Effects of different housing systems on haematological profile, salivary cortisol concentration, and behavioural stress responses in calves of different ages

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SUMMARY

Introduction - European animal welfare legislation (2008/119/EC) poses limitations on the current management practices of Valle d'Aosta breeders.

Aim - The haematological profiles, salivary cortisol concentrations, and behavioural responses of Valdostana calves housed in either tie-stalls (TS) or multiple pens (MP) were evaluated in relation to the age at enclosure: very young calves (VYC 7-39 days) or young calves (YC 40-80 days).

Materials and methods - Twenty-nine calves were divided into two age groups: VYC (12 females and two males) and YC (13 females and two males). Blood and saliva samples were collected at baseline, at 20 (T1) and at 40 days (T2) after enclosure in one of the two housing systems. Behaviour was analyzed by direct closed-circuit TV recording.

Results - Salivary cortisol levels measured at 40 days were significantly higher in the VYC housed in TS than those housed in MP ($P<0.01$). Differences in haematological profiles were observed only in the animals that had entered indoor housing (TS or MP) at age > 40 days. The VYC housed in TS spent more time in vigilant resting, tended to spend less time sleeping, and exhibited significantly more ruminant activity.

Discussion - The type of enclosure for housing calves can affect haematological profiles, cortisol concentrations, and behaviours. A multidisciplinary approach is more effective for evaluating animal welfare than assessment investigating a single parameter.

Conclusion - In this study, the hypothesis that MP housing could create stressful conditions in a breed selected for fighting behaviour was not confirmed by our data. The socioeconomic implications of different housing systems need to be studied in geographical areas with natural constraints, such as mountainous regions where local breeds are frequently reared. Animal welfare data on minor local breeds can be useful for informing practices and policies for maintaining biodiversity and breeding of local breeds.

KEY WORDS

Animal welfare; bovine; cortisol; haematological variables; Valdostana breed.

INTRODUCTION

Recent animal welfare legislation within the European Union (European Council Directive 2008/119/EC) has brought about large changes to the cattle management system, with far-reaching implications for the farming economy in some areas[1]. Animal welfare data on minor local breeds, reared not only for meat or milk production, is useful for informing practices and policies for maintaining biodiversity in the livestock system. In the Western Alps, the Valdostana chestnut and black-spotted cow breeds are kept especially for their tendency to fight[2]. Exploiting these breeds' natural propensity to fight, the farmers of the Valle d'Aosta (Italy) participate in the annual "Batailles de Reines" event, in which the cows 'fight' to become the 'Queen cow' of the year. The most important features besides milk and meat production that increase the economic value of these breeds are temperament and combativeness. According to their traditional housing system, producers raise the calves in tie-stalls during the winter period and on mountain pastures during the summer[3]. However, recent animal welfare legislation has posed certain limitations on the management practices of the Valle d'Aosta breeders. According to EEC Directive 91/629 (revised by 2008/119/EC) on the welfare of calves aged 2 to 6 months, tie-stalling practices do not comply with the minimum standards for animal protection.

Calves benefit from an environment that meets their needs. Measuring behavioural and physiological changes in a calf's state of well-being is an integral part of evaluating their stress conditions[4]. Animals respond to stressors th-

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rough a variety of mechanisms. An integrated approach to obtaining scientific and objective animal welfare data will assess anomalous behaviours[5], cortisol concentrations[6], and physiological variables[7], including the haematological profile (especially anaemia-related variables and the neutrophil:lymphocyte [N:L] ratio). Changes in the N:L ratio in response to stress occur more slowly than increases in circulating cortisol concentration[8]. Moreover, the locomotion allowed by group housing has been shown to stimulate erythropoiesis[9,10].

Cortisol, a marker of hypothalamic-pituitary-adrenal axis activity, is associated with stress regulation and used as an indicator of an animal's well-being[11]. Cortisol is released in response to stress, excitement and exercise[11,12]. Saliva cortisol determination is a simple, non-invasive method to measure a direct reflection of the free fraction of this hormone in the blood. Saco et al. (2008) reported that the marked variability in serum cortisol concentration due to physical restraint during blood collection could render it a misleading indicator in stress evaluation[13]. Negrão et al. (2004) suggested that salivary sampling could be a reliable option when studying cortisol responses to normal physiological events[14].

The imposition of unnatural physical or social configurations for animals can impinge on their welfare and profoundly affect their behaviour and physiology[11,15]. The Valdostana breed is selected for its fighting behaviours. This criterion of selection contrasts with the criteria used for other cattle breeds. The hypothesis of the present study was that multiple-pen housing could lead more stressful conditions in some breeds selected for their fighting behaviours.

The aim of the study was to assess the effect of two different types of enclosure (tie-stalls *vs.* multiple pen) based on indicators of welfare (haematological profile, cortisol concentrations, and behaviours) in Valdostana calves at different age levels (very young calves [VYC] 7-39 days old and young calves [YC] 40-80 days old).

MATERIAL AND METHODS

All procedures were conducted according to EU Commission Recommendations (EU Commission, 2007) on the guidelines for the accommodation and care of animals used for scientific purposes. For the purposes of this study, the use of tie-stalls was approved by special authorisation from the local veterinary health service (before that derogation to European Council Directive 2008/119/EC coming into effect).

Pre-experimental conditions and animals

The study was conducted in a mountainous region in the north of Italy (Valle d'Aosta) from February to May 2010. Twenty-nine Valdostana breed calves were enrolled. Since breeders prefer to keep females for competing them in the annual "Batailles de Reines" event, 25 female and only 4 male calves were selected for the study. Calves were selected from four farms affiliated with the National Association of Aosta Valley Cattle Breeders (ANABoRaVa) that provides farmers with standardized guidelines on calf housing and management. The Association is recognised and regulated by the Italian Ministry of Agriculture, Food and Forestry. All subjects were separated from their mothers within one day of birth and they were transferred to the research facilities the same day. In the research facilities, they spent a week of adaptation period to their new environment. Because the delivery date was not the same for all calves, the age between calves was different. The health status was assessed by veterinarians and all calves were declared clinically healthy before entering the study. To create two fairly homogeneous age groups, the sample was divided into very young calves ([VYC] 12 females and 2 males, age range 7-39 days, median 15) and young calves ([YC] 13 females and 2 males, age range 40-80 days, median 50).

Housing environment

From the first day of the adaptation period through to the end of the study, all calves were allocated to the experimental housing conditions and randomly assigned to either tie-stall ([TS] n = 13) or multiple pen ([MP] n = 16) housing conditions. The TS housing conditions reflect the traditional Valdostana breed management while MP reflect the legislative requirement for calves management. The calves housed in the two MPs were subdivided by age: one MP contained six (5 females and 1 male) VYC calves and the other one contained ten (9 females and 1 male) YC calves. In each MP, the available surface area was double that recommended by EC Directive 2008/119/EC (3.4 m^2 per calf). The pens were constructed of galvanised steel rail panels with a concrete floor covered with straw. Located adjacent to the pens were the stalls to allow the visual contact between the groups. Similarly, the calves housed in the stalls were subdivided by age: the VYC group was composed of eight animals (7 females and 1 male) and the YC group of five animals (4 females and 1 male). All wore a collar and were tethered with a double rope. The stalls measured 110 x 80 cm. Each stall had an elevated wooden grid on the concrete floor and was covered with straw. Feed mangers were placed in front of the stalls and along two sides of the pens. The pens and stalls were cleaned every morning immediately after feeding, and clean straw was added to provide soft bedding. Temperature and humidity were monitored continuously (mean temperature 20 °C, range 16-25 °C, 55% humidity, range 50-65%).

Feeding plan and management

The feeding plan was the same for all animals; it began at the start of the adaptation period and was calculated according to age. The VYC were fed by milk replacers (hay was available) and the YC were weaned. The feed amount was calculated based on calf nutrient requirements established by the National Research Council[16]. Feed was served twice (7:00 and 17:00) daily by the same two operators. Starting from 10 days of age, the quantity of milk replacer (23% protein, 19% fat, 6.5% ash; dry matter basis; dilution 1:10) was gradually decreased from 7 to 3 litres, and the amount of calf concentrate (20% protein, 3.2% fat, 8.6% fibre, 7.4% ash; dry matter basis) was increased. Milk replacers were offered in a calf bucket with a nipple and concentrate was offered in feed mangers. The calves were weaned at age 40 days, when they consumed at least 800 g of concentrate/day. Most calves will have sufficient rumen development to be weaned by about 4 weeks of age[16]. Hay and water were offered in separate buckets by the farmer. Weaned calves were fed with concentrate (the same used for weaning) and hay (*ad libitum*).

Behavioural observations

Behaviour was recorded by CCTV (Sony Hc 30e and Canon mv 550i video cameras). Each camera was positioned to record the entire pen and 4 to 5 calves in the stalls over 8-hour periods from 10:00 to 18:00. Only the recordings between 12:00 and 16:00 were used in the behavioural analysis to avoid potential confounding behaviours at mealtime. The video recordings were then converted into a form suitable for viewing on a computer screen and analysed at real speed by two separate examiners to identify behavioural variables for each animal. Animals were video-recorded and observed at the beginning of the study period, i.e., the week after arrival at the research facility (baseline, T0), 20 days after (T1), and 40 days after (T2). Behaviours were classified as *postures* and *activities* (Table 1), as described elsewhere[17-20], and summarized for the three time points (Tables 5, 6, 7).

Blood and saliva sample collection

Blood and saliva samples were collected at T0, T1, and T2. Saliva samples were collected using cotton wool buds tightly wound around a pair of thin surgical staple pliers[14]. Calves were induced to suck the cotton buds for 3 minutes. The saliva was then pressed out of the cotton bud using a 10 mL syringe, yielding 2 to 5 mL of saliva delete per calf, immediately refrigerated in ice, and stored at -20 °C within 2 hours of collection. Blood samples (10 mL) were collected into EDTA tubes by left jugular vein puncture, immediately refrigerated in a cool bag, and brought to the lab within 2 hours for analysis of haematological profiles. Blood and saliva samples were collected in the same sequence (first saliva, then blood) in the morning (between 9:00 and 11:00) of the day after the behavioural observations. Blood samples to determine haematological profile were analysed using ADVIA 120 Hematology System (Siemens Healthcare GmbH, Germany) for erythrocytes (RBC x 10^6/mm^3), haemoglobin (Hb g/dL), packed cell volume (PCV %), mean corpuscular haemoglobin concentration (MCHC %), mean corpuscular haemoglobin (MCH pg), mean corpuscular volume (MCV fl), neutrophils (10^3/mm^3), and lymphocytes (10^3/mm^3). Cortisol was analysed using a competitive immunoassay kit specifically designed for the quantitative *in vitro* measurement of salivary cortisol concentrations (Salimetrics™ Salivary Cortisol ELISA Kit, Salimetrics, Carlsbad, CA, USA) as described in Tarantola et al. (2016)[21]. Analyses were performed in triplicate and the results were expressed in µg/dL of saliva. The intra- and inter-assay coefficient of variation was 3.45% and 6.4%, respectively, and the minimal detectable concentration of cortisol was 0.003 µg/dL. The standard curve had a detectable range of 0.012-3.000 µg/dL. In brief, a microtitre plate is coated with anti-cortisol monoclonal antibodies. The cortisol within the saliva samples competes with the cortisol-linked peroxidase for the antibody binding sites. After incubation, unbound components are washed away and bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme with the substrate tetramethylbenzidine (TMB), producing a blue colour. A yellow colour is formed after stopping the reaction with sulphuric acid, and the optical density of the product was read using a standard plate reader at 450 nm. The amount of cortisol-peroxidase detected is inversely proportional to the amount of cortisol present.

Table 1 - Description of postures and activities during observations (ethogram only includes behaviour that could be performed by both MP and TS calves).

Behaviour		Description
Posture	Sternal decubitus	% of time spent in sternal position
	Lateral decubitus (DL)	% of time spent in lateral position with legs extended
	Total decubitus (TD)	% of time spent lying down with the body in contact with the ground
	Standing (ST)	% of time standing with all hooves in contact with the ground
	Vigilant resting (VR)	% of time in decubitus with head lifted up and supported by the neck
	Sleeping (S)	% of time in decubitus with the head not supported by the neck
	Lying down (LD)	moving from the standing position to the decubitus position (n° of times)
Activity	Ruminating (R)	% of time spent chewing cud in any position
	Feed intake (FI)	% of time spent eating feed at the feeding trough
	Urination (U)	(n° of times)
	Bar biting (BB)	chewing any equipment for more than 5 s (n° of times)
	Bar licking (BL)	bar licking for at least 5 s (n° of times)
	Rubbing head (RH)	rubbing the head against the stall structures (n° of times)
	Allogrooming (ALL)	licking another calf (n° of times)
	Self grooming (SG)	licking own body (n° of times)
	Social sniffing (SS)	sniffing another calf (n° of times)
	Bedding sniffing (BDS)	sniffing the bedding (n° of times)
	Bar sniffing (BRS)	sniffing any equipment in the stall for more than 5 s (n° of times)
	Tongue playing (TP)	twisting the tongue either inside or outside the open mouth for at least 5 s (n° of times)
	Tongue rolling (TR)	rolling the tongue either inside or outside the open mouth for at least 5 seconds (n° of times)

Statistical analysis

Statistical analyses were performed using GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA, USA). Prior to performing statistical tests, the data were tested for normality using the Shapiro-Wilk test. Normally distributed data were tested using two-tailed unpaired t-tests with Bonferroni correction after checking the homogeneity of the variances. The effects of the two housing systems (TS *vs.* MP) on the haematological profile, cortisol concentrations, and behavioural variables in each age group (VYC and YC) were evaluated at three time points. Non-normally distributed data were analysed using the Wilcoxon-Mann-Whitney rank-sum test. Results are expressed as means and standard deviations for normally distributed variables, and as medians plus 25th and 75th percentiles for the non-normally distributed variables. Means were considered statistically significantly different at a probability level of $P<0.05$.

RESULTS

Haematological profile

All recorded values were within the normal range for healthy calves at each sampling time. The effects of the two housing systems differed depending on the age at which the animals were initially housed. Haematological profiles differed only in the YC according to housing system between T1 and T2, and at T0 (Table 2), whereas no differences in haematological profiles in the VYC were observed between the two housing systems (Table 3). MCV ($P<0.01$) and MCH ($P<0.01$) differed significantly in the MP- and the TS-housed VYC at T1. This may have been due to the different initial states of the animals on arrival at the research facility (T0), though the baseline values fell within the normal range. Notable differences were found in the animals enclosed at ages older than 40 days (YC) (Table 2): both PCV and Hb were lower in the TS-housed groups ($P<0.01$ and $P = 0.03$, respectively). These differences persisted at 20 days (T1) and at 40 days (T2) and they were already identifiable at T0, even if not statistically significant. Significant differences between housing systems were also evident at 40 days: MCV was lower ($P = 0.03$) and MCHC was higher ($P<0.01$) in the TS-housed calves. The statistical differences in neutrophil and white blood cell count and N:L ratio, measured at T0 and T1, between the calves housed in pens and those housed in stalls reflected the differences in the initial conditions of the animals on arrival from different farms and were not related to the housing conditions.

Salivary cortisol concentrations

The salivary cortisol concentration measured at 40 days (T2) was significantly higher in the VYC housed in TS than in those housed in MP ($P<0.01$). No statistically significant differences in salivary cortisol concentrations were observed between the YC housed in MP and those housed in TS (Table 4).

Behaviour

Video analysis of postural behaviours at T2 showed statistically significant differences in time spent in vigilant resting between the calves housed in pens and those housed in stalls. The percentage of time the MP-housed VYC spent in vigi-

Table 2 - Effect of enclosure type on haematological profile in young calves (YC) (n = 15) at three different time points (means ± standard deviations).

	Normal Range*		T0	±SD	P	T1	±SD	P	T2	±SD	P
RBC 10^6/µL	4.9-7.5	TS MP	6.77 7.52	1.24 0.63	ns	6.21 7.45	0.85 0.73	<0.01	6.54 7.04	0.54 1.53	ns
PCV %	24-46	TS MP	27.96 31.21	5.88 3.50	ns	24.84 30.85	3.94 3.64	<0.01	26.04 30.17	2.78 2.34	<0.01
WBC 10^3/µL	5.1-13.3	TS MP	9.02 7.20	1.27 1.23	0.02	9.00 7.42	2.19 0.99	ns	7.32 7.04	2.28 1.53	ns
Hb g/dl	8.4-12.0	TS MP	9.34 10.43	2.01 1.15	ns	8.74 10.52	1.55 1.29	0.03	8.94 10.13	1.09 0.81	0.03
MCV fL	36-50	TS MP	41.13 41.45	2.18 1.33	ns	39.91 41.35	1.65 1.52	ns	39.79 41.36	1.59 0.94	0.03
MCH pg	14-19	TS MP	13.72 13.85	0.78 0.42	ns	14.03 14.09	0.96 0.56	ns	13.65 13.89	0.74 0.35	ns
MCHC g/dl	38-43	TS MP	33.36 33.42	0.29 0.13	ns	35.16 34.09	2.00 0.60	ns	34.29 33.57	0.57 0.28	<0.01
NEU 10^3/µL	1.7-6.0	TS MP	4.67 2.83	1.80 0.84	0.02	4.51 2.62	1.66 1.24	0.03	2.19 2.63	0.75 1.33	ns
LYMP 10^3/µL	1.8-8.1	TS MP	3.51 3.76	1.09 0.60	ns	3.95 4.47	0.72 1.18	ns	4.46 2.03	2.04 0.88	ns
N/L	0.5	TS MP	1.57 0.77	0.99 0.25	0.03	1.15 0.70	0.41 0.63	ns	0.55 0.69	0.21 0.36	ns

MP = multiple pens; TS = tie stalls.
T0 = baseline sample; T1 = after 20 days; T2 = after 40 days.
RBC = red blood cells, PCV = packed cell volume, WBC = white blood cells, Hb = haemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, NEU = neutrophils, LYMP = lymphocytes, N/L = neutrophil to lymphocyte ratio.
ns = not significant ($P > 0.05$).
*Normal Range from Weiss D.J., Wardrop K.J. (2010) Schalm's Veterinary Hematology. 6th ed. Ames, IA: Wiley-Blackwell Publishing.

Table 3 - Effect of enclosure type on haematological profile in very young calves (VYC) (n = 14) at three different time points (means ± standard deviations).

	Normal Range*		T0	±SD	P	T1	±SD	P	T2	±SD	P
RBC 10^6/µL	4.9-7.5	TS MP	7.49 7.05	1.98 1.30	ns	6.94 6.45	1.21 0.59	ns	6.56 6.74	0.49 0.81	ns
PCV %	24-46	TS MP	33.70 28.75	9.89 6.43	ns	29.08 25.25	5.85 2.79	ns	26.25 26.33	2.21 3.63	ns
WBC 10^3/µL	5.1-13.3	TS MP	10.16 8.32	1.36 2.60	ns	7.79 7.45	1.29 2.29	ns	7.90 7.07	1.02 2.54	ns
Hb g/dl	8.4-12.0	TS MP	11.31 9.60	3.23 2.10	ns	10.10 8.53	2.02 1.02	ns	8.88 8.93	0.79 1.17	ns
MCV fL	36-50	TS MP	44.84 40.55	2.43 1.68	<0.01	41.73 39.13	1.70 0.89	<0.01	39.98 39.01	1.22 0.81	ns
MCH pg	14-19	TS MP	15.07 13.55	0.75 0.51	<0.01	14.50 13.22	0.90 0.38	<0.01	13.51 13.25	0.48 0.19	ns
MCHC g/dl	38-43	TS MP	33.62 33.42	0.26 0.39	ns	34.75 33.77	1.63 0.52	ns	33.80 33.95	0.33 0.31	ns
NEU 10^3/µL	1.7-6.0	TS MP	5.90 4.99	0.21 0.20	ns	2.71 3.19	1.14 1.25	ns	2.92 2.15	0.54 1.27	ns
LYMP 10^3/µL	1.8-8.1	TS MP	3.40 2.68	1.59 0.84	ns	4.58 3.84	9.21 1.12	ns	4.24 4.49	0.79 1.52	ns
N/L	0.5	TS MP	2.27 1.98	1.57 0.87	ns	0.64 0.83	0.40 0.29	ns	0.71 0.49	0.19 0.29	ns

MP = multiple pens; TS = tie stalls.
T0 = baseline sample; T1 = after 20 days; T2 = after 40 days.
RBC = red blood cells, PCV = packed cell volume, WBC = white blood cells, Hb = haemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, NEU = neutrophils, LYMP = lymphocytes, N/L = neutrophils to lymphocyte ratio.
ns = not significant (p>0.05).
*Normal Range from Weiss D.J., Wardrop K.J. (2010) Schalm's Veterinary Hematology. 6th ed. Ames, IA: Wiley-Blackwell Publishing.

Table 4 - Effect of enclosure type on salivary cortisol levels in function of calf age (mean ± standard deviation) at three different time points (T0 = baseline sample; T1 = after 20 days; T2 = after 40 days).

Cortisol µg/dL	T0±SD		T1±SD		T2±SD	
VYC TS (n = 8)	0.14	0.07	0.14	0.06	0.18 ^{A}	0.08
VYC MP (n = 6)	0.13	0.04	0.12	0.05	0.06 ^{B}	0.02
YC TS (n = 5)	0.13	0.03	0.13	0.02	0.11	0.03
YC MP (n = 10)	0.13	0.04	0.12	0.02	0.09	0.03

VYC = very young calves; YC = young calves; TS = tie stall; MP = multiple pen.
^{A,B} Means with different superscript letters identify statistical differences between rows (groups of enclosure - MP vs TS), P<0.05.

lant resting was significantly less (P = 0.02), whereas it was higher in the MP-housed YC (P = 0.001). The VYC housed in stalls spent more time in ruminating activities (P = 0.02) and less time self-grooming (P = 0.008) and bedding sniffing (P = 0.008) than the VYC housed in pens. The YC housed in pens exhibited significantly more head rubbing activity (P = 0.03) and feed-intake behaviours (P = 0.02) than those housed in stalls (Table 7). No fighting behaviours were observed in either the YC or the VYC housed in pens.

DISCUSSION

The study of the effect of different farming systems on physiological and behavioural changes in animals can help to assess their state of well-being and the onset of stressful conditions[4] in other minor breeds, like Valdostana, which are reared not only for milk and meat production. Animal welfare legislation has posed limitations on the current management of Valle d'Aosta breeds, which is based on extensive mountain grazing from spring through to autumn and the use of tie-stalls during the winter, when the calves are born. Selection for fighting and dominance in the Valdostana breed can lead to differences in dominance and aggressiveness[22] as well as in their physiological responses to environmental factors, such as the system employed to house them. Many breeders feel that the multiple-pen housing system is not particularly suited to the Valdostana breed. Previous research comparing tie-stall and multiple-pen housing in calves has yielded contradictory results on animal-based indicators of welfare. Terosky et al. (1997) and Sabbioni et al. (2005) found no effect of the housing system on haematological variables in veal calves[22,23]. We, however, studied a particular breed: Valdostana calves which, because of genetic selection, could exhibit physiological differences depending on the type of housing they are exposed to[22]. Since the effects of age and diet on haematological variables are well-known, they were not included in the design of the present study[25-27].
Our study showed, for the first time, that the housing condition can significantly affect Hb and PCV levels (P = 0.03 and P<0.01, respectively) in young calves at 20 and 40 days after initial enclosure (T1 and T2, respectively). Moreover, Hb and RBC indices (MCV, MCHC) differed depending upon the housing system the calves were exposed to. While tie-stalls confine movement and limit social contact with other calves[29], multiple-pen housing can allow for more physical acti-

Table 5 - Effect of housing system (TS and MP) and age (YC and VYC) on posture and activity behaviours (observation over a 4 hour period) at time T0. Data are expressed as the median plus inter-quartile range (25th and 75th percentile) (-- = not applicable). The observed postures and the activities, rumination and feed intake behaviours were assessed as time durations and reported to the nearest minute, then expressed as the percentage of time spent in that posture or performing that activity during the four hours of assessed observation. For the other activity behaviours, their frequency of occurrence was expressed as the number of times during the four hour assessed recording period.

POSTURE	YC TS	YC MP	P	U	VYC TS	VYC MP	P	U
Sternal decubitus	26.25 (20.83 to 28.34)	28.96 (7.18 to 35.31)	0.57	20.00	26.46 (14.27 to 44.69)	17.09 (8.33 to 35.42)	0.39	17.00
Lateral decubitus	27.50 (22.09 to 35.42)	41.46 (32.19 to 50.32)	0.01	5.00	31.46 (24.38 to 60.73)	26.05 (15.94 to 29.38)	0.16	13.00
Total decubitus	78.54 (68.75 to 84.58)	67.81 (58.54 to 70.83)	0.12	12.00	61.25 (45.84 to 75.00)	52.29 (47.92 to 60.42)	0.36	16.50
Standing	47.92 (39.59 to 53.96)	30.21 (27.08 to 37.719	0.03	8.00	30.63 (12.81 to 48.13)	47.71 (39.59 to 52.09)	0.08	10.50
Vigilant resting	5.83 (2.91 to 12.71)	7.50 (5.62 to 10.84)	0.65	21.00	14.16 (5.42 to 22.50)	12.92 (10.63 to 14.80)	0.72	21.00
Lying down	4.00 (3.00 to 5.00)	4.00 (3.75 to 5.00)	--		1.25 (1.00 to 2.00)	1.00 (0.93 to 1.31)	0.27	15.50
Sleeping	25.42 (15.42 to 27.50)	35.94 (25.42 to 39.58)	0.55	20.00	16.05 (7.81 to 29.89)	18.75 (12.50 to 29.38)	0.91	23.00
ACTIVITY	YC TS	YC MP	P	U	VYC TS	VYC MP	P	U
Ruminating	29.17 (22.29 to 42.05)	21.87 (20.31 to 27.08)	0.15	13.00	13.13 (9.99 to 30.11)	8.33 (6.66 to 17.19)	0.32	16.00
Feed intake	24.17 (12.71 to 25.21)	11.25 (9.16 to 18.41)	0.05	9.00	20.21 (9.58 to 25.62)	12.71 (6.77 to 15.42)	0.24	14.50
Urination	0.00 (0.00 to 0.50)	0.00 (0.00 to 1.00)	0.60	20.00	0.00 (0.00 to 0.00)	0.00 (0.00 to 1.00)	0.16	16.00
Bar biting	0.00 (0.00 to 0.50)	0.00 (0.00 to 2.50)	0.32	18.00	0.37 (0.00 to 1.00)	0.00 (0.00 to 0.75)	0.34	17.00
Bar licking	1.00 (0.00 to 2.00)	6.50 (1.50 to 12.25)	0.04	9.00	0.37 (0.06 to 2.43)	1.37 (0.00 to 2.06)	0.82	22.00
Rubbing head	0.00 (0.00 to 1.00)	0.00 (0.00 to 2.00)	0.60	21.00	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	--	
Allogrooming	0.00 (0.00 to 1.50)	0.50 (0.00 to 2.25)	0.68	21.00	1.00 (0.00 to 1.75)	0.00 (0.00 to 2.25)	0.50	18.50
Self grooming	16.00 (8.00 to 24.50)	5.00 (1.75 to 12.25)	0.13	12.50	0.62 (0.00 to 1.56)	1.62 (1.37 to 2.81)	0.08	10.50
Social sniffing	2.00 (0.37 to 2.00)	2.50 (1.00 to 3.25)	0.15	13.50	0.75 (0.56 to 3.18)	0.25 (0.00 to 0.50)	0.008	4.00
Bedding sniffing	0.00 (0.00 to 1.00)	1.00 (0.00 to 2.00)	0.33	16.00	0.00 (0.00 to 0.18)	0.00 (0.00 to 0.06)	--	
Bar sniffing	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	--		0.00 (0.00 to 0.00)	0.12 (0.00 to 0.25)	0.24	15.00
Tongue playing	0.00 (0.00 to 0.37)	0.00 (0.00 to 3.50)	0.32	18.00	0.00 (0.00 to 0.62)	0.00 (0.00 to 0.31)	0.49	19.50
Tongue rolling	0.00 (0.00 to 0.00)	1.00 (0.00 to 4.00)	0.10	12.50	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.25)	0.42	20.00

Table 6 - Effect of housing system (TS and MP) and age (YC and VYC) on posture and activity behaviours (observation over a 4 hour period) at time T1. Data are expressed as the median plus inter-quartile range (25th and 75th percentile) (-- = not applicable). The observed postures and the activities, rumination and feed intake behaviours were assessed as time durations and reported to the nearest minute, then expressed as the percentage of time spent in that posture or performing that activity during the four hours of assessed observation. For the other activity behaviours, their frequency of occurrence was expressed as the number of times during the four hour assessed recording period.

POSTURE	YC TS	YC MP	P	U	VYC TS	VYC MP	P	U
Sternal decubitus	20.83 (16.67 to 17.29)	22.50 (17.29 to 34.17)	0.42	18.00	22.50 (11.15 to 67.81)	41.67 (34.38 to 50.21)	0.43	17.50
Lateral decubitus	38.33 (31.25 to 42.71)	40.84 (35.21 to 46.67)	0.38	17.50	40.00 (20.10 to 55.84)	22.50 (17.08 to 28.75)	0.15	12.50
Total decubitus	61.67 (57.29 to 68.75)	59.17 853.34 to 64.80)	0.38	17.50	53.75 (44.17 to 79.90)	77.50 (71.25 to 82.92)	0.11	11.50
Standing	43.33 (40.83 to 47.50)	33.96 (22.50 to 41.25)	0.03	8.00	28.33 (4.27 to 31.57)	34.58 (28.54 to 40.42)	0.05	9.00
Vigilant resting	17.50 (14.17 to 18.75)	4.37 (2.08 to 6.87)	0.007	0.00	12.92 (4.89 to 16.46)	13.34 (7.08 to 14.58)	0.92	23.00
Lying down	4.00 (2.50 to 5.00)	3.50 (2.00 to 4.00)	0.54	19.50	2.00 (2.00 to 2.00)	3.50 (1.75 to 4.25)	0.16	13.50
Sleeping	14.17 (0.83 to 22.50)	26.04 (21.14 to 32.19)	0.07	10.50	4.16 (0.00 to 36.46)	42.29 (37.39 to 48.65)	0.05	9.00
ACTIVITY	YC TS	YC MP	P	U	VYC TS	VYC MP	P	U
Ruminating	19.16 (14.17 to 42.08)	18.13 (13.80 to 25.83)	0.49	19.00	29.58 (24.79 to 31.98)	15.42 (10.73 to 20.31)	0.01	6.00
Feed intake	23.33 (22.71 to 25.83)	16.25 (9.68 to 21.88)	0.03	8.00	27.71 (10.83 to 30.31)	11.88 (9.16 to 14.26)	0.13	12.00
Urination	0.00 (0.00 to 3.00)	0.00 (0.00 to 1.25)	0.69	22.00	1.75 (1.00 to 2.00)	1.00 (0.50 to 1.00)	0.48	18.00
Bar biting	0.00 (0.00 to 1.00)	1.50 (0.00 to 4.75)	0.15	13.50	0.00 (0.00 to 0.00)	0.00 (0.00 to 2.00)	0.16	16.00
Bar licking	6.00 (0.00 to 14.00)	8.00 (3.50 to 15.75)	0.57	20.00	0.00 (1.00 to 3.50)	0.50 (0.00 to 3.00)	0.73	21.00
Rubbing head	0.00 (0.00 to 0.00)	0.00 (0.00 to 2.00)	0.50	17.50	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	--	
Allogrooming	4.00 (1.50 to 11.00)	2.00 (0.75 to 3.25)	0.21	14.50	2.00 (0.00 to 4.75)	0.00 (0.00 to 1.25)	0.29	16.00
Self grooming	14.00 (5.50 to 22.00)	8.50 (4.75 to 13.00)	0.15	13.00	3.00 (1.00 to 7.50)	8.00 (5.00 to 10.00)	0.09	11.00
Social sniffing	6.00 (2.50 to 11.00)	2.50 (1.50 to 7.75)	0.39	17.50	2.00 (0.50 to 7.50)	4.50 (1.75 to 7.00)	0.65	20.00
Bedding sniffing	0.00 (0.00 to 2.00)	2.50 (1.00 to 4.25)	0.05	9.00	0.00 (0.00 to 0.75)	0.00 (0.00 to 1.25)	0.93	22.00
Bar sniffing	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	--		0.00 (0.00 to 0.00)	0.00 (0.00 to 0.25)	--	
Tongue playing	3.50 (1.00 to 4.00)	1.50 (0.0 to 5.00)	0.51	19.50	0.00 (0.00 to 4.25)	0.00 (0.00 to 0.50)	0.41	18.00
Tongue rolling	0.00 (0.00 to 1.00)	2.50 (0.00 to 4.00)	0.07	10.00	0.00 (0.00 to 3.25)	0.00 (0.00 to 0.00)	0.20	15.00

Table 7 - Effect of housing system (TS and MP) and age (YC and VYC) on posture and activity behaviours (observation over a 4 hour period) at time T2. Data are expressed as the median plus inter-quartile range (25th and 75th percentile) (-- = not applicable). The observed postures and the activities, rumination and feed intake behaviours were assessed as time durations and reported to the nearest minute, then expressed as the percentage of time spent in that posture or performing that activity during the four hours of assessed observation. For the other activity behaviours, their frequency of occurrence was expressed as the number of times during the four hour assessed recording period.

POSTURE	YC TS	YC MP	P	U	VYC TS	VYC MP	P	U
Sternal decubitus	36.04 (32.29 to 46.67)	40.83 (33.33 to 47.08)	0.59	20.50	33.13 (30.83 to 36.88)	34.58 (26.25 to 41.67)	0.66	20.00
Lateral decubitus	17.08 (9.17 to 33.33)	20.83 (16.25 to 21.67)	0.59	20.50	33.75 (19.79 to 43.96)	41.67 (33.33 to 51.25)	0.28	15.50
Total decubitus	61.25 (45.42 to 71.04)	61.67 (49.58 to 68.75)	0.95	24.00	66.88 (58.96 to 74.79)	77.50 (67.92 to 83.33)	0.10	13.00
Standing	38.75 (28.96 to 54.58)	38.33 (31.25 to 50.42)	0.95	24.00	33.13 (25.21 to 41.04)	22.50 (16.67 to 32.08)	0.18	13.00
Vigilant resting	4.36 (0.83 to 7.50)	14.17 (13.33 to 18.75)	0.001	1.00	16.25 (14.48 to 17.29)	13.33 (5.83 to 14.58)	0.02	6.00
Lying down	3.00 (2.00 to 4.00)	4.00 (1.50 to 5.00)	0.77	22.50	2.00 (1.50 to 3.00)	3.50 (1.50 to 4.50)	0.35	16.00
Sleeping	26.04 (13.33 to 32.71)	14.17 (2.92 to 22.50)	0.16	13.50	13.54 (8.33 to 27.92)	42.29 (36.46 to 50.63)	0.06	9.00
ACTIVITY	YC TS	YC MP	P	U	VYC TS	VYC MP	P	U
Ruminating	30.42 (13.75 to 36.67)	19.17 (15.00 to 35.83)	0.68	21.00	29.17 (25.46 to 2.08)	15.42 (10.21 to 21.46)	0.02	6.00
Feed intake	18.12 (5.21 to 21.04)	23.33 (21.67 to 25.83)	0.02	6.00	2.97 (2.35 to 4.33)	2.97 (2.29 to 3.70)	0.95	23.00
Urination	0.25 (0.00 to 0.38)	0.00 (0.00 to 0.75)	0.86	23.50	0.50 (0.00 to 1.00)	0.50 (0.00 to 1.00)	0.85	22.50
Bar biting	1.00 (0.00 to 3.00)	0.00 (0.00 to 1.00)	0.25	15.00	0.00 (0.00 to 0.00)	0.00 (0.00 to 3.00)	--	
Bar licking	10.00 (5.00 to 17.00)	6.00 (0.50 to 14.00)	0.51	19.50	1.00 (0.00 to 2.50)	0.50 (0.00 to 4.00)	0.81	22.00
Rubbing head	0.00 (0.00 to 0.50)	2.00 (0.50 to 3.00)	0.03	7.00	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.50)	0.95	23.00
Allogrooming	2.50 (0.50 to 5.00)	4.00 (2.00 to 11.00)	0.20	14.00	0.50 (0.00 to 1.50)	0.00 (0.00 to 0.50)	0.57	19.00
Self grooming	4.00 (2.00 to 12.00)	14.00 (5.50 to 22.00)	0.13	12.50	2.00 (1.00 to 5.00)	8.00 (5.00 to 10.00)	0.008	4.00
Social sniffing	2.00 (0.00 to 4.00)	0.00 (0.00 to 0.50)	0.08	10.00	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.50)	0.95	23.50
Bedding sniffing	2.00 (0.50 to 8.50)	6.00 (2.00 to 11.00)	0.44	18.00	0.00 (0.00 to 1.00)	4.50 (1.50 to 7.00)	0.008	4.50
Bar sniffing	0.00 (0.00 to 2.00)	1.00 (0.00 to 2.00)	0.68	21.00	0.00 (0.00 to 0.50)	0.00 (0.00 to 1.50)	0.85	22.00
Tongue playing	0.50 (0.00 to 1.50)	1.00 (0.00 to 4.00)	0.51	19.50	0.00 (0.00 to 0.50)	0.00 (0.00 to 1.00)	0.85	22.50
Tongue rolling	1.50 (0.00 to 3.50)	0.00 (0.00 to 1.50)	0.21	14.50	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	--	

vity. Indeed, exercise has been shown to modify haematological profiles (increase in total leukocyte count, haemoglobin, erythrocytes, and packed cell volume) in horses, with an increase in MCV and a decrease in MCHC[28].

Though the nutritional plans were balanced and forage was given *ad libitum,* Hb values were lower in the TS-housed YC at both 20 and 40 days (T1 and T2). Decreased appetite has been reported in calves with haemoglobin levels less than 7.0 g/dL[7]. In this group of young calves, however, lower values of food-intake behaviour were observed (18% *vs.* 23%; P = 0.02), even though the haemoglobin level was 8.9 g/dL, which is higher than the 7.25 g/dL required by Council Directive 2008/119/EC (18 December 2008). We observed no changes in blood variables in the VYC enclosed before 40 days of age or differences in haematological patterns with respect to housing condition. This may have been due to the younger age of the animals: they spent 42% of their time sleeping even though the MP system allowed them more room to move around. No differences in the N:L ratio, WBC or neutrophil count were recorded in either the YC or VYC at T2. Since T2 was the first time point at which differences in cortisol levels were found, this effect on WBC count may be explained by the fact that the N:L ratio increases more slowly than circulating cortisol concentrations[8].

Cortisol concentrations have been used as stress indicators in calves[27,30,31]. Because cortisol is a potent glucocorticoid, its immunosuppressive effects may serve as physiological downregulators of initiated immune responses[32]. Differences in posture and activity behaviour between the two housing conditions can be identified when we take cortisol as the prime stress indicator and evaluate the behavioural phenomena of the VYC in relation to the higher cortisol values recorded in the TS-housed calves. The TS-housed VYC spent more time in vigilant resting (16% *vs.* 13%; P = 0.02) and tended to spend less time sleeping (P = 0.06). They also exhibited more ruminant activity (29% *vs.*15%; P = 0.02). The reason why they spent less time sleeping is probably because the collar kept the head in an uncomfortable posture for sleeping. In contrast, the MP-housed VYC tended to spend more time sleeping (42% *vs.* 14%; P = 0.06) perhaps because of the reassuring environment.

Maintaining age-related biorhythms is fundamental for basic physiological functions. Just as for humans, so too for animals, adequate sleep is key to health and well-being, particularly during growth and development[33]. Sleep regulates the secretion of several essential hormones, including growth hormone and glucocorticoids[34]. Cortisol secretion is inhibited during certain sleep stages[35]. Sleep also has modest, but clearly detectable, modulatory effects on hypothalamic-pituitary-adrenal axis activity. Sleep onset exerts an inhibitory effect on cortisol secretion, while awakenings and sleep offset are accompanied by cortisol stimulation[36]. The total time spent sleeping was reduced in the TS-housed VYC probably because the enclosure system disrupted their sleeping behaviour. Management practices should interfere as little as possible with normal circadian patterns[37].

We observed higher cortisol concentrations at T2 in the TS-housed VYC than in the MP-housed VYC. Such differences

could stem from the reduced ability of calves enclosed at a very young age to cope with the emotional and environmental stress caused especially by isolation[4]. The TS-housed VYC were noted to spend more time in vigilant resting and exhibit more ruminant activity (70% *vs.* 37%; P = 0.02). In calves, the total time spent ruminating is inversely proportional to that spent sleeping, and the amount of time gradually shifts from one activity to the other as the animals grow[38]. The MP-housed VYC also spent more time bed sniffing (0 *vs.* 4.5; P = 0.008). Enclosing calves in pens modifies the amount of space allowed each calf, and bed sniffing could contribute to individual recognition. In their natural environment, calves exhibit "crèche behaviour" beginning at 3 weeks of age, i.e., they spend the majority of their time with other calves; before that age, however, the number of calf-calf social interactions is usually limited[39]. Sniffing is primarily an exploratory behaviour exhibited in response to novel environments[40] and it can be altered when the space allowed per calf is limited by the size of the enclosure. Moreover, the tendency for calves in wider stalls or pens to conduct more self-grooming is probably a result of their being able to self-groom while in a more comfortable postural position[20]. Excessive self-licking, however, can also be interpreted as a response to deprivation situations or frustration, such as lack of roughage sources or housing in tie-stalls[3]. The higher frequency of this behaviour we observed in the MP-housed calves, especially those enclosed at a very young age (8 *vs.* 2 times/4 h; P = 0.008), may not be a real increase but rather should be put in relation to the lower frequency of this behaviour in the TS-housed calves due to deprivation of free movement. The differences in some posture and activity behaviours seen in the VYC were not observed in the YC, i.e., animals first enclosed at an older age. This difference might reflect the better ability of the older calves to cope with their environment, and it could also explain why we observed no difference in cortisol concentrations between the TS- and the MP-housed YC. While there were no differences in time spent sleeping, the time spent in vigilant resting was greater in the MP-housed calves (4% *vs.* 14%) and in feeding-intake behaviour, as described above. The YC spent more time at the feeding trough during the observation period: the MP-housed calves spent more time in feed-intake behaviour. This might have been a sign of the better welfare conditions they were exposed to, but only when the age at enclosure was started later. Of note is that reducing the feeder space to animal ratio can increase the effort to obtain feed and competition to access feed[41].

An increase in behaviours related to space allowance and social contact was also observed in the YC. The MP-housed YC exhibited head rubbing against stall structures more often (2 *vs.* 0 times/4h; P = 0.03), which may have been partly related to the greater amount of space available in the pens. Confinement housing reduces the expression of highly active movements in calves, like head rubbing against stall structures. No fighting behaviour, one of the major concerns of breeders, was seen, probably because the calves were too young to show aggressive behaviours. Possible aggressiveness manifestations appear later with growth and puberty[39]. We observed no differences in the expression of oral stereotyped behaviours, like bar biting and licking and tongue playing and rolling, between the TS- and the MP-housed calves in either age group. Stereotypical behaviours have gained particular interest as welfare indicators in farm animals. These behaviours are also influenced by calf age and the weaning process. Seo et al. (1998) observed tongue playing in calves after forced weaning at 42 days of age[40]. We also noted this behaviour especially in the VYC, albeit at a low frequency.

CONCLUSIONS

Our findings should be interpreted in a specific traditional context, concerning a minor breed selected for fighting ability, like the Valdostana. Local and rural breeds have well-known peculiar fighting abilities (agonistic interactions generating a within-group hierarchy of social dominance) that are not taken into account by legislation. We found that the type of enclosure for housing calves can affect haematological profiles, cortisol concentrations, and behaviours. A multidisciplinary approach to evaluating animal welfare was more effective than assessment investigating a single parameter. Indeed, no one variable alone was able to describe the stressful condition. When calves are tethered at a very young age, they can experience difficulty in coping with their environment, even after 40 days of enclosure. They sleep less and spend more time in vigilant resting and less time in other activities such as self-grooming and bedding sniffing. This could result in higher cortisol concentrations that influence immune response over time. While no differences in cortisol concentrations were seen in the calves enclosed at older ages, marked differences in haematological profiles were observed between TS-housed and MP-housed calves. Moreover, we observed less feed-intake behaviour in the TS-housed calves, although the haemoglobin levels remained higher than the recommended minimum of 7.25 g/dL.

Based on these results, however, the hypothesis that MP housing could create stressful conditions in a breed selected for fighting behaviour was not confirmed, but at the same time, do not clearly demonstrate more stressful conditions related to TS. The socioeconomic implications of the transition to a different housing system need to be studied in geographical areas with natural constraints, such as mountainous regions where local breeds are frequently reared, because our results reflect only the management of Valdostana in wintertime. Collecting and analysing animal welfare data on minor local breeds, farmed mainly with extensive mountain grazing from spring to autumn, are key to maintaining the biodiversity and breeding of local breeds.

LIST OF ABBREVIATIONS

TS = tie-stall
MP = multiple pen
VYC =very young calves (<40 days)
YC = young calves (>40 and<80 days)
T0 = baseline sample
T1 = at 20 days
T2 = at 40 days
Hb = haemoglobin (g/dl)
PCV = packed cell volume (%)
MCHC = mean corpuscular haemoglobin concentration (%)
MCH = mean corpuscular haemoglobin (pg)
MCV = mean corpuscular volume (fl)
HPA = Hypothalamic-Pituitary-Adrenal axis

AUTHORS'CONTRIBUTIONS

LP, RB, and EV carried out the studies and performed the statistical analysis. MCO carried out the interpretation of the video-recordings of the calves' behaviour. EP participated in the design of the study and drafted the manuscript. LP and AS conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

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References

1. EU Commission 2007/526/EC (2007) Commission Recommendation of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes. Official Journal of the European Union.
2. Sartori C., Mantovani R. (2010) Genetics of fighting ability in cattle using data from the traditional battle contest of the Valdostana breed. J Anim Sci, 88:3206-3213.
3. Mattiello S., Arduino D., Tosi M.V., Carenzi C. (2005) Survey on housing, management and welfare on dairy cattle in tie-stalls in western Italian Alps. Acta Agr Scand A-An, 55:31-39.
4. Von Borrel E.H. (2001) The biology of stress and its application to livestock housing and transportation assessment. J Anim Sci, 79:260-267.
5. Dantzer R., Mormede P., Bluthé R.M., Soissons J. (1983) The effect of different housing conditions on behavioural and adrenocortical reactions in veal calves. Reproduction Nutrition Developpement, 23(3):501-508.
6. Stilwell G., Lima M.S., Broom D.M. (2008) Comparing plasma cortisol and behaviour of calves dehorned with caustic paste after non-steroidal-anti-inflammatory analgesia. Livest Sci, 119:63-69.
7. Stull C.L., McDonough S.P. (1994). Multidisciplinary Approach to evaluating Welfare of Veal Calves in Commercial Facilities. J Anim Sci, 72:2518-2524.
8. Smith L.C. (2011) Neutrophil:lymphocyte Ratio as a possible indicator of chronic anthropogenic stress in Bats (Mammalia: Chiroptera). PhD thesis, Auburn University.
9. Reece W.O., Hotchkiss D.K. (1987) Blood studies and performance among calves reared by different methods. J Dairy Sci, 70:1601-1611.
10. Andrighetto I., Gottardo F., Andreoli D., Cozzi G. (1999) Effect of type of housing on veal calf growth performance, behaviour and meat quality. Livest Prod Sci, 57:137-145.
11. Bayazit V. (2009) Evaluation of Cortisol and Stress in Captive Animals. Australian Journal of Basic and Applied Sciences, 3:1022-1031.
12. Cross N., Pines M.K., Rogers L.J. (2004) Saliva Sampling to Assess Cortisol Levels in Unrestrained Common Marmosets and the Effect of Behavioral Stress. Am J Primatol, 62:107-114.
13. Saco Y., Fina M., Gimènez M., Pato R., Piedrafita J., Bassols A. (2008) Evaluation of serum cortisol, metabolic parameters, acute phase proteins and faecal corticosterone as indicators of stress in cows. The Veterinary Journal, 177:439-441.
14. Negrão J.A., Porcionato M.A., de Passillé A.M., Rushen J. (2004) Cortisol in saliva and plasma of cattle after ACTH administration and milking. J Dairy Sci, 87(6):1713-1718.
15. Brscic M., Leruste H., Heutinck L.F.M., Bokkers E.A.M., Wolthuis-Fillerup M., Stockhofe N., Gottardo F., Lensink B.J., Cozzi G., Van Reenen C.G. (2012) Prevalence of respiratory disorders in veal calves and potential risk factors. J Dairy Sci, 95:2753-2764.
16. National Research Council (2001) Nutrient Requirements of Dairy Cattle, 7th rev. ed. National Academy Press, Washington DC, USA.
17. Haley D.B., Rushen J., de Passillé A.M. (2000) Behavioural indicators of cow comfort: activity and resting behaviour of dairy cows in two types of housing. Can J Anim Sci, 80:257-263.
18. Martin P., Bateson P. (2001) Measuring Behaviour. An Introductory Guide, Cambridge University Press, UK.
19. Tuomisto L., Ahola L., Martiskainen P., Kauppinen R., Huuskonen A. (2008) Comparison of time budgets of growing Hereford bulls in an uninsulated barn and in extensive forest paddocks. Livest Sci, 118:44-52.
20. Wilson L.L., Terosky T.L., Stull C.L., Stricklin W.R. (1999) Effects of individual housing design and size on behavior and stress indicators of special-fed Holstein veal calves. J Anim Sci 77:1341-1347.
21. Tarantola M., Valle E., De Marco M., Bergagna S., Dezzutto D., Gennero M.S., Bergero D., Schiavone A., Prola L. (2016) Effects of abrupt housing changes on the welfare of Piedmontese cows. Italian Journal of Animal Science 15:103-109.
22. Terosky T.L., Wilson L.L., Stull C.L., Stricklin W.R. (1997) Effects of individual design and size on special-fed Holstein veal calf growth performance, hematology, and carcass characteristics. J Anim Sci 75:1697-1703.
23. Sabbioni A., Beretti V., Bertocchi A., Zanon A., Soffiantini C.S., Superchi P. (2005) Effect of Housing type on Veal Calf Performance. Annali della Facoltà di Medicina Veterinaria di Parma, 25:111-130.
24. Knowles T.G., Edwards J.E., Bazeley K.J., Brown S.N., Butterworth A., Warriss P.D., (2000) Changes in the blood biochemical and haematological profile of neonatal calves with age. Vet Rec, 147(21):593-8.
25. Lynch E.M., Earley B., McGee M., Doyle S. (2010) Characterization of physiological and immunological responses in beef cows to abrupt weaning and subsequent housing. BMC Vet Res, 6 (37):1-8.
26. Piccione G., Assenza, A., Fazio, F., Giudice E., Caola G. (2001) Different periodicities of some haematological parameters in exercise-loaded athletic horses and sedentary horses. J Equine Sci, 12:17-23.
27. Mattiello S., Canali E., Ferrante V., Caniatti M., Gottardo F., Cozzi G., Andrighetto I., Verga M. (2002) The provision of solid feeds to veal calves: II. Behavior, physiology, and abomasal damage. J Anim Sci, 80:367-375.
28. Grasso F., Napolitano F., De Rosa G., Quarantelli T., Serpe L., Bordi A. (1999) Effect of pen size on behavioral, endocrine, and immune responses of water buffalo (*Bubalus bubalis*) calves. J Anim Sci, 77:2039-2046.
29. Yayou K., Ito S., Yamamoto N., Kitagawa S., Okamura H. (2010) Relationships of stress responses with plasma oxytocin and prolactin in heifer calves. Physiol Behav, 99:362-369.
30. Hickey M.C., Drennan M., Earley B. (2003) The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocytes, acute-phase proteins and in vitro interferon-gamma production. J Anim Sci, 81:2847-2855.
31. Hänninen L., Mäkelä J.P., Rushen J., de Passillé A.M., Saloniemi H. (2008) Assessing sleep state in calves through electrophysiological and behavioral recordings. A preliminary study. Appl Anim Behav Sci, 111:235-250.
32. Steiger A. (2002) Sleep and the hypothalamo-pituitary-adrenocortical system. Sleep Med Rev 6:125-138.
33. Weitzman E.D., Zimmerman J.C., Czeisler C.A., Ronda J. (1983) Cortisol Secretion Is Inhibited during Sleep in Normal Man. J Clin Endocr Metab, 5:352-358.
34. Balbo M., Leproult R., Van Cauter E. (2010) Impact of Sleep and Its Disturbances on Hypothalamo-Pituitary-Adrenal Axis Activity. Int J Endocrinol, 1-16.
35. Sisto A.M., Friend T.H. (2001) The effect of confinement on motivation to exercise in young dairy calves. Appl Anim Behav Sci, 73:83-91.
36. Kerra S.G.C., Wood-Gusha D.G.M. (1987) A comparison of the early behavior of intensively and extensively reared calves. Anim Prod, 45:181-190.
37. Bouissou M.F., Boissy A., LeNeindre P., Vesseier I. (2001) The Social Behaviour of cattle. In: The Social Behaviour in Farm Animals, Ed. Keeling L. and Gonyou H.V., 113-147, CABI Publishing, Wallingford, UK.
38. De Passillé A.M., Rushen J., Martin F. (1995) Interpreting the behaviour of calves in an open-field test: a factor analysis. Appl Anim Behav Sci, 45:201-213.
39. Chua B., Coenen E., van Delen J., Weary D.M. (2002) Effects of Pair Versus Individual Housing on the Behavior and Performance of Dairy Calves. J Dairy Sci, 85:360-364.
40. Seo T., Sato S., Kosaka K., Sakamoto N., Tokumoto K., Katoh K. (1998) Development of tongue-playing in artificially reared calves: effects of offering a dummy-teat, feeding of short cut hay and housing system. Appl Anim Behav Sci, 56:1-12.
41. Verdú M., Bach A., Devant M. (2015) Effect of concentrate feeder design on performance, eating and animal behavior, welfare, ruminal health, and carcass quality in Holstein bulls fed high-concentrate diets. J Anim Sci, 93:3018-3033.

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SOCIETÀ ITALIANA VETERINARI PER ANIMALI DA REDDITO

ASSOCIAZIONE FEDERATA ANMVI

19° CONGRESSO INTERNAZIONALE

10-12 MAGGIO 2017

Cremona, Centro Studi EV

PROGRAMMA PRELIMINARE

MERCOLEDÌ 10 MAGGIO

COMMUNITY YOUNG & DAIRY E YOUNG & EXPERIENCED VETS			
9.00	Registrazione dei partecipanti	**18.00**	Termine lavori

in collaborazione con zoetis

GIOVEDÌ 11 MAGGIO

	PROFESSIONALE	BOVINI 1	BOVINI 2	BOVINI 3 SESSIONE AZIENDALE
9.30	BENESSERE ANIMALE	PROBLEMATICHE COMPORTAMENTALI E BENESSERE ANIMALE NEL BOVINO DA CARNE	IL SISTEMA IMMUNITARIO NELLA BOVINA DA LATTE AD ALTA PRODUZIONE	
11.30	BENESSERE ANIMALE	PROBLEMATICHE COMPORTAMENTALI E BENESSERE ANIMALE NEL BOVINO DA CARNE	IL SISTEMA IMMUNITARIO NELLA BOVINA DA LATTE AD ALTA PRODUZIONE	
	OVICAPRINI	**BOVINI 1**	**BOVINI 2**	**BOVINI 3 SESSIONE AZIENDALE**
14.30	RIPRODUZIONE E FECONDAZIONE NELLE CAPRE	PROBLEMATICHE COMPORTAMENTALI E BENESSERE ANIMALE NEL BOVINO DA CARNE	QUALITÀ DEL LATTE	
18.00	TERMINE DELLA GIORNATA			

VENERDÌ 12 MAGGIO

	BOVINI 1	BOVINI 2	SUINI	BOVINI 3
8.30	CHIRURGIA DEL VITELLO	SALUTE E PERFORMANCE DEL PERIODO DI TRANSIZIONE E DEL VITELLO: RUOLO DEI SALI MINERALI, DELLE VITAMINE E DELLA PROTEINA	ANTIBIOTICI 2.0: DAGLI ADEGUAMENTI NORMATIVI ALLE NUOVE FRONTIERE DELLA NUTRACEUTICA	sivar ACADEMY
	BOVINI 1	**BOVINI 2**	**SUINI**	**BOVINI 3**
14.00	ZOPPIA NELLA VACCA DA LATTE E NELLA PECORA	SALUTE E PERFORMANCE DEL PERIODO DI TRANSIZIONE E DEL VITELLO: RUOLO DEI SALI MINERALI, DELLE VITAMINE E DELLA PROTEINA	ANTIBIOTICI 2.0: DAGLI ADEGUAMENTI NORMATIVI ALLE NUOVE FRONTIERE DELLA NUTRACEUTICA	sivar ACADEMY
17.00	TERMINE DEI LAVORI			

Gli organizzatori dell'evento si impegnano a rispettare il programma pubblicato che rimane comunque suscettibile di variazioni dovute a cause di forza maggiore.

RESPONSABILE CONGRESSUALE: PAOLA ORIOLI

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