Journal of Advanced Veterinary Research Volume 8, Issue 3 (2018) 60-65

Journal of Advanced Veterinary Research

http://advetresearch.com/index.php/avr/index

Effects of Probiotic on Comfort and Body Care Behaviors of Broilers Reared at Different Stocking Densities

Rasha R. Ibrahim[1], Fatma Khalil[1*], Ahmed S. Mostafa[2], Hosny H. Emeash[1]

[1]Animal and Poultry Management and Wealth Development, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.
[2]Animal and Poultry Management and Wealth Development, Faculty of Veterinary Medicine, El Menia University, El Menia, Egypt.

ARTICLE INFO

Original Research

Received:
04 July 2018

Accepted:
21 July 2018

Keywords:

Behavior, Broilers, Comfort, Probiotic, Stocking density

ABSTRACT

This study was conducted to investigate the effect of probiotic on comfort and body care behaviors of broilers reared at two different densities. Two hundred and forty chicks were subdivided into four groups, two groups were reared under low stocking density (LSD); 10 bird/m^2 and another 2 groups were reared under high stocking density (HSD); 15 bird/m^2. In each density, one group was supplemented with probiotic in the drinking water and the other was not supplemented. Normal behavior of birds was recorded throughout the growing cycle twice a week, using video camera (Sony, Japan). Then after, comfort (standing, lying and sleeping), body care (preening, dust bathing, wing/leg stretching and head scratching) and aggressive behaviors of birds were analyzed. HSD reduced comfort behavior compared to low density without probiotic group. Standing frequency was significantly increased at the 6th week of the growing cycle. In addition, interrupted lying bouts frequency was increased and the non-interrupted lying bouts (frequency and duration) were decreased significantly. Moreover, preening frequency was decreased. However, aggressive behavior was not observed in all groups. Probiotic supplementation didn't alter comfort, aggressive and body care behaviors of broilers at both densities in comparison to low density without probiotic group. However, birds stocked at high stocking density and supplemented with probiotic showed no alterations in all behavioral patterns compared to HSD group without probiotic supplementation. Data suggested that the used probiotic in this study was unable to improve comfort and body care behaviors of broilers reared at low stocking density and failed to alleviate impacts of high density stress on these behaviors.

J. Adv. Vet. Res. (2018), 8 (3), 60-65

Introduction

In the recent decades, intensive broiler rearing systems were increased to face the growing demand of poultry meat. Though this intensification increases production of broiler farms, behavioral alterations may occur, especially in uncontrolled environmental rearing system (Estevez, 2007). Consequently, more or less severe performance and welfare problems may be resulted (Marchewka *et al.*, 2013).

Indeed, there is a scientific debate about the appropriate and stressful stocking densities. Thomas *et al.* (2004) found no difference in feed intake of broilers reared at 10, 15 and 20 bird/m^2 densities. In addition, Turkyilmaz (2008) reported no alteration in feed intake of birds 15, 20, 25 bird/m^2 rearing densities. Moreover, feed consumption was not significantly influenced by increasing stocking density from 11 to 14 or 17 bird/m^2 (Zhao *et al.*, 2009).

Broilers spend most of their time lying down (Bessei, 1992). This time is controlled by total space availability (Murphy and Preston, 1988). Furthermore, as the birds grow and get heavier spending more time lying and visit the feeders less often (Savory and Lariviere, 2000; Weeks *et al.*, 2000).

Comfort behavior is closely linked with poultry meat carcass quality (Cornetto *et al.*, 2002). Therefore, fragmentation of comfort behavior leads to less sleeping time, which may retard growth and performance of birds (Malleau *et al.*, 2007). In addition, chickens require space to accommodate normal body functions and also for maintaining normal behaviour such as body care. Moreover, adequate floor space is required for performance of birds' social behavior (Albentosa and Cooper, 2004). Reduction of social behavior performance may increase aggression between birds. Feather pecking and aggressive encounters are considered as important welfare and management concern in intensive poultry farms (Marchewka *et al.*, 2013). Hence, decreasing floor space restricts social interactions and body care behavior that may increase incidence of welfare problems occurrence.

There is a close correlation between brains and bowels that allows the individual's gut bacteria to steer their behavior (Yong, 2011). Furthermore, probiotic bacteria can reduce stress and depression related behaviors (Sudo, 2006; Messaoudi *et al.*, 2011). Therefore, our goal was to investigate effects of the

*Corresponding author: Fatma Khalil
E-mail address: fatma.khalil@vet.bsu.edu.eg

used probiotic (Protexin®) on comfort and body care behaviors of broilers reared at two different stocking densities (10 and 15 birds/m^2).

Materials and methods

Experimental design

This study was conducted in the poultry house belonging to Department of Animal and Poultry Management and Wealth Development, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

Two hundred and forty unsexed one-day old (Cobb type breed) chicks were purchased from a commercial hatchery and brooded at 33ºC using electric heaters for the first week of age. Then, they were distributed into four groups at the end of the first week (two low density groups and other two high density groups). In each density, one group supplemented with probiotic and the other did not supplemented with probiotic. Ninety six birds were used in low stocking density (LSD) groups (10 bird/m^2); 48 chicks in each group; three replicates for each (16 bird/ replicate). For high stocking density (HSD) groups (15 bird/m^2), 144 birds were divided into two equal groups; three replicates (24 bird / replicate) in each.

The study was approved by Institutional animal care and use committee of Beni-Suef University (BSU-IACUC), Egypt.

Birds accommodation and management

All chicks were reared in 12 floor pens (1m × 1.6 m), a new wood shaving litter material was used. The ventilation was maintained using windows, fans and exhausting fans. Heating during brooding was maintained by the electric heaters, with a decrease in the temperature 2ºC each week. Lighting program was set as continuous lighting for the first week, 23 hrs light and 1 hr dark till the end of the experiment by well- distributed red bulbs. All birds were reared under the same environmental conditions. Feed and water were provided ad libtum. The birds were fed using a two-phase broiler-feeding regime; a starter (23% protein) crumble for the first 21 days, followed by a grower pellet (21% protein) until the end of the study at day 42.

Protexin® probiotic supplementation

Protexin® probiotic {Enterococcus faecium 2 x 10^9 CFU/g (2 x 10^{12} CFU/kg)} was purchased from probiotic International Smorest, UK. It was added to the drinking water of birds starting from the second week according to the manufacturer recommendation.

Measurement of behavior

The behaviour of chicks was recorded twice a week, in the morning (9:00 – 12:00 am) for 5 consecutive weeks. Behavior of each replicate was recorded using video camera (Sony, Japan) for 20 minutes. Then after, behavior of birds was analyzed using scan method of observation that adopted according to Maria *et al*. (2004). Comfort behavior was analyzed including standing lying and sleeping. Lying bouts were classified into non-interrupted lying bouts (NILBs) and interrupted lying bouts (ILBs). The later was analyzed as ILB1 (lying bouts interrupted by other birds, ILB2 (lying bouts interrupted by standing) and ILB3 (lying bouts interrupted by adjusting the bird's position)

Comfort behavior analysis was expressed as weekly frequency of standing, interrupted and non-interrupted lying bouts weekly frequency, weekly non-interrupted lying total duration and weekly frequency of sleeping behavioral pattern. In addition, frequency of body care (preening, dust bathing, wing or leg stretching), and behavioral patterns throughout the growing cycle was analyzed:

Statistical analysis

Data was analyzed by one-way ANOVA test (Dunnet test) using SPSS (Statistical Package for Social Science, 2011). Results considered significant at $P<0.05$. Results of the four groups at the same week of the growing cycle were compared statistically while, the differences of data within groups were not analyzed.

Results

By comparing results of the 4 groups using One Way ANOVA test, it was found that comfort and body care behaviors were significantly impaired in birds reared at high group size with and without probiotic supplementation (group 3 and 4. Standing frequency was significantly ($P<0.05$) increased at the 6th week of the growing cycle (Table 1).

Though weekly frequency of total lying bouts was not significantly altered, ILBS frequency was increased in expanse of non-ILBs frequency (Table 2). There was a significant increase ($p<0.05$) in weekly frequency of ILB2 and ILB3 at the 6th week and at the 5th and 6th weeks of the growing cycle, respectively (Table 2). On contrary, weekly frequency of non-ILBs of group 3 and 4 was decreased significantly ($P<0.05$) at 2nd, 3rd, 4th, 5th, 6th week of the growing cycle (Table 2). In addition, non-ILB duration/sec was significantly shortened at the 2nd ($p<0.05$), 3rd ($P<0.05$), 4th ($P<0.05$), 5th ($p<0.01$) and 6th ($P<0.05$) weeks of the growing cycle (Table 3). Moreover, sleeping frequency

Table 1. Effect of probiotic supplementation on weekly frequency of standing behaviors at different stocking densities.

		Weekly Standing frequency "SF"				
		2nd	3rd	4th	5th	6th
Groups	1	9.67±0.88ᵃ	8.00±1.55ᵃ	8.00±2.00ᵃ	6.00±0.58ᵃ	6.00±1.00ᵇ
	2	9.33±1.20ᵃ	8.00±1.00ᵃ	7.33±1.45ᵃ	5.67±0.67ᵃ	5.67±0.67ᵇ
	3	12.00±0.58ᵃ	8.67±1.33ᵃ	9.33±0.88ᵃ	8.33±0.88ᵃ	9.67±0.33ᵃ
	4	12.33±2.19ᵃ	10.00±2.65ᵃ	7.67±0.67ᵃ	8.67±0.67ᵃ	10.00±0.58ᵃ

Results were expressed as Means ± Standard Error (S.E.).

a,bSuperscripts within columns indicate significant difference

1= Gp1= 10 bird/m2 rearing density without probiotic supplementation

2=Gp2=10 bird/m2 density with probiotic supplementation

3= Gp3=15 bird/m2 rearing density without probiotic supplementation

4= Gp4=15 bird/m2 rearing density with probiotic supplementation showed no significant decrease in both high group size (with and without probiotic supplementation) (Table 4). Furthermore, frequency of preening behavior throughout the growing cycle was reduced significantly (P<0.05) in birds stocked at high density (Table 5). By comparison of low stocking density groups (1 and 2), it was clear that probiotic supplementation did not significantly improve comfort and body care behaviors of broilers. Similar results were noticed when data of high stocking density groups (3 and 4), In all groups, aggression and abnormal behaviors were not observed between birds.

Table 2. Effect of probiotic supplementation on the weekly frequency of different lying bouts at different stocking densities.

Groups	Week	Total LBs	Non-interrupted LBs	Interrupted LBs (ILBs) ILB1	ILB2	ILB3
1	2nd	25.00±2.52^{a}	18.67±1.20^{a}	2.33±0.89^{a}	ND	4.00±0.58^{a}
	3rd	22.33±2.03^{a}	15.00±1.73^{a}	2.33±0.67^{a}	0.67±0.67^{a}	4.33±0.33^{a}
	4th	18.00±1.00^{a}	11.00±0.57^{a}	2.00±0.66^{a}	1.00±0.58^{a}	4.00±1.15^{a}
	5th	15.67±1.45^{a}	9.33±0.88^{a}	2.00±1.15^{a}	1.33±0.67^{a}	3.00±1.15^{b}
	6th	16.00±1.15^{a}	8.67±0.88^{a}	1.67±1.20^{a}	0.33±0.33^{b}	5.33±0.88^{b}
2	2nd	23.33±0.67^{a}	17.33±1.45^{a}	3.00±0.87^{a}	ND	3.00±1.00^{a}
	3rd	22.00±2.64^{a}	15.67±2.33^{a}	2.33±0.33^{a}	1.00±0.58^{a}	3.00 ± 0.58^{a}
	4th	16.33±1.20^{a}	11.33±1.86^{a}	2.00±0.57^{a}	0.67±0.67^{a}	2.33±0.33^{a}
	5th	17.33±0.67^{a}	11.00±1.15^{a}	3.00±0.58^{a}	1.33±0.33^{a}	2.00±1.00^{b}
	6th	16.33±1.76^{a}	10.00±1.15^{a}	2.33±0.88^{a}	0.67±0.33^{b}	3.33±1.33^{b}
3	2nd	21.67±1.21^{a}	11.00±0.58^{b}	5.33±1.67^{a}	1.00±0.57^{a}	4.33±0.88^{a}
	3rd	18.33±0.88^{a}	7.00±1.00^{b}	5.67±1.76^{a}	1.00±0.00^{a}	4.67 ± 0.88^{a}
	4th	19.00±1.53^{a}	5.67±0.67^{b}	6.00±0.88^{a}	1.33±0.33^{a}	6.67±1.67^{a}
	5th	20.33±1.20^{a}	4.67±0.88^{b}	5.67±0.33^{a}	1.00±1.00^{a}	9.00±1.00^{a}
	6th	24.00±2.89^{a}	4.00±1.00^{b}	4.00±0.58^{a}	4.00±0.57^{a}	12.00±1.52^{a}
4	2nd	22.67±3.38^{a}	11.67±1.45^{b}	6.00±1.00^{a}	1.33±0.89^{a}	3.67±0.67^{a}
	3rd	16.33±2.91^{a}	6.33±1.20^{b}	4.33±1.33^{a}	1.33±0.33^{a}	4.33±0.66^{a}
	4th	19.33±2.33^{a}	5.33±0.88^{b}	5.33±1.33^{a}	1.67±0.33^{a}	7.67±1.45^{a}
	5th	19.00±3.06^{a}	4.67±0.33^{b}	4.33±1.45^{a}	1.33±0.88^{a}	8.67±1.20^{a}
	6th	23.33±1.86^{a}	3.33±0.88^{b}	4.67±1.20^{a}	4.33±0.88^{a}	11.00±0.58^{a}

Results were expressed as Means ± Standard Error (S.E.).
^{ab} Superscripts within columns indicate significant difference
1= Gp1= 10 bird/m^2 rearing density without probiotic supplementation (control)
2=Gp2=10 bird/m^2 density with probiotic supplementation
3= Gp3=15 bird/m^2 rearing density without probiotic supplementation
4= Gp4=15 bird/m^2 rearing density with probiotic supplementation
LBs: Lying bouts; NILB: non-interrupted lying bouts; ILBs: Interrupted lying bouts; ILB1: caused by other birds
ILB2: caused by standing; ILB3: caused by adjusting the bird's position; ND: Not detected

Table 3. Effect of probiotic supplementation on the weekly non-interrupted lying total duration at different stocking densities

Groups	Weekly total duration of NIL (sec) 2nd	3rd	4th	5th	6th
1	3218.33 ±238.30^{a}	2850.00 ±350.19^{a}	2200.00 ± 136.14^{a}	1988.33 ±149.51^{a}	1771.67 ±337.30^{a}
2	3100.00 ±228.11^{a}	2920.00 ±472.90^{a}	2283.33 ±536.45^{a}	2340.00 ±287.46^{a}	2045.00 ±253.20^{a}
3	1870.00 ±232.45^{b}	1210.00 ±101.49^{b}	996.67 ±131.19^{b}	850.00 ±132.29^{b}	640.00 ±185.20^{b}
4	1996.67 ±265.60^{b}	1112.67 ±212.85^{b}	990.00 ±150.99^{b}	843.33 ±33.33^{b}	530.00 ±177.76^{b}

Results were expressed as Means ± Standard Error (S.E.).
^{a,b} Superscripts within columns indicate significant difference
1= Gp1= 10 bird/m^2 rearing density without probiotic supplementation
2=Gp2=10 bird/m^2 density with probiotic supplementation
3= Gp3=15 bird/m^2 rearing density without probiotic supplementation
4= Gp4=15 bird/m^2 rearing density with probiotic supplementation
NIL= non-interrupted lying

Table 4. Effect of probiotic supplementation on the weekly frequency of sleeping behavioral pattern at different stocking densities.

		Weekly sleeping frequency				
		2nd	3rd	4th	5th	6th
Groups	1	24.33±4.37ª	12.67±2.33ª	12.00±1.73ª	11.33±1.86ª	15.67±6.23ª
	2	23.33±6.01ª	14.00±2.52ª	13.33±1.67ª	12.33±0.33ª	11.00±2.08ª
	3	20.00±1.15ª	11.33±2.34ª	8.00±1.02ª	9.67±1.84ª	4.00±1.00ª
	4	17.67±1.45ª	10.67±3.48ª	7.67±1.45ª	11.00±2.52ª	4.33±1.20ª

Results were expressed as Means ± Standard Error (S.E.)

[a,b] Superscripts within columns indicate significant difference

1= Gp1= 10 bird/m^2 rearing density without probiotic supplementation

2=Gp2=10 bird/m^2 density with probiotic supplementation

3= Gp3=15 bird/m^2 rearing density without probiotic supplementation

4= Gp4=15 bird/m^2 rearing density with probiotic supplementation

Table 5. Effect of probiotic supplementation on the frequency of body care behavioral patterns throughout the growing cycle at different stocking densities.

		Body care behavioural patterns			
		Preening	Dust bathing	Wing or leg stretching	Head scratching
Groups	1	16.133±1.43ª	0.80±0.12ª	2.53±0.18ª	2.60±0.31ª
	2	15.40±0.75ª	0.67±0.18ª	2.40±0.31ª	2.20±0.50ª
	3	10.13±1.25ᵇ	0.33±0.13ª	1.47±0.07ª	1.13±0.18ª
	4	10.33±0.98ᵇ	0.47±0.13ª	1.80±0.35ª	1.20±0.40ª

Results were expressed as Means ± Standard Error (S.E.)

[a,b] Superscripts within columns indicate significant difference

1= Gp1= 10 bird/m^2 rearing density without probiotic supplementation

2=Gp2=10 bird/m^2 density with probiotic supplementation

3= Gp3=15 bird/m^2 rearing density without probiotic supplementation

4= Gp4=15 bird/m^2 rearing density with probiotic supplementation

Discussion

The results of our study revealed the impacts of increasing group size of broiler on comfort and body care behaviors in addition to the role of probiotic supplementation in alleviation of these effects.

Results of this study indicated that HSD induced significant increase in standing frequency only at last week of the growing cycle. This was agreeable with Buijs *et al.* (2010, 2011), while, Andrews *et al.* (1997) found that standing duration wasn't affected by stocking density.

The obtained results in this study indicated the marked disturbance in lying behavior of birds reared at HSD at the end of the growing cycle. Similarly, Lewis and Hurnik (1990) and Buijs *et al.* (2012) observed shorter lying bouts of birds tucked at high density compared to control. On the other hand, Andrews *et al.* (1997) stated that total time spent lying was not significantly affected by stocking density and Martrenchar *et al.* (1997) mentioned that the duration of LB (lying bouts) did not relate to stocking density.

It is worthy noted that, the effect of HSD stress on comfort behavior was prominent at the end of the growing cycle. This might be attributed to increase size of the birds and consequently floor space relatively decreased by age.

The recorded increase in standing frequency resulted in the increase of ILBs frequency because standing of birds during lying is an interruption of the lying bouts. In addition, increase of ILB frequency suggested that birds were restless rather than resting (Murphy and Preston, 1988). There are three possible explanations of increasing birds' ILB frequency. The first is the disturbances caused by other birds (Lewis and Hurnik, 1990). This was confirmed by Martrenchar *et al.* (1997), who declared that when a bird walks to a feeder or drinker in an overcrowded shed, it is likely to pump into other, remove away or move their heads away from the interference whilst they remain sitting. The second explanation is that the birds may frequently stand as cooling strategy to alleviate heat stress (Murphy and Preston, 1988; Lewis and Hurnik, 1990; Zulkifli and Sti nor Azah, 2004). This is because increased contact with neighboring birds at HSD limited the area available for heat transfer to 33% of a bird's total surface (Murphy and Preston, 1988). Thus, birds tend to adjust their sitting posture (Buijs *et al.*, 2012). The third suggested explanation is the high litter content of ammonia that may cause irritation in respiratory tract of the birds, hence they stand as an attempt to decrease its inhalation.

This restless behaviour (interrupted lying), which was a reflection of cooling strategy or due to pumping into other birds on the floor may have implications on bird welfare and production efficiency. For example, increases the risk of carcass scratching and bruising (Murphy and Preston, 1988).

The observed fragmentation of resting behavior resulted in corresponding decrease in sleeping frequency was agreeable with Malleau *et al.* (2007). However, Andrews *et al.* (1997); Bessei (1992) and Sanotra *et al.* (2002) found that birds stocked at HSD spent more time dozing and sleeping than those at lower density.

The reported reduction of frequency of body care behavior especially preening is similar to the result recorded by Andrews *et al.* (1997). This was because chickens require adequate space to perform normal grooming (Albentosa and Cooper, 2004). This space was diminished by increasing stocking density, which consequently reduces performance of preening behavior.

Moreover, aggressive behavior was not recorded in this study neither in high nor low stocking densities. Similar results were recorded by Murphy and Preston (1988) and Preston and Murphy (1989). On the other hand, many studies reported aggression in broiler reared at HSD (Puron *et al.*, 1995; Feddes *et al.*, 2002). Mean a while, Keeling and Duncan (1991) reported that aggressiveness is relatively higher in small flocks than in large flocks, as birds adopt strategies to avoid negative social interactions.

In the present study, protexin® supplementation didn't alter comfort, aggressive and body care behaviors of broilers at low density. In addition, birds stocked at high stocking density and supplemented with Protexin® showed no alterations in all behavioral patterns compared to HSD group It means that, the used probiotic was not effective in mitigating stressful effect of HSD on comfort and body care behaviors broilers.

Stressful effect of HSD on the bird's behaviors may be attributed to some factors such as the competition of birds on feed and water (Rashidi *et al.*, 2010), increasing litter content of moisture and ammonia (Dawkins *et al.*, 2004) and the increased air carbon dioxide (Yardimci and Kenar, 2008). These factors may be prevented or corrected by probiotic supplementation (Endo and Nakano, 1999; Zhang and Kim, 2013). This was unlike our study results. Hence, the used probiotic (Protexin®) could not correct or prevent these stressful effects of HSD on broilers.

Conclusion

Based on our results it may be concluded that, Protexin® was unable to improve comfort and body care behaviors of broilers reared at low stocking density and failed to alleviate impacts of high density stress on these behaviors.

Acknowledgement

We acknowledge Projects Funding and Granting unit belonging to the Scientific Research Developing Unit in Beni-Suef University, Egypt for funding this study.

References

Albentosa, M.J., Cooper, J.J., 2004. Effects of cage height and stocking density on the frequency of comfort behaviors performed by laying hens housed in furnished cages. Animal Welfare 13, 419–424.

Andrews, S.M., Omed, H.M., Phillips, C.J.C., 1997. The effect of a single or repeated period of high stocking density on the behavior and response to stimuli in broiler chickens. Poultry Science. 76, 1655-1660.

Bessei, W., 1992. The behaviour of broilers under intensive management conditions. Arch. Geflügelk 56, 1–7.

Buijs, S., Keeling, L.J., Vangestel, C., Baert, J., Tuyttens, F.A.M., 2011. Neighbourhood analysis as an indicator of spatial requirements of broiler chickens. Applied Animal Behavior Science 129, 111–120.

Buijs, S., Keeling, L. J., Vangestel, C., Baert, J., Vangeyte, J., Tuyttens, F.A.M., 2010. Resting or hiding? Why broiler chickens stay near walls and how density affects this. Applied Animal Behavior Science 124, 97–103.

Buijs, S., Van Poucke, E., Van Dongen, S., Lens, L., Baert, J., Tuyttens, F.A.M., 2012. The influence of stocking density on broiler chicken bone quality and fluctuating asymmetry. Poultry Science 91, 1759–1767.

Cornetto, T., Estevez, I., Douglass, L.W., 2002. Using artificial cover to reduce aggression and disturbances in domestic fowl. Applied Animal Behaviour Science 75, 325-336.

Dawkins, M. S., Donnelly, C. A., Jones, T. A., 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature 427, 342-344.

Endo, T., Nakano, M., 1999. Influence of a Probiotic on Productivity, Meat Components, Lipid Metabolism, Caecal Flora and Metabolites, and Raising Environment in Broiler Production. Animal Science Journal 70 (4), 207-218.

Estevez, I., 2007. Density allowances for broilers: where to set the limits? Poultry Science 86, 1265–1272.

Feddes, J.J.R., Emmanuel, E.J., Zuideft, M.J., 2002. Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities. Poultry Science 81, 774–779

Keeling, L.J., Duncan, I.J.H., 1991. Social spacing in domestic fowl under semi-natural conditions: the effect of behavioural activity and activity transitions. Applied Animal Behavior Science 32, 205- 217.

Lewis, N.J., Hurnik, J.F., 1990. Locomotion of broiler chickens in floor pens. Poultry Science 69, 1087–1093.

Malleau, A.E., Duncan, I.J.H., Widowski, T. M., Atkinson, J.L., 2007. The importance of rest in young domestic fowl. Applied Animal Behavior Science 106, 52–69.

Marchewka, J., Watanabe, T.T.N., Ferrante, V., Estevez, I., 2013. Review of the social and environmental factors affecting the behavior and welfare of turkeys (Meleagris gallopavo), Poultry Science 92, 1467–1473.

Maria, G.A., Escos J., Alados, C.L., 2004. Complexity of behavioural sequences and their relation to stress conditions in chickens (Gallus gallus domesticus): A non-invasive technique to evaluate animal welfare. Applied Animal Behavior Science 86, 93-104.

Martrenchar, A., Morisse, J.P., Huonnic, D., Cotte, J.P., 1997. Influence of stocking density on some behavioural, physiological and productivity traits of broilers. Veterinary Research 28, 473–480.

Murphy, L.B., Preston, A.P., 1988. Time-budgeting in meat chickens grown commercially. Br. Poult. Sci. 29, 571–580.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., Bisson, J. F., Rougeot, C., Pichelin, M., Cazaubiel, M., Cazaubiel, J.M., 2011. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and Bifidobacterium longum R0175) in rats and human subjects. British Journal of Nutrition 105, 755-764.

Preston, A.P., Murphy, L.B., 1989. Movement of broiler chickens reared in commercial conditions. British Poultry Science 30, 519–532.

Puron, D., Santamaria, R., Segura, J.C., Alamilla, J.L., 1995. Broiler performance at different stocking densites. Journal of Applied Poultry Research 4, 55–60.

Rashidi, A.A., Ivari, Y.G., Khatibjoo, A., Vakilia, R., 2010. Effects of dietary fat, vitamin E and zinc on immune response and blood parameters on broiler reared under heat stress. Research Journal of Poultry Sciences 3, 32–38.

Sanotra, G.S., Lund, J.D., Vestergaard, K.S. ,2002. Influence of light-dark schedules and stocking density on behaviour, risk of leg problems and occurrence of chronic fear in broilers. British Poultry Science 43, 344-354.

Savory, C.J., Lariviere, J.M., 2000. Effects of qualitative and quantitative food restriction treatments on feeding motivational state and general activity level of growing broiler breeders. Applied Animal Behaviour Science 69, 135–147.

Sudo, N., 2006. Stress and gut microbiota: Does postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response? Int. Congr. Ser. 287, 350-354.

Thomas, D.G., Ravindran, V., Thomas, D.V., Camden, B.J., Cottam, Y.H., Morel, P.C.H., Cook, C.J., 2004. Influence of stocking density on the performance, carcass characteristics and selected welfare indicators of broiler chickens. New Zealand Veterinary Journal 52, 76-81.

Turkyilmaz, M.K., 2008. The effect of stocking density on stress reaction in broiler chickens during summer. Turkish Journal Veterinary Animal Science 32, 31–36.

Weeks, C.A., Danbury, T.D., Davies, H.C., Hunt, P., Kestin, S.C., 2000. The behaviour of broiler chickens and its modification by lameness. Applied animal Behaviour Science 67, 111-125.

Yardimci, M., Kenar, B., 2008. Effect of stocking density on litter microbial load in broiler chickens. Arch. Zootech. 11, 75–81.

Yong, E.D., 2011. From guts to brains–eating probiotic bacteria changes behavior in mice. Discover Magazine. https://blogs.discovermagazine.com/notrocketscience/2011/08/29/fro.m-guts-to-brains-eating-probiotic-bacteria-changes-behavior-in-mice.

Zhao, F.R., Geng, A.L., Li, B.M., Shi, Z.X., Zhao, Y.J., 2009. Effects of environmental factors on breast blister incidence, growth performance, and some biochemical indexes in broilers. Journal of Applied Poultry Research 18, 699-706.

Zulkifli, I., Sti Nor Azah, A., 2004. Fear and stress reactions, and the performance of commercial broiler chickens subjected to regular pleasant and unpleasant contacts with human being. Applied Animal Behavior Science 88, 77-87.

Zhang, Z.F., Kim, I.H., 2013. Effects of probiotic supplementation in different energy and nutrient density diets on performance, egg quality, excreta microflora, excreta noxious gas emission, and serum cholesterol concentrations in laying hen. Journal Animal Science 91,4781–4787.