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Influence of Repeated Regrouping on Productivity and Physiological Parameters of Dairy Cows

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ABSTRACT

The effect of social stress caused by the repeated regrouping of cows of different breeds (Holstein, red steppe, and Simmental) on milk productivity and quality indicators (milk yield, fat, protein) and physiological parameters: hormones (cortisol and adrenaline) and general blood parameters (number of leukocytes, lymphocytes, red blood cells, and hemoglobin) were studied. It was found that the effect of social stress caused by repeated regrouping of cows - in different breeds is heterogeneous. According to the set of physiological stress biomarkers, Holstein cows were the most susceptible, manifested in their increased adrenaline levels by 48.87% and hemoglobin by 15.37%. Also, the Simmental breed of cows characterized a significant increase in adrenaline concentration (an increase of 73.53%). In comparison, relatively stable animals of the red steppe breed can be considered (excess of cortisol by 18.23%). At the same time, the regrouping of cows did not affect changes in productivity and milk quality, and the detected deviations of physiological indicators were within the norm. The results indicate that social stress from regrouping affects breeds differently. Holstein cows were most susceptible, showing increased adrenaline and hemoglobin levels. Simmental cows also had a significant adrenaline rise, while Red Steppe cows were relatively stable with minor cortisol changes. Regrouping did not impact productivity (milk yield) or milk quality (fat, protein), and physiological changes remained within normal limits.

Key words: Cattle, Social stress, Cortisol, Adrenaline, Hemoglobin, Milk productivity.

INTRODUCTION

One of the reserves for increasing productivity in dairy cattle breeding is the realization of the genetic potential of animals based on the rational use of their behavioral reactions (Jakupov et al. 2024). In particular, the study of social stress in cows of different breeds makes it possible to find additional ways to increase their productivity under specific conditions of feeding and housing (Mussayeva et al. 2021; Beishova et al. 2024; Il et al. 2024).

It is known that frequent changes in herd composition, herd instability and crowding, rank interactions, low status, and isolation cause stress reactions in cows. This leads to a decrease in productivity and fertility, weakening of muscle tone, and violation of physiological functions (Dobson et al. 2001; Durham 2010; Ermakova 2017; Chernenko et al. 2018; Tallo-Parra et al. 2018) and affects the general state of animal health.

In addition, in all forms of stress in animals, slowing and intensification of intestinal peristalsis, decreased and loss of appetite, tachycardia, arrhythmia, muscle tension, muscle tremors, increased body temperature, dilated pupils, increased respiration and heart rate, urination and defecation are noted (Zelenkov et al. 2005; Yakhnik et al. 2024). An increase in salivary cortisol concentration during regrouping in dairy cattle was observed in a study, and a large display of aggressive and displacement behaviors during regrouping, thereby supporting the notion that regrouping does cause physiological signs of distress in dairy heifers (Denham and Progar 2023).

However, it is impossible to completely avoid regrouping in a herd. Most are carried out before, during, and after the dry period. During the lactation period alone, a herd of dairy cows undergoes regrouping up to four or more times (Smith et al. 2001; Bøe and Færevik 2003). When such regroupings occur, cows face a new

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environment (Schirmann et al. 2011), and new hierarchical relationships inevitably arise (Chebel et al. 2016). All this leads to the formation of agonistic behavior in animals, which increases sociohierarchical instability in the herd (von Keyserlingk et al. 2008) and creates competitive dominance relations in it (Kondo and Hurnik 1990). At the same time, the duration of the instability state may vary depending on the breed, individual characteristics, and previous experience of regrouping (Bøe and Færevik 2003; Soonberg 2021). However, according to other information, the number of regroupings in a herd does not change cow behavior and does not reduce the intensity of competition for dominance (Raussi et al. 2005; Raketsky et al. 2021).

However, all authors, without exception, have noted characteristic ethological signs associated with herd regrouping, manifested in the fact that animals spend more time standing without moving, are less often in a lying position, sniff the pen for a long time, and more often than usual demonstrate aggressive behavior (Raussi et al. 2005; von Keyserlingk et al. 2008).

Nevertheless, despite the importance of the problem of social stress, there are very few studies investigating the effect of regrouping, especially repeated regrouping of cows, on their productivity and physiological parameters. This could be due primarily to the complexity of the diagnostic evaluation of the stress state (Zaitsev et al. 2024). At the same time, milk productivity (milk yield and amount of protein), changes in hormonal background (cortisol, androgen, thyroxine, somatotropin, etc.), hematological parameters (red blood cells, hemoglobin, etc.), as well as glucose and fructosamine are most often considered as its possible markers (Möstl and Palme 2002; Bristow and Holmes 2007).

As a solution to the problem of social stress, Lyu et al. (2022), in their study aimed at investigating acute physiological and behavioral variations of individually- or group-housed calves after being introduced into a mixed group, proposed a hypothesis that social integration before regrouping may relieve stress, but more evidence is needed to verify this hypothesis. Lindner et al. (2021) concluded in their study that timely grouping, especially from birth, positively affects social behavior, displays no detrimental effect on performance, and, contrary to existing beliefs, does not affect the health of the calves.

Our study aimed to investigate the effect of social stress caused by the repeated regrouping of cows of different breeds (Holstein, Red Steppe, and Simmental) on hormonal status, indices of general blood analysis, and milk productivity.

MATERIALS AND METHODS

Ethical approval

All studies were conducted following the European Convention for the Protection of Pet Animals (1987), the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (2006), the Universal Declaration on Animals (1978), as well as following the national legislation of Kazakhstan (On responsible treatment of animals. Law of the Republic of Kazakhstan dated December 30, 2021 No. 97-VII LRK).

The research was conducted from August 2023 to November 2023. 27 adult Holstein cows, 31 red steppe cows, and 28 Simmental were selected for the experiment. In each breed group, the animals were approximately the same age, had similar lactation periods, and had previously participated in regrouping.

All animals were clinically healthy, had a well-established social hierarchy, and no ethological signs of stress were observed; prior to regrouping, the cows were kept on the farm untethered and had resting places (stalls) and free access to feed and water. The main ration consisted of corn silage, haylage, and grass hay; premixes were used to compensate for mineral deficiency. Cows were milked twice: in the morning - at 5 o'clock and in the evening - at 17 o'clock.

The stress factor in the experiment was the addition of 36 new cows of the same breed, approximately of the same age and lactation period, to the experimental groups of animals. At the same time, one day before exposure to the stress factor, the cows of the experimental groups had a control milk yield with the determination of fat and protein content in milk. Blood samples were taken to analyze the level of hormones (cortisol and adrenaline) and general blood parameters (total number of leukocytes, lymphocytes, red blood cells and hemoglobin) (Hopster et al. 1999; Caroprese et al. 2010). Similar studies were carried out within 30 days after regrouping the animals. Blood samples from cows were taken from the tail vein in the morning before feeding.

Quantitative determination of cortisol and adrenaline was investigated in blood serum by solid-phase enzyme immunoassay on a Tecan Infinite F50 microplate reader (Tecan, Austria) using a Bovine Cortisol Elisa kit and Bovine Epinephrine/Adrenaline (EPI) ELISA kit (BlueGene Biotech, China). Blood analysis of experimental animals included counting the number of erythrocytes, leukocytes, and lymphocytes in a Goryaev chamber and hemoglobin determination on a photometer KFK-3-01 ZOMZ (Russia). At the beginning and the end of the experiment, the milk yield of experimental animals was determined. Milk quality parameters (fat and protein content) were obtained on a Lactan-700 device (SibagroPRIBOR LLC, Russia). Sample data were analyzed with a preliminary determination of normality of distribution by the Kolmogorov-Smirnov and Shapiro-Wilk criteria. In case of failure to fulfill this provision, the nonparametric Mann-Whitney U-test (M-W U-test) was used, otherwise - Student's t-statistic (Triolo et al. 2018).

The standard error (Sx) was determined as an indicator of variability of the mean value of the trait (X). Grouping of primary data and biometric calculations were performed using Microsoft Excel and STATISTICA programs.

RESULTS

Stress is a set of complex physiological reactions occurring in the body. Considering that any stress, including social stress, affects the endocrine processes in the animal body, hormones are usually used as the main biomarkers of stress state. One of the indicators of the presence of stress is an increase in the level of cortisol, increased concentrations of which are associated with the occurrence of anxiety. In our case, reliable differences in this indicator were obtained only in red steppe cows, in which the concentration of this glucocorticoid hormone

increased by 18.23% compared to the state before the stress factor exposure (Table 1).

Table 1: Changes in serum cortisol and adrenaline concentrations in cows of different breeds under the influence of stress

Cattle breed	Regrouping	Cortisol (nmol/mL)	Adrenaline (pg/mL)
Holstein	A	67.43±5.106	45.71±4.588
(n=27)	B	79.72±5.861	68.05±9.489*
Red steppe	A	63.08±4.390	40.35±4.022
(n=31)	B	75.21±4.168*	57.61±9.068
Simmental	A	71.63±4.400	38.54±4.203
(n=28)	B	72.60±4.400	66.88±9.557**

A=Before; B=30 days after. Significance level: *P<0.05; **P<0.01.

In our experiment, the stress induced by regrouping cows led to a notable rise in adrenaline levels in the blood serum. This increase was observed exclusively in Holstein cows, with a 48.87% rise compared to their pre-stress levels, and in Simmental cows, the increase reached 73.53%.

In addition to the increase in the concentration of hormones, the total number of erythrocytes and hemoglobin levels increased in most cases under stress. Along with mental overload caused by regrouping, other stress factors, such as infectious diseases and traumatic injuries, also cause an increase in hemoglobin. According to our results, a significant deviation was obtained among the general blood parameters for hemoglobin and only in Holstein cows (excess of 15.37%) (Table 2). However, in our case, the regrouping of cows did not affect the decrease in milk yield or shifts in protein and fat content (Table 3). In other words, we can see certain similarities in the response to stress caused by the regrouping of cows by the absence of changes in their productive parameters and differences in physiological parameters.

At the same time, the impact of social stress caused by cow regrouping turned out to be heterogeneous, depending on breed affiliation. In particular, Holstein cows were the most susceptible to stress according to the set of biomarkers detected, manifested in an increase in adrenaline and hemoglobin levels. Also, the Simmental breed of cows was characterized by a significant increase in adrenaline, while the animals of the red steppe breed can be considered relatively stable.

DISCUSSION

Cows are inherently social animals and establish complex hierarchical relationships among themselves (Raussi et al. 2005; Sailo et al. 2017). In this regard, the regrouping of animals causes social stress in them, accompanied by anxiety, which activates the hypothalamic-pituitary-adrenal axis, which leads to an increase in the production of glucocorticoid hormones, including cortisol (Negrão et al. 2004), which we can observe in red steppe cows. In addition to an increase in the level of glucocorticoid hormones, the consequence of the stress response is the activation of the sympathetic nervous system with the release of noradrenaline and adrenaline into the blood (Lefcourt and Elsasser 1995). In our study, regarding the influence of repeated regrouping on dairy cows' productivity and physiology, we applied biological markers and milk yield as determinants, unlike the popular behavioral markers. For our study, we also measured the adrenaline content in the blood, a less common practice among research trends. Our methods agree with the work of Koenneker et al. (2023), who used milk yield and blood samples to detect cortisol as determinants.

Our findings showed that Holstein cows exhibit the highest stress levels, which aligns with recent research by Denham and Progar (2023), which recorded high cortisol concentrations in Holstein cows under stress after regrouping. In addition to cortisol, a steady state of anxiety is accompanied by the release of adrenaline and noradrenaline, mediators of the sympathetic nervous system, into the blood. During their separation, an increase in adrenaline concentration was observed in cows and calves (Lefcourt and Elsasser 1995). At the same time, early weaning steers were more stress-resistant during transportation and transfer to the feedlot and had a better feed conversion ratio (Arthington et al. 2005).

In addition to this, our results displayed an increase in cortisol levels after regrouping. This data contradicts with the results of Marumo et al. (2024), who concluded that there was no change in cortisol after regrouping. This may be due to the duration of the study, a pre-developed resilience, or a familiarity developed among the breeds. Foris et al. (2021) concluded that to reduce the effect of regrouping, the breed should be considered. Regrouping had little to no effect within subgroups of familiar cows.

Table 2: Changes in hematologic indices in cows of different breeds under stress

Cattle breed	Regrouping	RBC (10^6/μL)	WBC (10^3/μL)	LYM, (10^3/μL)	HGB (g/L)
Holstein breed (n=27)	Before	6.53±0.105	9.18±1.45	5.46±1.198	104.93±1.718
	30 days after	6.74±0.132	8.75±1.988	5.31±1.471	121.06±1.901*
Red steppe breed (n=31)	Before	6.68±0.108	9.04±1.988	4.91±1.330	109.23±1.645
	30 days after	6.73±0.095	7.82±1.781	3.76±0.908	107.65±1.396
Simmental breed (n=28)	Before	6.72±0.128	9.41±2.127	4.71±1.246	108.68±1.588
	30 days after	6.79±0.127	7.16±1.055	3.14±0.734	100.04±1.630

Significance level: *P<0.05.

Table 3: Changes in milk yield and milk quality indices in cows of different breeds under the influence of stress

Cattle breed	Regrouping	Milk yield (kg)	Fat (%)	Protein (%)
Holstein breed (n=27)	Before	28.86±1.509	3.87±0.153	3.22±0.005
	30 days after	26.03±2.895	3.59±0.133	3.24±0.015
Red steppe breed (n=31)	Before	17.88±1.288	3.92±0.102	3.42±1.330
	30 days after	17.56±1.091	3.63±0.104	3.44±0.908
Simmental breed (n=28)	Before	16.18±1.004	3.80±0.119	3.39±0.036
	30 days after	15.07±1.300	3.67±0.099	3.35±0.019

Thus, small groups of familiar individuals can help alleviate social stress associated with the regrouping of dairy cows, especially in large-scale dairy farms.

Adrenaline released into the bloodstream activates the sympathetic nervous system. As a result, the number of heartbeats and blood pressure increase, and rapid breathing appears. The body begins to feel the need for more oxygen and nutrients. Under these conditions, the number of red blood cells increases, and the hemoglobin level rises.

In our experimental model, a reliable increase of adrenaline concentration relative to the pre-stress state in Simmental and Holstein cattle and in the case of cortisol in red steppe cattle was within the normal range. In this case, the increase in hemoglobin in Holstein cows, together with a jump in adrenaline, can be considered as a manifestation of a prolonged reaction to stress (Lefcourt and Elsasser 1995). The changes in adrenaline levels in dairy cows align with the works of Fiol et al. (2024), who showed that social regroupings provoked endocrine responses, leading to a range of behavioral actions based on the hierarchy of the dairy cow in the social setting. These endocrinal responses also played a role in determining the milk yield.

It is important to note that cortisol decreases milk protein synthesis and, together with adrenaline, inhibits oxytocin release, which leads to a decrease in milk yield (Buryakov et al. 2016). In parallel with the physiological changes caused by animal regrouping, behavioral competitive relationships for forage resources also increase (Dobson and Smith 2000), increasing the probability of milk yield, especially in low-ranking cows. For example, introducing a new cow into a group reduces its average milk yield by about 5% (Lefcourt and Elsasser 1995).

However, according to our results, the average milk yields of cows of all three breeds under consideration changed unreliably both before and 30 days after regrouping. This may be because the animals studied have been previously subjected to regrouping. Moreover, repeated regroupings affected not only dairy (Raussi et al. 2005) but also beef productivity of cattle (Gupta et al. 2005). Our study showed that regrouping did not significantly affect the breeds' milk yield. These results disagree with the works of Marumo et al. (2024), who concluded that there was a noticeable reduction in the milk yield, protein content, and composition after regrouping. This may be due to the duration and nature of the experiment or the breed under study, advancements in management practices, and the animals' pre-exposure to similar stressors, contributing to their developed resilience.

In addition to the repetition and intensity of exposure to a stress factor, resistance to it is determined by the adaptive capabilities of animals (Lacetera et al. 2002; Salak-Johnson and McGlone 2007), which, in addition to individual characteristics, are also determined by their breed affiliation. Although we failed to establish interbreed differences in the studied stress biomarkers, the Holstein breed of cows was the most sensitive to rearrangements according to the set of indicators manifested in the stress response.

The conclusion that the effect of social stress is breed-dependent creates room for further research and genetic analysis to understand why an indigenous breed like the Red steppe breed remained stable. At the same time, the Holstein cow displayed many biomarkers.

Nevertheless, despite the large number of available physiological biomarkers of stress, the simplest and most effective way to detect stress in cattle is still considered to be a decrease in milk yield (Grelet et al. 2022). At the same time, prolonged exposure to stress in dairy cattle can lead to the development of mastitis (Heikkilä et al. 2012) and impaired lactation function (Wenzel et al. 2003).

As for the diagnosis of stress state in dairy cattle, it is suggested that cortisol levels in hair and fructosamine levels in blood be determined as reliable physiological markers (Grelet et al. 2022). According to our data, when detecting stress response in cows, it is preferable to conduct studies on the content of cortisol and adrenaline in blood serum, as well as hemoglobin in whole blood. Our results also support the global adoption of breed-specific breeding and management strategies since cattle breeds have differences in tolerance to social stress. Theoretically, this could be extended further to include breed-specific techniques for stress management that provide additional value to overall productivity at the herd level, especially for large-scale dairy enterprises where regrouping is popular. Further research should also investigate long-term physiological adaptations to repeated stress exposures, including hormonal patterns, immune responses, and the development of chronic stress markers.

Conclusion

Exposure to social stress caused by re-grouping of cows is heterogeneous across breeds. According to the set of physiological stress biomarkers, Holstein cows were the most susceptible, manifested by an increase in their adrenaline and hemoglobin levels. A significant increase in adrenaline concentration also characterized the Simmental breed of cows, while animals of the red steppe breed can be considered relatively stable (insignificant increase in cortisol). At the same time, the regrouping of cows did not affect the change in productivity (milk yield), and milk quality (fat, protein), and the detected deviations of physiological indicators were within the norm.

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