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Effects of stocking density on welfare indicators, rumen fill score, and systemic physiological responses in dairy cows

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Research Article

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Abstract

In this study, the effects of different stocking densities on welfare indicators, rumen fill score, haematological parameters, oxidative stress markers, immunoglobulin levels, and biochemical profile in dairy cows were evaluated. The cows were divided into three groups based on the area per animal (< $7m^2$, 7-$9.1m^2$, and > $9.1m^2$). Welfare assessment included hind leg dirtiness, hip-knee dirtiness, udder-abdominal dirtiness, body condition score (BCS), and rumen fill score. In addition, haematological parameters (WBC, RBC, HGB, HCT), oxidative stress markers (TAS, TOS, OSI, GPx, MDA), immunoglobulins (IgA, IgG, IgM), and biochemical parameters were analysed.

Significant effects of stocking density on hygiene scores were observed, with the hind leg, hip-knee, and udder-abdominal dirtiness scores significantly higher in Group 1 ($P < 0.00$). BCS were higher in the medium stocking density group ($P < 0.00$), while rumen fill scores increased in the high stocking density groups ($P < 0.00$). No statistically significant differences were found among the groups for haematological parameters, oxidative stress markers, and immunoglobulin levels, although minor numerical differences were observed in TAS and IgA values. However, significant differences were determined in biochemical analyses for Alkaline phosphatase (ALP), total protein (TP), albumin (ALB), globulin (GLOB), and the ALB/GLOB ratio ($P < 0.05$).

In conclusion, it was observed that stocking density had a significant effect on welfare indicators, such as hygiene status and rumen fill, in dairy cows, while oxidative stress and haematological parameters remained largely stable. However, protein metabolism and protein fraction balance were affected by stocking density. The findings indicate that optimal stocking density in shelter management is critically important for animal welfare and metabolic balance.

Introduction

In modern dairy operations, limiting the space per animal is a common management strategy to increase production efficiency and optimise housing capacity. However, increased stocking density can lead to significant changes in cows' behavioural patterns, social interactions, and physiological responses. Stocking density is one of the critical environmental factors that directly affects key components of welfare, such as access to feed and resting areas, lying behaviour, social competition, and hygiene conditions (Krawczel et al., 2012). Increased stocking density can lead to stress responses, particularly by increasing competition for limited resources. Overstocking practices have been reported to cause behavioural changes, reduced lying time, and increased physiological stress indicators in dairy cows (Huzzey et al., 2012). It has been shown that high stocking density during the dry period increases cortisol secretion and affects energy metabolism (Fustini et al., 2017). These findings indicate that stocking density can affect not only behavioural parameters but also systemic physiological balance. Lying behaviour is considered one of the strongest indicators of welfare in dairy cows. Adequate lying time is critically important for hoof health, metabolic stability, and overall physiological well-being. It has been reported that increased stocking density reduces lying time and disrupts resting behaviour (Tucker

et al., 2021). Restrictions in resting behaviour can lead to increased stress levels and, in turn, to impaired immune function.

Hygiene scores are among the practical and reliable indicators used to assess the effects of stocking density on welfare. Increased stocking density can lead to higher levels of dirtiness, particularly in the lower extremities and udder region, thereby increasing the risk of mastitis and hoof diseases (Fulwider et al., 2007). The Welfare Quality® assessment protocol recommends separate scoring of the udder, flank, and lower leu areas and defines hygiene indicators as fundamental components of welfare assessment (Welfare Quality, 2009).

Body condition score (BCS) and rumen fill score are commonly used in assessing nutritional status and metabolic health. Increased social competition due to higher stocking density can affect feed intake and feeding behaviour, thereby altering energy balance (Grant and Albright, 2001). The rumen fill score has been reported to be related to dry matter intake and to be a reliable tool for evaluating nutritional management in the field (Burfeind et al., 2010).

Intensive housing conditions and stress factors can also trigger oxidative stress mechanisms. Oxidative stress is defined as the disruption of the balance between reactive oxygen species and antioxidant defence systems and has significant effects on immune functions and metabolic processes (Sordillo and Aitken, 2009). Total antioxidant capacity (TAC) and total oxidant status (TOS) are commonly used parameters in the comprehensive assessment of oxidative balance, and standard references for these methods have been defined by Erel (2004, 2005).

The effects of stress related to stocking density on the immune system remain a controversial topic. It has been reported that social stressors and environmental constraints may affect immune responses and health status in dairy cows during transition periods (Chebel et al., 2016).

The aim of this study was to comprehensively evaluate the effects of stocking densities on welfare indicators, hygiene status, rumen fill score, haematological parameters, oxidative stress markers, immunoglobulin concentrations and biological profiles in dairy cows. It was hypothesised that increased stocking density would negatively affect welfare-related parameters and may induce measurable physiological and metabolic responses.

Materials and Methods

Animal material and experimental design

This study was conducted in three commercial dairy farms with free-stall housing systems. Holstein dairy cows in similar lactation stages and clinically healthy were used in the study. Animal welfare and hygiene assessment were conducted for all cows housed in the visited shelters (a total of 73 shelters), and the space allowance per animal was determined:

Group 1: <7m^2/animal

Group 2: 7–9.1m^2/animal

Group 3: >9.1m^2/animal

The stocking density groups were established considering the farm's existing housing capacity and animal distribution. All animals received the same ration throughout the trial period, and feeding and management conditions were standardised across groups.

Welfare and hygiene assessments

The following parameters have been determined for the assessment of animal welfare:

Hind leg dirtiness

Hip-knee dirtiness

Udder-abdomen dirtiness

Body condition score (BCS)

Rumen fill score

Hygiene scores were determined by visual assessment of the udder, flank, and lower extremities, following the Welfare Quality® protocol (Welfare Quality®, 2009). The same observer performed scoring.

Body condition score was assessed using a 1–5 scale system (Edmonson et al., 1989).

Rumen fill score was determined by visual assessment on a 1–5 scale (Burfeind et al., 2010).

Collection of blood samples

Blood samples were collected from 10 cows representing different sherlters within each stocking density category. Blood samples were collected from the jugular vein using sterile vacuum tubes. EDTA tubes were used for haematological analyses. Tubes with gel separators were used for biochemical, oxidative stress, and immunoglobulin analyses. Samples were stored under appropriate conditions until analysis.

Haematological analyses (White blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), hematocrit (HCT)) were performed using the Mindray BC 10 haematology analyser.

Oxidative stress analyses

Within the scope of oxidative stress assessment: Total antioxidant status (TAS), Total oxidant status (TOS) measured according to the methods defined by Erel (2004, 2005).

The oxidative stress index (OSI) was calculated using the following formula:

$$OSI = \left(\frac{TOS}{TAS} \right) x\ 100$$

Glutathione peroxidase (GPx) activity and malondialdehyde (MDA) level were determined using Mindray BS 240 automatic biochemical analyser.

Immunoglobulin analyses

Serum IgA, IgG, and IgM concentrations were determined using commercial ELISA kits (Relassay, Mega Tıp,Gaziantep, Türkiye).

Biochemical analyses

Serum biochemical parameters (Alanine aminotransferase (ALT), Gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP), Total cholesterol (TC), Creatinine (CREA), Calcium (Ca), Inorganic phosphatase (IP), Total protein (TP), Albumin (ALB), Lactate dehydrogenase (LDH), Triglyceride (TG), Total bilirubine (TBil), Glicose (GLU), Blood urea nitrogen (BUN)) measured using Mindray BS 120 automatic biochemical analyser.

The globulin (GLOB) value was calculated according to the following formula:

$$GLOB = TP - ALB$$

The ALB/GLOB ratio was also calculated.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to evaluate the data (MINITAB V20). An appropriate Tukey's test was used to determine differences among the groups. The level of statistical significance was set at $P < 0.05$.

Results

Welfare and hygiene parameters

The effects of stocking density on hygiene parameters are presented in Table 1. Statistically significant differences were determined between groups in terms of hindfoot dirtiness, hip-knee dirtiness, and udder-abdominal dirtiness scores ($P = 0.000$). The hindfoot dirtiness score was highest in Group 1 and lowest in Group 3. Similarly, hip-knee and udder-abdominal dirtiness scores were found to be

significantly higher in Group 1. Significant differences were observed among the groups in BCS (P = 0.000), with higher BCS values in Group 2. A statistically significant difference was found among the groups in terms of rumen fill score (P = 0.000), and an increase in rumen score was observed in Group 3.

Table 1
Effects of stocking density on welfare-related scores

	Mean ± S. E.			
Parameters	Group 1	Group 2	Group 3	P-value
Hind legs hygiene	2.819 ± 0.021^{a}	2.520 ± 0.019^{b}	2.418 ± 0.021^{c}	0.000
Hip-knee hygiene	2.775 ± 0.020^{a}	2.445 ± 0.022^{b}	2.249 ± 0.018^{c}	0.000
Udder-abdomen hygiene	2.819 ± 0.021^{a}	2.449 ± 0.022^{b}	2.256 ± 0.018^{c}	0.000
BCS	1.948 ± 0.016^{b}	2.034 ± 0.020^{a}	1.950 ± 0.012^{b}	0.000
Rumen fill score	2.990 ± 0.022^{b}	3.075 ± 0.020^{a}	3.119 ± 0.015^{a}	0.000

^{abc} Different superscripts indicate significant differences among means within the same row.

Blood parameters

No statistically significant differences were detected among the groups for haematological parameters (WBC, RBC, HGB, and HCT; Table 2).

Table 2
Effects of stocking density on haematological parameters

	Mean ± S. E.			
Parameters	Group 1	Group 2	Group 3	P-value
WBC ($x10^3$/μL)	8.380 ± 0.434	14.090 ± 3.540	8.770 ± 2.990	0.280
RBC ($x10^6$/ μL)	4.198 ± 0.124	4.224 ± 0.134	4.072 ± 0.356	0.880
HGB (g/dL)	9.300 ± 0.212	9.120 ± 0.188	7.940 ± 0.849	0.170
HCT (%)	18.258 ± 0.587	19.402 ± 0.709	18.480 ± 1.070	0.590

Similarly, no statistically significant differences were observed among groups for oxidative stress markers (TAS, TOS, OSI, GPx, and MDA; Table 3). A numerical decrease trend in TAS values was observed in Group 2 (P = 0.060).

Table 3
Effects of stocking density on oxidative stress markers

	Mean ± S. E.			
Parameters	Group 1	Group 2	Group 3	P-value
TAS (mmol/L)	1.066 ± 0.035	0.926 ± 0.039	1.072 ± 0.053	0.060
TOS (µmol/L)	10.130 ± 2.000	6.750 ± 1.870	18.82 ± 9.910	0.370
OSI	0.965 ± 0.201	0.709 ± 0.171	1.617 ± 0.787	0.410
GPx (U/L)	242.800 ± 13.000	287.200 ± 13.100	337.600 ± 86.800	0.450
MDA (nmol/mL)	56.130 ± 9.160	73.440 ± 9.430	79.560 ± 9.080	0.220

No statistically significant differences were detected among groups for serum immunoglobulin concentrations (IgA, IgG, and IgM; Table 4). However, a numerically increased trend in IgA levels was observed in Group 2 (P = 0.090).

Table 4
Effects of stocking density on immunoglobulin concentrations

	Mean ± S. E.			
Parameters	Group 1	Group 2	Group 3	P-value
IgA (mg/dL)	1.200 ± 0.346	4.520 ± 1.550	2.322 ± 0.556	0.090
IgG (µg/mL)	215.700 ± 12.900	223.300 ± 16.000	248.000 ± 18.700	0.360
IgM (mg/dL)	5.284 ± 0.708	3.880 ± 1.170	2.980 ± 0.616	0.210

Biochemical analysis results are presented in Table 5. Most parameters (ALT, GGT, TC, CREA, Ca, IP, LDH, TG, TBil, GLUC, and BUN) were not significantly affected by stocking density and were therefore not shown in the table. In contrast, ALP levels differed significantly among groups (P = 0.010). Significant differences were also observed for TP, GLOB, ALB, and the ALB/GLOB ratio (P < 0.05), with the highest TP and GLOB values in Group 2 and elevated ALB in Group 1.

Table 5
Effects of stocking density on biochemical parameters

	Mean ± S. E.			
Parameters	Group 1	Group 2	Group 3	P-value
ALP	57.333 ± 0.667^{a}	33.670 ± 2.730^{b}	49.000 ± 4.730^{a}	0.010
TP	8.410 ± 0.089^{b}	9.893 ± 0.445^{a}	8.827 ± 0.003^{ab}	0.020
ALB	3.107 ± 0.065^{a}	2.387 ± 0.098^{b}	2.670 ± 0.080^{b}	0.020
GLOB	5.303 ± 0.096^{c}	7.507 ± 0.528^{a}	6.157 ± 0.083^{b}	0.010
ALB/GLOB	0.586 ± 0.019^{b}	0.323 ± 0.037^{a}	0.434 ± 0.019^{ab}	0.000

^{abc} Different superscripts indicate significant differences among means within the same row.

Discussion

This study comprehensively evaluated the effects of stocking density on welfare, hygiene, rumen function, and systemic physiological responses in dairy cows. The findings revealed that stocking density had significant effects, particularly on welfare and hygiene parameters, while haematological and oxidative stress indicators remained largely stable. These findings are consistent with previous reports indicating that the primary consequences of increased stocking density are expressed through behavioural, hygiene, and comfort-related responses rather than overt systemic physiological disruption (Krawczel et al., 2012a; Krawczel and Lee, 2019).

The hygiene scores showed that contamination levels in the hind legs, hip-knee region, and udder-abdomen region increased significantly in Group 1. This finding is consistent with previous studies that revealed the relationship between stocking density and hygiene. It has been reported that increased stocking density increases the risk of contamination on shelter surfaces and prolongs the duration of contact between animals and manure (Fulwider et al., 2007; Krawczel et al., 2012b). Beaver et al. (2021) further highlighted that stall design and space organisation significantly influence stall hygiene and space-use behaviour, emphasising the importance of environmental layout in maintaining cleanliness and cow comfort. Consistent with this, Robles et al. (2021) demonstrated that freestall cleanliness is strongly associated with lying behaviour, hygiene status, and health risks, emphasising the importance of adequate space allowance and stall management. Poor hygiene is a significant risk factor, particularly for udder health and hoof problems. Therefore, the hygiene findings in our study support the direct effects of stocking density on welfare.

Body condition score results revealed higher BCS values in Group 2. This is consistent with a study indicating that increased social competition and stress under high-density conditions may negatively

affect feeding behaviour and energy balance (Grant and Albright, 2001). In addition, Wang et al. (2016) reported that increased stocking density reduces comfort indices and alters feeding and resting behaviour, which may, in turn, indirectly influence body condition. On the other hand, it is thought that increased activity levels under very low density conditions may increase energy expenditure and limit fitness differences. In this context, the increase in BCS observed in Group 2 may be related to the maintenance of behavioural and metabolic balance.

The increase in rumen fill scores in Group 3 reflects the indirect effects of stocking density on feed intake. Increased competition for access to forage areas can limit dry matter intake and may result in a decrease in rumen fill score (Burfeind et al., 2010). Stocking density and time-budget stress alter feeding opportunities and rumination behaviour by reducing lying time and increasing competition (Krawczel et al., 2019). Our findings suggest that the decrease in rumen score observed in Group 1 may be related to social competition and changes in feeding behaviour.

The absence of significant differences among groups in haematological parameters indicates that stocking density did not markedly affect systemic inflammatory response or haematological balance. Comparable findings were reported by Creutzinger et al. (2021), who observed that variations in prepartum stocking density did not consistently result in marked changes in physiological biomarkers, emphasising the importance of adaptive capacity. Similarly, oxidative stress markers did not differ significantly among groups, suggesting that stocking density did not induce a pronounced oxidative imbalance. Sordillo and Aitken (2009) report that oxidative stress responses are generally associated with severe metabolic loads or pathological conditions. Nevertheless, minor numerical variation in antioxidant capacity may reflect subtle physiological adjustments rather than oxidative dysfunction. However, elevated stocking density can contribute to oxidative stress under a specific physiological context. For instance, Lin et al. (2019) reported increased oxidative stress and altered mammary gland permeability in early lactating dairy cows exposed to higher stocking densities. The discrepancy between studies may indicate that oxidative responses to stocking density are context-dependent and influenced by physiological stage and management conditions.

The absence of statistically significant differences among groups in immunoglobulin concentrations suggests that stocking density did not markedly suppress the humoral immune response. Although not significant, IgA levels were numerically higher in Group 2, which may indicate subtle variation in immune responsiveness. IgA plays a central role in mucosal immune defense, and such variability may reflect adaptive physiological responses rather than pathological alterations. It has been reported that the effects of social stress and environmental stressors on the immune parameters are complex and may depend on stress duration, intensity, and adaptive capacity (Chebel et al., 2016). Supporting this interpretation, Matković et al. (2020) demonstrated that welfare and physiological outcomes under varying stocking densities can be modulated by management and environmental factors. Collectively, these findings suggest that the stocking density conditions evaluated in the present study did not exert a pronounced detrimental effect on humoral immune status.

Significant differences observed in biochemical analyses, particularly in parameters related to protein metabolism (TP, ALB, GLOB, and ALB/GLOB ratio), are defined as sensitive indicators of inflammatory conditions, physiological stress, and metabolic adaptation. The combination of environmental and nutritional stressors can activate immune-metabolic-inflammatory pathways, leading to changes in metabolic and protein markers (Cavallini et al., 2021). The ALB/GLOB ratio is a sensitive biomarker widely used to assess both clinical and subclinical inflammatory processes. A low ALB/GLOB ratio is generally associated with a positive acute-phase response, increased GLOB fraction, and increased inflammatory activity, whereas a high ALB/GLOB ratio has been reported to be linked to lower systemic inflammation and better physiological adaptation (Cattaneo et al., 2021). Similarly, changes in serum TP, GLOB, and ALB/GLOB ratio have been reported to differ significantly in various stress and inflammation conditions; particularly in conditions associated with physiological stress, such as complicated pregnancy, abortion, and dystocia, TP, GLOB, and ALB/GLOB parameters have been reported to be sensitive biochemical indicators (Ercan et al., 2022). Taken together, these findings support the view that protein fraction parameters, particularly the ALB/GLOB ratio, may represent sensitive indicators of subtle physiological and inflammatory adjustments to environmental and management-related stressors (Cattaneo et al., 2021; Ercan et al., 2022). In light of these findings, when the increased TP and GLOB levels observed in Group 2 and the elevated ALB values in Group 1 are considered together, these observations suggest that stocking density may influence systemic protein fraction dynamics, potentially reflecting adaptive metabolic responses rather than overt pathological alterations.

Conclusion

This study has demonstrated that stocking density significantly affects welfare-related parameters in dairy cows. A decrease in space per animal was found to be associated with significant deterioration in hygiene scores and clearly demonstrated a direct relationship between stocking density and environmental cleanliness. Similarly, the higher rumen fill scores observed under low stocking density conditions indicate that space adequacy plays a critical role in maintaining feeding behaviour and rumen function.

Haematological parameters, oxidative stress markers, and serum immunoglobulin levels were not significantly affected by stocking density. These findings suggest that the stocking densities evaluated in the study did not cause significant systemic stress or an inflammatory response. However, significant changes have been detected in biochemical parameters related to protein metabolism. The differences observed in total protein, albumin, globulin, and the albumin/globulin ratio suggest that stocking density may affect systemic protein balance and metabolic adaptation mechanisms. Protein metabolism-related biomarkers appeared to be more responsive to stocking density variations compared to other systemic parameters.

This study demonstrated that stocking density significantly influenced welfare-related hygiene parameters and rymen fill scores in dairy cows. In contrast, haematological, oxidative stress, and immunoglobulin parameters remained largely stable, suggesting that the evaluated stocking densities

did not induce pronounced systemic stress responses. Notably, protein metabolism-related biomarkers were affected by stocking density, indicating potential metabolic adaptations under varying housing conditions..

Declarations

Financial Support

This research received no specific grant from any funding agency, commercial entity, or non-profit organisation.

Ethical Approval

All experimental procedures involving animals were reviewed and approved by the Burdur Mehmet Akif Ersoy University Local Ethics Committee for Animal Experiments (Approval No: 29.03.2023/10-1051).

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors’ contributions

Hacı Düzen was responsible for stocking measurements, determination of stocking density parameters, and animal welfare and hygiene evaluations. Serkan Özkaya conceptualised and designed the study, contributed to the animal welfare and hygiene assessment, interpreted the data, and wrote the manuscript. Elif Rabia Şanlı performed blood sampling procedures and laboratory analyses, and interpreted blood data.

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Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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