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The effects of environmental enrichment objects on behaviors of Japanese quails at different cage stocking densities

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ABSTRACT

The aim of this study is to investigate the behaviors of and monitor the changes on behaviors of Japanese quails reared in different stocking density, through using different objects, (small red balls, bunch of rope, mirrors and beads) providing environmental enrichment (EE) in the cages of quails. Six hundred of 1 day old Japanese quail chicks (mixed sex) were used in this study. Birds were reared in battery cages with four floors, each of which has two separations. The quails were placed in cage dimension in the quantities of 10 quails (lower density: LD) and of 20 quails (higher density: HD) per separation. Our hypothesis was that EE would improve the welfare of quails. The birds were observed in their cages naturally in accordance with one-zero sampling, and home cage avoidance test was performed for these birds. While the applications have significant effect on feeding and relaxation behaviors ($P<0.05$ levels) in LD environment, the effect of comfort, social and other behaviors was realized at $P<0.01$ levels in the same environment. However in HD environment, only rest behavior was affected significantly in $P<0.05$ level, the other properties were affected in $P<0.01$ levels. The live weights weren't affected from EE (for LD, $P=0.134$ and for HD $P=0.216$). It was determined in the result of this study that the objects hung in the quail cages had potential effect to improve the welfare of quails.

Key words: Captive, Enrichment objects, Welfare, Quail.

INTRODUCTION

Quails are migratory birds they spend most of the time on the ground. Their normal habitat is farmland, where their call can be heard among the crops and pastures (Zucca *et al.,* 2005). Rearing in cage may have a stress increasing effect on animals. Behaviors of quails in captive conditions do not reflect their behavior under more natural conditions. Domestication may have resulted in unnaturally aggressive male quail. Perhaps males only harass females when confined with them in a small space. Obviously, caution is required when extrapolating from domesticated to wild populations and from artificial to natural environments (Persaud and Galef, 2005). There may be serious welfare problems due to head-banging in domesticated quails reared in cages.

Environmental Enrichment (EE) can be defined as the addition of one or more factors, improvement of behavioral capabilities of animals, hosted outside their natural environment, through modification of the environment and thus contributing to the development of animals biologically. (Newberry, 1995; Young, 2003). EE exerts beneficial effects on brain, behavior and cognition, for instance by affecting endocrinologic, immunologic, and neuroplastic parameters (Buscherta *et al*., 2016; Brenes *et al*., 2016). EE applications are often discussed within the context of welfare of animals hosted in cages (Wolfie, 2005). According to general opinion, EE, which could be created using different methods, plays an important role on the welfare of animals. EE primarily helps the animals to exhibit normal behavior patterns throughout the day. But the primary goal of environmental enrichment is to prevent abnormal behaviors of animals (Poggiagliolmia *et al.,* 2011). The purpose of environmental enrichment (EE) is reduce the stress of animals and increase the welfare level of those (Laurence *et al.,* 2015). EE not only improve locomotor activity by supporting it, but also can be expected to decrease the damages resulted from aggression of the animals, through these applications (Letzguß and Bessei, 2009). The use of some objects with enrichment purposes in cages can reduce social stress of animals (Tauson, 1998).

EE programs are in advantage of animals in respect of efficiency, health and welfare as well as providing good management of stock farming and has the potential to develop these properties (Estevez, 2007). EE can be used to forestall, or for recovery from, induced behavioral disorders (Meehan *et al.,* 2004). It was hypothesized that EE would reduce nervousness and stress and thus increase welfare level of quails. During this study, the quails were monitored without letting them out of their cages, for the purpose of interpreting their behaviors (Hawkins *et al.,* 2001).The general purpose of this study, is to increase the welfare level

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of the poultry hosted in cages. The particular purpose of this study is to determine the effects of the objects used for EE purposes, on Japanese quails, hosted in the cages enriched with various objects and in two stocking densities including lower density (LD: 200 cm^2/quail) and higher density (HD: 100 cm^2/quail).

MATERIALS AND METHODS

Animal materials: This study was carried out in Poultry Unit of the Animal Science Department of the Ahi Evran University. All experiments performed in this study followed the recommendations within the regulations on the welfare of farm animals. Six hundred 1-day-old quail chicks (mixed sex) were used as animal material in the study. The quails for the study have be chosen randomly in a bigger group. The chicks obtained from flock of quails with sixteen weeks of age, and without any selection being implemented. At the beginning of the EE, 1-day-old quail chicks were placed and randomly divided into five groups (ball, rope, mirror and beads and control). For the placement of birds groups, size cage for quail (50x40x30 cm WDH) available in poultry unit were used. In this study, two different stocking densities (SD), higher density (HD) = 100 cm^2/quail, and lower density (LD) = 200 cm^2/quail were applied. Each treatment was replicated four times. First, the birds were allowed to adapt to the environment, in which the related test would be performed, for the first two weeks. The same observer monitored the animals on weekly basis until the test got over. Warmth, moisture and light intensity in the cages were controlled using Data Logger (HOBO brand).

Water and food (high protein cereal diet (2,900 kcal ME and 26% CP) in the form of mixed pellets for chicks and granulates for adults) were provided to animals as ad libitum during the study. The fresh water requirement of the quails in the cages was supply by nipple drinkers. The study was conducted over seven weeks. The reason why the investigation lasted until the end of seventh week is that the sexual activity of Japanese quail reaches peak level in this period. The birds were weighed with an accurate scale with 0.01 g susceptibility. The environment temperature which was 37.8°C at the beginning of test, was decreased gradually until it reaches to room temperature (20±2°C and 50-60% humidity) on twenty-fourth day. In the same period, while lighting program primarily was arranged as light for 24 hours, then it was rearranged as light for 15 hours and dark for 9 hours (Miller and Mench, 2005). When they were 21 days old their sex was determined relying on feather dimorphism, through checking female-male ratio in each separation, it was seen that, there is no significant difference between the groups.

Enrichment treatments: Environmental enrichment consisted of objects designed to encourage pecking and locomotor activity in cages. Four enrichments objects were suspended from overhead wires such that they dangled from the cage ceiling (Jones *et al.,* 2000). Object's properties are as follows:

Ball: a red ball is made of plastic, with a diameter of 40 millimeters. Rope: bunches of green plastic rope totally 1 cm diameter to simulated grass. Mirror: a double-faced mirror (5 x 4 cm). Beads: shiny silver plastic beads strung together on wire.

Behavioral observations: Behavioral observations were carried out three days a week. Daily observations were performed in the mornings (AM) between 10:00-11:00 hours and in the afternoons (PM) between 14:00-15:00 hours. Total duration of observation in this study was 22 hours. The activities of the animals examined in this study were categorized as follows: 1- Feeding behaviors: a) Feeding with feed. b) Water drinking. c) Wire pecking. 2- Comfort behaviors: a) Feather cleaning. b) Wings stretching. 3- Rest Behavior: a) Sitting. 4- Social Behavior: a) aggressive pecking. Apart from these behaviors, standing behavior was included in the other behaviors classification.

Time sampling is a recording rule where behaviors are recorded periodically, involving either instantaneous sampling or one-zero sampling. One- zero sampling also includes intervals and with this method specific behaviors performed during an interval are registered only once (Martin and Bateson, 2007). While the behavior properties being observed, the behaviors were recorded every five minutes in accordance with One- zero sampling method, within an hour of observation. Aggressive pecking, because of realized instant, differing from other behaviors relatively, was recorded at the moment of appearance. The observer standing in front of the cages during the period of at least fifteen minutes, just before starting the behavior observations. Thus, it was accepted that the birds got used to observer. The first two weeks of test were accepted as adaptation duration for the new environment. Observations performed between three to seven weeks, were subjected to the analysis.

The avoidance reaction of quails

Avoiding the strike of an approaching predator requires rapid visual detection of a looming object, followed by a directed escape maneuver (Temizer *et al.*, 2015). Approach–avoidance conflict results in behaviors that have been correlated with increases in physiological stress indicators (Holmes *et al.,* 2003). The avoidance reaction of birds depends on the physiological conditions of the birds, and cage design (OECD, 2003).

In this part of the study investigated that the birds' responses against to an approaching object. For this purpose, avoidance test in the cage was applied. A pencil was brought closer to the birds in cage. The responses of the birds were categorized as follows: 1: Pen pecking (PP). 2: Stay motionless (SM). 3: Withdrawal to side of the cage (WS). 4: Withdrawal to the rear of the cage (WR). 5: Sudden escape (SE).

Statistical Analysis: Before the statistical evaluation, data recorded by time-sampling method for individual behavioral activities were arranged to percentage values, which give the proportion of each behavioral activity within total frequency of feeding and individual behavioral activities. And then normality test of data for these traits were tested by preliminary analyses. Data, are not normally distributed, were normalized by logarithmic transformation. GLM procedures at SPSS 16.0 statistical program was used to determine the effects of environmental enrichment (Different objects), stock density and day periods and their interaction on behavioral characteristics, LSD method was used for significance control. Repeated measurement of ANOVA was also used for data of stock density parameters. The model of repeated ANOVA statistics included sampling time (at AM and PM) LSD method was used in order to assess significance control. Statements of significance are given based on $P<0.05$ for all variables. Spearman's correlation coefficients were calculated for the direction and degree of correlation between variables (Seber and Wild, 1989).

RESULTS AND DISCUSSION

As shown in Table 1 the effects of time, stocking density and treatments on behavioral traits. According to the table, it was seen that the effects of the applications, were determined as statistically significant for all traits, in the assessments carried out in the mornings in LD environment. In terms of effects of treatments on feeding behavior, ball application with control group has resulted similar effect and so has got higher value than the other applications. In terms of comfort behaviors, mirror application with control group has got the highest value and has been in the same group. Ball application is clearly separated from the others and has got the lowest value.

In terms of resting behavior, while ball application has got higher value than the others, rope, mirror, bead applications with control group have been in the same group.

Table 1: The effects of time, stocking density and treatments on behavioral traits.

Stocking Density	Time	Treatment	Behaviors				
			Feeding	Comfort	Resting	Social	Other
High Density	AM	Ball	46.04±2.34 a	20.21±2.44 c	12.92±1.03 a	7.68±0.35 a	13.12±1.33 a
(100 cm^2/quail)		Rope	44.79±2.18 b	32.29±2.56 b	10.83±1.11 b	5.20±0.28 c	6.87± 1.27 b
		Mirror	43.75±2.67 b	35.21±2.75 a	10.42±1.42 b	5.42±0.67 c	5.21±1.07 c
		Bead	45.41±1.98 b	32.50±2.16 b	10.21±0.88 b	6.71±0.44 b	5.20±0.95 c
		Control	46.20±2.08 a	35.00±2.32 a	10.80±0.92 b	4.11±0.28 d	3.92±0.42 d
	SEM	0.766	0.812	0.423	0.228	0.043	
	P	0.002	0.000	0.004	0.000	0.002	
	PM	Ball	46.89±3.14 a	19.88±2.75 c	14.79±1.44 a	8.77±0.66 a	9.62±0.53 a
		Rope	45.02±2.78 b	30.98±2.82 b	10.85±1.62 b	5.83±0.48 c	7.29±0.48 b
		Mirror	44.38±3.56 b	34.37±2.54 a	11.04±1.38 b	4.79±0.47 d	5.41±0.62 c
		Bead	46.87±3.16 a	30.41±2.15 b	10.62±1,03 b	6.85±0.39 b	5.21±0.25 c
		Control	46.20±2.88 a	35.00±2.37 a	10.80±1.26 b	4.16±0.52 d	3.95±0.33 d
	SEM	0.615	0.702	0.504	0.124	0.041	
	P	0.006	0.002	0.018	0.003	0.005	
Lower Density	AM	Ball	48.12±3.59 ab	16.67±2.43 d	13.23±1.32 a	8.96±0.78 b	12.98±1.13 a
(200 cm^2/quail)		Rope	47.18±3.17 b	22.9±2.16 c	13.22±1.01 a	7.28±0.43 c	9.45±1.25 a
		Mirror	47.29±3.91 b	23.86±2.87 b	13.05±1.42 a	6.49±0.71 d	9.23±0.96 b
		Bead	49.35±4.02 a	16.79±2.69 d	12.46±1.33ab	11.90±1.04 a	9.52±0.84 b
		Control	49.06±3.55 a	26.36±2.45 a	11.88±1.08 b	6.25±028 d	6.45±1.01c
	SEM	0.712	0.694	0.477	0212	0.038	
	P	0.023	0.002	0.016	0.000	0.007	
	PM	Ball	45.94±3.24 c	16.36±2.47 d	12.9±1.37 a	9.96±0.93 a	14.8±1.32 a
		Rope	48.12±3.76 b	21.62±2.16 b	12.6±1.72 a	7.86±0.46 b	9.85±1.16 b
		Mirror	47.91±4.12 b	24.16±2.21 a	12.71±1.54 a	6.35±0.67 bc	8.84±1.03 c
		Bead	50.21±4.28 a	18.96±2.13 c	12.19±1.15 a	10.11±1.14 a	8.57±1.18 c
		Control	50.10±4.05 a	25.96±2.61 a	11.14±1.06 b	5.42±0.64 c	7.44±0.93 d
	SEM	0.612	0.542	0.318	0.157	0.219	
	P	0.003	0.003	0.041	0.001	0.006	
High Density	AM	GM	45.24 b	31.04 a	11.04 a	5.77 a	6.93 a
	PM		45.74 b	30.08 a	11.62 a	5.96 a	6.59 a
Lower Density	AM		48.20 a	21.92 b	12.71 a	7.79 a	9.42 a
	PM		48.46 a	21.42 b	12.19 a	8.29 a	9.61 a

SEM: standard error of mean; AM: *ante meridiem* (before midday), PM: *post meridiem* (afternoon). [a,b]Mean values within a column differ significantly from each other ($P<0.05$).

In terms of social behaviors and other behaviors, ball application has got a higher value. All applications in terms of these two behavior patterns have got higher values than control group's. In LD environment, similar results in the measurements made in afternoon, were obtained with the measurements made in morning. It was determined that bead application had significant effect on feeding behavior as well as ball application. When LD was evaluated in general, it was seen that, the ball application, except for comfort behavior, affected all behaviors significantly. Comfort behavior was affected by only ball application. There has been a striking point here. Both measurements performed in morning and in afternoon, it was seen that, ball application has a significant effect on feeding behavior, and mirror application has a significant effect on comfort behavior. But since both applications took place within the same statistical group, the effect of applications has become controversial. Similarly, the results are in accordance with Laurence *et al.,* (2015), it was reported that EE affected the activities of the quails. It was reported that EE, which was applied on Beijing ducks, caused a reduction in undesired behaviors comparing to ones, on which EE wasn't applied (Colton and Fraley, 2014), and might reduce conspecific pecking behavior at laying hens, thus weakening the injuries (Daigle *et al.,* 2014), and also EE, created using straw bales, had potential necessary to increase welfare of animals (Bailie and O'Connell, 2015).

The variability in the results obtained through observations in mornings, related with animals in HD environment, was relatively increased. When the effects of applications on nutrition behavior were examined, it was determined that, there wasn't any significant difference statistically between bead application, ball application, and control group. Comfort behavior has yielded similar results with LD environment. However, there were changes in the resting behavior. It has been reported that male quails showed the resting behavior more than the females in non-enriched environment (Karabayir and Tolu, 2008).

Ball, rope and mirror applications have got high effect on rest behavior. It was seen a change in social behaviors comparing to LD, and it was determined that the effect of bead application on social behaviors is higher than the other applications. The string application as well as the ball application has got a significant effect on other behaviors. In the measurements performed in the afternoon, the bead application has got the same effect with control group. The ball and the bead applications on social behaviors, the ball application on the other behaviors have yielded better results. When evaluated in general, while the nutrition behavior in HD has got higher value than that in LD, the comfort behavior has yielded lower value. HD has yielded higher effect on resting, socializing, and other behaviors.

When an evaluation was performed regardless measurement times, it was seen that, the bead application has got a significant effect on HD and LD (Table 2). The ball application has got a significant effect (resting: 17.40±1.89, social: 7.81±1.14 and other behaviors: 11.35±1.65) on HD. The ball application in HD has got a significant effect on comfort behavior (and the mirror application in LD has got a significant effect on comfort behavior. The ball application is important in LD in respect of rest, socialization and other behaviors. The rope, mirror and bead applications in HD has got a significant effect on the rest behavior, the bead application has got a significant

Table 2: The effects of stocking density and treatments on behavioral traits.

Stocking Density	Treatment	Behaviors				
		Feeding	Comfort	Resting	Social	Other
High Density	Ball	44.14±4.15 b	19.30±2.54 c	17.40±1.89 a	7.81±1.14 a	11.35±1.65 a
(100 cm^2/quail)	Rope	44.89±3.94 b	31.83±2.88 b	10.83±1.72 b	5.41±1.32 c	7.08±1.12 b
	Mirror	44.06±3.17 b	34.79±3.01 a	10.73±1.53 b	5.10±0.77 c	5.31±0.93 c
	Bead	46.61±4.05 a	31.46±2.69 b	10.42±2.01 b	6.46±0.92 b	5.50±1.01 c
	Control	45.10±3.66 ab	35.52±2.61 a	10.83±2.12 b	4.48±0.78 c	4.06± 0.73d
	SEM	0.723	0.612	0.449	0.284	0.272
	P	0.033	0.004	0.014	0.000	0.000
Lower Density	Ball	49.68±3.76 a	26.15±2.24 a	11.46±1.88 b	5.84±0.72 d	6.83±0.34 d
(200 cm^2/quail)	Rope	47.65±4.12 b	22.29±2.57 c	12.92±1.82 a	7.49±0.56 b	9.43±0.48 a
	Mirror	47.61±4.23 b	24.21±2.71 b	12.87±1.46 a	6.41±0.90 c	8.91±0.99 b
	Bead	49.79±4.18 a	19.38±2.14 d	12.79±1.60 a	10.05±1.03 a	7.97±0.68 c
	Control	49.56±3.79 a	26.14±2.17 a	11.46±1.92 b	5.99±0.91 d	6.83±0.81 d
	SEM	0.813	0.762	0.442	0.207	0.194
	P	0.007	0.002	0.041	0.006	0.002
High Density	GM	45.27 b	30.71 a	12.58 a	5.39 b	6.08 b
Lower Density	GM	48.48 a	23.65 b	12.43 a	7.45 a	7.99 a

SEM: standard error of mean; GM: General mean. [a,b]Mean values within a column with no common superscript differ significantly from each other ($P<0.05$).

effect on the socialization behavior, and the rope application has got a significant effect on the other behaviors. While feeding, socialization, and other behaviors were observed mostly in HD. LD was take a pew in the forefront in respect of comfort behavior. Both animal densities have got similar effects on rest behavior.

When correlations were examined, it was seen that, in terms of the relationships between applications and Pen pecking (PP), the correlations have been positive for all applications. But this case wasn't considered as important statistically. The most positive relationship was observed between comfort and bead applications. However, it was seen that, the effects of the applications started to be increased along with PP. The mirror and bead applications has affected stay motionless (SM) positively and significantly (r=0.430* and r=0.439*). However, comfort behavior (r=-0.409*) and rest behavior (r=-521*) have affected SM adversely and significantly. In other words, the more the comfort behavior and the rest behaviors increased, the more SM value decreased. However, it was determined that the effects of other applications on SM are positive, but statistically insignificant. It was observed that, all features, except for the resting behavior, has affected WS (withdrawal to side of the cage) significantly. While comfort behavior WS, (r=-0.442*) affected WS adversely but significantly, rest behavior has also affected it adversely and (r=-0.327) and was considered as statistically insignificant. The ball (r=0.561**), the mirror (r=0.507**), the bead (r=0,653**), feeding behavior (r=0,572**), and social behaviors (r=714**) have got a positive effect on SM and also more significant effect in P<0.01 values. It was determined that the string behavior (r=0.453*) and other behaviors (r=0.400*) had positive effect and significant relationships (P>0.05) on WS.

When the effects on WR (withdrawal to the rear of the cage) examined, it was seen that, the string behavior(r=0.672**), the mirror behavior (r=0.712**), and bead behavior (r=0.653**) had positive and significant effect on WR. The ball (r=0.417*) had positive and significant effect on WR in P>0.05 values. However, the comfort behavior (r =-0.552**) had negative and significant effects on WR. The effects of the other applications on WR weren't considered as significant statistically. When relationships between the applications and SE (Sudden escape). SE examined, it was seen that, there were positive correlation with the ball behavior (r=0.629**), the string behavior (r=0.588**), and the mirror behavior (r=0.704**), and the bead behavior (r=0.671**) and significant effect in P>0.01 values. However, the comfort and nutrition behaviors, like in others, had negative but significant relationship with SE too. Although other applications had positive relationship they hadn't a significant effect.

When the effects of applications on behaviors were examined, it was seen that, the ball application had positive and significant effect (r=0.442*) on the behaviors comparing to other applications. While the nutrition behavior was affected adversely and insignificantly, other applications were affected positively but insignificantly. When examined in general, it was understood that, the string application hadn't any effect. The mirror effect was changed according to the behaviors. While the mirror had a positive and significant effect on the comfort behavior (r=0.531*), negative but significant effect on nutrition behavior (r=-0.412*) and the rest behavior (r=-0.448*). While the mirror had a negative and insignificant effect on social behavior, positive but insignificant effect on other behaviors. When the bead application was examined in general, it was seen that, it hadn't a significant effect on the behaviors. While it has negative and insignificant effect on feeding behavior and rest behavior, on the other hand it had positive and insignificant effect on the other properties.

When the interactions of the applications were examined, it was seen that, nutrition behavior caused comfort behaviors (r=0.721**) and rest behaviors (r=0.477*) to be increased, but on the other hand, socialization behaviors to be decreased significantly (r = 0.632**). It was seen that, comfort behavior affected rest behavior (r=0.772**) and other behaviors (r=0.502**) positively and significantly. But comfort behavior (r=-0.458*) affected socialization behavior adversely and significantly. In other words, the more comfort behavior increased, the more socialization behavior of animals decreased. Similarly, rest behavior affected socialization behavior (r=0.-402*) adversely and significantly.

In terms of avoidance reactions, the effects of the applications are given (Figure 1, Figure 2 and Figure 3). When Figure 1 examined, it was seen that the greatest effect was obtained in WS. Mirror application in WS had the greatest effect. Ball application had the minimum effect. While bead application and ball application had the greatest effect in SM level, the mirror application had the minimum effect. The minimum test results were observed in SE. Here, string application had a greater effect on animals comparing to other applications. In this respect the ball, bead and mirror applications had lower effects. Similar results were obtained in both densities for SE. When an evaluation was performed in general, all applications except for bead application had significant and similar effects on SE in HD. However, only string application had a significant effect on SE in LD.

It was seen that the applications had different effects on animals. This case was considered to be induced from reaction differences in HD and LD densities. While higher values were obtained in HD for PP and SM, higher values were obtained in LD for WS and WR. It was observed in general that, rope application hadn't a significant effect on

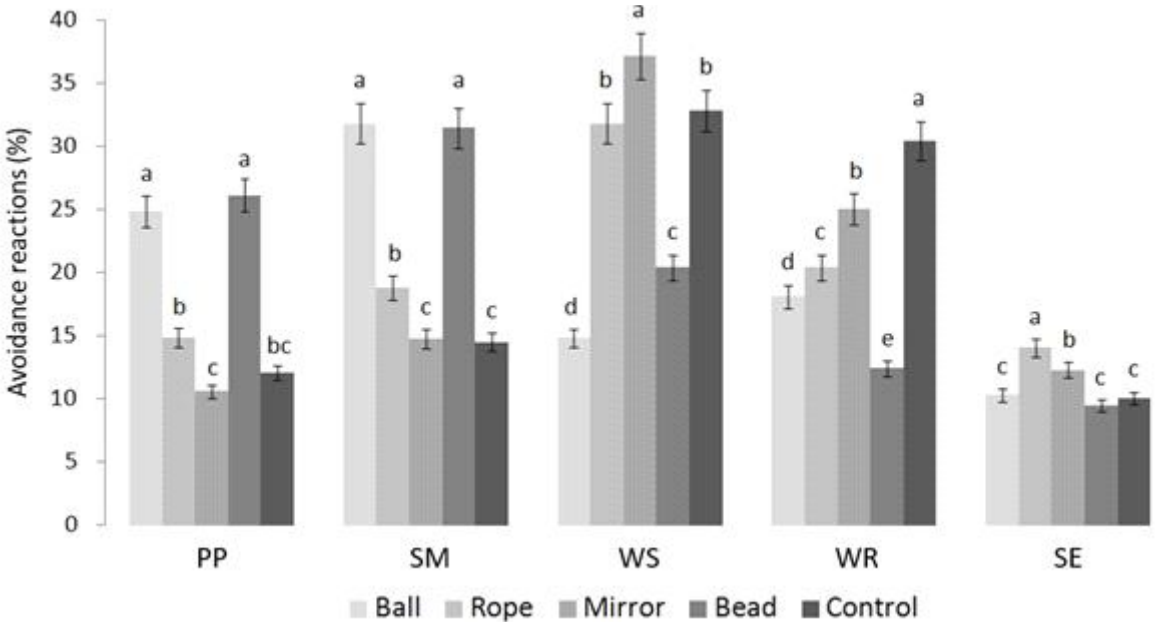

Fig 1: Avoidance reactions (%) for LD (200 cm^2/quail). SEM values of PP, SM, WS, WR and SE: 0.342, 0.493, 1.205, 0.941 and 0.815 respectively for LD. (Pen pecking (PP), Stay motionless (SM), Withdrawal to side of the cage (WS), Withdrawal to the rear of the cage (WR), Sudden escape (SE)).

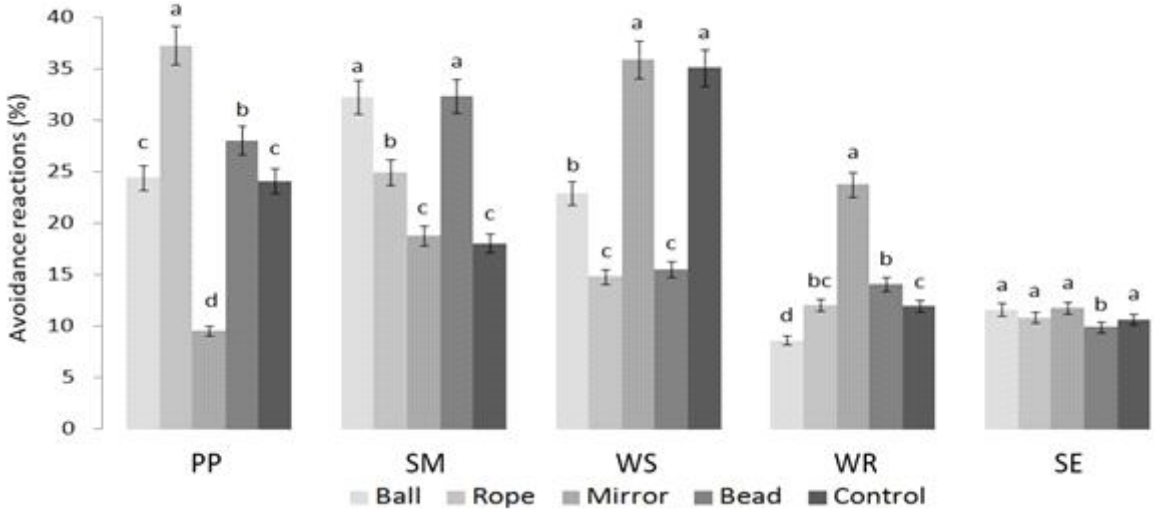

Fig 2: Avoidance reactions (%) for HD (100 cm^2/quail). SEM values of PP, SM, WS, WR and SE: 0.451, 0.588, 0.502, 0.428 and 0.390 respectively for HD. (Pen pecking (PP), Stay motionless (SM), Withdrawal to side of the cage (WS), Withdrawal to the rear of the cage (WR), Sudden escape (SE)).

LD. It was determined that although string application take a pew in the forefront on PP, on the other hand the mirror application had more effect on WS, WR, and SE behaviors in HD.

In this study used four different types of enrichment with the objects. The fact that any enrichment may have a positive effect on welfare of caged birds is well known. At the same time it is well known that the animals loose quickly interest in an object put in the cage; probably could be interesting analyze the effect over the time of these object in order to verify if one of these attract the quails more than another. To sum up, we can say that, the objects used for EE purposes had an effect potential increasing the welfare of the animals, although further studies are still needed to elucidate the effects of enrichment objects on quail behaviors in different stocking densities. Because meaningful and positive correlations were determined between the objects used and the behaviors in general.

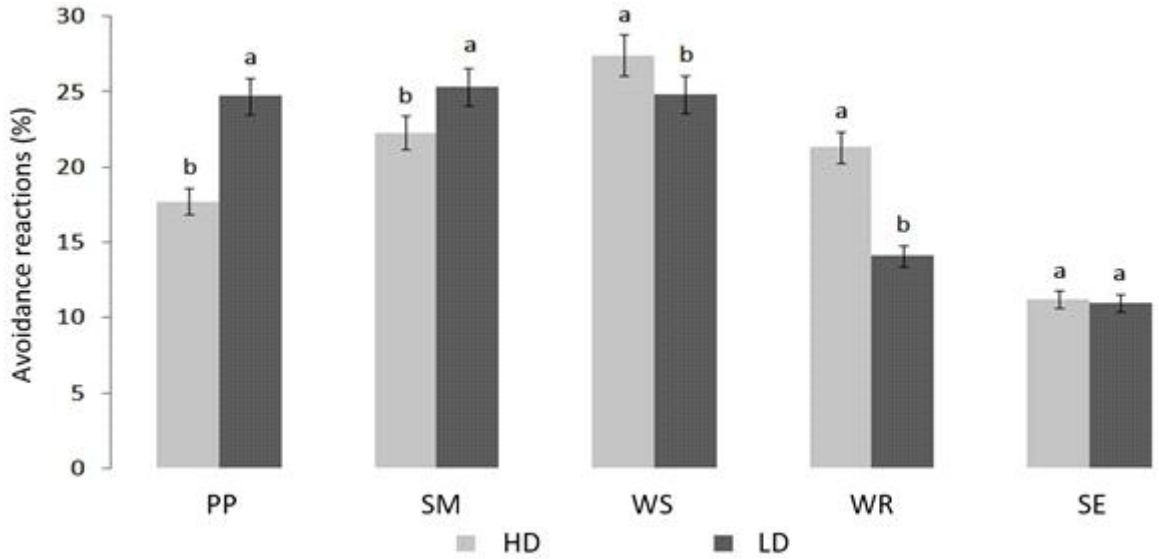

Fig 3: Avoidance reactions (%) HD: 100 cm^2/quail, and LD: 200 cm^2/quail. (Pen pecking (PP), Stay motionless (SM), Withdrawal to side of the cage (WS), Withdrawal to the rear of the cage (WR), Sudden escape (SE)).

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