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Effects of Stocking Rate on Behavior, Serum Cortisol and Heart Rate of Hanwoo (*Bos taurus coreanae)* Heifers.

Moon SH[1], Kim DH[1], Park JH[1], Kim SJ[1], Oh MR[1], Kim DH[2], Cho WM[3], Jeon JH[3], Jang SY[1], Jeon BT[1], and Kim NY[1*]

[1]Division of Food Bio Science, Korea Nokyong Research Center, Konkuk University, Chungju, 380-701, Republic of Korea
[2]Gyeongnam National University of Science & Technology, Jinju, 660-758, Republic of Korea
[3]National Institute of Animal Science, RDA, Wanju, 565-851, Republic of Korea

ABSTRACT

This study aimed to provide basic information to allow a proper feeding environment for Hanwoo heifers by determining the effect of different stocking rates on behavioral and physiological responses. Six heads of heifers aged 18months and weighing 242.08kg (±19.93kg) were used for the experiment. Heifers were studied using three different stocking rates (controls: 10.07m^2/head; T1: 5.33m^2/head; T2: 16m^2/head) to measure behavior, heart rates, and cortisol level in the blood. Time spent lying down at rest was highest for controls and lowest for the T1 ($P<0.05$). Time spent standing at rest was highest for the T1 and lowest for controls ($P<0.05$). Eating, sniffing and self-grooming for control and T2 were significantly high, while fighting was low compared with the T1 ($P<0.05$). According to analysis of the blood cortisol level, a significant difference was observed between the T1 (0.89ug/dL) and the other groups ($P<0.05$). Heart rates during lying down resting, lying down rumination, standing rumination and fighting were significantly high in the TI ($P<0.05$). Overall, heart rates were high during fighting and eating for all groups ($P<0.05$). Consequently, in terms of animal welfare and productivity, a space allowance of around 10m^2/head is considered suitable for feeding Hanwoo heifers.

Keywords: Hanwoo, Stocking rate, Behavior, Blood cortisol, Heart rate,

Corresponding author

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INTRODUCTION

An intensive production system focused mainly on productivity of livestock has caused several critical issues, such as widespread chronic infectious disease, reduction of disease resistance, abuse of antibiotics and drugs, and decrease in quality of animal products [17]–There has also been an increase in consumer' dissatisfaction and a decrease in reliance on animal products.

Recently, some researchers have become more interested in using behavioral responses of farm animals as a way to evaluate their welfare [10]. Behavior can be a form of commentary by an animal regarding its own internal state, and it can make an important contribution to our understanding of the biology of the animal being studied [9]. Studies of cow behaviors are being extended to include the sizes and forms of the livestock to determine whether the welfare of the cows living in the artificial environment can be improved [7]. The provision of adequate space allowance during the housing period was important for animal productivity and welfare in most cases [3].

There was some obvious evidence regarding the relationship of space allowance and behavioral response of the animals. Increasing stocking rate caused an increase in resting behavior [13]. As space allowance for cattle decreases, the reduction in available space for lying might reduce resting times, and aggressive behavior might increase [27]. It is thought that low-ranked animals in the social order, in particular, would have more reduced resting with decreased space allowances [5]. Thus, because of complex social relationships among animals in the cattle group, individual and social behaviors of cattle such as eating, resting, fighting, and grooming are influenced by stocking rate [8], [20]. However, a change in the rearing environment can alter the circadian rhythm [20], [26] and increase the mean heart rate [19] in cattle, and it can increase serum cortical concentration in pigs [4]. Cortisol, an adrenocortical hormone, is known as the stress hormone and is important in inducing defense responses against external stimulation [6]. Continued stress decreases immune functions and reduces resistance to various diseases.

Hanwoo (*Bos taurus coreanae;* Korean native cattle) are mainly raised for beef production and have good quality meat. Interest in customer' needs, which are mainly focused on high-quality and eco-friendly animal production systems [11], and in animal welfare are required. Since cattle grazing is limited in Korea as its territory is small, most of Hanwoos are generally raised in cattle house. Therefore, it is necessary to determine behavioral and physiological characteristics related to feeding conditions in the Hanwoo. However, there are scarce studies regarding behavioral and physiological approaches to investigate whether Hanwoo are allowed proper feeding space and, especially the stocking rate.

This study aimed to provide basic information to allow proper feeding environments for Hanwoo heifers by determining the effects of different stocking rates on their behavior, heart rate, and serum cortisol levels.

MATERIALS AND METHODS

Experimental animals

Six heads of Hanwoo heifers aged 18 months and weighing between 215kg and 268kg (242.08 ± 19.93 kg) were used for the experiment and the field trial was performed at Hanwoo farm (Saebom farm, Icheon, Korea) from July to November 2011. Rice straw and corn-based concentrates (Table 1) were supplied twice per day (at 08:00 and 18:00) during the trial period. Animals had free access to fresh water and mineral blocks at all times. All animal-based procedures were approved by the Institutional Animal Care and Use Committee at Konkuk University (KU11002).

Experimental design

To determine the effects of stocking rate on behavioral and physiological responses of the same individuals, heifers were sequentially arranged into groups with three different stocking rates (control: 10.07m^2/head, T1: 5.33m^2/head, T2: 16m^2/head; control→T1→control→T2) to measure behavior, heart rate and the level of the hormone cortisol in the blood. The trial consisted of 20 days of adaptation, a 10-day preliminary period, and a 3-day collection period for each stocking rate group (Table 2).

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Table 1: Chemical composition of experimental diets and feed ingredients

Items	Rice straw	Basal concentrates
Chemical composition		
Dry matter (%)	85.79	83.83
Crude protein (%DM)	4.63	13.28
Ether extract (%DM)	1.45	3.04
Crude fiber (%DM)	27.91	5.86
Crude ash (%DM)	11.25	5.05
Ingredients (%)		
Ground corn		47.8
Wheat bran		41.0
Soy bean residue		5.0
Rapeseed residue		2.0
Molasses		2.0
Calcium phosphate		1.5
Salt		0.4
Vitamin-mineral additive		0.2
Lasalocid		0.1
Total		100.0

Table 2: Heifer allocation and experimental design of the trial

Item	Group		
	Control	T1	T2
Pen size	8×8 m	4×8 m	12×8 m
Animals per pen (n)	6	6	6
Space area per individual	10.07m^2	5.33m^2	16m^2

Behavioral observation

Behaviors of heifers were continuously recorded for 24 hours with three replications by digital video cameras during each collection period. The cameras (SDC-435, Samsung, Seoul City, Korea) and multiplexers (PDR-XM3004, Egpis, Goyang City, Korea) were used for monitoring the behaviors of each heifer. Cameras were arranged so that there were no blind spots. Nine behaviors, lying down resting (LD), lying down rumination (LDR), standing resting (ST), standing rumination (STR), Eating (EA), walking (WA), sniffing (SF), self-grooming (SG) and fighting (FT), of focal animals were observed. Expressed behaviors were recorded as a unit of time (minute).

Heart rate determination

Heart rate was measured for 48 hours to determine a relationship between heart rate and behavior. Heart rate was recorded with heart rate meter (Bodypro 100, Du-sung Technology, Daejeon City, Korea) in the same six heifers depending on stocking rate. The heart rate monitoring device was activated at the start of the trial and synchronized with video recording of behavior to match behavioral and physiological data. Heart rate was analyzed considering total heart rate and changes in mean heart rate according to each behavior.

Measurement of serum cortisol concentration

In this experiment, blood samples of approximately 10 ml were drawn from the caudal vein of all heifers at the end of the trial for analysis of serum cortisol concentration. Collected samples were immediately centrifuged at 3000 rpm for 30 minutes to separate serum. The serum samples were analyzed using an endocrinology analyzer (A-360, TOSHO, Minato-ku, Japan) to determine cortisol concentration.

Statistical analysis

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The main effects between treatments were subjected to analysis of variance (ANOVA) using the general linear model procedure of SAS (version 6.0, 1989). The differences between means were assessed by Student's *t* test and statistical significance was defined as P<0.05.

RESULTS ANDDISCUSSION

Effect of stocking rate on behavior of Hanwoo heifers

Time spent during various behavioral activities with different stocking rates are given in Figure1. Time spent LD was highest for controls (774.9min) and lowest for the T1 group (538.9min) (P<0.05). In contrast to LD, time spent ST was highest for the T1 (901.2min) and lowest for controls (665min) (P<0.05). Time spent STR in the T1 (126.3min) was higher than for controls (102.9min) and the T2 (113.9min). Interestingly, EA times for controls (144.4 min) and the T2 (148.3min) were significantly high compared with that for the T1 (105.6min) (P<0.05). The SG time was highest for controls (28min) and lowest for T1 (4.7min) (P<0.05). The WA time was not significantly different among groups, but it was highest for the T2 (27.3min) and lowest for the T1 (10min). Time spent SF in the T1 (5.2min) was lower than that of the control (10.6min) and the T2 (9.8min) (P<0.05). Contrary to the SF, time spent FT in the T1 (7min) was higher than that of the control (4min) and the T2 (4.1min) (P<0.05).

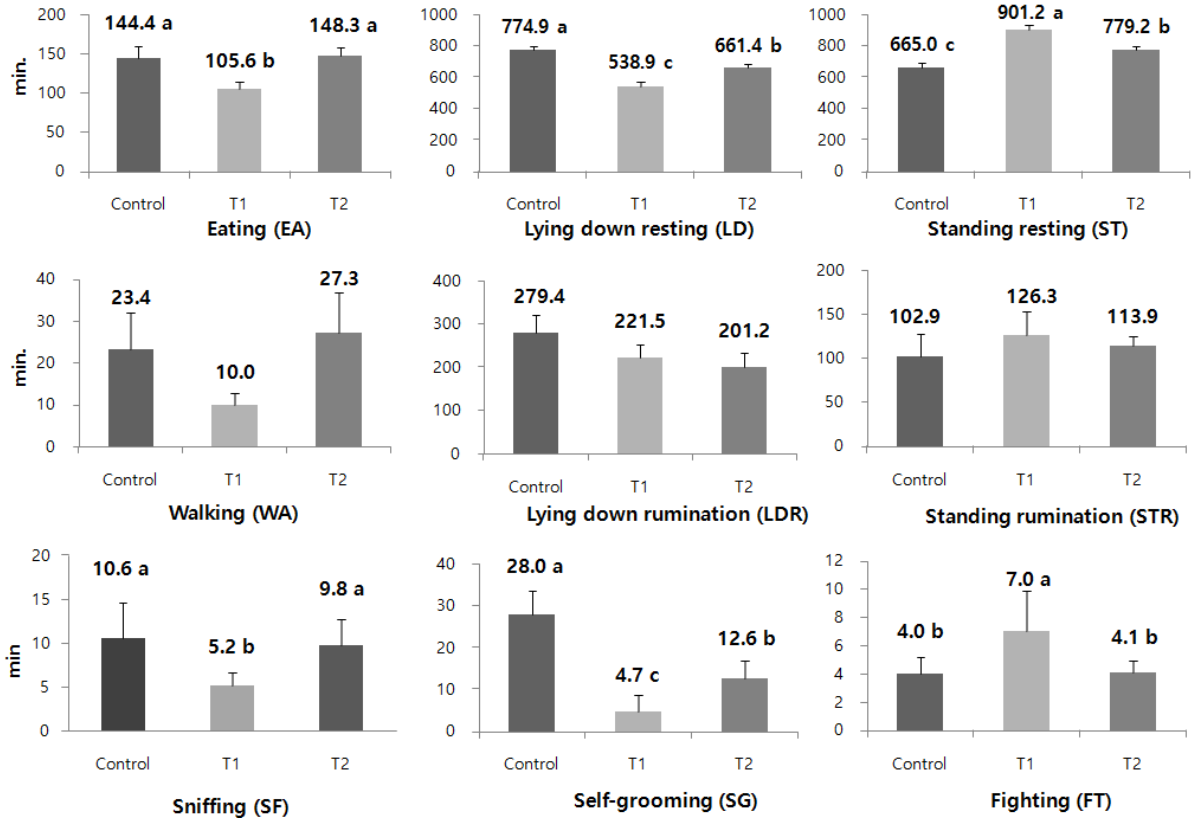

Figure 1: Time spent on each behavior by different stocking rate in Hanwoo heifers.
[a, b,c]Means with different superscripts among the groups are different(P<0.05)

The main finding of this experiment was that the lowest space allowance for T1 reduced LD time while increased ST time. It means, comfortable resting space was decreased by less than 10m²/head, and there was also a decrease in LD behaviors. The cattle spent their time mostly in the lying state during rest. The bulls spent approximately 12 hours per day in the lying state [14], whereas milk cows and prepartum cows spent 9 hours in the lying state [22]. According to recently found cows spend an average of around 12h/d lying down [15]. The calves in the cowshed also spent 71% of their total time in the lying state [25]. The results of this study indicate that the LD time was highest for control; the T1 showed the lowest effects (P<0.05).

Social behavior of pair wise grooming is a good measure of the growth or health of the cow [1]. Furthermore, it is important to determine the social structure in their group [18]. Behavioral patterns that

indicated good feelings in cows were cleaning behaviors such as biting, scratching and SG [16]. Moreover, parasites and contaminants would remove the function of SG which actually protects cattle from disease. The experiment of restricted space allowance for groups of housed heifers resulted in reduced SG time.

The SF is a very important means for social relationships [15]. Cattle could distinguish themselves and convey their emotions through the use of pheromones. In this experiment, it was understood that those in the control and T2 had more interest in social relationships than did those in the T1.

In this experiment, the stocking rate was also related to FT. There was significant difference between groups in FT ($P<0.05$). As space allowance decreases, aggressive behavior might increase [27]. Once the social ranking is determined, it continues for more than 8 years [21]. A cow needs 4.9m² of space to avoid other cows and, considering the transfer, each cow needs 7m² of area inside the paddock [23]. This minimum space is supposed to be individual space.

In this experiment, stocking rate influenced LD, ST, EA, SF, SG and FT. Strictly restricted space allowance by less than 10m²/head of housed heifers reduced time spent LD, EA, SF and SG, while it increased ST and FT. These measurements appear to be the most useful for evaluating the response to stocking rates in the Hanwoo heifers.

Effect of stocking rate on blood cortisol of Hanwoo heifers

The blood levels of cortisol according to stocking rate are shown in Figure2. The blood cortisol level was significantly higher in the T1 (3.37ug/dl) than in the control (0.66ug/dl) and T2 (0.89ug/dl) groups ($P<0.05$). Cortisol is an indicator of an animal's endocrine response to environmental changes, and its concentration has been used as a reliable physiological end point for determining stress response [2]. Importantly, the release of cortisol is often increased in response to stress, therefore, the hormonal change due to the stress of the livestock is well-known as the factor changing the serum cortisol level. Concentrations of serum cortisol in cattle are increased when they get stressed. Cortisol, a hormone that affects muscle tension and nerve tissue stimulus, has also been known to increase in the circulation as a result of non-specific biologic responses to stress.

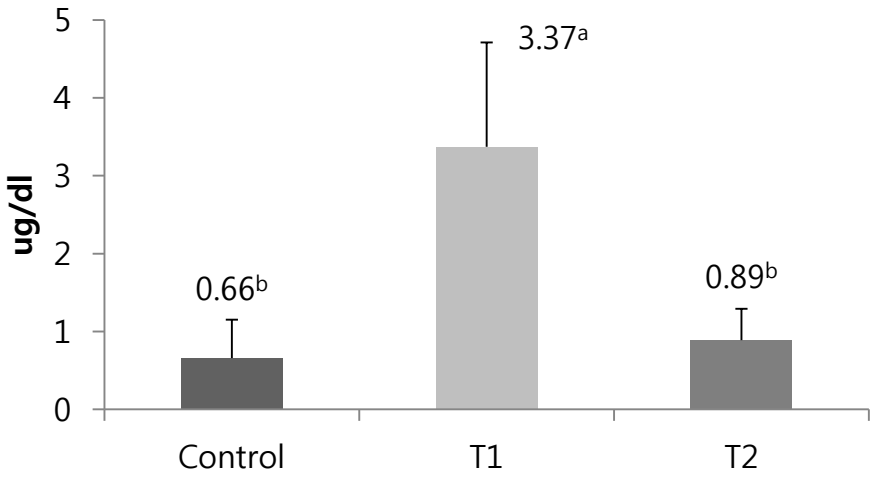

Figure 2: Serum cortisol concentration by different stocking rate in Hanwoo heifers.
[a,b]Means with different superscripts among the groups are different($P<0.05$)

The hypothalamo-pituitary-adrenal axis is activated by stressors, resulting in increased concentrations of plasma catecholamines and cortisol [24]. Catecholamine and cortisol are essential components of adaptation to stress. Having a wide living space would reduce stress [10]. Therefore, this can explain why the cattle would be stressed if not provided with a certain level of stocking rate.

Effect of stocking rate on heart rate of Hanwoo heifers

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Heart rate changes during various behavioral activities according to the stocking rate are given in Figure3. Average heart rate during STR was higher for the T1 (71.9bpm) than for the control (67.8bpm) and T2 (68.7bpm) (P<0.05). Average heart rate for the T1 group (64.4bpm) was higher than for the control (62.4bpm) and T2 (59.5bpm) during LD (P<0.05). Heart rate during LDR was shown to have a similar pattern during LD (P<0.05). Average heart rate for the T1 (86.6bpm) was higher than for the control (84.2bpm) during EA (P<0.05). Also heart rate during SF for the T1 (65.7bpm) was higher than for the control (61.2bpm) (P<0.05). The group with the highest heart rate during FT was expressed in the T1 group (91.6bpm), and the control was identified as having significantly low heart rates (84.1bpm) (P<0.05).

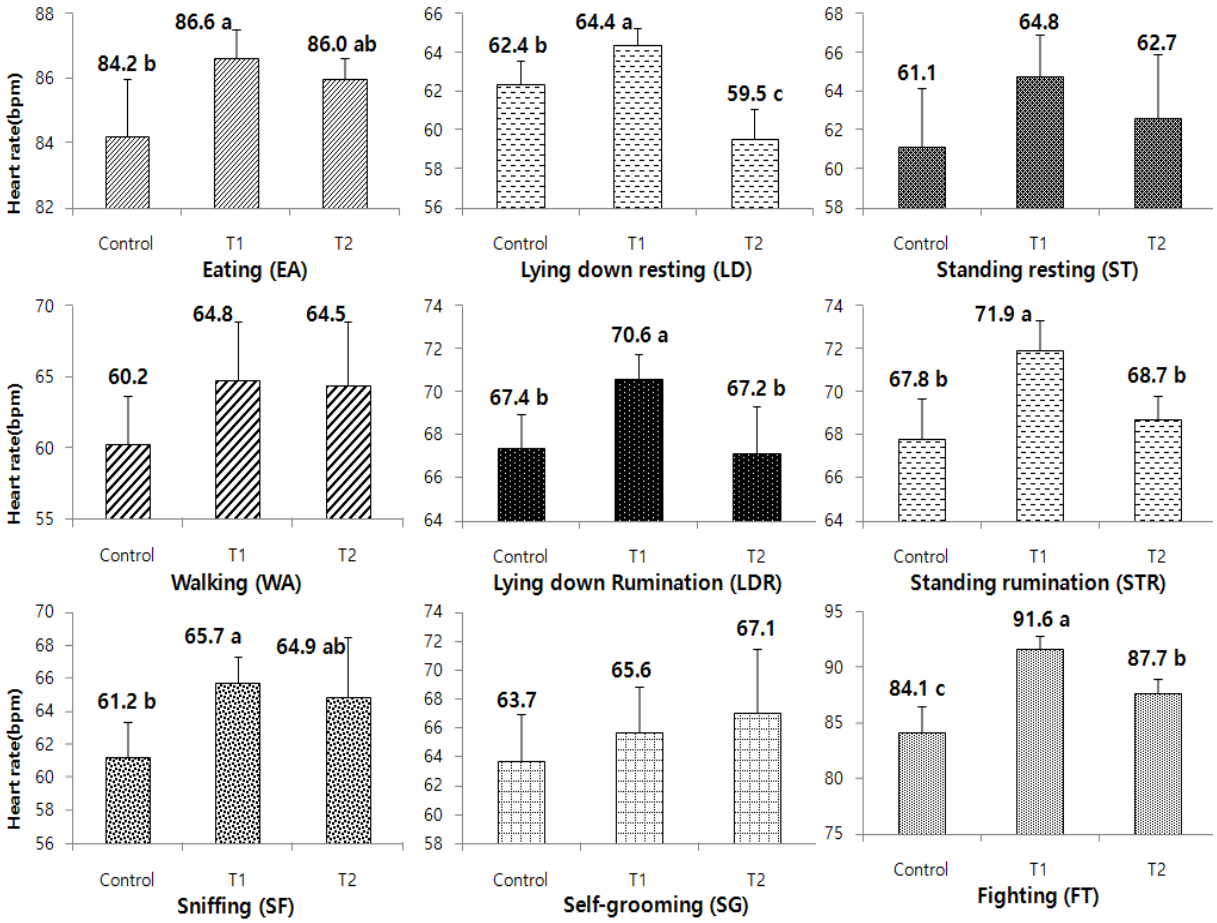

Figure 3: Heart rate depending on each behavior by different stocking rate in Hanwoo heifers.
[a, b,c]Means with different superscripts among the groups are different(P<0.05)

According to Figure 4, sections 51 to 60 of the control had the highest heart rate (47 %), and sections 51 to 60 in the T2 group had a similar trend (highest heart rate at 53%), in sections 61 to 70 in the T1, 36% showed the highest heart rate. For total average heart rate, the T1 (64.7bpm) was identified as the group with the highest heart rate. The heart rate of the control was 58.7bpm and the heart rate of the T2 was 62.8bpm. Using the regress expression (0.042*X–1.766=Y) [12], heat production during FT behavior was calculated as 1.73 cal/kg/hour for controls, as 2.08 cal/kg/hour for the T1, and 1.88 cal/kg/hour for the T2 group. During LD activity, heat production was calculated as 0.84 cal/kg/hour for controls, as 0.94 cal/kg/hour for the T1, and as 0.72 cal/kg/hour for the T2. Also it was calculated as 1.13 cal/kg/hour for controls, as 1.23 cal/kg/hour for the T1, and as 1.11 cal/kg/hour for the T2 group during STR activity. According to these results, the T1 had the most expendable unnecessary energy.

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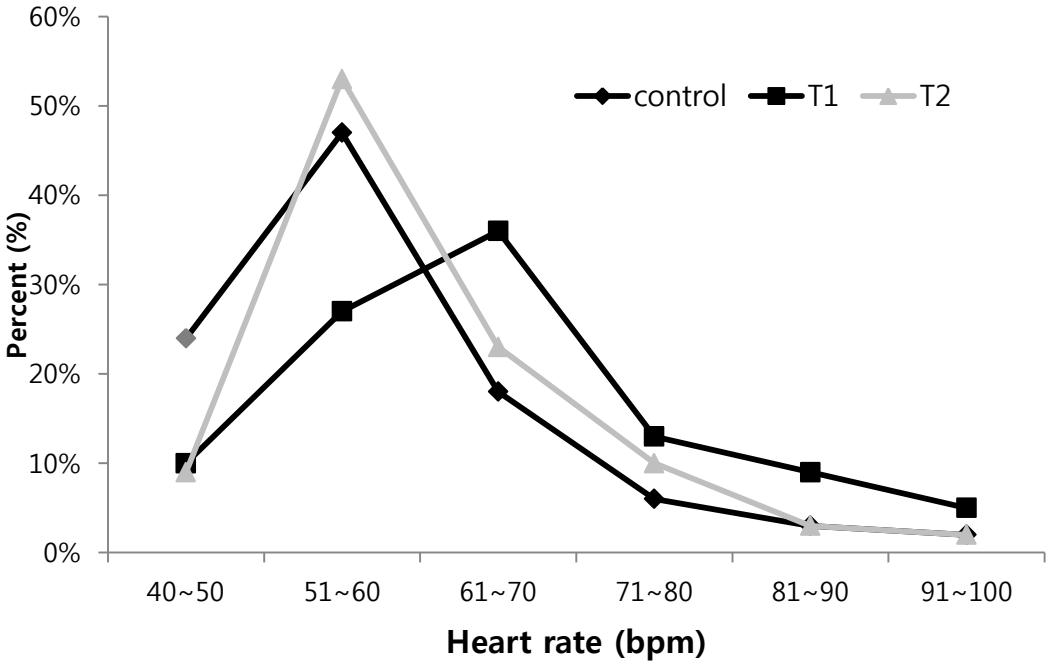

Figure 4: Heart rate distribution by different stocking rate in Hanwoo heifers.

Thus, the T1 had the highest heart rate during LD, LDR, STR and FT ($P<0.05$). When comparing the same behaviors, most of the T1 had a higher heart rate than the other groups. This means that the heart rate is affected by the space allowance for cattle.

CONCLUSIONS

In this experiment, strictly restricted space allowance by less than 10m²/head of housed heifers reduced time spent LD, EA, SF and SG, while it increased ST and FT ($P<0.05$). These measurements appear to be the most useful for evaluating the response to stocking rates in the heifers. According to the analysis of blood cortisol levels, which were targeted to determine the effect of stocking rate on stress, a significant difference was observed between the T1 (0.89ug/dL) and the other groups ($P<0.05$). Heart rates during LD, LDR, STR and FT were significantly high for the T1 ($P<0.05$). When heifers exhibited the same behavior, the T1 showed higher heart rates than the other groups. This means that the heart rate would be affected by the width of the space allowed for the cattle. Therefore, in terms of animal welfare, around 10m²/head of space allowance is considered suitable for feeding Hanwoo heifers.

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