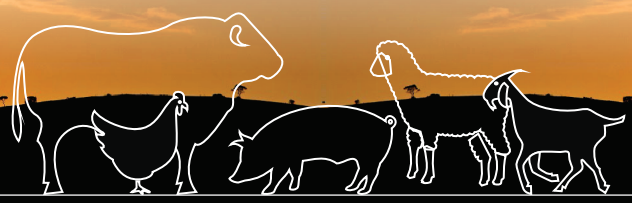
ISBN 978 - 86 -82431 - 71 - 8

4th INTERNATIONAL CONGRESS

PROCEEDINGS

NEW PERSPECTIVES AND CHALLENGES OF SUSTAINABLE LIVESTOCK PRODUCTION



Belgrade, Serbia 7th - 9th October 2015

Proceedings of the 4th International Congress
New Perspectives and Challenges of Sustainable Livestock Production
October 7 – 9, 2015

HOUSING CONDITIONS AND WELFARE OF DAIRY COWS IN SERBIA

D. Ostojić Andrić[1], S. Hristov[2], M. M. Petrović[1], V. Pantelić[1], J. Bojkovski[3], Ž. Novaković[4], M. Lazarević[1], D. Nikšić[1]

[1]Institute for Animal Husbandry, Auto put 16, 11080 Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Republic of Serbia
[3]Faculty of Veterinary Medicine, Bulevar oslobođenja 18, 11000 Belgrade, Republic of Serbia
[4]Institute for Science Application in Agriculture, Bulevar despota Stefana 68b, 11000 Belgrade, Republic of Serbia
Corresponding author: andricdusica.iah@gmail.com
Invited paper

Abstract: Research conducted on 16 dairy farms in Serbia has shown that poor housing conditions represent one of the major dairy cows' welfare problem in our country. This is also an area of animal welfare in which the greatest difference in relation to the situation in EU countries can be observed. Poor comfort conditions are estimated based on a high share of cows that lie outside their lying area (36.5%) as a consequence of inadequate or insufficiente size of lying areas. Investigated the farms showed a very poor state of hygiene of dairy cows, with a high percentage of cows with the contaminated lower parts of the legs (84.6%), rump (71.3%) and udder (60.0%), which indicates the inadequate hygiene of lying areas and facilities, insufficient amount of bedding but also disorders of rumen digestion. The biggest welfare problems are present on farms with tied system, also the presence of grazing in our conditions, unlike the EU, is also insufficient from the standpoint of ensuring the welfare of dairy cows.

Key words: welfare, dairy cows, housing conditions, comfort, freedom of movement, hygiene

Introduction

The general concept of welfare is based on the concepts of adaptation, stress, animal needs and their rights. The most important question in terms of providing welfare is certainly the question of animal needs. According to *Broom and Johnson (1993)* the need is the request, part of the biological basis of the animal, to provide adequate resources or responses to specific stimuli from the surrounding environment or its body. Animals in the absence of resources to meet their basic needs are becoming more prone to numerous welfare risks. In the report,

EFSA (2009) highlighted four key risks to the welfare of dairy cows: housing, feeding, management and genetic selection. The conditions for housing of dairy cows, according to research by other authors (*Regula et al., 2004; Ostojić - Andrić et al., 2011)* significantly affect the ensuring of their welfare and include a wide range of conditions that need to be provided in order to ensure the animal needs and protect welfare. The authors of the *Welfare Quality Protocol (Welfare Quality®, 2009),* as key factors to ensure satisfying of the animal needs in regard to housing conditions, include freedom of movement and comfort of the animals.

As a form of behavior, movement is an integral part of all other forms of behavior and enables the animal to react appropriately in terms of space, time and stimulus which meets their needs for maintaining the homeostasis, or physical, psychological and genetic integrity (*Vučinić, 2006*). If the animal is highly motivated to satisfy a need, and due to the inability of movement is prevented in doing so, it leads to the development of frustration *(Vučinić, 2006).*

According to many authors (*Krohn and Munksgaard,* 1993; *Ristov et al., 2006; Ostojić - Andrić et al., 2011*), the housing system is strongly reflected on the quality of the welfare of dairy cows, especially in terms of health status and expression of behavior. In Serbia, like in most countries, the tied system prevails that allows individual treatment of each animal but is a strong contrast to the natural habitat of the cattle (*Ostojić - Andrić et al., 2011*). Free system is more and more present in modern rearing because the freedom of movement and separation of functions (feeding, watering, lying and milking) have a positive influence on the overall state of health, fitness of the animal, length of exploitation and production performance. Easier way to ensure the proper microclimate and zoohygienic conditions are also benefits of the free system.

Growing cows in the pasture system is the most natural form of rearing of cattle since it enables the expression of various forms of behavior characteristic of the cattle as a species (*Von Keyserlingk et al., 2009*). *Tucker (2009)* states that cattle grazing have more harmonious daily rhythm compared to farmyard kept cows, and that their daily feeding and lying pattern is better synchronized. The advantages of growing cattle on pasture are reflected primarily in lower incidence of laminitis, teat injuries (*Regula et al., 2004*) and abnormal behaviors (*Krohn, 1994*) as well as enhanced comfort while resting (*Krohn and Munksgaard, 1993*).

Providing comfort to cows is of great importance from the aspect of meeting their needs in terms of rest, behaviour and health. Studies have shown that the hygiene of cows and their behavior on the laying area during their resting can be considered as reliable indicators of comfort.

Hygiene of cows mirrors the environment in which they are grown and influences many aspects of health (*Schukken et al., 1990*). Some studies the behaviour of cows show that they have an aversion to unclean environment,

especially when first dealing with it, and that it is preferably avoided (*Phillips and Morris, 2002).* Soiling of various bodily parts of cows shows the different sources of contamination. For example, dirty lower legs suggest that cows are walking through mud or manure, whereas dirty hips suggest dirty and damp lying areas. Specific, splattered manure patterns on the hips are a sign that a cow kicked with a tail that was dirty with faeces and watery. This combination of behaviour and consistency of feces can be an indicator of disturbed function of the rumen (*Huxley and Whay, 2006).*

Lying is the behavior of high priority (*Munksgaard et al., 2005*), because the cows mainly rest during lying, so every change of this aspect is of great significance for the state of welfare. In studies *Brörkens and al. (2009a)* have found that the duration/time of lying down (in seconds), the percentage of collision with equipment when lying down and the percentage of cows that lie partially or completely outside the lying are can be considered as the most reliable indicators of welfare.

Taking into account the above-mentioned research in this field in the world, and the need for assessing the state of welfare on dairy farms in our country, the main objective of this study was to determine the quality of welfare in relation to housing conditions in order to define the most important welfare risks and propose measures for its improvement.

Materials and Methods

For the assessment of welfare in dairy farms in Serbia the protocol for evaluating the quality of the welfare of cattle (*Wefare Quality® Assessment Protocol for Cattle, 2009*) was used, specifically designed to assess relevant indicators of welfare from the viewpoint of the animals themselves. The protocol includes 29 indicators that are used to determine the 12 criteria: the absence of long-term hunger and thurst, comfort, thermal comfort, freedom of movement, lack of injuries and illness, absence of pain due to mutilation, expressing social and other behaviours, good human - animal relations and a positive emotional state. By aggregation of these criteria the values of 4 basic principles of welfare are determined: good nutrition, good housing, good health and appropriate behavior. Finally, based on the overall score farms are classified into one of four categories of quality of welfare: unacceptable, acceptable, appropriate and excellent. The study was conducted on a total of 16 farms with different housing (tied, free) and different capacity (small, medium, large) in which the cattle of Simmental and Holstein - Friesian breeds were reared. Minimum number of cows in the sample was 30 and the average per farm was 64 animals in two repetitions - during winter and summer season. Data processing and categorization of welfare quality of the

investigated dairy farms was conducted using software specially developed under the Protocol, and the respective statistical parameters were analyzed with the program *StatSoft.Inc (2004), Statistica for Windows version 7*.

Results and Discussion

Overall welfare assessment on dairy farms in Serbia

Welfare quality assessment on dairy farms was conducted by collecting data relating to the ensuring of good nutrition, good housing, good health and appropriate behaviour. The research results show that half of the surveyed farms was classified as acceptable (score 2) and the other half into the category of welfare of adequate/appropriate quality (grade 3) on the basis of which it can be argued that the observed farms, on average provided conditions that are of fundamental animals' needs in terms of nutrition, health, comfort, housing and expression of behaviour. Research conducted according the same methodology in farms in the EU (*Welfare Quality Network, 2012*) showed a great similarity with the results obtained in the present study. In 2011, the share of farms with an acceptable quality of welfare in the EU was 47%, with 51% of the appropriate quality and 2% of farms with unacceptable quality of welfare, while in our country none of the evaluated farms were classified in the latter category.

Table 1 shows the average rating of the quality of the welfare of the analyzed farms. The principle of good nutrition is rated satisfactory with the highest score in relation to other principles which indicates that the welfare of dairy cows in Serbia is not threatened by long-term starvation and thirst. Under this principle, the highest variability was established, so in certain farms (score $\leq$ 20) shortcomings were evident which, given the importance of this principle, need to be timely removed. Sums of other principles on average were in the range from 20 to 55 points, which corresponded to category of acceptable welfare, i.e. indicated that housing conditions, health and behaviour requirements were ensured and meet the minimum needs of animals in terms of their welfare. However, the survey results indicate that there is room for improvement, especially when it comes to housing conditions and the provision of appropriate behaviour.

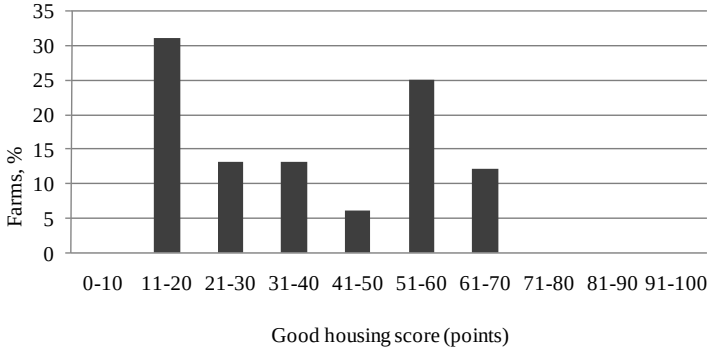
Table 1. Principle scores and overall assessment of welfare quality on dairy farms in Serbia

WF*	Welfare principles, score	$\bar{x}$	*SD*	S^2	*Min*	*Max*
En	Good feeding	75,97	25,31	640,39	12,20	100,00
Ac	Good housing	36,59	19,37	375,18	7,30	65,40
Ac	Good health	41,17	8,11	65,78	23,90	56,60
Ac	Appropriate behaviour	31,93	13,77	189,50	15,40	81,10
Overall welfare assessment (1-4), average score		2,47	0,51	0,26	2,00	3,00

*Welfare category (WF): Ex-excellent (>80 points); En-enhanced (55-80 points); Ac-acceptable (22-55 points) and Nc-not classified (< 20 points)

Welfare quality in relation to housing conditions

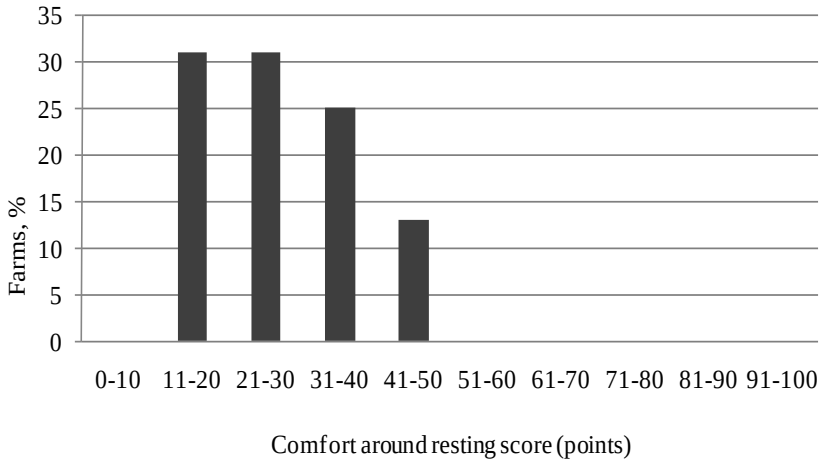
Distribution of the examined farms in Serbia according to the value of the principles of good housing (Graph 1) shows that the highest number of dairy farms (31.25%) was rated as very poor, in the range from 11 to 20 points, and the remaining 68.75% of the farms was rated in the range from 21 to 70 points. The average value of the principle was 36.59 points compared to the score of this principle in EU countries (*Welfare Quality Network, 2012*) where the highest number of farms (50%) is estimated in the range from 51 to 70 points with an average principle value of 56.5 points. It is obvious that dairy cows in our country are provided with significantly poorer growing/housing conditions compared to those that exist in European farms. This observation is further confirmed by the fact that in our study, no farm is estimated to be in the range from 81 to 100 points whereas in the EU 2% of farms are within these values. Also, only 2% of the farms in the EU are valued in the range from 11 to 20 points, in contrast to Serbia, where the highest number of farms are estimated within this range.



Graph. 1. Distribution of farms according to principle of good housing

Farms in Serbia on average were mostly (62.5%) scored in the range from 11 to 30 points for the criterion of comfort in housing (Graph 2), with an average value of 25.77 points as opposed to farms in the EU (*Welfare Quality Network, 2012*) where the average value was 45.1 points on 44% of farms scored in the range from 31 to 40 points and 4% estimated in the range from 1 to 10 points. This indicates that the comfort conditions in the housing of cows in Serbia on average is worse than the conditions that are provided for cows in EU countries.

In relation to the value of criterion freedom of movement, for the highest number of farms in the survey – 43.75% (Graph 3) were very highly rated (90 to 100 points), which means that the animals are provided with adequate capacity to move in stables, ranges or on pastures. However one third of the surveyed farms was estimated in the range from 11 to 20 points, which indicates a significant limitations in terms of freedom of movement.

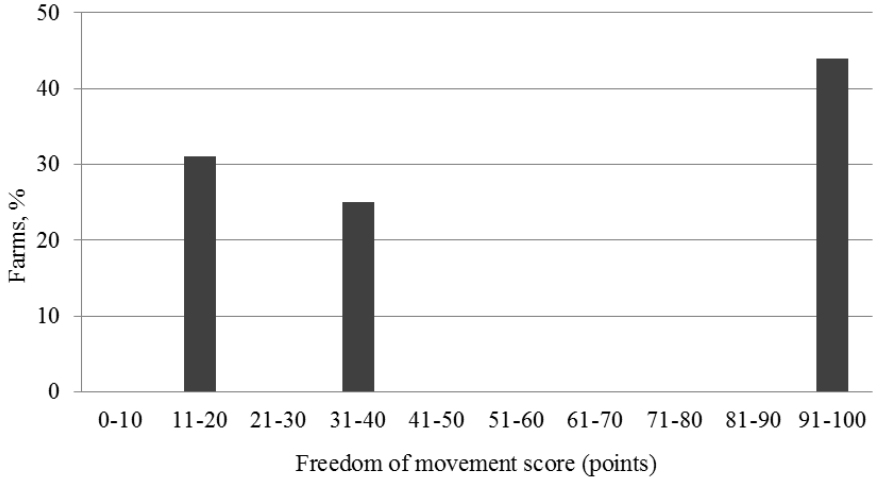


Graph. 2. Distribution of farms according to comfort around resting

Results of research of freedom of movement on dairy farms in the EU indicate that this parameter is significantly improved in the past five years. In 2008, the share of farms with a score of freedom of movement in the range from 11 to 20 points was 18%, while in 2012 all studied farms had the highest value of the criteria of 90 to 100 points (*Welfare Quality Network, 2012*).

Housing/rearing conditions are factors of great importance for the cow welfare. The value of the principle of appropriate growing conditions on farms in Serbia is defined by the values of criteria comfort and enabled freedom of movement (Table 2).

Survey results (Table 2) show that the cows in tested farms on average are given greater freedom of movement than is the case with the provision of comfort. It was determined that the average duration of lying down of 6.25 seconds is outside the range of acceptable values according to recommendations *Forkman and Keeling (2009)* and indicates the limited comfort of cows due to inadequate lying areas, problems with diseases of legs and pathological changes on the skin, hair and joints (*Algers et al., 2009*).



Graph. 3. Distribution of farms according to Freedom of movement

The collision with the equipment when resting was below the critical value of over 20% as recommended by *Forkman and Keeling (2009),* while the phenomenon of lying outside the lying area was recorded at an average 36.54% of cows which seriously undermines the comfort of cows. In addition, cows lying partially (in tied) or fully (in the free system) out of the lying area has a negative impact on their hygiene, behaviour and health (*Algers et al., 2009*). The most common cause of this phenomenon in the tied system are inadequate and short lying areas (*Bartussek, 1985*) but also attempts of cows to cool in the slurry during the summer months in case of free range system *(Curtis, 1982).*

Hygiene of cows on observed farms was very poorly scored (Table 2). An average proportion of cows with dirty lower parts of the legs, the hips and udders far exceeds the critical ranges in terms of the welfare of 20 - 50%, 10 - 19% and 10 - 19% respectively (*Forkman and Keeling, 2009*). The share of cows with dirty feet (Table 2) of 60% and 95% was very close to the share identified in the research of *Vučemile et al. (2012).*

Cook (2002) has identified inadequate - short lying areas and insufficient amount of bedding on them as the most common causes of high soiling of the lower parts of the legs. In fact, a high proportion of cows with dirty feet is not uncommon even on the farms of high welfare quality category.

Table 2. Assessment of housing conditions in relation to welfare assurance

No of farms, N	N=16				
Principles, criterions and indicators of welfare quality	$\bar{x}$	*SD*	S^2	*Min*	*Max*
II Good housing, score	36,59	19,37	375,18	7,30	65,40
1. Comfort around resting, score	25,77	12,50	156,32	2,70	45,10
Time needed to lie down, in sec.	6,25	0,72	0,52	4,50	7,58
Colliding with housing equipment during lying down, %	9,93	11,58	134,13	0,00	37,00
Lying partly or completely outside the lying area, %	36,54	33,83	1144,38	0,00	100,00
Dirty legs,%	84,64	20,39	415,77	14,81	100,00
Dirty udders,%	60,07	23,99	575,54	9,26	100,00
Dirty flank/ upper legs,%	71,34	20,42	417,05	3,70	100,00
2. Freedom of movement, score	56,94	39,25	1540,58	15,00	100,00
No. of days cows are kept tethered, per year	174,84	170,29	28997,56	0,00	365,00
No. of days with access to outdoor loafing area, per year	107,66	120,49	14517,72	0,00	365,00
No. of hours with access to outdoor loafing area, daily	9,00	10,11	102,19	0,00	24,00
No. of days with access to pasture, per year	16,88	52,76	2783,47	0,00	210,00
No. of hours with access to pasture, daily	2,25	6,42	41,23	0,00	24,00

In the study by *Webster (2005*), the ratio mentioned ranged on farms from 65% to 96%. However, in the same survey, filthy rumps were observed in 0 - 7% of cows from the best farms and in 26 -78% of cows from lowest scored farms while in our study, this ratio was 71%.

Soiling of the udders on farms in Serbia amounted to an average of 60%, which corresponds to the range of 24 - 70% on farm of the worst welfare quality category in the research of *Webster (2005*). In a study of *Vučemila et al. (2012)* the

soiling of rump was even more pronounced (75%). Extremely poor hygiene of cows usually is associated with poor facility hygiene and ninadequate and irregular manure removal but may be associated with disfunction of the rumen (*Huxley and Whay, 2006).* Soiling of rump can be a reliable indicator of the said conditions. It can be concluded that inadequate hygiene of cows on farms in Serbia threatens their welfare by increasing the risk of diseases of legs and udders (*Schukken et al., 1990)* while at the same time it can cause changes in the behaviour of cows (*Phillips and Morris, 2002*).

The movement is an essential element for expression of natural behaviours and satisfaction of innate need or instinct of animals. In our conditions the cows are, on average, held tied 175 days per year while access to ranges is enabled 108 days and pastures only 17 days (Table 2). Converted into hours, cows spend outdoors a total of 968 hours per year on free ranges discharges or 38 hours in the pasture. It can be concluded that the time cows are in the outdoors (in ranges and in the pasture) is very short and insufficient, taking into account the survey by *Krohn et al. (1992)* where cows, with the possibility of free choice, each year spent 4046 hours in the open air.

In our research, freedom of movement was acceptable, but with large variation (min. 15; max. 100) between the analyzed farms. On six of the sixteen examined farms, tied system of keeping was applied throughout the year which represents a major risk to the welfare of animals and reflects negatively on their comfort while resting (*Krohn and Munksgaard, 1993*) and the state of health of cattle in terms of increased incidence of laminitis and mastitis (Regula et al., 2004), as well as abnormal behaviours *(Krohn, 1994).*

Conclusion

Average score of the welfare quality on the tested farms corresponds to the descriptive assessment acceptable to appropriate welfare which leads to the conclusion that rearing/housing conditions satisfy more of the basic needs of animals in terms of nutrition, health, comfort, housing and expression of the behaviour, and there is room for improvement of welfare. Similar results were found on dairy farms in the EU.

Conditions of keeping/housing of dairy cows in Serbia were deemed acceptable, but still significantly worse than the conditions that exist on European farms. On the tested farms, cows on average were given greater freedom of movement than is the case with the provision of comfort. Indicators of comfort in keeping point to significant problems with hygiene in the facilities, insufficient bedding and too short lying areas. Freedom of movement in the average was assessed as adequate. The biggest problems are certainly present on farms with tied

system, where on more than half of the farms cows were not given possibility of movement during the year. Representation of grazing in our conditions, unlike the EU, is also insufficient from the standpoint of ensuring the welfare of dairy cows.

Results of testing the quality of welfare in relation to housing conditions, generally indicate that the quality of the welfare on the studied farms is acceptable and appropriate, and that there are opportunities for improvement. With regard to the identified major risk factors and current trends in the dairy cattle production, recommendations for improvements in welfare are related to the provision of adequate space and comfort in keeping/housing of dairy cows, the provision of adequate size, build quality and hygiene of lying areas as well as providing greater freedom of movement of cows through the application of free housing system and grazing.

Uslovi držanja i dobrobit mlečnih krava u Srbiji

D. Ostojić Andrić, S. Hristov, M. M. Petrović, V. Pantelić, J. Bojkovski, Ž. Novaković, M. Lazarević, D. Nikšić,

Rezime

Istraživanje sprovedeno na 16 mlečnih farmi u Srbiji pokazalo je da loši uslovi držanja predstavljaju jedan od najznačajnijih problema dobrobiti mlečnih krava u našoj zemlji. Ovo je ujedno oblast dobrobiti u kojoj postoje najveća odstupanja u odnosu na stanje u zemljama EU. Nezadovoljavajući uslovi komfora procenjeni su na osnovu visokog učešća krava koje leže van ležišta (36,5%) kao posledice neodgovarajuće veličine odnosno prekratkih ležišta. Na posmatranim farmama utvrđeno je veoma loše stanje higijene krava, sa visokim učešćem krava zaprljanih donjih delova nogu (84,6%), sapi (71,3%) i vimena (60,0%) što ukazuje na neodgovarajuću higijenu ležišta i objekata, nedovoljnu količinu prostirke ali i poremećaje buražnog varenja. Najveći problemi dobrobiti prisutni su na farmama sa vezanim sistemom držanja dok je i zastupljenost ispaše u našim uslovima, za razliku od zemalja EU, takođe nedovoljna sa stanovišta osiguranja dobrobiti mlečnih krava.

References

BROOM D.M. AND JOHNSON K.G. (1993): Approaching questions of stress and welfare. In: Stress and Animal Welfare. Kluwer Academic Publishers, p 1-7.

EFSA (2009): Scientific opinion on welfare of dairy cows in relation to metabolic and reproductive problems based on a risk assessment with special reference to the impact of housing, feeding, management and genetic selection. The EFSA Journal, 1140, p. 1-75.
FORKMAN B., KEELING L.(2009): Assessment of Animal Welfare Measures for Dairy Cattle, Beef Bulls and Veal Calves. Welfare Quality Reports. Cardiff University. Sweden. p. 1-314.
HUXLEY J. AND WHAY H.R. (2006): Cow based assessments: Part 1. Nutrition, cleanliness and coat condition. UK vet, 11(3), p 18-23.
KROHN C.C., MUNKSGAARD L., JONASEN B. (1992): Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments I. Experimental procedure, facilities, time budgets — diurnal and seasonal conditions. Applied Animal Behaviour Science, Vol. 34, Issue 1, p. 37-47.
KROHN C.C. (1994): Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments: III. Grooming, exploration and abnormal behaviour. Applied Animal Behaviour Science, 42, (2), p. 73-86.
KROHN C.C. AND MUNKSGAARD L. (1993): Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments II. Lying and lying-down behaviour. Applied Animal Behaviour Science, 36, p. 1-16.
WELFARE QUALITY NETWORK (2012): Welfare Quality® scoring system. Retrieved August, 2012 from http://www1.clermont.inra.fr/wq/index.php?id=farms
TUCKER C.B. (2009): The Behaviour of Cattle, In: The Ethology of Domestic Animals, 2nd Edition. Edited by Jensen P. © CAB International. Walingford, UK. p. 151-159.
ALGERS B., CANALI E., BAROLI D, WESTIN R. (2009): Resource - based Parameters in Cattle, In: Assessment of Animal Welfare measures for dairy cattle, Beef Bulls and Veal Calves. Welfare Quality Reports No.11. Edited by: Forkman B., Keeling L. Cardiff University, Uppsala, Sweeden. p. 273-294.
BARTUSSEK H. (1985): Vorschlag für eine Verordnung der Steiermärkischen Landesregierung für den Bereich der Intensivtierhaltung. Der österreichische Freiberufstierarzt, 9, p. 4-15.
COOK N.B. (2002): The influence of barn design on dairy cow hygiene, lameness, and udder health. Proceedings of the 35th Ann. Conv. Amer. Assoc. Bov.Pract. Madison, USA, p. 97-103.
CURTIS S.E. (1982): Perception of Thermal Comfort by Farm Animals. In: Farm Animals Housing and Welfare. Edited by: Baxter S.H., Baxter M.K., MacCormack J.A.D. p. 59-66.
HRISTOV S., VUČINIĆ M., RELIĆ R., STANKOVIĆ B. (2006): Uslovi gajenja, dobrobit i ponašanje farmskih životinja. Biotehnologija u stočarstvu, 22, 73-84.

MUNKSGAARD L., JENSEN M.B., PEDERSEN K.J., HANSEN S.W., MATTHEWS L. (2005): Quantifying behavioural priorities—effects of time constraints on behaviour of dairy cows, Bos taurus. Applied Animal Behaviour Science., vol. 92, 1, p. 3-14.

OSTOJIĆ - ANDRIĆ D., HRISTOV S., NOVAKOVIĆ Ž., PANTELIĆ V., PETROVIĆ M. M., ZLATANOVIĆ Z. , NIKŠIĆ D. (2011): Dairy Cows Welfare Quality In Loose Vs. Tie Housing System. 3rd International Congress "New perspectives and Challenges of Sustainable Livestock production"Belgrade, Republic of Serbia, 5-7th October 2011. Biotechnology in Animal Husbandry, vol 27, 3, Book 2, p.975-984.

PHILLIPS C.J. AND MORRIS I.D. (2002): The ability of cattle to distinguish between, and their preference for floors with different levels of friction and their aviodance of floors contaminated with excreta. Animal Welfare, 11, p. 21-29.

REGULA G., DANUSER J., SPYCHER B., WECHSLER B. (2004): Health And Welfare Of Dairy Cows In Different Husbandry Systems In Switzerland. Preventive Veterinary Medicine, 66, p.247–264.

SCHUKKEN Y.H., GROMMERS F.J., ERB VAN DER GEER D.H.N., BRAND A (1990): Risk factors for clinical mastitis in herds with low bulk milk somatic cell count. 1. Data and risk factors for all cases. Journal of Dairy Science, 73, p. 3463-3471.

VUČINIĆ M. (2006): Ponašanje, dobrobit i zaštita životinja. Fakultet veterinarske medicine Beograd.

WEBSTER J. (2005): The assessment and implementation of animal welfare: theory into practice. Rev.sci.tech.off.int.Epiz., 24 (2), p.723-734.