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Social Behavior and Productive and Stress Parameters in Holstein Steers Fattened in Three Contrasting Production Systems

Oscar Blumetto[1], Andrea Ruggia[1], Jessica T. Morales Pyñeyrúa[1] & Aranzazu Villagrá García[2]

[1] National Institute of Agricultural Research (INIA), Rincón del Colorado, Canelones, Uruguay

[2] Animal Technology Centre CITA-IVIA, Segorbe, Castellón, Spain

Correspondence: Oscar Blumetto, INIA Las Brujas, Ruta 48 km 10, Rincón del Colorado, 90200, Canelones, Uruguay. Tel: 598-2367-7641. E-mail: oblumetto@inia.org.uy

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Abstract

Beef production with Holstein male calves is becoming more intensive in Uruguay. Some of the new systems with confined animals could improve productivity but also could compromise animal welfare. The aim of this study was to compare animal welfare, stress evidence and productivity of castrated young males reared in three different productive systems. The traditional pastoral system (T3) was compared with, a confined fattening system (T1) and an alternative one with confinement and six hours diary access to pastures (T2). The experiment was performed for evaluating the first phase of fattening period (initial mean live weight 93±20.3 kg; and 112±11 days of age). No differences ($p = 0.1254$) between treatments were detected for live weight gain 0.756±0.829, 0.757±0.676 and 0.730±0.762 kg day^{-1} for T1, T2 and T3, respectively. There was no evidences of increasing stress or health problems in any production system, according to the obtained serum profile of enzymes and hormones, however permanent confined animals increased agonistic behavior, which could reflect some welfare problems that might increase in the following phases of the fattening process.

Keywords: animal welfare, animal husbandry, plasma biochemical profile, cortisol

1. Introduction

Beef production in Uruguay is mainly based on grazing systems, which occupy more than 70% of the country's territory (Agricultural Statistics Direction [DIEA], 2015). Feedlot production represents nowadays only 9,1% of total slaughter but it is expected to continue growing (Agricultural Planning and Budget Office [OPYPA], 2015). Livestock production systems combining grazing and grain supplementation are well known in dairy cattle production and increasingly common in beef production, consequently for dairy calves fattening, some alternatives which combine characteristics of both grazing systems and high use of grain supplementation, have been developed (Ruggia et al., 2014).

The intensive production systems modify animal conditions, by reducing their space allowance, changing feeding and resting conditions, and influencing social behavior (Kondo et al., 1989; Tuomisto et al., 2015). Subsequently, competition for resources such as food or attractive resting places may cause aggression and social stress (Purcell & Arave, 1991).

One of these aspects, insufficient space allowance, induces a repeated state of stress that alters the activity of the pituitary-adrenal axis, immune function, behavior and growth rate (Fisher et al., 1997a). Average daily gain is reduced and cortisol blood concentration increases as a result of restricted space allowance (Gupta et al., 2007). Diet in finishing steers has been found as affecting cortisol levels as well (Larraín et al., 2008).

Animal health is one of the most important aspects considered to evaluate welfare (Broom, 2006) and biochemical serum profile is a common method to assess it (Chorfi et al., 2007; Adams et al., 2008). Some blood parameters such as glucose or creatin kinase (CK), are used as stress indicators (Bonacic et al., 2006). Elevation of their plasma concentrations reflects alterations in tissue function or indicates cell damage or necrosis (Scientific Committee on Animal Health and Animal Welfare [SCAHAW], 2000). In addition, plasma activities CK is sign of stress-induced tissue damage (Hocking et al., 1994) or skeletal muscle lesions attributable to trauma or vigorous exercise (IDEXX, 2014).

Despite intensive systems are normally associated to worse welfare status, conditions associated with extensive livestock production could also create a substantial number of welfare problems (Petherick, 2005). This makes the study of different effects of each production system on social and welfare aspects crucial.

The main objective of the present study was to characterize three different Holstein steers' production systems, throw their effect on productive traits (live weight gain and feed intake), physiological stress indicators (cortisol and plasma biochemical profile) and social interaction and evaluate if differences between systems affects these indicators.

2. Materials and Methods

The experiment was carried out at Las Brujas Experimental Centre of the National Agricultural Research Institute (INIA) of Uruguay (34º40′S lat, 56º20′W, 36 masl). The experimental period lasted 133 days, from August to December 2010. Results for feed quality and behavioral characterization of the steers have been reported in detail by Blumetto et al. (2016). In the present paper we focus on performance, stress parameters and social behavior.

2.1 Animals and Housing

Experimental design was established according to Manninen et al. (2007). Forty-eight Holstein castrated males were randomly divided into three groups (16 calves in each group) corresponding to three treatments:

(T1) confined in a yard of 210 square meters;

(T2) confined in a yard of 210 square meters with six hours of access to pasture;

(T3) permanently placed at pasture.

Initial live weight (LW) was 92.6±21.2 kg for T1, 93.4±20.0 kg for T2 and 93.0±21.3 for T3. In these groups, initial age was 111±12 days, 113±10 days and 112±11 days for T1, T2 and T3 respectively.

All steers were individually identified by ear tag (number ID), and within each group, different color collars were used for each animal to help identify individuals in the behavior studies. All experimental measurements started on 15 September, after an adaptation period of 43 days. This period was considered necessary for avoiding de effect of changes in group composition, diet and handling, and finished when assured no evidence of digestive dysfunction.

The experimental yards (treatments T1 and T2) consisted on an outdoor 21 × 10 m yard, built with electric fencing were feed and water trough were placed. The pasture parcels were also built with electric fencing, and the surface area was calculated depending on the dry matter (DM) forage offered to reach 8% of average live weight per animal. The average area was about 2000 square meters.

Animals assigned to T1 were maintained for this experiment in the yard with an automatic water trough and were fed *ad libitum* with alfalfa hay. Twice a day, at 9:00 and 16:00, humid sorghum grain silage (2% of average live weight) and sunflower expeller (0.35% of average live weight) and soybean expeller (0.15% of average live weight) were supplied. Steers assigned to T2 were placed in the yard with an automatic water trough, fed *ad libitum* with alfalfa hay and humid sorghum grain silage (1.5% of average live weight) supplied a single time per day at 16:00. In addition, for six hours per day (10:00 to 16:00) they were moved to a pasture parcel with an additional water trough. Steers in T3 were maintained on pasture, with alfalfa hay and water freely available. The pasture offered was a mix of alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*) and ryegrass (*Lolium multiflorum*). Chemical composition of offered foodstuff is presented in Table 1.

Table 1. Composition of feeds used in the experiment (mean ± SD)

	n	DM %	CP*	ADF*	NDF*	OMD*	Ash*
Pasture	12	20.8±4.1	17.5±3.4	40.0±2.8	54.4±3.2	74.1±4.6	12.8±0.6
Sorghum grain silage	5	71.0±2.8	7.9±0.4	10.7±0.3	14.9±0.5	76.8±4.5	2.1±0.3
Alfalfa hay	9	88.7±2.9	13.4±1.1	45.0±4.5	53.6±6.7	62.0±4.6	9.1±2.5
Sunflower expeller	3	91.8±0.8	35.5±0.4	26.2±0.6	39.1±0.8	65.6±1.1	6.8±0.2
Soybean expeller	3	89.9±1.6	45.2±0.4	8.2±1.0	15.9±1.6	88.2±1.5	6.5±0.4

Note. * % of dry matter; n: number of samples; DM: dry matter; CP: crude protein; ADF: acid detergent fibre; NDF: neutral detergent fibre; OMD: organic matter digestibility.

2.2 Production Measures

Animals were individually weighed every two weeks. Average daily gain was calculated by dividing total weight gain in the measured period by total days in that period. Provided feed per group was weighed every day and then, food intake of each group was weekly measured for hay and grain supplement. Pasture intake was estimated as the difference between offering (forage mass availability at the moment of opening a new grazing parcel) and remaining (forage mass availability at the moment of taking out the animals of the parcel) with an standard method (Coates & Pennining, 2000), taken 7 samples in each grazing parcel before opening and after closing parcels. Dry matter content of the pasture was estimated by freeze-dried in pooled herbage samples for 48 hours at 60 ºC. Hay and supplement DM was determined by drying at 80 ºC for 24 hours and nitrogen (N) content of pasture, hay and supplement were analyzed by Kjeldahl method.

In addition, feed conversion rate for each group was calculated by dividing total DM intake by the total individual weight gain.

Total N intake was calculated as the DM intake multiplied by this calculated N content and the proportion (%) of N in total intake, was obtained by the following equation: N(%) = Kg total N intake/total DM intake × 100. Feed efficiency was calculated as kg LW gain per kg DM intake. All intake and efficiency determinations are referenced to the animals group as measured unit.

2.3 Social Behavior

Calves' interactions were directly observed during twelve hours a day (from 7:00 to 19:00), three days per week in four weeks distributed throughout the experiment (weeks 7, 10, 13 and 16). Six people were trained to perform the behavioral observations, and then, there was one observer for each treatment, in three hours turns. Observers were randomly assigned to each treatment and timetable every day. An interaction was considered when physical contact between two animals was produced. They were registered continuously and the considered activities are described in Table 2. Complementary, two new variables were created: positive interactions (PI) by integrating all non-agonistic social behavior (licking a group mate, smelling a group mate and Scratching with other) and negative interaction (NI) by integrating all agonistic social behavior (mounting, displacing, pushing with chest and head knocking).

Table 2. Observed interaction between calves and its respective descriptions

Behaviour	Description
Mounting (M)	Calf clasping or trying to clasp other calves back with both legs
Displacing (D)	One calf displacing another, with shoulder, side, flank or rump from its standing or lying place
Pushing with chest (P)	One calf pushing with the chest to another calf from its standing place
Head Knocking (H)	One calf knocking another with the head in any part of its body
Licking a group mate(L)	Calf licking another at any part of its body
Smelling a group mate (S)	Calf smelling another with contact with its skin
Scratching with other (SO)	Calf scratching with the body of another calf

2.4 Cortisol and Biochemical Profile

Nearby the end of the experiment (day 120) while weighting routine, eight animals per group were randomly chosen and blood samples were taken from jugular vein puncture. Samples were collected in 7 mL Vacuum tubes without any anticoagulant and immediately refrigerated and taken to the laboratory, where they were centrifuged at 3000 rpm for 15 min at 4 °C, as described by (Titto et al., 2010). Serum was then removed and passed to Eppendorf tubes (1.5 mL) for storage at -40 ºC until the analyses were made. Each sample was divided into three tubes: one for cortisol, one for biochemical profile and the other for backup.

Serum samples were assayed in the Laboratory of Nuclear Techniques, Veterinary Faculty, Montevideo, Uruguay. Cortisol concentrations were determined by a direct solid-phase radioimmunoassay (RIA) using DPC kits (Diagnostic Product Co., Los Angeles, CA, USA). The RIA had a sensitivity of 0.52 ug/dL. All samples were determined in the same assay. The intra-assay coefficients of variation for low (1.28 ug/dL), medium (5.91 ug/dL) and high (17.05 ug/dL) were 10.89%, 7.13% and 2.58% respectively.

Finally, twelve biochemical parameters were determined by IDEXX VetTest® Chemistry Analizer: Alanine Trasnpherase (ALT), Alkaline Phosphatase (ALKP), Gamma Glutamine Transferase (GGT), Albumin (ALB),

Glucose (GLU), Total Protein (TP), Urea (BUN), Total Bilirubin (TBIL), Creatinine Kinase (CK), Calcium (Ca), Phosphates (PHOS), Globulin (GLOB).

2.5 Statistical Analysis

No statistical analysis was possible for food intake data as it was measured for each group so results are presented as absolute values determined, using tables and bar diagrams.

The rest of data were analyzed by Statistical Analysis System package (SAS, 2008). Live weight was analyzed using the Mixed Procedure (PROC MIXED) with repeated measures within animals, with initial weight as covariate. The model used was:

$$y = \mu + T + PS + PS \times T + e \quad (1)$$

Where, y is the measurement on animal in the treatment (production system); μ is general term; T is the effect of weeks since start measures; PS the effect of the production system; $PS \times T$ the interaction between system and period and e is residual error. Tukey-Kramer adjustments were used for post-hoc comparisons.

Data from the biochemical profile and cortisol were transformed throw LN(1+value) in order to normalize residual errors, as cited by (Tadich et al., 2005) and variance analyzed by General Linear Model Procedure (PROC GLM).

Interactions between animals were expressed as a count and logarithmic transformation (Ln) were used as cited by (Coutellier et al., 2007) and analyzed using the Mixed Procedure (PROC MIXED) according to (Faerevik et al., 2008), using date as repeated factor and the effect of the period of the day and the week of observation was also assessed.

The model was $\text{Ln}(y) = \mu + T + P + W + P \times T + W \times T + e$; where y is the response variable; T is the effect of production system (T1, T2 and T3); P the effect of the period (corresponding to the four observation turns); W the effect of the week of observation; $P \times T$ the interaction between system and period; $W \times T$ the interaction between system and week of observation, and e is the residual error. Tukey-Kramer adjustments were also used for post-hoc comparisons.

3. Results

3.1 Production Measures

Figure 1 shows average live weight evolution for each treatment. Initial live weight was 107.6±30.0 kg, 101.4±28.3 kg and 96.9±23.9 and the final live weight was 206.8±52.5 kg, 202.1±41.9 kg and 194.0±37.0 kg for T1, T2 and T3 respectively. The average of live weight across the experiment was similar for the three treatments (Figure 1). Average daily gain (ADG) was 0.756±0.829, 0.757±0.676 and 0.730±0.762 kg day^{-1} for T1, T2 and T3, respectively (p = 0.1254).

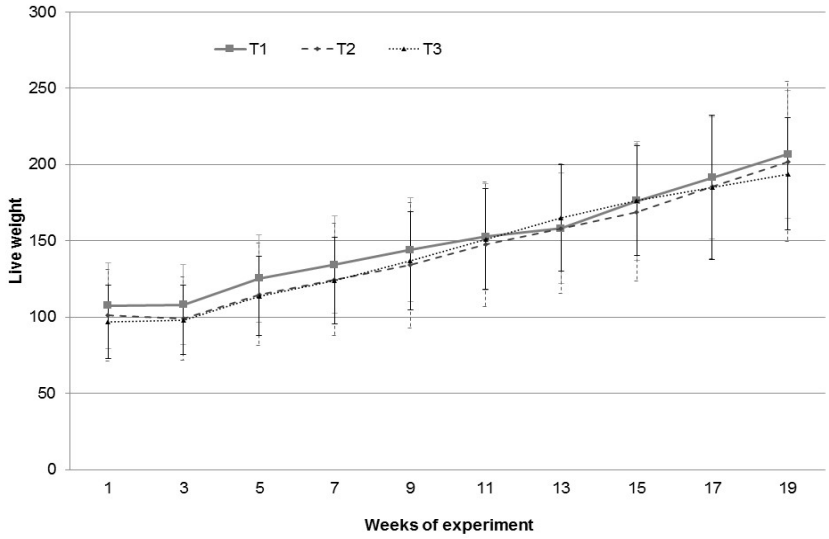

Figure 1. Live weight evolution (kg) from the end of adaptation period to the finish of the experiment

As regards to total DM and total N intake from the different feeding sources, results are presented in Table 3. It is important to remark that these data were calculated per group. In addition, feed conversion rates (kg DM/kg ADG) for each group were 9.5, 13.0, and 10.0 for T1, T2, and T3, respectively.

Table 3. Total dry matter (DM) and total nitrogen (N) intake (kg) for each treatment from the beginning (end of adaptation period) to the end of the experiment

Treatment	T1	T2	T3
Pasture DM (Kg)	0	12 302	14 494
Pasture N (Kg)	0	347	405
Hay DM (Kg)	10 324	5198	1711
Hay N (Kg)	221	111	37
Sorghum silage DM (Kg)	3459	3410	0
Sorghum silage N (Kg)	44	44	0
Soy+sunflower DM (Kg)	1530	0	0
Soy + sunflower N (Kg)	88	0	0
Total DM (Kg)	15 313	20 910	16 205
Total N (Kg)	354	502	442

Table 3 shows that total DM and total N intake were higher for T2 compared with the other treatments. However, N content of total dry matter intake (DMI) was similar, being 2.3%, 2.4% and 2.5% for T1, T2 and T3, respectively. Analyzing the components of this DMI for T1 and T2, very similar pasture intakes can be observed, while hay intake is sensibly higher for T2. The highest hay dry matter intake was registered for T1.

An estimate average individual intake for the experiment was also calculated by dividing the total DMI by the number of animals per group and the days the experiment lasted, resulting in 7.2, 9.8 and 7.6 kg DM for T1, T2 and T3, respectively.

3.2 Social Behavior

Daily average number of interactions for the three treatments is presented in Table 4. In general terms, positive interactions did not differ significantly between treatments ($P = 0.1496$) whereas negative interactions (aggressions) resulted higher in T1 ($P < 0.0001$) than T2 and T3. The same situation can be observed for all separated aggressions, except for M, which did not differ significantly within treatments. H was the most frequent aggression in T1 while for T2 and T3 it had similar values to those found for M. Regarding PI, L was the most frequent for all treatments.

Table 4. Average daily interactions within calves of each treatment (means ± S.D.)

Treatment	T1	T2	T3	p
H	5.8±6.3[b]	2.7±2.5[a]	1.9±2.3[a]	< .0001
P	3.2±5.6[b]	1.1±1.8[a]	0.8±1.2[a]	0.0002
D	2.2±4.0[b]	0.9±1.5[a]	0.8±1.8[a]	0.0038
M	2.2±2.1	2.7±3.6	2.5±2.6	0.9981
L	8.7±7.2	7.2±8.4	6.7±6.9	0.1936
SO	1.3±2.1	1.4±2.2	0.8±1.7	0.3138
S	3.3±5.1	2.0±3.4	2.0±4.0	0.0937
PI	13.3±12.2	10.5±11.4	9.5±10.4	0.1496
NI	13.4±13.6[b]	7.4±5.4[a]	5.9±4.6[a]	0.0001

Note. Mounting (M), Displacing (D), Pushing with chest (P), Head Knocking (H), Licking a group mate (L), Smelling a group mate (S), Scratching with other (SO), Positive Interactions (PI) and Negative Interaction (NI).

[a], [b] Means with no common superscript differ significantly ($P < 0.05$).

On the other hand, related to the interaction between the moment of the day and treatments, no significant differences were found for any studied behavior ($p > 0.9272$).

Nevertheless, the period of the day itself showed some effect for M and NI, where higher number of interactions were registered from 7:00 to 10:00 in relation to 10:00 to 13:00 period (p = 0.0118 and 0.0151) and near significance if compared with 13:00 to 16:00 (p = 0.0542 and 0.0686, respectively). Regarding the effect of the week of observation, daily average interactions for each week are presented in Table 5.

Table 5. Average daily interactions within calves for each observation week (means ± S.D.)

Week	H	P	D	M	SO	L	O	NI	PI
7	3.9±5.5	2.7±5.9	1.9±4.4	3.0±3.7^{b}	1.2±1.7	11.6±8.5^{c}	2.9±3.8	11.5±13.4	15.7±12.0^{b}
10	4.4±5.4	1.9±3.4	1.1±2.0	3.3±3.0^{b}	1.2±2.3	7.9±7.7^{b}	2.8±4.8	10.7±10.4	11.9±12.3^{ab}
13	2.7±2.3	1.1±1.2	0.5±1.1	2.5±2.1^{b}	1.4±2.5	6.8±6.9^{ab}	2.5±5.1	6.8±4.2	10.8±11.9^{ab}
16	2.7±3.5	1.1±1.9	1.6±2.2	1.1±1.4^{a}	0.9±1.3	3.8±4.6^{a}	1.42.9±	6.5±5.7	6.2±7.0^{a}
p	0.1717	0.2475	0.0570	0.0023	0.9257	< .0001	0.1447	0.0523	0.0002

Note. Mounting (M), Displacing (D), Pushing with chest (P), Head Knocking (H), Licking a group mate (L), Smelling a group mate (S), Scratching with other (SO), Positive Interactions (PI) and Negative Interaction (NI).

^{a}, ^{b} Means with no common superscript differ significantly (P < 0.05).

As it is observed, significant differences were detected for M, L and PI. M was reduced during the last observation week (16) and L and PI presented the highest values in week 7 as well as a tendency to decrease at the end of the experiment. Nevertheless, the interaction between the week of observation and treatment did not show any difference.

3.3 Cortisol and Biochemical Profile

Average cortisol concentration did not show statistically significant differences (P = 0.7189), and means for T1, T2 and T3 were 2.15±1.69, 2.54±1.54 and 2.05±0.81 ug/dL, respectively.

Biochemical profile of blood serum is presented in Table 6. As it is displayed, average values were inside the reference ranges except for CK and GLU, which levels exceeded them in the three treatments.

Table 6. Biochemical profile (serum concentration means ± SD)

	Units	Reference values	T1	T2	T3	p
ALT	U/L	4-11*	93.44±6.31	98.14±7.15	89.75±6.69	P = 0.6969
ALKP	U/L	10-149**	115.60±9.92 a	102.75±11.10 a	67.88±11.10 b	P = 0.0125
GGT	U/L	0-80**	15.11±2.86	19.22±2.86	13.43±3.24	P = 0.3859
ALB	g/dL	2.5-3.6**	1.17±0.18	0.89±0.20	0.78±0.23	P = 0.3792
GLU	mg/dL	46.0-93.2**	127.20±12.79	157.88±14.30	144.38±14.30	P = 0.2919
TP	g/dL	5.80-8.00**	8.01±0.73	7.86±0.81	8.09±0.81	P = 0.9802
BUN	mg/dL	7.0-17.2**	5.60±0.66 b	6.38±0.74 b	11.83±0.86 a	P < 0.0001
TBIL	mg/dL	0-0.73**	0.32±0.02	0.36±0.02	0.37±0.02	P = 0.1352
CK	U/L	0-110**	239.00±116.69	355.67±116.69	321.00±132.32	P = 0.7723
Ca	mg/dL	7.8-10.46**	11.17±0.64	11.90±1.21	10.90±0.70	P = 0.7765
PHOS	mg/dL	4.29-7.89**	6.96±0.41	6.74±0.41	6.60±0.50	P = 0.8511
GLOB	g/dL	2.70-3.80**	6.78±0.54	6.74±0.69	6.30±0.69	P = 0.8499

Note. Alanine Trasnpherase (ALT), Alkaline Phosphatase (ALKP), Gamma Glutamine Transferase (GGT), Albumine (ALB), Glucose (GLU), Total Protein (TP), Urea (BUN), Total Bilirubine (TBIL), Creatinine Kinase (CK), Calcium (Ca), Phosphates (PHOS), Globuline (GLOB). Mean ± S.E.

* (Research Animal Resourses, 2010); ** (IDEXX, 2014); ^{a}, ^{b} Means with no common superscript differ significantly (P < 0.05).

Even though all values were within the reference range, certain parameters presented statistically significant differences between treatments, and animals in T3 reached the highest concentration of BUN (p < 0.0001) as well as the lowest value of ALKP (P = 0.0125).

4. Discussion

4.1 Productive Aspects

Live weight gains in this experiment lie within the range obtained for this strain and age in similar management systems in the region (Fernández Mayer & Sastre, 2011; Ruggia et al., 2011). In addition, similar results, (0.733 kg/day) were also obtained for Friesian steers in the same range of weights, in a mixed system with concentrates and grazing (Keane & Drennan, 2008) and for Danish Holstein steers, grazing in similar pastures with a mix of ryegrass and white clover (Nielsen et al., 2004). In this sense, pasture system did not mean benefits or detriments in live weight as compared to more intensive systems. The same conclusions can be extracted from ADG, as those obtained in this experiment are within the same range in the three treatments. Nevertheless, these ADG are lower than those reported by other authors such as Brosh et al. (1995) and Nielsen et al. (2004), although they worked with wider ranges of live weight and its difficult to know in which point of the growing curve animals are. Nevertheless the feed conversion is one of the most economic traits and in this sense, feed efficiency rate was worse in T2. One possible factor affecting the feed conversion is the high voluminous feed intake (pasture and hay) that can promote a rapid intestinal transit and a reduction of its metabolic use.

At this point, it is important to remark the importance of N coming from pasture in total intake in T3, as the proportion of N in total DM that was 2.7%, in contrast with 2.3 and 2.4% of T1 and T2 respectively.

In brief, an excess in voluminous feed sources and inefficient use of food N for low energy level could not have consequences on growing performance, but it could reduce economical profit and increase N losses to the environment in the different production systems.

4.2 Social Behavior

As mentioned before, total NI and all social agonistic behaviors (except M) were higher in T1 than in the other two treatments. The increase of the agonistic behavior, as a consequence of a reduction in space allowance is reported by several authors (Kondo et al., 1989; Napolitano et al., 2004; Gygax et al., 2007), but in the present work it does not seem a valid explanation as there was a high space availability in all treatments as compared to European welfare legislation (European Union Council, 2008) and published references, *e.g.* Mogensen et al. (1997); Gygax et al. (2007); Gupta et al. (2007).

Nevertheless, the possibility of interaction between animals in T1 is higher due to a reduced space allowance (as compared to the other treatments), although positive interactions (non-agonistic behaviors) did not differ significantly among treatments. Another possibility for the increase of aggressions in T1 is the competence for resources such as feeders (Faerevik et al., 2007). This hypothesis is reinforced as those behaviors were increased from 7:00 to 10:00 when grain was supplied in T1, although we could not detect any interaction between treatment and the period of the day, so the relevance of the competence for feeders in these systems must be accurately studied.

Nevertheless, the experience while doing behavioral observation, indicated that competence increased in the hay expender zone, where animals of T1 spent a long time due to the importance of eating hay in total intake (Table 2). This situation could explain the higher number of NI in T1 although the place of aggressions was not registered in this study.

Regarding the decreasing trend of M, L and total PI along the day, it is in the line of patterns shown by Blumetto et al. (2016) where resting behaviors were less likely to be performed at the end of the day. However, more specific research should be carried out in order to establish the situations which cause each specific behavior (especially negative interactions), to be able to improve facilities or handling.

4.3 Cortisol and Biochemical Profile

In order to evaluate stress indicators, we consider that having an only moment of blood sampling limit the possibility of the analysis, although could give us some initial evidence. Cortisol levels did not bring any evidence of differences among treatments. One of the aspects which are commonly related to stress when studying production systems, is space allowance since it plays a key role for the social behavior of cattle (Boe & Faerevik, 2003). Its relationship with stress might be matched with higher blood cortisol concentrations.

In the current study, the surface used in the most restricted treatment (T1) was of 13.1 m^2 per animal, which is a higher space allowance in reference to cited works and considering the references established by European Union (1.5 to 1.8 m^2 per animal, (European Union Council, 2008). This high space allowance might be the cause for the lack of differences in cortisol levels between treatments, as probably it was not enough to affect animal's stress or cause changes in cortisol levels. In this sense, Fisher et al. (1997b) did not find either any effect on

stress parameters when working with space allowances between 2.0 and 3.0 m^2. On the contrary, Gupta et al. (2007) found that the lower space allowance, showed higher level of serum cortisol, in an experiment with spaces ranging between 1.2 and 4.2 m^2/animal.

Other aspect which must be taken into account, according to its relationship with stress levels, is the diet. For example, high-tannin sorghum instead of corn diets helps to reduce cortisol levels (Larraín et al., 2008). This type of sorghum was used in the present study for T1 and T2, and no differences were found as compared to T3, which did not include sorghum silage. In this case, probably the low proportion of sorghum in total diet (22.6 and 16.3% for T1 and T2, respectively) did not affect animals, but it is also important to remark that it is difficult to explain differences in stress responses by separated circumstances but in a whole way.

In general, animals in the three treatments looked healthy and no animal had to be removed from the experiment due to illness symptoms. This general good status is confirmed by the biochemical profile, which does not show evidence of health problems in any treatment. However, values of certain substances resulted remarkable. That is the case of CK, GLU, ALKP and BUN. Firstly, CK and GLU exceeded reference values for the three treatments whereas ALKP presented statistically significant differences between treatments. As was said, CK test is used to indicate stress-induced tissue damage (Grounds et al., 2008) or skeletal muscle lesions attributable to trauma or vigorous exercise (Brancaccio et al., 2007) and muscular stress and disorders increase its activity in the blood (Cardinet III, 1997). In the present experiment, the high activity of CK, can be explained by the management of the animals before the sample taking, as they had to walk about 1 km from the yards to the handling facilities where they were weighed. A similar situation was reported by (Ndlovu et al., 2009), with values above references, for grazing steers of three breeds (Angus, Bonsmara and Nguni) and they suggested that it was probably caused by a 2 km walk to the facilities where blood was sampled.

This circumstance is frequent when handling cattle in many regions of Uruguay, due to the average surface of the farms, where the infrastructure is centralized in one place. Then, more attention must be paid to handling, even when animals are adapted to walking as occurs in grazing production systems. In this direction, more information is required in order to discriminate between effects and their possible productive and welfare consequences.

Nevertheless, despite CK values were above the reference range for the category, similar CK activities were reported as reference values for young grazing Angoni cattle (Otto et al., 2000) or Nguni animals (Ndlovu et al., 2009). These results suggest that genetic component could also be affecting the values, and references could be variable for some races or local populations.

On the other hand**,** glucose concentration largely exceeded reference levels, as well as those reported by other authors (Arai et al., 2006; Swali et al., 2008). Similar high concentration of glucose near 144 mg/dL was obtained by (Tadich et al., 2005) in Frisian steers after 16 hours of transport. In the present experiment, transportation to handling facilities for weighing and blood sampling, could affect glucose concentration as well as CK (which might be confirmed by a little higher cortisol concentration) although it is difficult to compare the action of moving animals by walking and their transport by track.

Regarding ALKP, its differences are difficult to explain in the present study. High ALKP activity might indicate a rapid skeletal growth or a high bone:muscle ratio (Otto et al., 2000; Grunwaldt et al., 2005), but no arguments can be extracted from this work to support this hypothesis.

Relative to BUN concentrations, higher values obtained for T3 with may be matched with N content of the diet. Blood urea concentration is nearly related to protein levels of diet and its energy content (Hammond, 1997), increasing BUN values when crude protein in diet increases, maintaining energy levels (Hammond, 1983). When there is an excess of nitrogen in relation to energy in the rumen, ruminal ammonia concentration increases. Excess of ruminal ammonia enters to the blood torrent through the rumen wall and is transported to the liver where it is detoxified by conversion to urea (Tan & Murphy, 2004). Higher levels of BUN concentration is a reflect of higher ruminal degradability of protein (Hess et al., 2000; Razz & Clavero, 2004).

In this experiment, T3 might have promoted a less efficient use of N for ruminal microbiota, due to high ruminal degradability of pasture protein, and the absence of an energetic supplement. Although Hammond (1997) reported an association between BUN concentration and weight average daily gain, the results of our experiment did not show any difference. This suggests that total N intake exceeded its possibilities of being metabolized by animals, and more important proportion of N intake was excreted in a by animals in T3. These findings suggest that not only total DM and energy levels have to be controlled, but also N concentration in the diet, in order to assure a better conversion efficiency of diet. In this case, pasture becomes an important N source, and then a strategic supplementation could improve total diet utilization, as concluded by (Lund et al., 2008).

5. Conclusions

No differences in average daily gain among the three studied systems were found, although several aspects related to diet utilization must be deeply studied.

There were no evidences of increasing stress or health problems in any production system, however it has to be taken in account that permanent confined animals increased agonistic behavior, which probably reflect some welfare problems, even considering the relative big area of each yard.

General animal management needs to be further considered, in order to avoid any physical or physiological disorder caused by some routine handling.

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