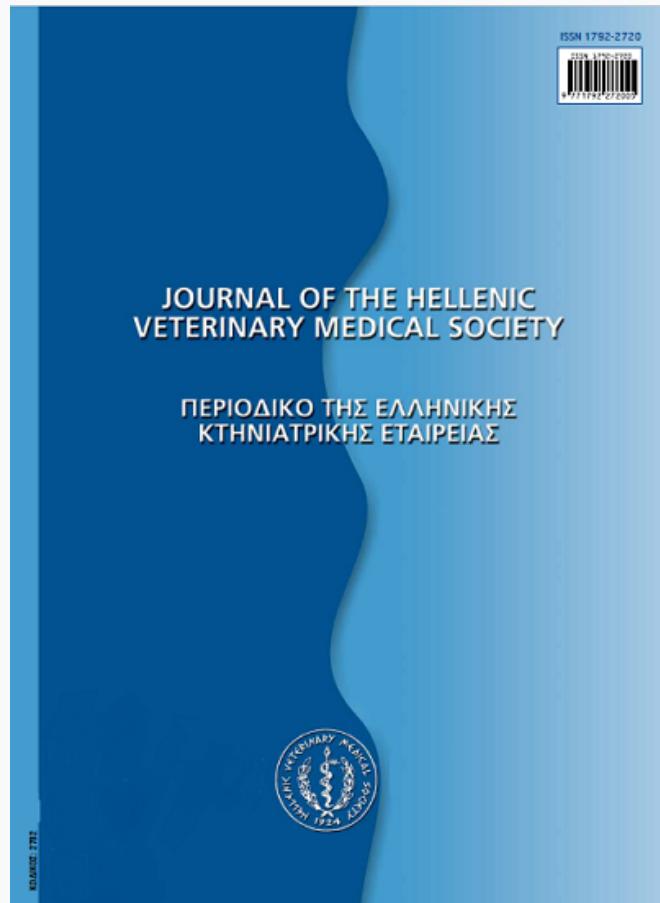


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Παράμετροι ευζωίας στις γαλακτοπαραγωγές αγελάδες που εκτρέφονται σε συστήματα εκτροφής κλειστού και ανοικτού ενσταβλισμού: πιλοτική μελέτη

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## ■ Welfare parameters in dairy cows reared in tie-stall and open-stall farming systems: pilot study

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**ABSTRACT.** Animals can experience pain or distress as a result of widely accepted management practices. In dairy cattle, housing system can affect animal welfare. The assessment of animal welfare requires the use of multiple indicators in order to analyse the heterogeneity of the aspects involved. The aim of this study was to compare the welfare of dairy cows reared in a tie-stall (TS) and open-stall (OS) system by metabolic, immunological and stress related parameters.

The study involved 80 pluriparous lactating cows belonging to eight dairy farms located in the area of Mugello (Florence, Italy) reared in TS and OS systems. Ten blood samples were collected at morning time to measure alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), blood urea nitrogen (BUN),  $\beta$ -hydroxybutyrate (BHBA), creatinine (Creat), non-esterified fatty acids (NEFA), total proteins (TP), calcium (Ca), phosphorus (P), and potassium (K), serum lisozyme (SL), bactericidal activity (SBA), haptoglobin (Hp), oxygen free radicals (OFR), and hair cortisol levels. At the same time a body condition score (BCS) was recorded. Statistical analysis was performed by ANOVA. The experiment was carried out in accordance with European Commission regulations (Directive 2010/63/EC and Directive 98/58/EC).

The results showed that the housing system affected various parameters such as ALT, BHBA, OFR, cortisol ( $P \leq 0.01$ ) and AST, BUN ( $P \leq 0.05$ ). Most of these parameters showed mean values within the range of reference without revealing any signs of suffering. An interesting outcome regarded the OFR levels, which was higher in the OS system ( $68.2 \pm 34.51$  U.Carr. vs  $36.1 \pm 21.39$  U.Carr.), probably as a consequence of the high productive effort. In conclusion, it is possible to state that TS did not show a comparable overall situation with serious signs of welfare impairment.

**Key words:** dairy cattle, welfare, farming system, metabolic parameters, immunological parameters

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## INTRODUCTION

Concern regarding animal welfare is widespread. It regards not only the absence of illness or injury, but also focuses on the pain or distress that the animals might experience as a result of managerial practices (Fraser, 2008), that in dairy industry mainly consist in animals reared in tie-stall (TS) and open-stall (OS) housing system (EFSA, 2015).

In terms of animal welfare, the TS housing system of dairy cows is controversial. According to some authors, this system, restricting voluntary movement and the social behaviour of cows, cannot be considered suitable (Popescu et al., 2013). Regarding the milk performance, health, fertility and behaviour (Zdziarski et al., 2002), some authors have not reported large differences between the two types of housing system, while others (Kara et al., 2015) report a direct effect on milk yield and animal health. Despite the criticism, TS housing systems are still widely used for dairy cows in many parts of the world (Popescu et al., 2013). In Italy, the TS system is mainly adopted on small sized farms where structural and economic constraints limit the possibility of reorganisation (Corazzin et al., 2010).

Animal welfare is a multidimensional concept (Fraser, 1995), its assessment can be performed by several approaches that can rely on farm resources and management, or on animal based indicators (EFSA, 2009; De Vries, 2015). Among the last, the health status of animals represents an important aspect that have to be taken into account. Biochemical investigation is used to check metabolic disorders (Radkowska and Herbut, 2014). Minerals have an important role for productive and reproductive performances (Galindo et al., 2014). The immune status of the animals can indicate a predisposition to developing diseases conditioned by stressful events. Some authors indicate that serum lysozyme, bactericidal activity, and haptoglobin are broader indicators of bovine non-specific immune reactivity in different breeding conditions (Bonizzi et al., 2003). Oxidative stress highlights the possible imbalance between reactive oxygen metabolite production and the neutralizing capacity of antioxidant mechanisms (Bernabucci et al., 2005), such oxidative stress may be involved in several pathological conditions, including those related to production and to the general welfare of the cows (Lykkesfeldt and Svendsen, 2007).

An evaluation of the pituitary-adrenal axis activity is very important because it regulates many biological processes such as energy balance, reproduction and immune responses (Comin et al., 2013). The measurement of glucocorticoids together with other indices of stress such as immune function, metabolism, and nitrogen balance reveal how animals perceive and adapt to their environment.

Furthermore, the body condition score (BCS) can indicate an effective nutritional management (Roche et al., 2009; 2013).

This study compared the animal welfare of dairy cows reared in TS and OS systems by means of metabolic, immunological, stress related parameters and BCS.

## MATERIALS AND METHODS

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

The study was carried out between April and May 2014 and involved 80 pluriparous, 3.5-6 years old, lactating cows belonging to eight dairy farms located in the area of Mugello (Florence, Italy): three farms reared animals in the TS system and the other five farms reared the animals in the OS system. The TS farms were smaller than the OS farms in terms of number of animals (mean  $22 \pm 10.2$  vs  $110 \pm 35.8$  heads) and farm size (mean  $33 \pm 12.1$  vs  $458 \pm 709.8$  ha). Feeding management based on the use of unifed was followed in the OS farms, and hay and meals in the TS farms.

No animal enrolled experienced a change in social group or had been affected by any diseases in the period before the study.

In each farm, ten blood samples were collected in the morning from the jugular vein using vacutainer tubes. The blood samples were kept in iceboxes and immediately sent to the laboratory of Rome, in the Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana (IZSLT). The following parameters were measured by an automated biochemical analyser (Olympus AU 400) using a commercial enzymatic test kits (Beckman-Coulter) and according to the manufacturer's instructions: alanine-aminotransfer-

ase (ALT), aspartate-aminotransferase (AST), blood urea nitrogen (BUN), creatinine (Creat), total proteins (TP), calcium (Ca), phosphorus (P), and potassium (K). Furthermore, non-esterified fatty acids (NEFA) and  $\beta$ -hidroxibutirrate (BHBA) were analysed by 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), and oxygen free radicals' levels (OFR) was monitored by a commercial colorimetric method (DIACRON) both according to the manufacturer's instructions. Finally, the serum lisozyme (SL) and bactericidal activity (SBA) determinations were performed according to validated procedures by bacteriological assay (Osserman and Lawlor 1966, Bonizzi et al. 1989, Ponti et al. 1989, Amadori et al. 2002).

Cortisol was analysed in a tail hair matrix following Accorsi et al. (2008) method. Hair samples were carefully cut from the tail switch using clippers and were frozen to -20°C to prevent lice, which are often found in this body area.

Blood and hair samples were collected during the daily routine in order not to disturb 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.

An ANOVA test was performed by JMP (The Statistical Discovery Software, SAS Institute, 2002). The model included the type of housing system and the farm nested in the type of housing system as variability factors. A 5% and 1% ( $P \leq 0.05$ ;  $P \leq 0.01$ ) significance levels were used.

## RESULTS

Some parameters displayed significant differences related to the housing system: ALT, AST, BUN, BHBA, OFR and hair cortisol. Most of the investigated parameters showed values within the reference range. Table 1 summarizes the results obtained in the study.

**Table 1.** Metabolic, immunological and stress parameters related to the two housing systems.

	Housing system						
	TS (n = 30)		OS (n = 50)		P	Normal range*	UM
	mean	SE	mean	SE			
ALT	42.4 $\uparrow$	12.42	35.4	7.59	0.0002	14-38	U/L
AST	87.4	19.16	97.8	29.24	0.0513	60-118	U/L
BUN	14.6 $\downarrow$	6.18	13.3 $\downarrow$	3.96	0.0395	20-30	mg/dl
BHBA	6.4	1.30	8.3	2.09	0.0001	<10.5	mg/dl
Creat	1.03	0.138	1.02	0.142	0.6212	1-2.7	mg/dl
NEFA	73.2 $\downarrow$	16.14	68.5 $\downarrow$	68.89	0.7051	89-618	mmol/L
TP	7.5	0.39	7.6	0.52	0.1228	5.7-8.1	g/dl
Ca	9.6	0.33	9.5	0.39	0.4956	8-10.5	mg/dl
P	5.3	1.20	5.3	0.77	0.8627	4-7	mg/dl
K	4.7	0.48	4.6	0.49	0.0989	3.9-5.8	mmol/L
SL	1.0	0.87	0.8 $\downarrow$	0.65	0.0591	1-3	ug/ml
SBA	81.2 $\downarrow$	16.31	81.5 $\downarrow$	11.35	0.8991	>90	%
Hp	0.1	0.44	0.1	0.24	0.9794	0.0-0.5	mg/ml
OFR	36.1	21.39	68.2	34.51	0.0001		U.Carr.**
Cortisol	2.8	1.43	1.6	1.05	0.0001		pg/mg
BCS	3.14	0.090	3.04	0.070	0.3996	1-5	

\*Reference values were provided by the laboratory of IZSLT

\*\*U.Carr. is an arbitrary unit; 1 U.Carr. is equivalent to 0.08 mg of H 202/100 mL.

$\uparrow$  Values over the threshold of the normal range;  $\downarrow$  Values under the threshold of the normal range.

Cows reared in the TS showed a significantly lower ( $P \leq 0.05$ ) serum AST activity ( $87.4 \pm 19.16$  U/L) than those reared in the OS system ( $97.8 \pm 29.24$  U/L). Radkowska and Herbut (2014) observed a similar trend in cows reared in stalls with access to an outdoor area compared with those reared without it. On the contrary, ALT values resulted higher in TS group ( $42.4 \pm 12.42$  U/L), contrasting the observations of the previous authors.

BUN also showed mean values under the normal range.

Creatinine resulted within the range of normality, revealing a proper kidney function.

NEFA values were under the normal range in both groups, highlighting that this parameter was not influenced by the housing system.

TP, Ca, P, K values fell within the normal range.

In our study SL, SBA and Hp did not vary within the groups; however, SL was close to significance. Mean SBA values were under the normal threshold in the two groups, while the SL mean value was low only in the OS group.

Hp was not affected by the housing system and the values were within the normal range.

OFR was significantly higher ( $P \leq 0.01$ ) in the OS group ( $68.2 \pm 34.51$  U.Carr), data regarding the normal range are not available.

Hair cortisol values showed a significant higher ( $P \leq 0.01$ ) mean values in TS ( $2.8 \pm 1.43$  pg/mg and  $1.6 \pm 1.05$  pg/mg).

The cows reared in the two housing systems did not differ in terms of BCS, which were 3.1 and 3.0 respectively for both systems.

## DISCUSSION

ALT values in the TS group slightly exceeded the normal range. Moreover, as AST, BHBA and Creat were within normal range, animal health and then welfare did not seem to be impaired.

Usually the plasma NEFA concentration increases in response to increased energy needs accompanied by inadequate feed intake (Overton and Waldron, 2004), while low NEFA concentrations are not to be considered biologically important (Oetzel, 2004).

Since NEFA and BHBA are indicators of negative

energy balance, the picture described in this study did not reflect such situation (Adewuyi, 2005).

The low level of BUN could be explained by a deficient protein intake (Lee et al., 1978) although in this study TP showed values within the normal range and did not differ between the two groups.

The interpretation of blood mineral concentrations varied according to each specific mineral: Ca is generally an ineffective means of assessing calcium intake, probably because of the sensitive homeostatic mechanism, while P and K blood concentrations are good measures of nutritional supply (Herdt et al., 2000).

SL, SBA and Hp represent a nonspecific cellular immune response. The alteration in these parameters may indicate inadequate hygienic and sanitary conditions of the herd or an inappropriate feed and management approach (Bonizzi et al., 2003). As SL is involved in the immune system, it is one of the most predictive parameters of disease. Variations in its levels have been found in response to inflammation or metabolic stress-related conditions in early lactation (Trevisi et al., 2012). Our results indicated a slightly altered immune response. Some authors have reported a decrease in SL in cows during the transition period (Bonizzi et al., 2003). On the other hand, others indicated SBA values of around 90% as being a significant alteration of the physiological conditions, thus indicating the predisposition to developing diseases conditioned by stressful events (Amadori et al., 2002).

The high level of OFR in the OS group is interesting given its influence on oxidative stress, which can lead to the modification of important physiological and metabolic functions. OFR values resulted unexpected in the OS farming system. Since failure in the adequate control of free radicals within metabolically active tissues results in oxidative stress and possibly increased health disorders in high-producing dairy cattle (Sordillo et al., 2009), our results would highlight the importance of antioxidants supplementation in the diet of dairy cows, particularly in those reared in OS.

It is likely that animals reared in OS are selected more for productive purposes than other animals, thus health problems could have a higher incidence together with animal welfare impairment.

In the present study the mean milk production of cows reared in OS reached a significantly higher level of milk production ( $P \leq 0.001$ ) than those reared in TS (respectively  $9558.68 \pm 384.803$  l and  $5996.76 \pm 496.779$  l).

Cortisol in the tail hair matrix normally shows a higher concentration compared to other parts of the body. The hairs also grow back in very short time in the tail, suggesting that this is the most suitable location to collect hair samples (Moya et al., 2013). The recorded values of hair cortisol were lower than found in the literature, but comparable with those reported by Rizzo et al. (2007) in pregnant cows ( $45.14 \pm 2.08$  and  $49.16 \pm 2.08$  U.Carr). Del Rosario et al. (2011) reported hair cortisol concentrations equal to  $12.1 \pm 1.85$  pg/mg in 2-year-old cows, and Burnett et al. (2014) found a cortisol level equal to  $11.0 \pm 1.2$  pg/mg in lactating dairy cows. These observations led us to consider that the obtained values of hair cortisol did not indicate a situation of chronic stress in the cows reared in TS.

Typically, psychological stress is associated with fear, such as that experienced during commingling or social mixing, exposure to new environments, loud and unusual noises, and restraint (Carroll and Forsberg, 2007). These conditions scarcely affected the animals reared in TS. The limitations of TS, such as the lack of areas for movement and the boredom, may

be compensated by the comforting and reassuring environment.

These moderate values, together with low plasma levels of OFR, NEFA and BHBA could indicate a reduced risk of metabolic disorders (Bernabucci, 2005).

The living conditions of cows reared in TS did not have a negative impact on the examined parameters, which in some cases were more suitable than those reared in OS.

## CONCLUSIONS

In conclusion, the study evidenced that some parameters significantly varied in the two farming systems but, in consequence of the fact that most of them are within the normal range, the welfare of the animals seem not to be impaired. Finally, animals reared in the TS system did not show alterations ascribable to evident signs of suffering.

## COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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