temperament was not related to HWP. Similar results were found by Randle (1998) who reported that cattle response to humans in general was not significantly associated with HWP.

When comparing initial and final Tindex within each diet, it was observed that animals from D2 were more excitable at the end of the experiment (Table 1). Even thought a strict protocol of good management practices was followed during the experiment, the degree of human contact was lower in D2 because these animals were not supplemented. Habituation to humans is considered to reduce the magnitude of the stress response arising from restraint and handling (Grandin, 1997). In D1 animals did not become calmer, but their reaction to handling remained stable (Table 1).

Table 1. Initial and Final Tindex by diet (and by breed within each diet).
Least square means ± Standard error.

	D1* *Pasture + Corn Grain		***D2* *High Quality Pasture***	
	Braford	Hereford	Braford	Hereford
Initial per breed	52.04 ^{bc} ± 7.6	65.4 ^{abc} ± 7.7	68.5 ^{ab} ±7.7	76.6 ^{a} ± 7.8
INITIAL	58.7 ^{B} ± 5.4		72.56 ^{A} ± 5.6	
Final per breed	62.01^{abc} ± 7.7	60.5 ^{abc} ± 7.9	44.2 ^{c} ± 7.7	63.9 ^{abc} ± 8.0
FINAL	61.2 ^{B} ± 5.6		54.1 ^{B} ± 5.6	

^{a,b,c} Values in lines and columns with different letters are different p<0.05
^{A,B} Values in lines and columns with different letters are different p<0.05

The increased excitability registered in D2 was mainly due to Braford steers (Table 1). Their reaction to handling increased with time in this diet, while Hereford reaction was stable throughout the experiment. Within the supplemented strategy, temperament evolution did not differ between Hereford and Braford steers (Table 1). As previously mentioned, there are several reports of breed differences in temperament, especially between the two cattle subespecies *Bos indicus and Bos Taurus*. Furthermore, within the British breeds, some authors reported that Herefords were the most docile (Stricklin et al., 1980; Tulloh, 1961). Frequent and proper handling therefore, appears to be even more important when working with more excitable animals like *Bos indicus* or it´s crosses. There was no difference in Final Tindex between finishing strategies (Table 1).

Health status

Health was compromised in 2 animals from D2 in the course of the experiment and no event was related to feed problems. In both cases, immediate and effective control measures were applied, with no incidence on ADG in the animals involved. Good physical health is undoubtedly a necessary condition for the well-being of animals. However, health is more than the absence of disease, and understanding the relationship between health and welfare depends on drawing inferences about subjective feelings such as pain, discomfort and distress (Appleby and Hughes, 2005). Based on productive and behavioural observations, it was considered that these events did not have a strong negative impact on welfare and both animals remained in the experiment. No deaths were registered during the experimental period.

3.2 Transport and slaughter plant

Physiology

Cortisol, CPK and NEFA

Finishing strategy did not have an effect on physiological indicators (except βHB). According to the genetic affiliation of steers, differences were found only for hematocrit, but all values were considered normal according to the literature. The physiological responses to water deprivation for periods of up to 48 hours in cattle and sheep generally indicate that ruminants can cope with this challenge (Ferguson and Warner, 2008). The results of the present experiment showed that differences in the diverse physiological indicators were mainly due to lairage duration and animal temperament.

Transport effect on cortisol

Figure 1 shows that each step (except transportation, Time B) involved higher stress in both slaughter groups. According to several authors, the major factors determining the well-being of cattle during road transport are: vehicle design, stocking density, ventilation, the standard of driving and the quality of the road (Broom, 2003; Hartung, 2003; Tarrant and Grandin, 1993). In our experiment, all these factors were standardised and optimised and, added to the calming role of animals herd-mates during transport, they could have been effective and could have contributed to our results. Similar results were reported by Ishiwata et al. (2008) who did not find differences in plasma cortisol concentration before and after travelling, suggesting that transport had no severe effects on cattle. Fazio et al. (2005) suggested that the effects of short-distance road transport on the increase in cortisol levels in cattle, probably depend on preliminary contact with staff during handling. Trunkfield and Broom (1990) reported a sharp response in cortisol levels in calves during the first 2 hours of transport, mainly due to the loading procedure. These authors suggested that cattle are stressed during the initial period of transportation (on short journeys), and that the degree of stress is greater after long-distance road transport. Villaroel et al (2003) also reported that cortisol was higher after 1-2 hours of transportation compared to journeys that were less than 1 hour or more than 2 hours long. After this initial period on short journeys (less than 4 hours), animals are thought to become accustomed to the new situation. In our experiment, animals from both slaughter groups showed a good habituation to transport (Figure 1, Times A and B). On the basis of the comparative response of circulating levels of cortisol before and after transportation, our data do not agree with results that consider transport to be one of the most potent stressors for cattle (Marahrens et al., 2003).

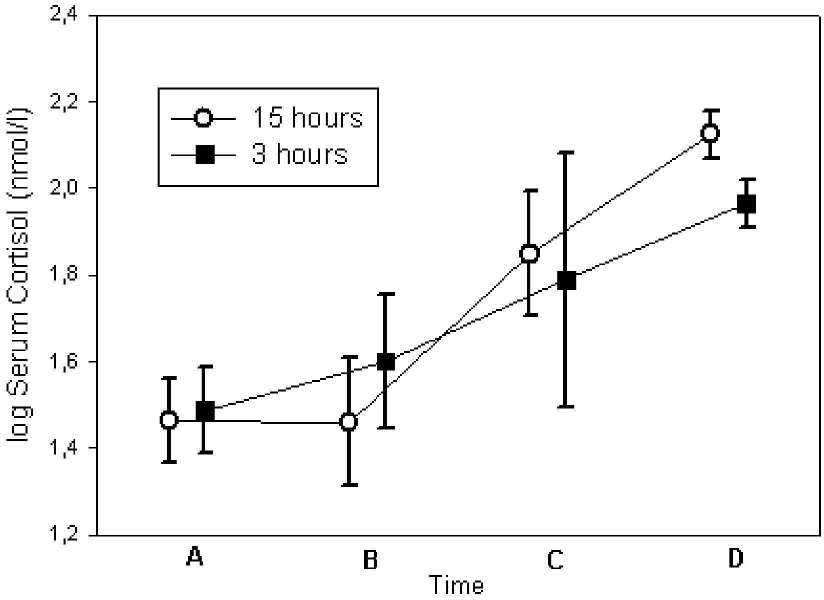

Figure 1. Serum cortisol (log) values at different times, within each slaughter group.
Lines represent media and confidence interval.
A: basal value in farm; B: after transport; C: after lairage; D: at slaughter.

<u>Lairage and preslaughter effect on cortisol</u>

Serum cortisol concentrations significantly increased with respect to basal values after lairage and at slaughter. Cattle have very low basal levels, often less than 15 nmol/l (Boissy and Le Neindre, 1997; Lay et al., 1992) but in the present experiment they were around 30 nmol/l in both slaughter groups. Cortisol concentration in serum was no different after 3 and 15 hours in lairage ($p<0.05$). All animals were stressed, probably due to the inherent noises and movement of animals and people in the yards during routine handling and because of the new environment. It is known that after a stressful event, haematological variables can return to basal levels within 30 minutes if animals are in their familiar environment (Sartorelli et al., 1992). In this study, probably due to the new environment, higher values of cortisol were registered even after 15 hours in lairage.

Both groups also had a considerable preslaughter stress response, increasing to 90 and 130 nm/L in the short lairage and the overnight group, respectively (Figure 1). This is consistent with results from Boissy and Le Neindre (1997) and Lay et al (1992), who reported that cortisol levels in response to a stressor could increase up to 60-200 nmol/L in cattle. Some authors believe that the increase in cortisol concentrations during bleeding are mainly a response to handling in the race when driving the steers to the stunning box (Tadich et al., 2005). It is worth noting that they could also represent the cumulative effects of all stages of the pre-slaughter handling. Moreover, due to food safety requirements, cattle are washed on their way to the stunning box to remove hide or fleece contaminants such as excreta and dirt. The process of handling and washing the animals would have elicited a stress response which could partially explain the cortisol rise in both slaughter groups. Although the distance between washing and stunning is short, it could have been enough to raise HPA axis activity. In addition, the effect of the process of stunning itself cannot be disregarded.

Calmer animals showed lower cortisol values in blood at slaughter, regardless of diet or slaughter group (see regression coefficients in Table 2; Figure 2; $p<0.05$).

Table 2. Effect of lairage time, Average Tindex and final live weight on serum cortisol concentration at slaughter.

	Parameter Estimator	***Pr > |t|***
Intercept	2.54328	<.005
Lairage time (Dummy)	-0.16518	<0.05
Average Tindex	-0.00176	<0.05
Final live weight	-0.00068	ns

*Lairage time (Dummy variables): 0= 15 hours; 1= 3 hours.

Calmer animals also showed lower cortisol concentrations in serum throughout the whole period (Estimator: -0.002, $p<0.05$). These results are consistent with those reported by Curley et al. (2008), who indicated that the functional characteristics of the HPA axis vary with animal temperament. Several authors reported elevated cortisol concentrations in cattle that are more agitated by human-animal interactions (i.e. exhibiting a faster EV) when compared to calmer animals (Curley et al., 2006; Fell et al., 1999). Our results seem to provide support to the recognized influence of temperament in modulating the adrenal response of cattle to different stressful situations.

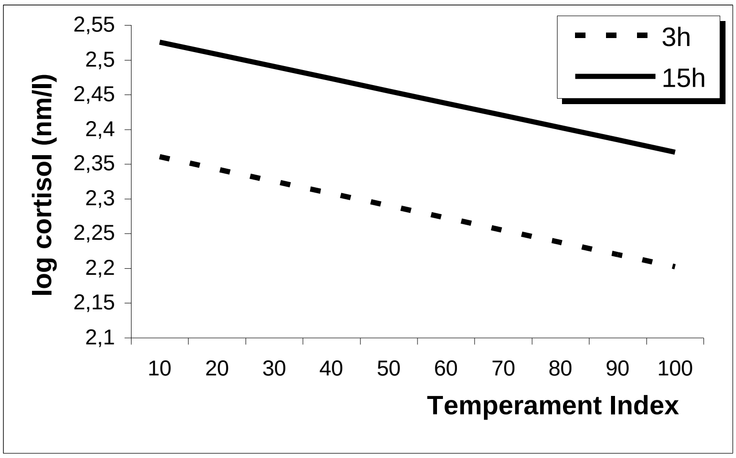

Figure 2. Average Tindex effect on cortisol (log) values at slaughter. Trendlines per slaughter group, estimated by regression analysis (R^2=0.30).

Transport effect on CPK and NEFA

Serum CPK concentrations significantly increased with respect to basal values after transportation in both slaughter groups (Table 3). Similar results were found by several other authors (Grasso et al., 1989; Groth and Granzer, 1977; Van de Water et al., 2003; Villaroel et al., 2003). Plasma creatine kinase is a muscle-specific enzyme whose activity in the blood is useful for indicating leakage from the muscle as a result of trauma, physical exercise or other damage (Lefebvre et al., 1996).

Table 3. CPK (log) values at different times within each slaughter group.
Least square means ± Standard error.

Log CPK (U/L)	***Basal value***	***After transportation***	***After lairage***	***At slaughter***
3 hours	$1.99^{c} \pm 0.50$	$2.48^{b} \pm 0.51$	$2.39^{b} \pm 0.52$ b	$2.71^{a} \pm 0.51$
15 hours	$2.08^{c} \pm 0.50$	$2.44^{b} \pm 0.51$	$2.40^{b} \pm 0.51$ b	$2.67^{a} \pm 0.50$

Values with different letters differ $p < 0.05$

The increased activity of the enzyme in this experiment could indicate possible trauma during loading, transport and unloading, or it could have increased as a result of behavioural interactions between steers (Anderson et al., 1976; Lefebvre et al., 1996). Even if driving is smooth, animals need to make a considerable physical effort during transportation to keep their balance (stability) and posture. Vibration and motion might also have caused stress, so that, travelling could have been an accumulation of the non-specific stress response and the physical effort (absolute values in U/L increased 2 times after transport and 4 times at slaughter with respect to basal values in both groups).

NEFA concentrations also increased in both slaughter groups after transportation (Table 4). Similar results were found by Warriss et al. (1995), who reported that transport of cattle for between 5 and 15 h was associated with increases in blood concentrations of free fatty acids indicating that the cattle mobilised body energy reserves. Changes in these blood metabolites are indicative of energy mobilization, a mechanism necessary to maintain homeostasis (Moberg and Mench, 2000). Fasting and stressful events are typically associated with increased energy demands and this leads to depletion of energy stores, in particular liver glycogens and body fat (Balm, 1990). In the present experiment, the higher NEFA concentrations after transport are consistent with CPK increases reported before. As mentioned, free fatty acids may increase during feed restriction as a result of fat reserves being mobilised to supply energy requirements, but they may also increase in response to catecholamine release following acute stress (Shaw and Tume, 1992). Although cortisol concentrations did not increase after transport, it is possible that sudden moments of extremely acute stress (like sudden truck movements or vibrations), provoked activation of the autonomic nervous system with the consequent increase in NEFA, although it was not enough to activate the HPA axis. According to Mellor and Stafford (1997) the relatively slow response time of the HPA axis may make it insensitive as a means of discriminating different level of stress within the first few minutes after a noxious stimulus. The physiological changes elicited by the sympathetic adrenomedullary system may be more accurate in assessing the early stages of distress response (Mellor et al., 2000). In our experiment, physical stress was evident after transport, according to CPK and NEFA concentrations, but results showed that the situation did not involve the HPA axis activity. The activation of the HPA-axis is mainly dependent on the emotional involvement of the animal; stressors do not necessarily activate the HPA system when the animal does not perceive the situation as stressful (von Borell, 2001).

We could not conclude from the results obtained that animals were suffering during transport. The physiological changes registered in this stage seem to be an indication that the adaptive mechanisms were functioning.

<u>Lairage and preslaughter effect on CPK and NEFA</u>

After lairage, CPK values did not increase in any slaughter group (Table 3). Similar results were found by Tadich et al. (2005) who found higher CPK activity after transport (with 0, 3 and 16 hours) but did not find an additional increase during lairage (in different combinations of transport: 0, 3, 16 hours; and lairage duration: 0, 3, 12, 16 and 24 hours). In our experiment, negative behaviour during lairage (mounting and fighting) were more frequent in the 3-hour group (see behavioural analysis), but this higher activity/exercise was apparently not enough to increase serum CPK concentrations. NEFA concentrations were higher after lairage but only in animals from the overnight group (Table 4), suggesting a greater energy demand to restore homeostasis because of the longer food deprivation (Gupta et al., 2005). These differences could therefore be explained as a result of fat reserves being mobilised to supply energy requirements, probably with the additional effect of psychological stress due to the new environment. However, as has been mentioned, HPA axis activity increased but did not differ between groups during lairage.

Here again, physiological results did not allow us to conclude that there was a higher degree of suffering in the long lairage group.

Preslaughter handling procedures had a significant effect on CPK values in both groups (Table 3). The higher presence of CPK implies constant muscle movement, both voluntary and those that are controlled by the autonomic nervous system (heart, lungs). Elevated plasma CPK activity is also associated with strenuous or unaccustomed muscular exercise

(Berg and Haralambie, 1978). For this reason we considered that the stunning process itself could have had a considerable effect on these results (tonic and clonic phases).

Calmer animals had lower CPK and NEFA values in serum throughout the experiment ($p<0.05$). The effect of temperament on the stress response in cattle has been already discussed in this paper.

Table 4. NEFA values at different times within each slaughter group.
Least square means ± Standard error.

NEFA (mmol/L)	***Basal value***	***After transportation***	***After lairage***	***At slaughter***
3 hours	$0.36^{d} \pm 0.02$	$0.55^{b} \pm 0.03$	$0.48^{bcd} \pm 0.08$	$0.43^{cd} \pm 0.03$
15 hours	$0.37^{d} \pm 0.02$	$0.49^{bc} \pm 0.03$	$0.68^{a} \pm 0.04$	$0.52^{b} \pm 0.03$

Values with different letters differ $p< 0.05$

Cattle kept in lairage overnight had greater NEFA concentration at slaughter than the 3-hour group, whose values remained unaltered after lairage and were no different from basal concentrations (Table 4). The short lairage group was only affected after transportation, suggesting that food deprivation was not long enough to cause a lasting rise in NEFA. Increases after transport could probably have been related to links with the adrenomedullary system activity. Although NEFA slaughter values in the long lairage group were lower than in the post-lairage, recovery was not enough to achieve basal values. Undoubtedly, overnight animals presented higher energy demands than the 3 hour-group. These results are consistent with those from Jarvis et al (1996) who reported higher concentrations of NEFA during bleeding ($p<0.05$) in animals that spent more than 16 hours in the abattoir (overnight) when compared to animals that spent 5 hours in lairage pens previous to slaughter (0.28 and 0.33 mmol/l respectively). Cockram and Corley (1991) also found that cattle held overnight in lairage had significantly greater plasma-free fatty-acid concentrations than those slaughtered on the day of arrival. However, because of the experimental design, those authors could not separate the specific lairage effect from the preslaughter handling effect. In our experiment, NEFA differences between both groups were established after lairage.

ß-hidroxibutirate (βHB) serum concentrations were higher ($p<0.05$) in animals from D2 (0.32 ± 0.02 vs 0.28 ± 0.01 in D1) with no differences between slaughter groups. Ketonic bodies, like βHB are excellent fuel for tissue respiration, in particular when glucose levels are limited (fastening). However, under these circumstances, these tissues can easily use NEFA energy sources. In the present study probably fasting was not long enough to cause a strong and clear βHB stress response or to determine differences between slaughter groups.

Lairage pen behaviour

The animals did not drink water during lairage. It is possible that this behaviour was suppressed as a result of unfamiliarity with the new environment. However, hematocrit values at slaughter showed that animals were not dehydrated. If cattle are fully hydrated and fed before transport, it is likely that food deprivation rather than water, will be the greater stressor over the initial 24 hours, since this is more likely to disrupt rumen function (Hartung et al., 2000). From practical experience in our particular abattoir, we have seen that animals do not drink water while in lairage pens. In the present experiment, animals did not perform social behaviour, self grooming or lie down during the scan. The time budget is shown in Figure 3.

According to the scan sampling technique, steers from both slaughter groups spent around 80% of total time in lairage, standing/ruminating ($p<0.05$; Figure 3) and no differences were registered in walking and negative behaviour. Results from the Glimmix procedure showed that animals from D2 spent more time ruminating than supplemented steers (D1) ($p< 0.05$). Animals are known to ruminate while resting (Tribe, 1955) so these results suggested that animals from D2 were probably calmer than those from D1. Temperament and breed did not have any affect on the time budget.

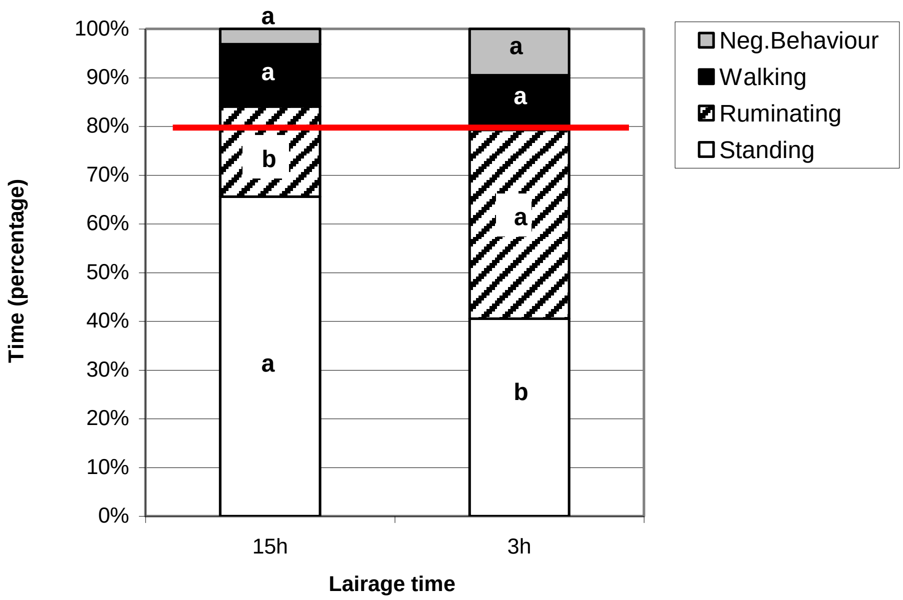

Figure 3. Percentage of the total time spent on each of the observed behaviour, by slaughter group.
Note: The same activity with different letter (between bars) differs with $p < 0.05$.

Figure 4 describes each behaviour evolution in the long lairage group, suggesting that the first hour was the most critical, with less rumination and more walking and negative behaviour.

As mentioned, negative behaviour would have been missed by scan sampling (Martin and Bateson, 1993). In fact, according to the *Behaviour sampling technique*, fighting frequency (fights/hour) was significantly higher in the short lairage group ($p<0.05$) and for Braford steers ($p<0.05$). Results from the Glimmix procedure also showed that supplemented steers (D1) were more aggressive than those from D2, and these results were consistent with the higher rumination time registered in animals from D2.

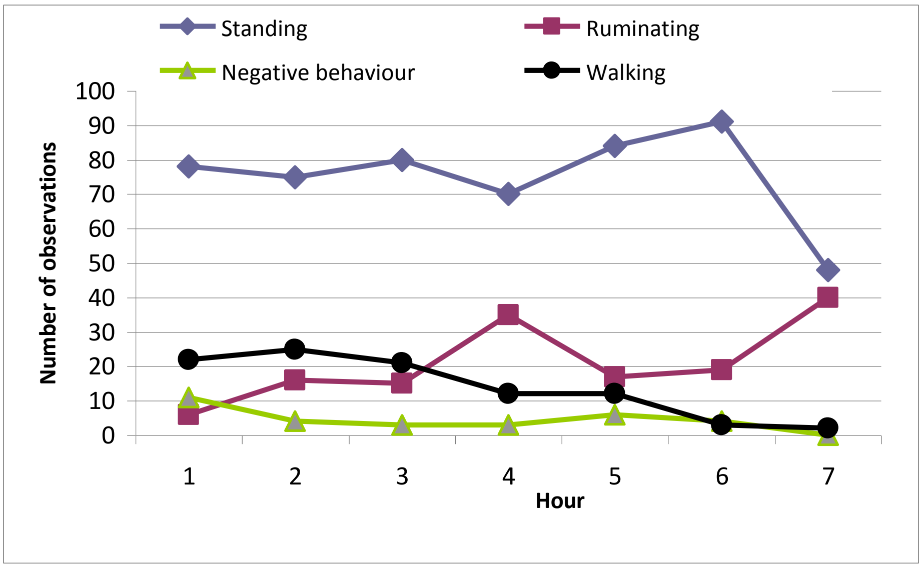

Figure 4. Frequency of different behaviours at each hour of lairage (long lairage group).

When analyzing fighting frequency during the first hour in lairage, no differences were found between slaughter groups (Figure 5; $p<0.05$). The frequency of this activity in consecutive hours (obviously in the long lairage group) was therefore compared to fighting frequency during the first hour. Results from each Binomial proportion comparison showed that fighting frequency in the first hour in pens was significantly higher than the second, third, forth, fifth, sixth and seventh hour, respectively (Figure 5; $p<0,05$). Based on these results, we could infer that the first hour in pens was a critical adaptation stage for both groups. The animals that remained in pens became calmer afterwards. According to these results we could have expected the same evolution in fighting frequency in the short lairage group. The lowest fighting frequency in the 15 hours group (with respect to the first hour) was registered during the 4^{th} and 7^{th} hour (Figure 5, $p<0.05$). Temperament and breed did not have an effect on fighting frequency during the first hour in lairage, suggesting that it was a stressful period for all animals.

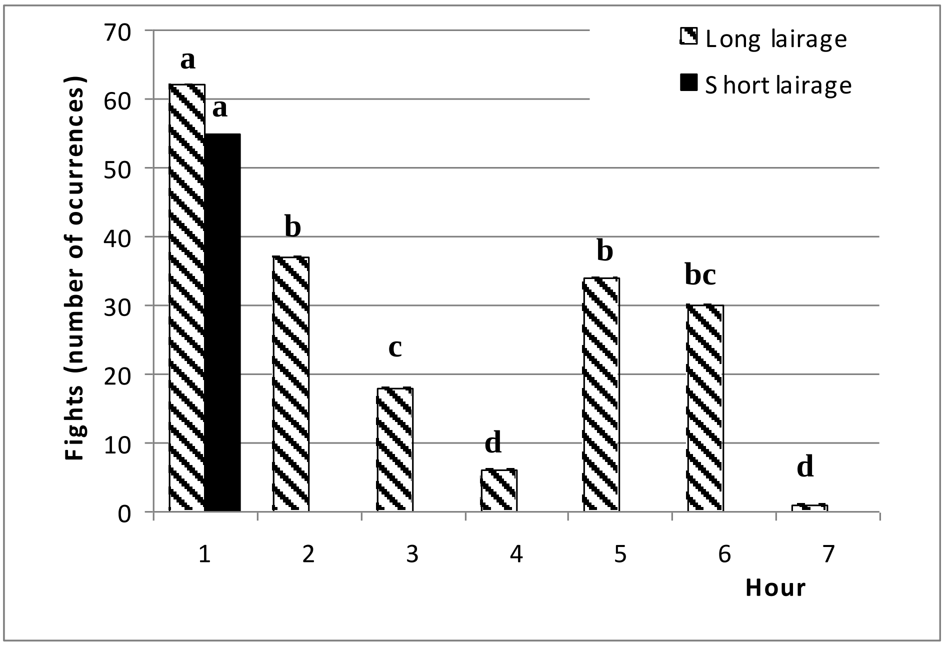

Figure 5. Number of fights during consecutive hours for each slaughter group.
Bars with different letter differ p<0.05

Both groups were situated in quiet environments far from the unloading facilities, but the 15-hour group waited overnight, with greater opportunities to rest. Noise generated by the normal abattoir activity was noticeably higher during the morning and mid-day because of the slaughter procedures. This could have contributed to a higher excitability in the 3-hour group, not having the opportunity to rest or to get used to the pens. However, considering that there were no differences between groups in fighting frequency during the first hour of observation, we consider that lack of resting time was probably the most important reason for these results. "Fights" could have been an adaptive mechanism for all steers, but even if they were showing adaptive behaviour, they may still be suffering in the process. Conflict may be beneficial in the long run, but will still be unpleasant while it lasts (Dawkins, 1980), especially considering those animals that did not have enough time to cope with the new situation (3-hour group). Based strictly on physiological results from times A to D, we could have concluded that animals from the overnight group were physically and emotionally more stressed than the 3-hour group. However, according to behaviour results, the short lairage group was more excited during lairage and carcasses showed higher pH values.

3.3. Carcass traits

pH

Carcasses from the short lairage group had higher values of final pH (5.83 ± 0.04 vs 5.68 ± 0.04 in the long lairage group; $p<0.05$). It seemed that their excitability without having the opportunity to recover, implied a significant depletion of muscle glycogen reserves with a profound effect on pH at 24 hours *post mortem*. There was thus, direct evidence that fighting activity during lairage resulted in a higher last muscle pH, as found by Grigor et al. (2004) and Warris et al. (1984). Stressors appear to be additive (Bray et al., 1989) so that multiple stressors without the opportunity to recover during lairage resulted in a greater elevation of muscle pH.

Bruising

Incidence of bruising was not significantly affected by lairage time, with 14 bruises registered in the overnight group and 15 in the short lairage group. These results are not consistent with those of Mc.Nally and Warris (1996) who reported higher bruise incidence in carcasses from cattle that remained for longer lairage periods. It is important to consider that bruise incidence in this study was registered according to welfare criteria. This means that minor bruises implied subcutaneous tissue remotion only, or any deterioration in the region affected. In spite of the relatively low incidence of bruising, we consider that the bleeding procedures (pre and post lairage) undoubtedly contributed to the presence of minor and especially major bruises. Major bruises affecting carcass and meat quality were only registered in the overnight group (1 bruise in two animals) and both steers had jumped through the chute while being bled. According to some authors, the vigorous avoidance response of cattle with poor temperament in confined areas during handling, transport and pre-slaughter increases the likelihood of falling and of collision with yard or stock crate structures, and also with other cattle, increasing the chance of bruising (Barnett et al., 1984). In the present study, results from a logistic regression analysis showed that temperament was not related to bruise incidence ($p<0.05$). The good management practices followed in the abattoir could have contributed to these results.

4. Conclusions

Considering average daily gains, environmental conditions, animal health performance and mortality rate, it is possible to make the preliminary inference that animal welfare was not compromised in any diet during the finishing period. Frequent and proper handling was considered to be very important, especially when working with excitable animals. Temperament appears to be an important feature with regard to its effect on productivity (ADG) and also on the individual stress response at different pre-slaughter stages. Transportation is, in general, inevitably associated with a stress response but its negative effects may be avoided or minimised after short travels (less than 4 hours) by proper handling and the use of suitable equipment and facilities. Increases in energy demands are unavoidable in fasting animals, especially with longer lairage, but adequate conditions and a calm environment may allow cattle to rest and recover while waiting in pens, with positive effects on pH values. Lairage and preslaughter handling induced a significant increase in the activity of the hypothalamus-hypophisis-adrenal axis, suggesting some degree of psychological stress. The first hour in lairage was a stressful period and animals became calmer afterwards. The insufficient resting period remained animals highly excitable before slaughter, contributing to glycogen depletion and to the consequent higher pH values registered. The results of this experiment suggest that more than 3 hours of resting in lairage appears to be desirable from a welfare point of view. We consider that an intermediate lairage duration would also be appropriate from the meat- quality perspective. Nevertheless, our data do not enable us to recommend a specific waiting time and further research is needed to clarify this issue.

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