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Welfare parameters in dairy cows reared in tie-stall and open-stall housing systems*

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The increasing attention paid by both the legislature and consumers concerning animal welfare has raised criticism regarding the tie stall housing system of dairy cattle, since it restricts voluntary movement and limits the social behaviour of cows. The aim of this study was to compare the welfare of dairy cows kept in a tie-stall (TS) and an open-stall (OS) system by assessing metabolic, immunological and stress-related parameters. The study involved 155 cows in 18 farms located in Tuscany. Blood samples were collected in the morning in order to measure: aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), betahydroxybutyrate (BHBA), blood urea nitrogen (BUN), non-esterified fatty acids (NEFA), total proteins (TP), creatinine (Creat), calcium (Ca), potassium (K), phosphorus (P), lysozyme (SL), haptoglobin (Hp), and oxygen free radicals (OFR). At the same time, hair samples were collected to measure cortisol levels and the body condition score was recorded. The results showed that the housing system affected AST, ALT, BHBA, BUN, SL and OFR levels. Most parameters showed values within the range of reference. However, the OFR level was higher in the OS system, probably due to the higher productivity than in TS. Cortisol did not raise particular concern related to chronic stress, since the values were lower than the data reported in literature. The study revealed that the evaluation of welfare based on physiological parameters showed no severe signs of impairment in cows reared in the TS system.

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Increasing concerns related to animal welfare worldwide have amplified public awareness of the conditions, in which animals raised for food production are kept. European policies also lean towards farming systems that limit the use of tethered housing facilities. Nevertheless, in Europe between 20% (lowland) and 80% (upland) cows are tethered at least in the winter [Popescu *et al.* 2013]. The perception of the tie stall (TS) housing system in terms of animal welfare has been widely debated. The major downsides involve limitation of movement and the impossibility of the animals to express their natural behaviour [Popescu *et al.* 2013]. However, some studies [Veissier *et al.* 2008] have reported no acute or chronic physiological stress responses in cows kept under a tied housing system.

Due to the ethical principles involved, the impartial evaluation of welfare of animals reared under various life conditions is an important issue. The welfare assessment of farm animals can be driven by different methods both animal- and resource-based. The metabolic profile can be a useful tool for the prediction of dairy cow problems, such as infertility, metabolic diseases, stress conditions and welfare impairment [Calamari and Bertoni, 2009, Calamari *et al.* 2016]. It has been reported that prolonged stress may compromise the metabolic function and the immunity system [Asres and Amha, 2014]. SL and Hp represent an innate cellular immune response; in fact, SL is one of the most predictive parameters of diseases and variations in its levels have been described as a result of inflammation or metabolic stress-related conditions in early lactation [Trevisi *et al.* 2012]. Alteration in these parameters may indicate inadequate hygienic and sanitary conditions of the herd or an inappropriate feeding management [Bonizzi *et al.* 2003]. Those authors also reported that SL and Hp provide a valuable broader indication of bovine non-specific immune reactivity under various breeding conditions.

Cortisol is considered as a biomarker in stress conditions [Burnett *et al.* 2014] and its measurement in the hair matrix allows in to detect factors undetcable in other sample types leading to short-term variations [Comin *et al.* 2012]. In turn, the BCS provides useful information on nutrient intake in relation to the physiological requirements [Roche *et al.* 2013].

The aim of this study was to evaluate the animal-based parameters of dairy cows reared in tie stall and open stall housing conditions.

Material and methods

The experiment was performed in accordance with the European Commission regulations and animal handling followed the recommendations of EU directive 98/58/EEC concerning the protection of animals kept for farming purposes.

A total of 18 dairy farms located in the provinces of Firenze (8), Livorno (4), Massa Carrara (3), Lucca (2) and Pisa (1) were enrolled in this study. Ten farms rear

animals in the TS system (28±21.6 heads) and eight farms use the OS system (94±48.7 heads). The TS system of the inspected farms implied that animals were tethered over their entire lifetime. Feeding management was based on the use of the Unifeed ration on the OS farms, and on hay and meal in the TS management system. A total sample of 155 multiparous, 3.5-6 year old lactating cows was selected. The study lasted for three months, from April to June 2016.

None of the animals enrolled experienced a change in social group or had been affected by any diseases in the period before the study.

Blood samples, 79 for the OS housing system (HS) and 76 for the TS housing system, were collected in the morning from the jugular vein using Vacutainer™ tubes. The blood samples were kept refrigerated and immediately sent to the laboratory of the Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana (IZSLT).

The following parameters were measured with an automated biochemical analyser (Olympus AU 400) using a commercial enzymatic test kit (Beckman-Coulter) and according to the manufacturer's instructions: aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (Creat), total proteins (TP), calcium (Ca), phosphorus (P) and potassium (K). Furthermore, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) were analysed using two different commercial enzymatic test kits, Randox and Catachem, respectively, according to the manufacturer's instructions. Haptoglobin (Hp) was determined by an ELISA commercial method (Tridelta), while oxygen free radical levels (OFR) were monitored by a commercial colorimetric method (DIACRON), both according to the manufacturer's instructions. Finally, the serum lysozyme (SL) and bactericidal activity (SBA) were determined according to validated bacteriological assays [Osserman and Lawlor 1966, Bonizzi *et al.* 1989, Ponti *et al.* 1989, Amadori *et al.* 2002]. The IZSLT laboratory provided reference values.

Cortisol was analysed in the tail-hair matrix following Accorsi *et al.* [2008]. Hair samples were carefully cut from the tail switch using clippers and were frozen to -20°C to eliminate lice and external parasites.

The tail-hair matrix is the most suitable location to collect hair samples because other matrices, such as blood, saliva, urine or faeces, can be affected by the stress of handling [Moya *et al.* 2013].

Blood and hair samples were collected during the daily routine in order not to disrupt the animals and in compliance with the current legislation on animal welfare.

At the same time, the body condition score (BCS) was recorded by the same observer using the 1-5 scale according to Ferguson *et al.* [1994], along with an increasing level of fattening.

All results were expressed as means and standard errors (SE). An ANOVA test was performed including the type of HS and the farm nested in the type of HS as the variability factor. As a discrete variable, BCS was analysed with a non parametric test (Wilkixon). Statistical analysis was performed by JMP [SAS Institute, 2002].

Results and discussion

Some parameters revealed significant differences related to the HS: AST, ALT, BHBA, BUN, OFR and SL ($P<0.05$), with most of the parameters investigated showing values within the reference range. Table 1 list the results obtained in the study.

Table 1. Metabolic, immunological and stress parameters related to the two housing systems (HS)

Item	HS				P[1]	P[2]	Reference range
	tie-stall (TS) n=76		open-stall(OS) n=79				
	mean	SE	mean	SE			
AST (U/L)	83.57	2.689	95.05	2.304	0.0003	0.0002	60-118
ALT (U/L)	40.16↑	1.145	34.51	0.981	0,0058	<0.0001	14-38
BUN (mg/dl)	13.22↓	0.362	13.82↓	0.310	0,0034	<0.0001	20-30
BHBA (mg/dl)	6.83	0.396	9.38	0.339	<0.0001	<0.0001	<10.5
Creat (mg/dl)	1.00	0.018	0.99	0.015	0.8256	<0.0001	1-2.7
NEFA (umol/L)	82.24↓	14.079	86.33↓	16.064	0.7703	0.0135	89-618
TP (g/dl)	7.62	0.078	7.67	0.067	0.4162	0.3180	5.7-8.1
Ca (mg/dl)	9.42	0.053	9.41	0.046	0.9278	0.0005	8-10.5
P (mg/dl)	5.45	0.130	5.32	0.112	0.5582	0.1227	4-7
K (mmol/L)	4.69	0.065	4.52	0.056	0.1912	0.0082	3.9-5.8
SL µg/ml)	1.19	0.103	0.89 ↓	0.089	0.0326	<0.0001	1-3
Hp (mg/ml)	0.18	0.047	0.11	0.040	0.3364	0.6371	0.0-0.5
OFR (U.Carr.*)	46.21	3.138	58.50	2.651	0.0106	<0.0001	
Cortisol (pg/mg)	2.17	0.170	1.45	0.145	0.1144	<0.0001	
BCS (1-5 scale)_	2.99	0.046	3.03	0.054	0.4860		

[1] Experimental factor: HS.
[2] Experimental factor: farm nested in HS.
*U. Carr. is an arbitrary unit; 1 U. Carr. is equivalent to 0.08 mg of H 202/100 mL.
¶Values over the threshold of the reference ran ge;
⇃Values under the threshold of the reference range.

Cows reared in the TS system showed significantly lower ($P<0.01$) serum AST values than those reared in the OS system, confirming the findings reported in previous studies [Benvenuti *et al.* 2016]. A similar trend was observed by Radkowska and Herbut [2014] in cows reared in stalls with access to an outdoor area compared with those reared without such access.

In the TS group, ALT values were slightly over the reference range (40.16 U/L) and differed significantly with the OS group, in contrast with previous observations [Radkowska and Herbut 2014].

Since ALT and AST are liver enzymes reflecting possible liver injury, these findings could be due to the different diet adopted in the two systems. In fact, ALT values in the TS group slightly exceeded the reference range; however, since AST, BHBA and creatinine levels were within this range, the metabolic condition of the animals did not seem to be impaired.

BHBA was significantly higher ($P\leq0.0001$) in the OS system, although still within the reference range, and it was consistent with previous findings [Benvenuti *et al.* 2016] for similar farming conditions.

Van Saun [2008] described that BHBA concentrations lower than 2.6 mmol/l and higher than 14 mmol/l may suggest subclinical ketosis or result from poorly fermented silage. This was not the case in our study, because the BHBA values were in the reference range.

BUN was below the reference range in both HSs and it was found to be affected by the HS (P=0.0034) with lower values in the TS group.

A poor protein intake could explain the low level of BUN [Lee *et al.* 1978]; however, TP values in this study were within the reference range and did not differ in the two groups.

The NEFA values were not influenced by the HS and were below the reference range in both groups, confirming the findings of Benvenuti *et al*. [2016]. Moreover, Oetzel [2004] reported that low NEFA concentrations should not be considered biologically significant.

Creatinine was slightly below the reference value (0.99 mg/dl) in cows reared in the OS system.

Blood mineral concentrations (Ca, K, and P) were within the reference range and showed no significant differences. Ca is not an effective measure to assess calcium intake, due to the homeostatic mechanism, while the other minerals are a good measure of the nutritional supply [Herdt *et al.* 2000].

Immune parameters, such as SL and Hp, are useful indicators of the health and welfare status of cows. SL is involved in crucial pathways of the homeostatic control of inflammation and tissue damage, suggesting that concentrations above and beneath an optimal range are correlated with poor or no control of the inflammatory response together with a decreased ability of the immune system to cope with environmental pathogens [Amadori *et al.* 1997, Starvaggi Cucuzza *et al.* 2014]. In our study SL was found to be within the reference range in the TS group and slightly below in the OS group, with significant differences between the two housing types (P=0.0326).

HP is one of the most important acute phase proteins and is an aid in the regulation of inflammation after tissue damage. In our study Hp was not affected by the HS and the values were within the reference range.

OFR had significantly different ($P \leq 0.0106$) values between the two groups, in agreement with the findings of Benvenuti *et al.* [2016]. The TS group data were comparable with those reported by Rizzo *et al.* [2007] in pregnant cows (45.14±2.08 and 49.16±2.08 U.Carr), whereas the higher OFR level in the OS group confirmed the results of Benvenuti *et al.* [2016]. In the present study the higher OFR values in the OS housing system highlight the higher productive performance required. In fact, the mean milk yield of OS cows was significantly higher ($P<0.001$) than the TS cows (9574.1±1228.67 l and 7011.0±750.08 l, respectively). However, considering the ability of OFR to damage biological macromolecules, disrupt physiological functions and consequently induce health disorders [Abd Ellah, 2010], these results contribute to a further understanding of the influence of housing on OFR.

Hair cortisol values showed no significant differences between the two groups and the moderate concentration of this hormone, similar to that reported by Benvenuti *et al.* [2016] and lower than those observed by other authors [Del Rosario *et al.* 2011, Burnett *et al.* 2014], indicates the absence of chronic stress.

BCS showed no significant differences, with appropriate values linked to the physiological status of the cows [Roche *et al.* 2013].

The "farm nested in the type of HS" variability factor significantly influenced most of the parameters investigated (Tab. 1), highlighting the role of all types of managerial practices in safeguarding animal welfare. In fact, the role played by the animal keeper is crucial in ensuring a quiet, clean and safe environment, thus guaranteeing reasonable living conditions for animals. In addition, the quality of life of tethered cows may be improved if they are provided with comfortable and clean stalls, adequate feeding and water supply in terms of both quantity and quality, access to an exercise area, and a good relationship with the stockperson. In any case, the OS system clearly enables the animals to express their behavioural patterns, but it can lead to hierarchical problems or environmental discomfort.

Our findings showed that the living conditions of cows reared in the TS system do not seem to have a negative impact on the parameters examined, particularly SL and OFR, where more suitable values were found than those observed in the OS group. There were no signs of evident distress in either of the groups tested.

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