Journal of Advanced Veterinary Research Volume 8, Issue 4 (2018) 73-78

Journal of Advanced Veterinary Research

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

Efficacy of Probiotic in Improving Welfare and Mitigating Overcrowding Stress in Broilers

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, Beni-Suef University, El Menia University, El Menia, Egypt*

ARTICLE INFO

Original Research

Received:
04 September 2018

Accepted:
27 September 2018

Keywords:

Behavior
Broilers
High stocking
Probiotic
Stress
Welfare

ABSTRACT

This study was conducted to investigate the efficacy of probiotic in improving welfare of broilers reared under low stocking density (LSD) and mitigating overcrowdings or high stocking density (HSD) stress. A total number of 240 chicks were sub divided into four groups with three replicates for each. Two groups were reared under LSD (10 bird/m^2); 48 chicks (16 bird/ replicate) for each and another 2 groups were reared under HSD (15 bird/m^2); 72 chicks (24 bird/ replicate). At each density, one group was supplemented with Protexin® and the other was not supplemented. Blood corticosterone (CS) level was measured and behavior of broilers was recorded weekly. At the end of the growing cycle, behavioral welfare, fear response test (time of first attempt to stand, number of attempts to stand and tonic immobility duration; TI) was performed and brain monoamines (serotonin, norepinephrine and dopamine) concentrations were measured. As a result, HSD stress increased the time birds spent to perform the first attempt to stand and prolonged TI duration. However, Protexin® supplementation only reduced the time of first attempt to stand at higher density without alteration of the number of attempts required to induce TI. No significant improvement in fear response in LSD birds supplied with Protexin®. Furthermore, HSD stress decreased feeding, drinking and walking duration. However, Protexin® supplementation improved feeding, drinking and walking behaviors at LSD and did not improve behaviors of birds at HSD. Moreover, HSD increased CS levels at the 4th, 5th and 6th week of the growing cycle. However, Protexin® supplementation had a decreasing effect on CS levels in the birds reared at HD at the last two weeks of the growing cycle. In addition, brain serotonin concentration was increased in birds reared at HD without Protexin® supplementation and showed no alteration in that supplemented with the probiotic at LSD and HSD. Data suggests that, Protexin® supplementation may be beneficial in improving welfare (behavioral indicator) of broilers reared at LSD and alleviate some effects of HSD stress on birds.

J. Adv. Vet. Res. (2018), 8 (4),73-78

Introduction

There is a marked increase in the global demand of poultry meat. By 2030, poultry meat consumption will increase to 25% than that of 2015 in developing countries. To meet this growing demand, many broiler farms were shifted from extensive to intensive rearing system (Wahyono and Utami, 2018). However, this intensification/ high stocking density (HSD) poses stress on birds.

In the uncontrolled environmental conditions, HSD is a multiple stressor because it results in unfavorable environmental conditions (Heckert *et al*., 2002; Estevez, 2007) such as high temperature and ammonia. In controlled environmental conditions, confliction between the birds is the main stressful effect of HSD (Ravindran *et al*., 2006). In additions, restriction of the floor space allowed for birds may hinder them to express their normal behavior and enjoy good health, hence bird's welfare is impaired (Welfare Quality®, 2009; Abudabos *et al*., 2013). In the poultry industry, the utmost goal is maximizing production without impairing bird's welfare caused by overcrowdings (Abudabos *et al*., 2013). Since stressors have negative effects on the gut microflora balance (Sohail *et al*., 2010; Guardia *et al*., 2011), the best approach to alleviate stress and improve welfare of birds is the activation of these flora using feed or drinking water additives.

Among these additives, probiotics, which activate gut microflora (Yu *et al*., 2008), performance (Cengiz *et al*., 2015), immunity (Teo and Tan, 2007), decrease excreta ammonia emission (Zhang and Kim, 2013) and improve the raising environment (Endo and Nakano, 1999) in poultry production.

Stress response could be assessed by measuring bird's behavior (Bruno *et al*., 2011), blood corticosterone concentration (Shakeri *et al*., 2014; Najafi *et al*., 2015) and brain monoamines (Chaouloff *et al*., 1999). Impairment of feeding, drinking and kinetic (walking) behaviors may retard growth and performance in poultry farms. Hence, behavior measurement is a useful tool of welfare indicator.

Based on our knowledge, there are many reports about

*Corresponding author: Fatma Khalil
E-mail address: fatmahs77@yahoo.com

ISSN: 2090-6277/2090-6269/

HSD stress (Endo and Nakano, 1999; Zhang and Kim, 2013) such as using probiotic to avoid physiological stress (Sohail *et al*., 2010, 2011). While, there is lack of studies that assess the role of probiotic in providing welfare and alleviating HSD stress on broilers.

Therefore, this study was designated to investigate efficacy of probiotic to improve welfare of birds reared at low stocking density; LSD (10 birds/m^2) and mitigate high stocking density; HSD (15 birds/m^2) stress on broilers using blood corticosterone, brain monoamines and behavior (fear response behavioral test, feeding, drinking and waking), as welfare indicators and stress assessment parameters.

Materials and methods

Chemicals and probiotic

Protexin® probiotic {*Enterococcus faecium* 2x10^9 CFU/g (2x10^{12} CFU/kg)} was purchased from probiotic International Smorest, UK., noradrenaline (NA), dopamine (DA) and serotonin (5-HT) HPLC standards and streptozotocin (STZ) were purchased from Sigma Aldrich Chemicals Company St. Louis USA. CoQ10 capsules were purchased from Arab Company for Pharm. & Medicinal Plants (MEPACO-MEDIFOOD) Enshas-Sharkeya-Egypt. All other chemicals were of HPLC grade and purchased from Sigma.

Experimental design

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

Two hundred and forty unsexed one-day old (Cobb type breed) chicks, purchased from a commercial hatchery at Beni-Suef. They were brooded at 33ºC using electric heaters for the first week of age. At the end of the first week, they were randomly distributed into four groups. Two groups of birds were reared at LSD (10 bird/m^2) groups, 96 birds were divided into two groups (one was not supplemented with probiotic (LDS) and the other was supplemented (LSDP), three replicates for each. Forty eight chicks were used in each group (16 bird/ replicate). The other two groups were reared at HSD (15 bird/m^2) groups, 144 birds were divided into two groups (one was not supplemented with probiotic (HSD) and the other was supplemented (HSDP); three replicates (24 bird/ replicate). This study was approved by Institutional Animal Care and Use Committee of Beni-Suef University (BSU-IACUC), Egypt.

Birds accommodation and management

Chicks were reared in 12 floor pens –of equal dimension (1m×1.6 m) with a new clean wood shaving litter material. The proper ventilation was maintained using windows, fans and exhausting fans. In addition, maintaining brooding heating was performed by the electric heaters, with a decrease in the temperature 2ºC weekly. Continuous lighting program was used for the first week and 23 hrs light and 1hr dark till the end of the experiment. Feed and water were provided *ad libtum* in well distributed manual plastic feeders and drinkers. Feeding of birds was divided into two-phase broiler-feeding regime; a starter containing 23% protein crumble for the first 21 days, then a grower pellet with 21% protein till the end of the study at day 42.

Protexin® supplementation

Protexin® was added to the water starting from the second week (according to the manufacturer recommendation).

Fear response (fearfulness) test

Birds were tested for fear response using fear test level; tonic immobility (Forkman *et al*., 2007) two birds/ replicate were caught and carried in an upright position to a separate neighboring room. A few seconds after the bird was caught, tonic immobility was induced by placing the bird on its back with the head hanging in a U-shaped wooden cradle (Jones and Faure, 1981). The bird was restrained for 10 seconds(s). The observer sat in full view of the bird, about 1 min. away, and fixed his eyes on the bird to give the fear-inducing properties of eye contact. If the bird remained immobile for 10 sec., after the experimenter removed his hands, a stopwatch was started to record: a) Latency Duration (s) until the bird righted itself. If the bird righted itself in less than 10 sec, then it was considered that tonic immobility had not been induced, and the restraint procedure was repeated (3 times maximum). If the bird did not show a righting response over the 10 min test period, a maximum score of 600 s was given for righting time. Thus, tonic immobility (TI) duration ranged from 0 to 600 sec. b) Number of attempts that were done by the bird to righted himself and the duration of the first attempt was calculated.

Measurement of ingestive and locomotor behaviours

The behavior of chicks was recorded once a week, in the morning (9:00 – 12:00 a.m.) for 5 consecutive weeks. Each pen was observed for 20 minutes. Scan method of observation was adopted in this study according to Bubier (1996) and Maria *et al*. (2004) to analyze ingestive (feeding and drinking) and locomotor (walking) behaviour of birds. Total feeding duration; TFD (when head extended towards available feed resources while beak in or above the feed trough appeared to be manipulating or ingesting), total drinking duration; TDD (Beak in contact with water in or above the drinker and appears to be drinking water) and total walking duration; TWD (moving forward taking one or more step) throughout the growing cycle were calculated.

Measurement of blood corticosterone level (µg/dl)

Five ml of blood were collected from wing vein of 6 randomly overnight fastened birds per replicate (2 from each replicate). Blood was collected without anticoagulant for serum separation. The samples were collected weekly for corticosterone (CS) throughout the growing cycle. All samples were kept at 4ºC then centrifuged at 3000-4000 rpm for 10-15 min, the obtained supernatant was stored at -20ºC. Corticosterone levels were measured using commercial ELISA kits.

Measuring brain monoamines concentrations (nmol/ml)

At the end of the growing cycle (6^{th} week), 12 randomly selected birds from (4 from each replicate) were fasted overnight then slaughtered and their brains were removed quickly and placed in iced normal saline, perfused with the same solution to remove blood cells, and frozen at -80°C for estimation of brain monoamines (Noradrenaline (NA), dopamine (DA) and serotonin (5-HT). or measuring brain monoamines concentration, the frozen tissues were cut into small pieces and homogenized in phosphate buffer (pH 7.4), then centrifuged at 4000 rpm for 15 minutes at 4°C and the supernatant was removed for chemical parameters estimation. Then, brain monoamines were measured in the obtained supernatants according to Abdel-Salam *et al*. (2011).

Statistical analysis

Data were presented as mean ± standard error of mean and analyzed by one-way ANOVA test using SPSS (2011). Data was considered significant at (P<0.05).

Results

HSD increased (P< 0.05) the time that birds spent to perform the first attempt to stand (Table 1) and prolonged (P<0.05) TI duration. However, Protexin® supplementation only reduced (P< 0. 05) the time of first attempt to stand at higher density without alteration of the number of attempts required to induce TI. Table 2, illustrated the effect of density and the role of probiotic on feeding, drinking and walking behaviors of broilers. LSDP group performed longer time feeding, drinking and walking compared to LSDP, HSD and HSDP at (P<0.05). Table 3, declared that HSD increased (P<0.05) CS levels at the 4th, 5th and 6th week of the growing cycle. However, protexin® supplementation had a decreasing effect on CS (P<0.05) levels in the birds reared at HD (HSDP) at the last two weeks of the growing cycle. Brain 5-HT level was significantly (P<0.05) increased in birds reared at HD (P<0.05) however, Protexin® supplementation did not affect serotonin level in HSDP group NE and DA levels were not altered in all groups

Discussion

The present study demonstrated that supplementation of broilers with probiotic may alter different welfare indicators and stress response of the birds.

The observed increase in fear response of birds in HSD group confirms that the intensification of birds impairs their welfare (Onbaşılar *et al*., 2008; Na-Lampang 2014). This result is in line with that of Onbaşılar *et al*. (2008) and Buijs *et al*. (2009), who reported significant increase in TI duration with increasing density. However, it is disagreeable with Ventura *et al*. (2010), who found that both duration and the number of attempts required to induce TI weren't affected with density. This may be attributed to high litter moisture content caused by overcrowding (Van Poucke *et al*. (2007) and Campo *et al*. (2007) and /or bird's confliction (Buijs *et al*. 2009).

Based on welfare behavioural test, our findings suggested that probiotic supplementation failed to decrease fear response in broilers subjected to stress. This data is in agreement with Ghareeb *et al*. (2008). Further study is needed to investigate the effect of other probiotics and medicinal plants and or combination of both on welfare indicators of broilers.

Our result indicated the prominent impairment of feeding and drinking behavior of birds stocked at HD. This result was in harmony with that reported by Cengiz *et al*. (2015). On the contrary, Uner *et al*. (1997) reported that highly stocked birds spent more time around feeders and drinkers. Meanwhile, Andrews *et al*. (1997) found no alterations in feeding and drinking behavior of birds stocked at HD. This difference in findings of researchers indicates that stress response of birds is gene and stressor-dependent.

The observed decrease of the walking behavior in response to HSD stress is also recorded by Sanotra *et al*. (2002)

Table 1. The effect of protexin® supplementation on fear levels of broiler chickens

	First attempt to stand (Sec)	Number of attempts	TI duration (Sec)
LSD	129.83±27.10^b	1.00±0.50	190.50±48.02^b
LSDP	120.83±12.82^b	1.17±0.60	169.00±21.73^b
HSD	492.33±79.57^a	0.33±0.17	587.67±12.33^a
HSDP	399.83±132.45^b	0.67±0.67	416.67±32.19^a

Results are expressed as Means ± Standard Error (S.E.).
^{a,b} superscripts within columns indicate significant difference at p<0.05
LSD = 10 bird/m^2 rearing density without probiotic supplementation (control)
LSDP =10 bird/m^2 density with probiotic supplementation
HSD =15 bird/m^2 rearing density without probiotic supplementation
HSDP =15 bird/m^2 rearing density with probiotic supplementation
TI= Tonic immobility

Table 2. The effect of protexin® supplementation behavior of broiler chicken

	TFD (Sec)	TDD (Sec)	TWD (Sec)
LSD	1366.93±77.17^b	155.33±4.5^b	89.53± 3.28^b
LSDP	1938.27± 145.69^a	190.87±3.9^a	97.6 ±6.00^a
HSD	1193.33±44.52^b	111.47±3.0^b	63.2±5.95^b
HSDP	1501.00±56.67^b	124.53±4.3^b	62.2 ± 2.55^b

Results are expressed as Means ± Standard Error (S.E.).
^{a,b} superscripts within columns indicate significant difference at p<0.05
LSD = 10 bird/m^2 rearing density without probiotic supplementation (control)
LSDP =10 bird/m^2 density with probiotic supplementation
HSD =15 bird/m^2 rearing density without probiotic supplementation
HSDP =15 bird/m^2 rearing density with probiotic supplementation
TFD =total feeding duration TDD =total drinking duration TDD =total walking duration

Table 3. The effect of protexin® supplementation on serum corticosterone levels (µg/dl)and brain monoamines concentration (nmol/ml)

	Weekly serum corticosterone levels (µg/dl)					Brain monoamines (nmol/ml brain homogenate supernatant)		
	2nd	3rd	4th	5th	6th	5-HT	NE	DA
LSD	0.56 ±0.05	0.55±0.01	0.67 ± 0.00^{b}	0.86±0.0^{c}	0.97±0.01^{c}	26.93±7.34^{b}	225.10±58.08	473.67±156.69
LSDP	0.52 ± 0.30	0.54±0.00	0.67±0.04^{b}	0.83±0.02^{c}	0.96± 0.01^{c}	26.57±8.38^{b}	217.40±26.53	460.00±42.55
HSD	0.62± 0.02	0.57±0.02	1.54±0.01^{a}	1.55±0.0^{a}	1.90±0.01^{a}	129.77±3.34^{a}	358.97±22.78	824.00±39.95
HSDP	0.57±0.02	0.54± 0.06	1.19±0.02^{a}	1.44±0.0^{b}	1.45±0.01^{b}	41.57±17.48^{b}	307.40±34.61	745.67±39.29

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

^{a,b} superscripts within columns indicate significant difference $p<0.05$

LSD = 10 bird/m^2 rearing density without probiotic supplementation (control)

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

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

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

NE= Norepinephrine DA= Dopamine 5-HT= Serotonin

and Buijs *et al*. (2009). It was noticed that the highest adverse effect of HSD on the birds was prominent at 5th and 6th weeks of the growing cycle. On the other hand, Andrews *et al*. (1997) recorded that obvious effect of HSD was at 4th week of age. Generally, birds' movements are negatively correlated to their age (Andrews *et al*., 1997; Kristensen *et al*., 2007) especially these stocked at HD. The observed increase in TFD in LSD group supplemented with probiotic is in consistence with data of Verdu *et al*. (2008) and Naglaa (2013) in mice and turkey, respectively. However, this result is in contrast to that reported by Koenen *et al*. (2004).

The reported increase of CS levels in response to HSD indicated the stressful effect of overcrowdings. This result was similar to that obtained by Beloore *et al*. (2010); Shakeri *et al*. (2014) and Najafi *et al*. (2015), who observed a trend of increasing corticosterone concentration with higher stocking density. On the other hand, this result is disagreeable with Turkyilmaz (2008); Tong *et al*. (2012) and Cengiz *et al*. (2015).

The obtained reducing effect of Protexin® supplementation on CS levels of birds stocked at HD is supported with Sohail *et al*. (2010), who found that probiotics were helpful in minimizing stress on broiler. However, Cengiz *et al*. (2015) reported that probiotics didn't affect CS levels at HSD.

HSD induced stressful effect because it was accompanied with several factors such as the competition on feed and water (Craig *et al*., 1986), increasing litter moisture and ammonia (Dawkins *et al*., 2004) and the increased carbon dioxide (Yardimci and Kenar, 2008), which were contributing in increasing CS levels. These factors may be prevented by Protexin® supplementation (Endo and Nakano, 1999; Zhang and Kim, 2013). Hence, probiotic may be effective in mitigating stressful effect on CS response.

The obtained increase in brain 5-HT level in the birds reared at HD is agreeable with Arborelius and Eklund (2007) and Cheng and Fahey *et al*. (2009), who recorded reduction in level of brain serotonin in layers and rodents exposed to different stressors. In additions, the reported high brain 5-HT concentration in birds stocked at HD with Protexin® supplementation is similar to data recorded by Adell *et al*. (1988) in rats and Liu *et al*. (2016) in mice supplemented with probiotic and exposed to stress.

Increasing serotonin may be urged to corticosterone action (Chalmers *et al*., 1993; Nishi and Azmitia, 1996). On the other hand, 5-HT was reported to stimulate the release of CRF and ACTH from the hypothalamus and pituitary of mammals (Dinan, 1996). Probiotics regulate central 5-HT metabolism by regulation of tryptophan metabolism (Desbonnet *et al*., 2008), and/or regulation of brain-derived neurotrophic factor which activate 5-HT (Benmansour *et al*., 2008). Therefore, Protexin® supplementation succeeded to alleviate HSD stress on brain 5-HT level in our study.

The recorded no alteration in brain NE, and DA levels is in agreement with Korte *et al*. (1997) and Cheng and Fahey *et al*. (2009), while, it is disagreeable with Rouge-Pont *et al*. (1995).

The conflicting monoamines data from different investigations may be related to different species, stressors, duration and frequency of stressor presentation as well as selected region of brain for measuring monoamines.

Brain 5-HT was suggested to play a role in regulating feeding (Yadav *et al*., 2009), walking (Kiehn and Kjaerulff, 1996), and neuroendocrine stress responses (Chaouloff, 1993). It had inhibitory effect on the responsiveness some behavioral patterns (Dallman, 1993). Thus, the observed increase of brain 5-HT concentration in our study was accompanied with reduction in the feeding behavior, feed intake and walking behavior in HSD group.

Conclusion

The results of this study suggested that HSD may pose serious alterations in behavior, blood CS and brain serotonin concentration, which may be alleviated by probiotic supplementation that also may improve welfare of the unstressed birds.

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

Abdel-Azeem, M., 2013. Do probiotics affect the behavior of turkey poults?. Journal of Veterinary Medicine and Animal Health 5(5), 144-148.

Abdel-Salam, O.M., Salem, N.A., Hussein, J.S., 2012. Effect of aspartame on oxidative stress and monoamine neurotransmitter levels in lipopolysaccharide-treated mice. Neurotoxicity Research 21(3), 245-255.

Abudabos, A.M., Samara, E.M., Hussein, E. O.S., Al-Ghadi, M.Q., Al-Atiyat, R.M., 2013. Impacts of Stocking Density on the Performance and Welfare of Broiler Chickens. Italian. Journal of Animal Science 12 (11), 66-71.

Adell, A., Garcia-Marquez, C., Armario, A., Gelpi, E., 1988. Chronic stress increases serotonin and noradrenaline in rat brain and sensitizes their responses to a further acute stress. Journal of neurochemical 50(6), 1678-1681.

Andrews, S.M., Omed, H.M., Phillips, C.J., 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(12), 1655-1660.

Arborelius, L., Eklund, M.B., 2007. Both long and brief maternal separation produces persistent changes in tissue levels of brain monoamines in middle-aged female rats. Neuroscience 145(2), 738–750.

Beloor, J., Kang, H.K., Kim, Y.J., Subramani, V.K., Jang, I.S., Sohn, S.H., Moon, Y.S., (2010). The effect of stocking density on stress related genes and telomeric length in broiler chickens. Asian-Australasian Journal of Animal Sciences 23 (4), 437-443.

Benmansour, S., Deltheil, T., Piotrowski, J., Nicolas, L., Reperant, C., Gardier, A.M., David, D.J., 2008. Influence of brain-derived neurotrophic factor (BDNF) on serotonin neurotransmission in the hippocampus of adult rodents. European Journal of Pharmacology 587(1-3), 90-98.

Bruno, L.D.G., Maiorka, A., Macari, M., Furlan, R.L., Givisiez, P.E.N., 2011. Water intake behavior of broiler chickens exposed to heat stress and drinking from bell or and nipple drinkers. Revista Brasileira de Ciência Avícola 13(2), 147-152.

Bubier, N.E., 1996. The behavioural priorities of laying hens: the effects of two methods of environment enrichment on time budgets. Behavioural Processes 37 (2-3), 239-249.

Buijs, S., Keeling, L., Rettenbacher, S., Van Poucke, E., Tuyttens, F.A.M., 2009. Stocking density effects on broiler welfare: Identifying sensitive ranges for different indicators. Poultry Science 88(8), 1536-1543.

Campo, J.L., Gil, M.G., Dávila, S.G., Muñoz, I., 2007. Genetic and phenotypic correlation between fluctuating asymmetry and two measurements of fear and stress in chickens. Applied Animal Behaviour Science 102 (1-2), 53-64.

Cengiz, Ö., Köksal, B.H., Tatlı, O., Sevim, Ö., Ahsan, U., Üner, A.G., Önol, A.G., 2015. Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. Poultry Science 94 (10), 2395-2403.

Chalmers, D.T., Kwak, S.P., Mansour, A., Akil, H., Watson, S.J., 1993. Corticosteroids regulate brain hippocampal 5-HT1A receptor mRNA expression. Journal of Neuroscience 13 (3), 914-923.

Chaouloff, F., 1993. Physiopharmacological interactions between stress hormones and central serotonergic systems. Brain Research Reviews 18 (1), 1-32.

Chaouloff, F., Berton, O., Mormède, P., 1999. Serotonin and stress. Neuropsychopharmacology 21(2), 28S-32S.

Cheng, H. W., Fahey, A., 2009. Effects of group size and repeated social disruption on the serotonergic and dopaminergic systems in two genetic lines of White Leghorn laying hens. Poultry Science 88(10), 2018-2025.

Craig, J.V., Craig, J.A., Vargas, J.V., 1986. Corticosteroids and other indicators of hens' well-being in four laying-house environments. Poultry Science 65(5), 856-863.

Dawkins, M.S., Donnelly, C.A., Jones, T.A., 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature 427(6972), 342.

Desbonnet, L., Garrett, L., Clarke, G., Bienenstock, J., Dinan, T.G., 2008. The probiotic Bifidobacteria infantis: an assessment of potential antidepressant properties in the rat. Journal of Psychiatric Research 43(2), 164-174.

Dinan, T.G., 1996. Serotonin and the regulation of hypothalamic-pituitary-adrenal axis function. Life Sciences 58 (20), 1683-1694.

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. Nihon Chikusan Gakkaiho 70(4), 207-218.

Estevez, I., 2007. Density allowances for broilers: where to set the limits?. Poultry Science 86(6), 1265-1272.

Forkman, B., Boissy, A., Meunier-Salaün, M.C., Canali, E., Jones, R.B., 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiology and Behavior 92 (3), 340-374.

Ghareeb, K., Awad, W.A., Nitsch, S., Abdel-Raheem, S., Böhm, J., 2008. Effects of Transportation on Stress and Fear Responses of Growing Broilers Supplemented with Prebiotic or Probiotic. International Journal of Poultry Science 7 (7), 678-685.

Guardia, S., Konsak, B., Combes, S., Levenez, F., Cauquil, L., Guillot, J., Gabriel, I., 2011. Effects of stocking density on the growth performance and digestive microbiota of broiler chickens. Poultry Science 90(9), 1878-1889.

Heckert, R.A., Estevez, I., Russek-Cohen, E., Pettit-Riley, R., 2002. Effects of density and perch availability on the immune status of broilers. Poultry Science 81(4), 451-457.

Jones, R.B., Faure, J.M., 1981. Tonic immobility ("righting time") in laying hens housed in cages and pens. Applied Animal Ethology 7(4), 369-372.

Kiehn, O., Kjaerulff, O., 1996. Spatiotemporal characteristics of 5-HT and dopamine-induced rhythmic hindlimb activity in the in vitro neonatal rat. Journal of Neurophysiology 75(4), 1472-1482.

Koenen, M.E., Kramer, J., Van Der Hulst, R., Heres, L., Jeurissen, S.H.M., Boersma, W.J.A., 2004. Immunomodulation by probiotic lactobacilli in layer-and meat-type chickens. British Poultry Science, 45(3), 355-366.

Korte, S.M., Beuving, G., Ruesink, W.I.M., Blokhuis, H.J., 1997. Plasma catecholamine and corticosterone levels during manual restraint in chicks from a high and low feather pecking line of laying hens. Physiology and Behavior 62 (3), 437-441.

Kristensen, H.H., Prescott, N.B., Perry, G.C., Ladewig, J., Ersbøll, A.K., Overvad, K.C., Wathes, C.M., 2007. The behaviour of broiler chickens in different light sources and illuminances. Applied Animal Behaviour Science 103(1-2), 75-89.

Marıa, G.A., Escós, 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 Behaviour Science 86 (1-2), 93-104.

Liu, Y.W., Liu, W.H., Wu, C.C., Juan, Y.C., Wu, Y.C., Tsai, H.P. Tsai, Y.C., 2016. Psychotropic effects of *Lactobacillus plantarum* PS128 in early life-stressed and naïve adult mice. Brain Research 1631, 1-12.

Najafi, P., Zulkifli, I., Jajuli, N.A., Farjam, A.S., Ramiah, S.K., Amir, A.A., Eckersall, D., 2015. Environmental temperature and stocking density effects on acute phase proteins, heat shock protein 70, circulating corticosterone and performance in broiler chickens. International Journal of Biometeorology 59(11), 1577-1583.

Na-Lampang, P., 2014. Productivity and Tonic Immobility Duration of Thai Crossbred Chickens Raised at Different Stocking Densities. Global Journal of Animal Scientific Research 2 (2), 72-75.

Nishi, M., Azmitia, E.C., 1996. 5-HT1A receptor expression is modulated by corticosteroid receptor agonists in primary rat hippocampal culture. Brain Research 722 (1-2), 190-194.

Onbaşilar, E.E., Poyraz, Ő., Erdem, E., Őztűrk, H., 2008. Influence of lighting periods and stocking densities on performance, carcass characteristics and some stress parameters in broilers. Arch. Geflűgelk 72, 193-200.

Shakeri, M., Zulkifli, I., Soleimani, A.F., o'Reilly, E.L., Eckersall, P.D., Anna, A.A., Abdullah, F.F.J., 2014. Response to dietary supplementation of L-glutamine and L-glutamate in broiler chickens reared at different stocking densities under hot, humid tropical conditions. Poultry Science 93(11), 2700-2708.

Ravindran, V., Thomas, D.V., Morel, P.C.H., 2006. Performance and welfare of broilers as affected by stocking density and zinc bacitracin supplementation. Animal Science Journal 77(1), 110-116.

Sohail, M.U., Ijaz, A., Yousaf, M.S., Ashraf, K., Zaneb, H., Aleem, M., Rehman, H., 2010. Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic: Dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. Poultry science 89 (9), 1934-1938.

Rougé-Pont, F., Marinelli, M., Le Moal, M., Simon, H., Piazza, P.V., 1995. Stress-induced sensitization and glucocorticoids. II. Sensitization of the increase in extracellular dopamine induced by cocaine depends on stress-induced corticosterone secretion. Journal of Neuroscience 15 (11), 7189-7195.

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(3), 344-354.

Sohail, M.U., Rahman, Z.U., Ijaz, A., Yousaf, M.S., Ashraf, K., Yaqub, T., Rehman, H., 2011. Single or combined effects of mannan-oligosaccharides and probiotic supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes, and serum trace minerals in cyclic heat-stressed broilers. Poultry Science 90 (11), 2573-2577.

Teo, A.Y., Tan, H.M., 2007. Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with Bacillus subtilis PB6 (CloSTAT). Journal of Applied Poultry Research 16(3), 296-303.

Tong, H.B., Lu, J., Zou, J.M., Wang, Q., Shi, S.R., 2012. Effects of stocking density on growth performance, carcass yield, and immune status of a local chicken breed. Poultry Science 91(3), 667-673.

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.

Van Poucke, E., Van Nuffel, A., Van Dongen, S., Sonck, B., Lens, L., Tuyttens, F.A.M., 2007. Experimental stress does not increase fluctuating asymmetry of broiler chickens at slaughter age. Poultry Science 86(10), 2110-2116.

Ventura, B.A., Siewerdt, F., Estevez, I., 2010. Effects of barrier perches and density on broiler leg health, fear, and performance. Poultry Science 89 (8), 1574-1583.

Verdu, E.F., Bercik, P., Huang, X.X., Lu, J., Al-Mutawaly, N., Sakai, H., Collins, S.M., 2008. The role of luminal factors in the recovery of gastric function and behavioral changes after chronic *Helicobacter pylori* infection. American Journal of Physiology-Gastrointestinal and Liver Physiology 295(4), G664-G670.

Welfare Quality®, 2009. Welfare Quality ®assessment protocol for poultry (broilers, laying hens). Welfare Quality® Consortium, Lelystad, Netherlands.

Yadav, V.K., Oury, F., Suda, N., Liu, Z.W., Gao, X.B., Confavreux, C., Tecott, L.H., 2009. A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure. Cell 138 (5), 976-989.

Yardimci, M., Kenar, B., 2008. Effect of stocking density on litter microbial load in broiler chickens. Archiva Zootechnica 11(3), 75-81.

Yu, B., Liu, J.R., Hsiao, F.S., Chiou, P.W.S., 2008. Evaluation of Lactobacillus reuteri Pg4 strain expressing heterologous β-glucanase as a probiotic in poultry diets based on barley. Animal Feed Science and Technology 141(1-2), 82-91.

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 hens. Journal of Animal Science 91(10), 4781-4787.

Wahyono, N.D., Utami, M.M.D., 2018, January. A Review of the Poultry Meat Production Industry for Food Safety in Indonesia. In Journal of Physics: Conference Series Vol. 953, No. 1, p. 012125. IOP Publishing.