MAE Vet Fak Derg, 7 (2): 67-76, 2022
DOI: 10.24880/maeuvfd.998320

Research Article

A study on behavior and welfare characteristics of Holstein cattle in manure and sand beddings in free-stall barns

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Key Words:
behaviour
cow welfare
holstein
manure
NEDAP smartted leg
sand bedding

Received : 21.09.2021
Accepted : 25.04.2022
Published Online : 31.08.2022
Article Code : 998320

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The research was summarized from a Master of the same name. The authors wish to gratefully acknowledge Aydin Adnan Menderes University (Aydın, Turkey) for financial supporting (Project No: VTF-17047) the present study.

ABSTRACT

The aim of the present study was to investigate the effects of manure and sand bedding on animal welfare and behavioral characteristics in Holstein cattle. A total of 110 Holstein dairy cattle were divided into two groups (manure and sand bedding) with a stall length of 125x250 cm and a walking area. At the end of the study, right and left carpal and tarsal joint lesions were scored. In addition, blood serum glucose, cholesterol, triglyceride and total protein levels were measured. During the study, behavioral characteristics of the animals (duration of standing, duration of lying/resting, number of steps, frequency of standing and lying) were recorded by NEDAP tracking system. Carpal and tarsal lesions of the joints, blood serum glucose, total protein, and triglyceride showed no-significant differences statistically between the groups. However, in terms of the standing time ($P<0.05$), lying/resting time ($P<0.001$), the number of steps ($P<0.001$) and standing/lying ($P<0.001$) frequency behavioural characteristics, neutrophil-lymphocyte ratio ($P<0.01$), and cholesterol levels ($P<0.05$) showed statistically significant differences between the groups. Lying/resting time was determined as 689 minutes in the sand bedding group and 638 minutes in the manure bedding group ($P<0.001$). In conclusion, sand bedding has beneficial effects to increase cow's welfare by increasing the lying/resting time. Lying/resting behavior is important to Holstein dairy cattle, but caution and a full understanding of the context and the variation of the blood parameters in question is needed before drawing firm conclusions about animal welfare from measures of biochemical blood parameters.

INTRODUCTION

Animal behaviors are taken as important criteria when determining the health level of animals. Therefore, changes in behavior and the presence/absence of particular behaviors can provide a valuable indication of the animal's welfare state (Yakan et al., 2007; Akbaş, 2013). The type of bedding used in loose-housing of dairy cows has a considerable effect on the animal comfort and well-being provided by the stalls. According to Endres (2022), Ozbeyaz & Unal (2018), while choosing the-barn floor, it is important to prefer materials which help the control of indoor temperature, and protect the animals from injuries due to ground friction, easy to clean and resistant but free of toxic substances. Additionally, a bedding material should be chosen that will not adversely affect the shelter air quality and the humidity level of the environment (Dimov & Marinov, 2021). According to Kumar Singh (2022), organic bedding materials as manure absorb moisture well, they are compatible with the manure processing systems, easily accessible, available in sufficient quantities in different regions of the world, and are not expensive. Dimov and Marinov (2021) reported that the sand bedding generally doesn't contain nitrogen or carbon required for growth of microorganisms.

In cattle farming, the bedding type should be chosen as dry, safe, comfortable, and durable. In the cases where the bedding is not chosen appropriately the dairy cows undergo stress, and lower productivity leading the cow to be affected negatively in terms of health and behavioral characteristics. According to Leach et al. (2014) the dry, soft, and with consistent quality and quantity bedding resembles the best floor characteristics. Also, for the udder health of the cows that spend most of the day resting, it is essential that the bedding is dry, clean, and soft. Demirci (2005) reported that sand bedding is more advantageous compared to others in terms of being easy to access, having low microorganism load and being the most preferable bedding of cows in hot regions. As cows lay on the ground from a 25-30 cm distance during lying/resting positions, the bedding used in the shelter ground should be soft considering the height of animals' live weight. Otherwise, knee

To cite this article:
Akdeniz M, Dereli Fidan E. A study on behavior and welfare characteristics of Holstein cattle in manure and sand beddings in free-stall barns. MAE Vet Fak Derg 2022; 7 (2) :67-76.

abrasions as well as wounds and lesions due to these abrasions may occur on animals (Weary & Tucker, 2003). Kocyigit (2014) reported that the most important reason for laminitis among animals is the behavior of remaining standing for a long time. Tucker et al. (2003) indicated that the lying/resting time for the cows is lower in sand beddings than to those which are provided sawdust beddings. Neutrophile-lymphocyte (N-L) ratio is accepted as an important indicator of stress in small and large ruminants (Broom, 2014). Kayar (2013) stated that it's an important indicator of stress when glucocorticoids increase the blood glucose level. Other blood parameters that are used to evaluate stress in animals are triglyceride, total protein and cholesterol levels (Bedanova et al., 2007).

Rushen et al. (2008) indicated that an increase in a number of the hoof diseases and lameness when there is absence of bedding. The housing of dairy cows only on a concrete floor is one of the main causes of lameness and deterioration of their welfare in general. Rushen et al. (2007) reported that the use of rubber mats contributes to the dairy cows' hooves health and reduces the lameness percentage in the cow herd. Dual chamber mattresses water beds reduce the leg diseases incidence, in particular the appearance of hock joints wounds due to pressure (Kour, 2022). Cows with a compost bedding show reduced incidence of lameness as compared to cows housed in free-stall barns with a sand bedding (Lobeck et al., 2011). Well-managed free-stall production systems farms with a bedding of manure and sand do not differ significantly in terms of hock joint health in cows (Eckelkamp et al., 2016).

As a topic that arises in the last decade, animal wealth has also brought some legislative regulations and applications along with it. Therefore, researches about indoor conditions of shelters, the impact of these conditions over animals, and the reactions the animals show towards these conditions have become very important. This study is carried out with the aim of providing a perspective on effects of manure and sand bedding on health and behavioral features of Holstein cattle. In this manner, it has aimed to measure the effect of manure and sand bedding on Holstein cows' behavioral characteristics such as carpal and tarsal joint lesions, standing and lying/resting time, and number of steps, frequency of standing/lying down and biochemical blood parameters, and N-L ratio.

MATERIALS and METHOD

Animals and Experimental design

The study was performed with the permission of the Aydin Adnan Menderes University Animal Experimentation Local Ethics Committee (No: 13.06.2017-64583101/048). The research was carried out in a 6-month-period between November 2017 and April 2018 at a semi-open free-stall dairy cattle business operating in Foca, İzmir. The sample of the research was derived from 110 dairy cows of the Holstein breed (Black Pied) between the ages of 2-6 being raised in this-farm. The animals were randomly divided into two groups (n=55). The research was carried out in two separate groups which have 50 station stall areas each. The size of the stall dimensions were 125x250 cm for each group. Manure was used on the first sample group as bedding whereas sand was used for the second group. One of the organic beddings, manure, was obtained by drying the stool after passing it through separators. Acquired dry and solid manure was used as dry manure bedding. On the other hand, fine sand with 40-50 cm depth was used in the sand bedding system. With this purpose, the edges of the soil floor were raised and sand was filled into it. The sand used as bedding material was observed daily when the cows were out for milking and lacking places of sand were filled in if there are any. The barn floor on the service road and all other surfaces of the farm is concrete. The manure scrapers only used in sand bedding group. The crawl spaces are made of aggregate insulating concrete at all stalls.

Husbandry

During the research, the animals had exactly the same care and feeding conditions under the care management conditions of the-farm. The animals were fed twice a day at the same time, once at 09:00 and secondly at 16:00. Animals were fed with mixed roughage and concentrate feeds. Feeding was performed as total mixed ration (TMR). The crude protein ratio of the feed was 18% and energy value vas 2900 kcal/kg. Feed and water was offered ad libitum to all cows in both groups during the study. The minimum temperature of the region where the study was performed was between 7.2-14.1 °C and the maximum temperature was 13.1-22.5 °C during the periods of November 2017-April 2018 (Anonyms, 2021).

Sampling and measurements

At the end of the study, carpal and tarsal joint lesions, neutrophil-lymphocyte (N-L) ratio and some biochemical blood parameters (glucose, cholesterol, triglyceride and total protein) were determined in all cows in the groups using manure and sand bedding. Right and left carpal and tarsal joint lesions of all animal groups were determined by scoring method. The scoring system between 0-5 was used for cows' carpal and tarsal joint lesion scoring (Demirci, 2005). In the description: score (0) no lesion observed, (1) bare, pale areas, (2) bare, red areas, (3) occurrence of serum and/or sore scabs, (4) open, infected wounds, (5) disorders/swellings (edema on knees or feet) on carpal/tarsal joints.

During the research, behavioral characteristics of the animals (duration of standing, duration of lying/resting, number of steps, frequency of standing and lying) were followed and recorded on an individual and daily basis using a wristband tracking system, NEDAP Smarttag Leg - Cow Control System, placed on their ankles (Nedap Livestock Management, DC Groenlo, The Netherlands). NEDAP smart wristband system identify each cow and registers the standing, lying, walking and inactive behaviors of all cows 24/7. In this study, data was received from the NEDAP system. The standing and lying/resting time as a minute, number of steps data as a number were recorded separately for two separate groups. And, standing/lying frequency data as a how many times the cow lying down and stands up daily was recorded. Neutrophile-lymphocyte (N-L) ratio and the biochemical blood parameters (glucose, cholesterol, triglyceride and total protein) were evaluated for all animals in both groups. For the N-L ratio, 1 ml of blood sample was taken from cows' jugular vein with EDTA tubes.

After blood smears made of these blood samples were printed with May-Grünwald-Giemsa, there were 100 cells counted on each preparation (O'Loughlin et al., 2011). In order to determine biochemical parameters, 5 ml of blood sample was taken into serum tubes without anticoagulant from animals' jugular veins. After taken blood samples were centrifuged in 4 ºC, 2000x g for 15 minutes, serum parts were separated and were put in Eppendorf tubes while the rest was stored in a –80 ºC fridge after the emergent analysis were completed for further evaluations. Blood glucose, cholesterol, triglyceride and total protein levels were determined using appropriate commercial kits (Archem Diagnostic Ind. Ltd., Istanbul, Turkey) with autoanalyzer (Sinnowa D280, Sinnowa Elec. Technology Co., Nanjing, China).

Statistical analysis

Statistical analysis of the collected data was made using SPSS 22.0 statistical package program (Inc., Chicago, II, USA). In the statistical analysis, significance test of the difference between two means (Student's t-test) was used to evaluate N-L ratio and biochemical blood parameters, and behavioral characters of Holstein cattle. Carpal and tarsal joint lesions score were classified into six scales: score 0, no lesion in the carpal/tarsal joint; good; score 5, disorders/swellings in the carpal/tarsal joint; poor. As the data of carpal and tarsal joint lesions did not meet the assumptions of normality data were analyzed with non-parametric tests. The Mann–Whitney U test was used to determine the effect of manure and sand bedding groups on carpal and tarsal joint lesions data. Mann–Whitney U test is based on the median, not mean (Tai et al., 2022). Thus, the data are presented as the median, minimum, maximum, rank average and rank total. Paired Samples t-Test was used to determine the differences in behavior characteristics among months depending on manure and sand bedding groups.

RESULTS

Carpal and tarsal joint lesions scoring in the manure and sand bedding groups, rank average and total values are provided in Table 1 and Figure 1a, b. Although it is determined that the carpal joint lesion scoring rank average for animals in the sand bedding group was higher than those in manure bedding group with a rank average of 58.39; the effect of bedding on carpal joint lesion was found statistically insignificant. Tarsal joint lesion rank average was determined as 54.00 in the manure bedding group and 57.00 in the sand bedding group.

Average time of standing, time of lying/resting, number of steps and frequency of standing/lying related to manure and bedding groups and standard errors were presented in Table 2. In December, time of standing was identified higher for manure beddings with 288 minutes than in sand bedding group with 285 minutes (P<0.05). In the study, the highest average lying/resting time in bedding groups was identified as 692 minutes in March, in manner groups it was identified as 642 in April. Between November-April period, general average value for time of lying/resting was identified respectively as 638 and 689 minutes in manner and sand bedding groups (P<0.001). During research period, the lowest average number of steps was identified in manure group as 2365 in January, the highest average for number of steps was identified as 2762 in sand group in March. Overall, the average of standing/lying frequency within the study period of November-April was identified as 9.40 and 7.97 in manure and sand bedding groups respectively (P<0.001).

The average N-L ratio for manure group was identified as 1.07 while it was identified as 1.47 in the sand bedding groups (P<0.01). Serum cholesterol level among manure and bedding groups was identified respectively, as 108.04 and 123.62 mg/dL (P<0.05) (Table 3).

DISCUSSION

Carpal and tarsal joint lesions are important indicators for evaluating cow health (Endres, 2022). In the study, the effect of bedding type was found statistically insignificant on carpal and tarsal lesion scores. The highest carpal joint lesions score was found in manure and sand groups as "2", the highest tarsal joint lesion score was found as "1" in manure and sand groups. Similarly, Chaplin et al. (2000) indicated that the relation between bedding groups was not significant in terms of carpal and tarsal joint lesions as well as laminitis. Also, they indicated that having no severe recorded lesions -no carpal and tarsal joint lesions of greater than score "2". The manure and sand bedding reduce the incidence of the tarsal joint lesions in cows (Van Gastelen et al., 2011; Eckelkamp et al., 2016; Dimov & Marinov, 2021). Due to the low carpal and tarsal joint scores in bedding groups, it can be said that the use of sand and manure bedding underlay does not cause a problem in terms of carpal and tarsal joint lesions in Holstein dairy cattle.

Livesely et al. (2002) founded that the incidence of carpal and tarsal joint lesions in Holstein heifers in the mat bedding group was significantly higher than in heifers on straw and mattress bedding. Rushen et al. (2007) stated that the incidence of carpal and tarsal joint lesions was significantly high on dairy cows which were housed on concrete bedding.

Table 1. Effects of manure and sand bedding on the carpal and tarsal lesions scores of the joints in Holstein cows

Examined parameters	Group of manure bedding					Group of sand bedding					
	n	Median	Min - Max	Rank average	Rank total	n	Median	Min - Max	Rank average	Rank total	P
Carpal joint	55	0.00	0.00-2.00	52.61	2893.50	55	0.00	0.00-2.00	58.39	3211.50	–
Tarsal joint	55	0.00	0.00-1.00	54.00	3970.00	55	0.00	0.00-1.00	57.00	3135.00	–

n: Number of cows. -: P>0.05.

Although the overall average standing time values within November-April periods were found significantly close to each other (respectively 287 and 286 minutes in manure and sand bedding groups), the difference between bedding groups

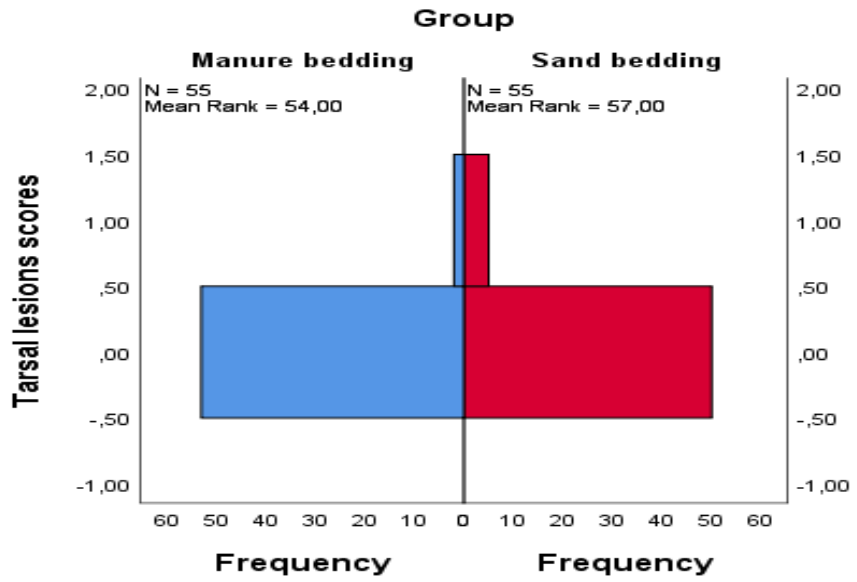

Figure 1. Effects of manure and sand bedding on the tarsal lesions scores of the joints in Holstein cows.

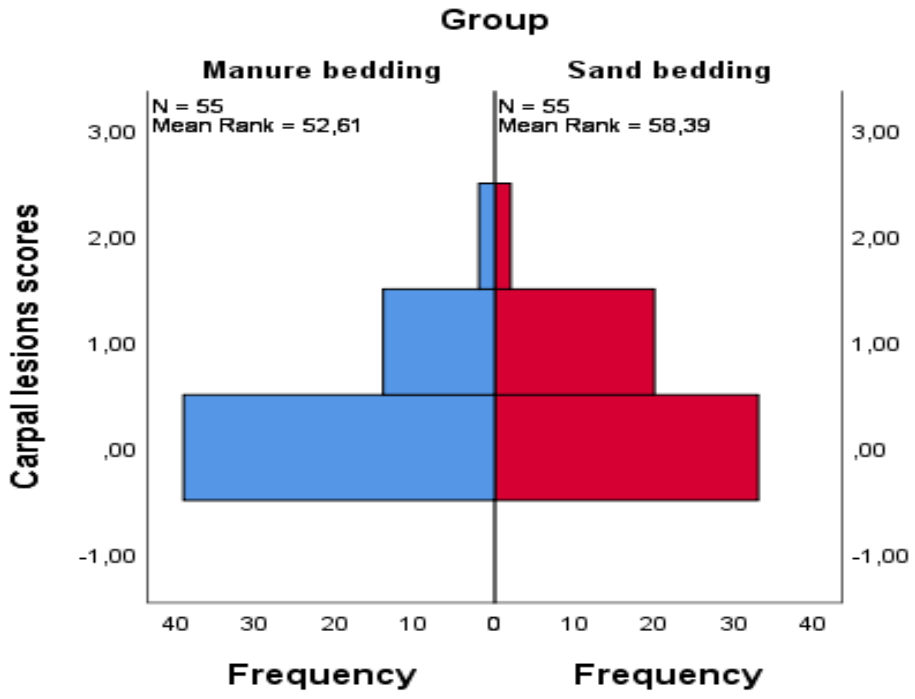

Figure 2. Effects of manure and sand bedding on the carpal lesions scores of the joints in Holstein cows.

were found statistically significant. Although they were small differences, this can only be explained by the fact that the large number of data set in the system obtained may have caused the value differences to be important between the groups which were measured with the NEDAP wristband system that had been following each animal 24/7 for six months. Kocyigit (2014) stated that one of the most important reasons for laminitis in dairy cows is long time of standing. According to Endres (2022), time of standing is used to evaluate cow health. Buyukok (2017) stated that in cases where concrete bedding is

used in dairy cow shelters, animals do not prefer to lie down on these types of beddings and spend most of their time standing. The fact that the overall average standing time in the manure and sand bedding groups were very close to each other in the study which suggests that the bedding was comfortable and the shelter is healthy in terms of animal welfare. The study findings (286 and 287 minutes) are in line with the results of other studies that reports the average standing time being between 0.3-13.0 hours/day (h/d) (Cook et al., 2004; Cook, 2008). The average free-stall cow spends 2.4 h/d standing in an alley socializing, moving between the feed bunk and stalls and returning from the parlor. Once in the stall, the average cow spends 2.9 h/d standing in the stall (Cook et al., 2004). Values determined by this study were found to be higher than the standing time (respectively 3.30 and 2.67 hours in free stall and free system shelters) that Grant (2007) stated in the study,

Table 2. Effects of manure and sand bedding on the carpal and tarsal lesions scores of the joints in Holstein cows

Bedding Groups- Standing Time (minute)						
		Manure Bedding		Sand Bedding		
Period (Month)	n	$\bar{x} \pm S^{\bar{x}}$	n	$\bar{x} \pm S^{\bar{x}}$	t value	P
November	55	287 ± 0.78	55	288 ± 0.80^{x}	-0.153	–
December	55	288 ± 0.80^{a}	55	285 ± 0.77^{b,y}	2.402	*
January	55	287 ± 0.79	55	285 ± 0.79^{y}	1.449	–
February	55	287 ± 0.81	55	286 ± 0.80^{x,y}	1.199	–
March	55	287 ± 0.79	55	287 ± 0.80^{x,y}	-0.055	–
April	55	287 ± 0.79	55	286 ± 0.79^{x,y}	0.876	–
Mean		287 ± 0.32^{a}		286 ± 0.01^{b}	2.324	*
P		-		*	–: P>0.05, *:P<0.05	
Bedding Groups- Lying/Resting Time (minute)						
		Manure Bedding		Sand Bedding		
Period (Month)	n	$\bar{x} \pm S^{\bar{x}}$	n	$\bar{x} \pm S^{\bar{x}}$	t value	P
November	55	634 ± 3.73^{b}	55	691 ± 4.09^{a}	-10.322	***
December	55	639 ± 3.56^{b}	55	684 ± 3.97^{a}	-8.528	***
January	55	638 ± 3.55^{b}	55	687 ± 3.83^{a}	-9.247	***
February	55	635 ± 3.62^{b}	55	690 ± 3.95^{a}	-10.332	***
March	55	638 ± 3.56^{b}	55	692 ± 3.90^{a}	-10.142	***
April	55	642 ± 3.55^{b}	55	689 ± 3.84^{a}	-9.054	***
Mean		638 ± 1.47^{b}		689 ± 1.60^{a}	-23.516	***
P		–		–	–: P>0.05, ***:P<0.001	
Bedding Groups- The Number of Steps						
		Manure Bedding		Sand Bedding		
Period (Month)	n	$\bar{x} \pm S^{\bar{x}}$	n	$\bar{x} \pm S^{\bar{x}}$	t value	P
November	55	2379 ± 15.82^{b}	55	2746 ± 10.98^{a,x,y}	-19.038	***
December	55	2407 ± 20.16^{b}	55	2744 ± 10.74^{a,x,}y	-14.738	***
January	55	2365 ± 8.84^{b}	55	2741 ± 10.52^{a,x,y}	-27.326	***
February	55	2369 ± 9.55^{b}	55	2737 ± 10.58^{a,x,y}	-25.788	***
March	55	2381 ± 9.06^{b}	55	2762 ± 10.66^{a,x}	-27.195	***
April	55	2379 ± 8.98^{b}	55	2724 ± 10.61^{a,y}	-24.866	***
Mean		2380 ± 5.27^{b}		2742 ± 4.36^{a}	-52.921	***
P		–		*	–: P>0.05, ***:P<0.001	

Table 2. cont. Effects of manure and sand bedding on the carpal and tarsal lesions scores of the joints in Holstein cows

	Bedding Groups- Standing/Lying Frequency					
		Manure Bedding		Sand Bedding		
Period (Month)	n	$\bar{x} \pm S^{\bar{x}}$	n	$\bar{x} \pm S^{\bar{x}}$	t value	P
November	55	9.44 ± 0.05[a]	55	7.93 ± 0.03[b]	26.555	***
December	55	9.41 ± 0.05[a]	55	7.96 ± 0.04[b]	25.441	***
January	55	9.38 ± 0.05[a]	55	7.99 ± 0.03[b]	24.394	***
February	55	9.39 ± 0.05[a]	55	7.98 ± 0.0[3]b	24.011	***
March	55	9.44 ± 0.05[a]	55	7.96 ± 0.03[b]	25.771	***
April	55	9.37 ± 0.04[a]	55	7.98 ± 0.03[b]	24.438	***
Mean		9.40 ± 0.02[a]		7.97 ± 0.02[b]	61.508	***
P		–		–	–: P>0.05, ***:P<0.001	

Table 3. Neutrophil-to-lymphocyte ratio and serum biochemical levels between groups and standart errors.

	Bedding groups					
		Manure bedding		Sand bedding	t value	P
Examined parameters	n	$\bar{x} \pm S^{\bar{x}}$	n	$\bar{x} \pm S^{\bar{x}}$		
N-L ratio	55	1.07 ± 0.06[b]	55	1.47 ± 0.10[a]	-3.217	**
Glucose (mg/dL)	55	45.78 ± 0.70	55	45.49 ± 0.78	0.279	–
Total protein (g/dL)	55	7.39 ± 0.06	55	7.30 ± 0.07	1.012	–
Cholesterol (mg/dL)	55	108.04 ± 4.77[b]	55	123.62 ± 4.72[a]	-2.322	*
Triglyceride (mg/dL)	55	24.09 ± 1.15	55	24.36 ± 1.07	-0.173	–

n: Number of animals in the groups. [a,b]: The difference between groups with different letters on the same line in terms of N-L ratio and biochemical blood parameters is statistically significant (P<0.05). *: P<0.05, **: P<0.01, -:P>0.05.

which analyzed the behavioral characteristics of cattle using the same approach. The result of the study for overall average being determined statistically significant among bedding groups in case of standing time during the study period (November-April), is compatible with other researches. According to the Rushen et al. (2007) in the study that was carried out in order to reveal the positive aspects of the soft shelter floor in terms of animal welfare, it was determined that animals had been spending much more time standing on concrete beddings than on rubber beddings. Haley et al. (2001) stated that animals had longer time of standing on concrete beddings while Calamari et al. (2009) stated they had longer time of standing on rubber mat and mattress beddings compared to sand and straw beddings.

In the months of November, December, January, February, March and April, it was determined that the animals in manure bedding group had tended to show longer lying/resting behavior than those in sand bedding group in terms of overall average value. This result can be explained by the fact that the frequency of standing up/lying down in the manure bedding group was higher than that of in the sand bedding group, which may have reduced time to lying/resting in cows. Lying/resting behavior was an important behavior for dairy cows which spend most of their lives in the semi-open barn system, and they spend about 45% of their time lying/resting on a daily basis (Haley et al., 2001). Longer time spent lying/resting behavior prolongs the rumination time, increases blood flow to the mammary gland, and lowers tension on the hoof (Rao et al., 2014). Cows tend to lying/resting, and if for some reason they are obstructed, they subsequently compensate the lying time at the expense of other activities, such as reducing social contact time, faster feeding, and more (Munksgaard et al., 2005).

Average lying/resting time found in study is (respectively 638 and 689 minutes in manure and sand bedding groups), and was compatible with the average values found by the studies of Haley et al. (2001), Cook et al. (2004), Tucker et al. (2021) while it was found a little less than the findings of Calamari et al. (2009) that state dairy cows spend approximately 50% of their days on lying/resting. The time the cow spends lying/resting behavior has been used in a number of studies as a measure of the comfort the resting area provides. Haley et al. (2001), reported that the lying time of lactating dairy cows which use mattress bedding in their beddings were much higher (12.3 h/d) than those with concrete (10.4 h/d). Once in the stall, the average cow spends 11.3 h/day lying in the stall (range 2.8-17.6) on average (Cook et al., 2004). Tucker et al. (2021) reported that the mean daily duration of time spent

lying down is around 11 h/d, but varies among cows from less than 6 h/d to more than 16 h/d. In study, the fact that the 45-50 cm thick sand layer on the suitably compacted soil floor served as a good cushion and provided drainage might have an impact on the result that the time of lying/resting was founded longer in terms of overall average in cows belonging to sand bedding group in all months examined within November-April period. In this case, it can be considered as the sand bedding being more comfortable for animals than manure bedding material. The results were in line with those reported previously by different researchers (Halet et al., 2000; 2001; Manninen et al., 2002; Tucker et al., 2003; Rushen et al., 2007; Koçyiğit, 2014). Tucker et al. (2003) determined the lying/resting time of dairy cows which use sand bedding in their beddings were much longer than those with sawdust. Manninen et al. (2002) found the time of lying/resting on cows which had 2-3 mm sand bedding without straw was much shorter than those with rubber bedding covered with straw and concrete bedding covered with lots of straw. Rushen et al. (2007) reported that the lying/resting time for dairy cows in mat bedding group was much higher than those in concrete bedding. Kocyigit (2014) reported that lying is an important behavior for dairy cows, and they spend respectively 32.57±0.67%, 36.79±0.91% and 37.66±0.68% in concrete, rubber mat and mattress bedding groups of their time lying down on a daily basis. According to the results of the study, it was expected that these differences in bedding groups in terms of lying down/lying time may be affected by conducting the studies under different environmental conditions and analyzing the effects of the diversity and different factors in the bedding types used in the study.

The NEDAP smart bracelet system based on the correct evaluation of the parameters monitored such as health, welfare and aggressive behavior. According to NEDAP-Livestock Management herd records, number of steps for healthy animals is 2500-3000 daily. In the study, it was determined that the number of steps in sand bedding groups were higher than those in manure bedding group in the months of November, December, January, February, March and April. The study results were found in line with the data obtained from NEDAP system average results and they provide information on number of steps which is one of the most important criteria of animal health level. Telezhenko & Bergsten (2005) examined the behavioral characteristics of dairy cows on five different bedding types: grid floor with or without 20 mm thick elastic rubber mattress cow, concrete floor with or without rubber and compacted sand. They found that the cows walk more slowly on the concrete grid floor with much shorter steps than on the sand bedding. It was determined that the cows on the concrete grid bedding took shorter steps than the cows on the sand bedding, however; they did not indicate a significant change in their walking speed. They found that the step lengths of the cows on the rubber bedding were longer than on the concrete grid bedding. As a result, it has been stated that the rubber bedding had a positive effect on the behavior of the cows.

In the study, it was determined that the animals in the group using manure bedding showed more standing/lying frequency compared to those in sand bedding group in the months. Tucker et al. (2003) found the lying frequency in cows which use sand bedding was much lower than those which use sawdust bedding. In the study, the fact that the standing/lying frequency being found higher in the manure group compared to the sand bedding group. This can be explained by the fact that the use of manure bedding increased the restlessness of the cows and the animals show frequent standing and lying behaviors during the day.

Davis et al. (2008) determined that the decrease in lymphocyte level and increase in neutrophil level causes N-L ratio to increase in animals under stress. In the study, N-L ratio in cows belonging to the ones in manure bedding group was found lower than those in sand bedding group. There are no researches found in literature review which evaluates the effect of bedding usage factor on N-L ratio. According to a study carried out by Hong et al. (2019) by examining the effects of stressed Holstein cows in terms of N-L ratio, they found the N-L ratio to be respectively as 2.32 and 1.27 in transported and non-transported cows. The study findings were in line with the results of other studies that reports the N-L ratio being above 1.00 (Hong et al., 2019) and between 0.6-1.4 (Kulberg et al., 2002). In the study, it can be said that administrative factors such as the diameter, dryness, thickness of the sand and how often it is changed should be reviewed considering that the high N-L ratio in the sand bedding group was affected by the stress of the cows in the sand bedding. In addition, the difference in the bedding groups may have been caused by the fact that the N-L ratio of cows was highly affected by some physiological factors such as estrus, pregnancy, lactation period, nutrition and health status, and the treatment of the animal before blood collection. Since biochemical blood parameters were not affected by a single factor, the variation in their measurements was quite wide as they have quantitative properties that can be observed under the influence of multiple factors. According to the studies that were conducted to determine biochemical blood reference levels in cattle, and the values for the range of change were reported as 40-80 mg/dL for glucose level, 80-120 mg/dL for cholesterol level, 15-45 mg/dL for triglyceride level and 6.7-7.5 g/dL for total protein level (Kayar, 2013). It has been observed that the biochemical blood values obtained as a result of the study were compatible with the results reported by Kayar (2013). In this study, bedding type had no significant affect, except for cholesterol level, on all blood parameters. Similarly, Kocyigit (2014) found no influence of bedding type groups (concrete, rubber and mattress) on serum glucose level in Brown Breed Cattle. Besides, he found that the effect of lactation order on glucose level was statistically significant ($P<0.05$). It was determined that serum cholesterol level was higher for the cows reared in sand bedding than those reared in manure bedding, although the difference between bedding groups in terms of serum cholesterol level was not statistically significant. In the light of these findings, it can be said that the biggest problem encountered in terms of blood parameters used to measure stress is the variation seen among animals in reactions to stress.

CONCLUSION

In conclusion, the type of bedding used in loose-housing of dairy cows has a considerable effect on the animal comfort provided by the free-stalls. When choosing bedding, first the comfort that it will provide to the animals must be considered. Results indicated that the use of sand and manure beddings in Holstein dairy cattle breeding is suitable in terms of protection against adverse effects on carpal and tarsal joint lesions. Cow behavior control provides the most precise and complete information about the health, welfare, and location status of individual cows and groups. The duration of lying is a good indicator of the comfort and bedding quality. The cows use sand bedding as compared with the manure bedding, which leads to increasing the length of the lying/resting time up to 689 minutes a day. Sand bedding have been shown to increase cow welfare by increasing the lying/resting time. Also, decreases time of standing and frequency of standing up/lying down behaviors with the sand bedding determine an improve in Holstein dairy cattle comfort. However, the manure bedding resulted in lower stress as indicated by the decreasing N-L ratio in cows. And, the use of manure bedding increased the blood cholesterol level, which is a biochemical parameter. In future studies, stress-related clues can be obtained when working with Elisa kits (such as total antioxidant capacity (TAS), total oxidant status (TOS), Superoxide dismutase (SOD)) directly related to stress. In order to reveal the effects of bedding type on some behavioral and welfare characteristics of cows, it is necessary to increase the knowledge on this subject by conducting new research.

DECLARATION

Ethics Approval

The Animal Care and Practice, Committee of Aydın Adnan Menderes University approved all procedures involved in this experimental study (13.06.2017-64583101/048).

Conflict of Interest

The authors declared that there are no conflicts of interest.

Consent for Publication

Not applicable

Author Contribution

Idea, concept and design: EDF, MA

Data collection and analysis: MA, EDF

Drafting of the manuscript: EDF, MA

Critical review: EDF, MA

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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