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Effects of abrupt housing changes on the welfare of Piedmontese cows

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ABSTRACT

The Italian Piedmontese cattle breed is traditionally housed in tie-stalls and, to a lesser extent, in loose housing systems with free stalls. The present study has compared the same group of 15 cattle before and after stable renovation (tie-stall vs free-stall housing) funded by Regulation (EU) No. 1305/2013. All the animals remained healthy during the trial, no clinical signs were observed and no mastitis occurred. The tested parameters were: locomotion scoring system, salivary cortisol, blood parameters, serum acute phase protein (albumin, haptoglobin, serum amyloid A and lysozyme). Samples were collected 3 times: before the change (T1), 3 days later (T2), 40 days later (T3). The change in housing determined noteworthy variations in the stress parameters: albumin and total protein displayed the lowest value at T2, while lysozyme displayed the lowest value at T3. Among the App, SAA and Hp were not affected by the sampling time. Salivary cortisol displayed the highest value at T1. This study suggests that tie-stall housing can endanger the welfare of animals, and it is hoped that this farming system will be abandoned in the future.

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Introduction

In recent years, European countries have been involved in the application of Regulation (EU) No. 1305/2013 on support for rural development by the European Agricultural Fund for Rural Development. Activities funded by this Regulation include the development of local infrastructures and local basic services in rural areas, including leisure and culture services, the renewal of villages. In this context, any activities aimed at the restoration and upgrading of the cultural and natural heritage of villages and rural landscapes are essential to realise the growth potential and to promote the sustainability of rural areas. In this wide scenario, the development of new rural structures that are more oriented towards animal welfare in livestock production is essential. The confinement and treatment of animals during the production process is an issue that is increasingly being discussed by people in developed countries. The inclusion of more measures for animals has been assumed to improve the welfare assessment system (Botreau et al. 2007).

The enhanced focus on animal welfare on commercial farms has resulted in a rise in interest in loose

housing systems for cattle. The housing system could play an important role in the welfare of cows. There is some evidence that loose housing (Weary & Taszkun 2000) and regular outdoor exercise (Gustafson & Lund-Magnussen 1995; Regula et al. 2004) have positive effects on the health and welfare of dairy cows. In a survey in Wisconsin, dairy producers mentioned 'cow comfort', hock injuries and teat injuries as the most important areas of improvement after changing from a tie stall to a loose-housing system (Bewley et al. 2001). Loose housing systems allow more freedom of movement than tether systems, and offer the animals the possibility of experiencing more natural behaviour including social behaviour (Rousing et al. 2000). In fact, the permanent tethering of dairy cows seems to change the normal activity pattern and increase their lying behaviour (Krohn 1994). If differences in housing can lead to important effects on cow comfort, for example reducing how long cows spend lying down, they might also affect cow health and productivity (Tucker & Weary 2001).

In the EU, the individual housing of veal calves and pregnant sow has officially been banned through

specific regulations (91/629/EEC; 97/2/EC and 2008/120/EC), but no explicit rules are currently in force for dairy cows or beef cattle, despite the publication of reference reports, such as “Risk factors for beef cattle welfare” by the Scientific Committee on Animal Health and Animal Welfare (SCAHAW 2001) and several recent scientific opinions by the European Food Safety Panel on Animal Health and Welfare, AHAW (EFSA 2006, 2009, 2012). However, although keeping cattle in tie-stall housing systems contradicts Council Directive 98/58/EC and some of the “five freedom rules” (FAWC 1992), that is, “freedom from discomfort by providing an appropriate environment”, “freedom to express normal behaviour by providing sufficient space” and “conditions and treatments which avoid mental suffering”, it is still common practice throughout Europe. Several different parameters are used as stress indicators for example: the hematological profile, salivary cortisol (Negrão et al. 2004), haptoglobin (Alsemgeest et al. 1995; Lomborg et al. 2008; Valle et al. 2015) and lysozyme (Salamano et al. 2010). All these parameters directly measure animal welfare and can be used to monitor stress in animals following abrupt changes in housing in both acute and chronic situations.

The “Piedmontese” breed is the most important Italian beef cattle breed and it has been utilised over the years as a dual-purpose animal. In Italy, “Piedmontese” cows are traditionally bred in a tie-stall housing system and less frequently in loose housing systems; these systems frequently adopt stalls, and this represents a critical husbandry situation from a welfare point of view, due to the extreme restriction of movement and because the normal behavioural repertoire of the cows is limited (Mattiello et al. 2005; Higashiyama et al. 2006; Popescu et al. 2013). Veissier et al. (2008) recommend that cows housed in tie-sheds should be given regular access to an exercise area. In fact, it has been shown that when periods of exercise are possible, some of the adverse effects are reduced.

Finally, as suggested in recent scientific publications by the European Food Safety Panel on Animal Health and Welfare (EFSA 2015), there are frequently no updated barns or sheds on traditional farms which are not suitable to satisfy the different behavioural needs of the animals and the management demands of the stockman.

The aim of the present study was to compare the animal-based parameters of a group of 15 cows first reared in a traditional tie-stall housing system, and then moved to a loose housing systems with free stalls. The tested blood parameters were:

hematological and biochemical profile, salivary cortisol, serum haptoglobin, Serum amyloid A, lysozyme and the Locomotion scoring system.

Materials and methods

Animals and housing

The study was conducted in Piedmont (North West Italy), in a typical tie-stall barn. The herd, before the transition, was composed of 135 “Piedmontese” cows.

A total of 15 animals were included in the study. They were selected on the basis of their health status; the cows were examined clinically using standard clinical examination procedures (Jackson & Cockcroft 2002). The chosen cows showed no health disorders such as diarrhoea or ketosis during the observation period. All the animals remained healthy during the trial, no clinical signs were observed and no mastitis occurred as the somatic cell count was below 100 000 cells/ml over the entire experimental period.

The selected animals were multiparous cows (parity = 3; mature equivalent production = 3050), between the first 45 days of lactation and 60 days after. The traditional barn, before the transition from tie-stall to free-stall housing system, was composed of single tie-stalls where the cows were kept in two rows, facing each other, divided by a feeding alley. The stall surface was a concrete floor covered with straw. The lactating cows were fed in the stall twice a day and had free access to drinker bowls near the tie-stall; the only movement possible was lying down and standing. In the new barn, the cows were kept in a free-stall housing system, where the animals had free access to 2.6 m × 1.2 m cubicles with a concrete floor covered with straw. The cows had free access to water troughs (9 cows each trough) and a central feeding alley. The total surface available in the stall allowed each cow to have 4 square meters of free surface for movement. The milking phase was conducted twice a day in a milking parlour.

The animals’ diet was adjusted according to the lactation stage and was based on total mixed rations. The mean content of the diet was on a dry matter basis and was composed of 53% of concentrate (44% corn, 25% soya bean meal, 17% corn distiller, 4% beet pulp, 4% gluten feed and 6% mineral and vitamin supplementation) and of 47% forage (61% corn silage, 12% Italian ryegrass, *Lolium Italicum* hay and 27% alfalfa, *Medicago sativa*). The cows had access to a feed bunk via a rail feed barrier, and were fed by means of a uni-feed system. A feed push-up system ensured that the feed was available to the cows.

Data collection

Observations were made and sampling was performed three times: the first time before restructuring (T1), the second time three days after restructuring (T2), and the third time 40 days after the change in the housing system (T3).

Locomotion score

All the cows were assigned a locomotion score, ranging from 1 to 4 (Sprecher et al. 1997): 1 (no gait abnormality); 2 (slight lameness); 3 (moderate lameness); 4 (severe lameness). The cows housed in tie stalls were scored as they were individually released from the barn in the morning after milking. The cows housed in free stalls were scored as they exited from the milk parlour. All the cows were scored while walking on a flat level concrete surface. Observations were performed three times: the first time before restructuring (T1), the second time three days after restructuring (T2), and the third time 40 days after the change in the housing system (T3).

Blood analyses

Blood samples were collected for each animal. All the samples were collected at T1, T2 and T3 and taken between 10.00 a.m. and 12.00 a.m. to avoid daily fluctuations of the analysed parameters. The blood samples were taken from the jugular vein and collected in two 10 ml tubes: one with added EDTA to determine the haematological profile, the other with no anti-coagulant factor to obtain serum.

A complete haematology test was carried out on all the samples using an automatic blood counter calibrated for cattle (HemaVet 3500, CDC Technologies Inc., Oxford, CT). From the haematological profile, the following parameters were considered: white blood cells (WBC), percentage of neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS), basophils (BASO), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), plateletcrit (PCT) and neutrophil-to-lymphocytes (N/L) ratio.

In addition, the serum from each blood sample was separated by means of centrifugation ($1560 \times g$ for 10 min) and frozen at -80°C . The following parameters were evaluated on the serum samples: total protein concentration, albumin, α -globulin, β -globulin, γ -globulin, albumin-globulin ratio (A/G), haptoglobin (Hp), serum amyloid A (SAA) and lysozyme. The total proteins were quantified by means of the "biuret method"

(Hospitex Diagnostics, Sesto Fiorentino, FI, Italy). The electrophoretic patterns of the serum were obtained using a semi-automatic agarose gel electrophoresis system (Sebia Hydrasys[®], Evry, France).

SAA and Hp were determined in duplicate using commercial assay kits (Tridelta, Greystones, Ireland). The serum lysozyme assay employed *Micrococcus lysodeikticus* cells as the substrate for the lysozyme, using the Osserman and Lawlor method (Osserman & Lawlor 1966). The serum concentrations were determined in duplicate using commercial assay kits (Tridelta Development Ltd, Kildare, Ireland).

Salivary cortisol determination

Saliva samples were taken from each animal at T1, T2 and T3 and by putting surgical forceps, with a cotton buffer on the top, into the bovine's mouth. The cotton buffer was then squeezed into a syringe, and the saliva was collected in bovine-processed tubes and then frozen to determine the salivary cortisol levels.

Cortisol was analysed using a competitive immunoassay kit specifically designed for the quantitative *in vitro* measurement of salivary cortisol levels (Salimetrics[™] Salivary Cortisol Kit, Philadelphia, PA), and which had previously been used by Pérez et al. (2004). Analyses were performed in triplicate, and the results were expressed in $\mu\text{g/dL}$ of saliva.

Statistical analysis

The statistical analysis was performed with SPSS 17 for Windows (SPSS 2008). Normality of the data distribution was assessed using the Shapiro–Wilk test. The data were then analysed using repeated measure one-way ANOVA followed by Tukey's *post-hoc* test, using the time of sampling as the variation source. The experimental unit was the individual animal. The results are presented as the mean and standard error of the mean (SEM). The results were considered statistically significant when associated with a lower probability than 5%.

Results

The locomotion scoring system was the same throughout the observation period (T1 = 1.7; T2 = 1.5; T3 = 1.8) and no statistical differences were observed. The majority of the blood cell parameters (Table 1) were not influenced by the sampling time, except for MONO and BASO: the lowest MONO value was observed at T3, while the highest BASO value was observed at T2.

Some of the serum parameters were affected by sampling time: albumin and total protein displayed the

Table 1. Red and white blood cell parameters according to the sampling time (mean and pooled error standard of means, SEM) ($n = 15$).

	T1	T2	T3	SEM	<i>p</i>
Red blood cells, K/ μ L	6.13	6.23	6.11	0.08	ns
Hematocrit, %	30.30	30.15	29.71	0.45	ns
Hemoglobin, g/dL	9.93	10.10	10.09	0.13	ns
MCV, fl ^c	49.19	48.46	48.21	0.53	ns
MCH, pg ^c	16.27	16.37	16.37	0.15	ns
MCHC, g/dL ^c	33.37	33.57	34.55	0.14	ns
White blood cells, K/ μ L	7.82	7.41	7.53	0.23	ns
Neutrophils, %	30.43	31.64	29.45	1.44	ns
Lymphocytes, %	54.01	52.16	57.12	1.40	ns
Monocytes, %	6.95 ^a	5.74 ^{ab}	5.06 ^b	0.31	<0.05
Eosinophils, %	8.58	10.34	8.55	0.73	ns
Basophils, %	0.02 ^a	0.11 ^b	0.03 ^a	0.01	<0.05
Plateletcrit, %	0.25	0.26	0.24	0.01	ns
Neutrophil-lymphocyte ratio	0.59	0.66	0.59	0.05	ns

T1 = tie-stall, T2 = 3 days after the housing change, T3 = 40 days after the housing change.

^{a,b}Mean values with different superscripts within the same row differ significantly with $p < 0.05$; ns: not significant.

^cMCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

Table 2. Salivary cortisol, serum protein and AAP according to the sampling time (mean and pooled error standard of means, SEM) ($n = 15$).

	T1	T2	T3	SEM	<i>p</i>
Salivary cortisol, μ g/mL	0.22 ^a	0.14 ^b	0.14 ^b	0.13	<0.05
Serum protein					
Albumin, g/dL	3.03 ^{ab}	2.73 ^a	3.07 ^b	0.05	<0.05
α Globulins, g/dL	1.54	1.47	1.48	0.02	ns
β Globulins, g/dL	1.29	1.13	1.33	0.02	ns
γ Globulins, g/dL	1.68	1.63	1.83	0.04	ns
Total proteins, g/dL	7.59 ^{ab}	7.00 ^a	7.76 ^b	0.10	<0.05
Albumin-globulin ratio	0.67	0.66	0.66	0.12	ns
Acute phase proteins					
Haptoglobin, mg/mL	0.15	0.15	0.15	0.00	ns
Lysozyme, μ g/mL	1.80 ^a	1.43 ^a	1.44 ^b	0.35	<0.05
Serum amyloid A, μ g/mL	110.45	176.20	128.50	15.51	ns

T1 = tie-stall, T2 = 3 days after the housing change, T3 = 40 days after the housing change.

^{a,b}Mean values with different superscripts within the same row differ significantly with $p < 0.05$; ns: not significant.

lowest value at T2, while lysozyme displayed the lowest value at T3. Among the App, SAA and Hp were not affected by the sampling time. Salivary cortisol (Table 2) displayed the highest value at T1.

Discussion

The new legislation on animal welfare, like that on animal health and food safety, is based on science. For this reason, it is important to offer objective scientific data regarding the improvement in cattle welfare when the housing system is changed to support the enactment of new laws concerning adult bovine.

The use of a locomotion scoring system to define lameness accurately has been described by several authors, who have attempted to determine the

prevalence of lameness at a single point in time. In this study, all the animals were given a score under 2, which points out that there was no claw disorder at any of the experimental observation points.

Following the suggestions found in the literature, some haemato-biochemical and salivary stress parameters have been monitored in cows transferred from tie-stalls to a free-stall housing system. Many blood parameters could be used to assess the stress conditions of cattle. Blood cells are sensitive indicators of physiological and patho-physiological responses in animals. A change in blood cell composition indicates a response to restore homeostasis in animals exposed to abrupt physical conditions (Radostits et al. 1994). Parameters related to red blood cells provide information on anemia (Jones & Allison 2007) or hemorrhaging (Roland et al. 2014), while the evaluation of leukogram is related to a common acute inflammatory state in adult cattle (Stockham & Scott 2013). Changes in the populations of WBC types in response to stressors, and in particular how the cortisol milieu of the blood can influence neutrophil development and immunity-related activities, have been studied in bovine (Burton et al. 2005). A study by Calamari et al. (2004) assessed that a greater N/L ratio than 1 can be considered a stress indicator. Although a change in housing involves handling, movement, separation from a familiar environment, a high level of adaptation, and the mixing of cattle that do not know each other, all of which are factors that can contribute to a stressful situation (Gebresenbet & Eriksson 1998), all the red and white blood parameters (Table 1) observed in the present study fell into the normal range for healthy cattle (George et al. 2010). Moreover, the housing change did not affect the N/L ratio. Although no differences in WBC concentration were detected for the different sampling times, a greater concentration of MONO was observed when the animals were tied (T1) and an increase in percentage of BASO was recorded during the restructuring (T2). Monocytosis may be observed in association with inflammation, tissue necrosis or occasionally as a part of a stress leukogram (Smith 2014).

The most common psychological events that affect physiological stress are fear and anxiety, with novelty probably being the greatest contributor (Grandin 1997). One of the most common fear and anxiety stress measures is the cortisol concentration (Koolhaas et al. 1999). Cortisol is one of the best stress indicators (Albertini et al. 2008). The concentration of cortisol in serum has often been used to measure stress, but this method shows a great variability, which is primarily caused by circadian rhythm, individual variability and stress induced by the sampling itself (Saco et al. 2008). In the

present study, salivary cortisol determination was chosen because it is influenced less by stress during the collection. Corticosteroids filter through saliva by means of passive diffusion. Thus, their concentrations are not influenced by the salivary flow (Negrão et al. 2004). Besides, it has been shown, that salivary cortisol levels are significantly correlated to the serum levels (Beerda et al. 1996; Negrão et al. 2004; Yates et al. 2010), although the stress-induced response is more appreciable in blood (Negrão et al. 2004). Corticosteroid can also be determined in faeces, and this procedure could be used for stressed animals because it does not require animal manipulation (Prola et al. 2013; Cornale et al. 2015). In our study, the salivary cortisol value was higher in cattle housed in tie-stall conditions than in loose housed cattle (Table 2). This reduction could be due to the cattle having a higher comfort situation, as suggested by Starvaggi Cucuzza et al. (2014).

APPs estimation may be used to help monitor the health and welfare of production animals on farms (Eckersall 2000). According to Alsemgeest et al. (1996), increased APP concentrations in cattle could be due to an increased concentration of cortisol as part of the response to stress. The albumin value, which is known to be one of the negative APPs, since its concentration decreases in response to challenge (Mackiewicz 1997; Paulina & Tadeusz 2011), was significantly lower during the restructuring (T2) and the reference range value suggested by Kaneko et al. (1997) (3.03–3.55 g/dL) and Cozzi et al. (2011) (37 g/L). Cattle with chronic debilitating disease, attributable to multiple causes, may be hypoalbuminemic with a low or normal total protein concentration (Russell & Roussel 2007), as in the present study where the total protein decreased significantly at T2, but the albumin-globulin ratio was not influenced and was always below the reference value (0.84–0.94; Kaneko et al. 1997). Hp and SAA are important APPs in cattle (Alsemgeest et al. 1995; Gruys et al. 2005). The measurement of these proteins is therefore recommended to detect inflammation processes in general (Alsemgeest et al. 1994). Hp is a major APP in cattle and it is released in response to tissue damage, inflammation, infection and bacterial components, as well as stress (Murata et al. 2004; Gruys et al. 2005; Lomborg et al. 2008). It is considered a reliable candidate for monitoring stress responses in normal conditions, as it is absent or present in low levels in the blood. Hp is classified as a positive APP, as its concentration in serum increases in response to inflammation, infection, trauma, immune disorders, neoplasia or stress (Eckersall 2000; Slocombe & Colditz 2005; Salamano et al., 2010). SAA is also classified as a positive APP, and it has been suggested to be more useful in

distinguishing between acute and chronic inflammation than neutrophil counts or white blood cell counts (Horadagoda et al. 1999). In calves, stress caused by housing on a slippery floor has no effect on the plasma Hp concentration, but plasma concentrations of SAA increase (Alsemgeest et al. 1995). SAA has also been found to be a marker of experimentally induced and naturally occurring mastitis (Eckersall et al. 2001). In the present study, the Hp and the SAA were not affected by the housing conditions, although SAA showed the highest numerical value during restructuring. Lysozyme participates in many immune responses, and its content in serum is an important indicator of the innate immune function of an animal. Increased tissue and body fluid lysozyme activity has been reported for cattle with acute inflammatory response to injuries (Radostits et al. 1994). Lysozyme is bactericidal against Gram-positive bacteria (Rausch & Moore 1979), and is associated with phagocytes and neutrophils. It is essentially related to the function of the macrophage system and basically indicates the presence of inflammation. An increase in lysozyme has been shown in laying hens during a stressful situation induced by changes in housing (Salamano et al. 2010). In light of this evidence, it can be concluded that, among the APPs, serum lysozyme could also give information on the adaptability to a new environment and it could be a useful stress-indicator parameter. During the present study, the tethered cattle showed a higher lysozyme concentration. Since this parameter can be influenced by stress conditions, the authors' hypothesis is that lysozyme levels were higher during the tethering period because the cows were under a stressful situation.

The present study has compared animal-based parameters pertaining to the same group of cattle reared in two different housing systems and during restructuring. The differences observed in various blood and salivary parameters during the tie-stall period and during restructuring, but not in the free-stall housing period would seem to suggest that tethering for the entire rearing period is not a good way of maintaining an acceptable standard of welfare for cattle. The housing change determined notable variations in certain stress parameters, that is, salivary cortisol, albumin, total protein and lysozyme.

Conclusions

Cattle are highly gregarious animals, and housing them in groups instead of individually is a step toward their improved welfare (Bouissou et al. 2001). This study suggests that tie-stall housing can endanger the welfare of animals, and it is hoped that this farming

system will be abandoned in the future. However, further studies with larger numbers of animals are required to investigate cattle welfare as well as to evaluate other stress parameters and production indicators.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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