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Original

Stocking density, sex and season on tonic immobility in broiler chickens

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ABSTRACT

Objective. To assess the effect of stocking density, season of the year, and sex on latency time and number of attempts required to induce tonic immobility in broiler chickens in an industrial production system. **Material and methods**. A total of 216.000 commercial hybrid Cobb500 chickens were used, reared in mixed batches, 54.000 in each of the four seasons of the year, in dark houses with forced tunnel ventilation, under two stocking densities: standard (14 chickens/m^2) and reduced (12 chickens/m^2). On day 35 of the productive cycle, 90 birds of each sex, of each density and in each season of the year were sampled and the tonic immobility test was performed, recording the latency time in seconds (s) and the number of induction attempts. **Results**. Stocking density significantly affected ($p<0.005$) latency time with values (median and interquartile range) of 72 s (38-143) for standard density and 63 s (36-127) for reduced density. The difference was marginally significant for the number of attempts. Non-significant differences were observed between sexes and among seasons in any of the two variables. **Conclusions**. The duration of tonic immobility is a useful tool as an indicator of fear in specific situations. Decreasing the stocking density decreases the latency period and more induction attempts are required in chickens, while sex and season do not produce similar effects.

Keywords: Environment; animal welfare; poultry meat; stress; induction; fear (*Source: CAB thesaurus, AIMS*).

RESUMEN

Objetivo. Evaluar el efecto de la densidad de alojamiento, la época del año y el sexo sobre el tiempo de latencia y el número de intentos de inducción de la inmovilidad tónica en pollos parrilleros en un sistema de producción industrial. **Material y métodos.** Se empleó un total de 216.000 pollos híbridos comerciales Cobb500, criados en lotes mixtos, 54.000 en cada una de las cuatro estaciones del año, en galpones oscurecidos de ventilación forzada tipo túnel, bajo dos densidades de alojamiento: estándar (14 pollos/m^2) y reducida (12 pollos/m^2). El día 35 se extrajeron 90 aves

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de cada sexo, de cada densidad y en cada estación del año y se realizó la prueba de inmovilidad tónica, registrándose el tiempo de latencia en segundos (s) y el número de intentos de inducción. **Resultados.** La densidad afectó significativamente ($p<0.005$) el tiempo de latencia, con valores de mediana (Mna) y rango intercuartílico (RI) de 72 s (38-143) para la densidad estándar y 63 s (36-127) para la densidad reducida. En el número de intentos la diferencia fue marginalmente significativa. No se observaron diferencias significativas entre sexos ni entre estaciones en ninguna de las dos variables. **Conclusiones.** La duración de la inmovilidad tónica es una herramienta útil como indicador de miedo en situaciones específicas. La disminución de la densidad reduce el período de latencia y se requieren más intentos de inducción en pollos, en tanto que el sexo y la estación del año no producen el mismo efecto.

Palabras clave: Ambiente; bienestar animal; carne de aves; estrés; inducción; miedo (*Fuente: CAB thesaurus, AIMS*).

INTRODUCTION

Similarly, to other chains involved in intensive animal husbandry, industrial poultry production considers the importance of the five domains of animal welfare. These are related to a physical component that includes nutrition, environment, behavior and health, as well as a mental health component in relation with mental states, such as fear (1). Fear is an undesirable state of suffering which animals use to protect themselves from potential hazards, such as predators, human interaction, or environmental changes. It can be expressed in violent attempts to escape, panic, wounds, pain, or even death. The animal's usual response to threat includes attempts to escape, flight-or-fight, or immobilization. Different tests have been used to assess fear in birds (2), including tonic immobility, as first described by Schwenter in 1636 (3). It is an unlearned behavioral response to physical restraint, characterized by a catatonic state with reduced responsiveness and variable duration (4), being one of the most widely used indicators to assess stress responses in chicken. This allows the detection of fear in chicken, as a previous step towards the implementation of actions to reduce and/or avoid fear.

It is well known that the stress response generated by fear can affect production and reduce profitability in intensive poultry systems (5). Tonic immobility testing has been used to assess sex-specific, life-long differences in stress responses, monitoring the reaction of chickens after being exposed to early stress in short periods, though the results are not conclusive (6).

Moreover, tonic immobility is a useful indicator to assess adaptability to variations in the environmental conditions of husbandry in poultry. Fear reactions under different environmental situations cause the deployment of defensive behaviors with varying degrees of intensity according to the animal's emotional situation as a response to the environment (7). It has been shown that immobility time is influenced by the stocking density used in each stage of production. Besides considering current data regarding the effect of these factors on tonic immobility, it is necessary to analyze them under the extreme conditions of the humid subtropical climate in the northeast area of the province of Santa Fe.

The objective of this work was to assess how the stocking density, the seasons of the year and sex affect latency times and the number of attempts to induce tonic immobility in broiler chickens in an industrial production system.

MATERIALS AND METHODS

Location and animals. This study was performed at a commercial farm inside the sphere of influence of INTA Reconquista (National Agricultural Technology Institute), in Santa Fe, Argentina, 28°45'11"S latitude and 59°28'05"W longitude. A total of 216.000 commercial hybrid Cobb500 chickens were used, reared in mixed batches, 54.000 in each season of the year, in dark houses with forced tunnel ventilation, under two stocking densities: standard (14 chickens/m^2) and reduced (12 chickens/m^2).

Variables. On day 35 of the productive cycle in each season of the year, 180 previously unhandled birds —90 of each sex— from each density were sampled. The tonic immobility test

was performed in accordance with the methods described by Jones and Faure (8). After being caught, each bird was placed on top of a plastic surface in supine position, with its head hanging. During 15 seconds, the operator kept the bird in this position, applying light pressure on the sternum. The observer was sitting in front of the bird, at approximately 1 m away, and stared directly in its eyes using eye contact to induce fear. Should the bird remain immobile for at least 10 s after the operator removed his hand, the induction was successful. In such case, the observer counted the time it took the bird to right itself, known as latency time. Should the bird right itself in less than 10 s, the attempt to induce tonic immobility was considered null and the restraint procedure was repeated. This sequence was repeated three times maximum and the total number of attempts was recorded. If the induction was not successful after the third attempt, a 0 value was recorded, for the purposes of this study. Should the bird show no response to right itself for a 5-minute period, the latency time was assigned the top value 300 s. Thus, the values assigned to the variables — duration of immobility and number of induction attempts— range between 0-300 s and 1-3, respectively.

Statistical analysis. The effects on the latency time caused by stocking density and sex were assessed separately, using the Mann-Whitney U test to compare medians. Meanwhile, for the effects caused by the seasons of the year, the Kruskal-Wallis one-criterion variance analysis, by rank, was used. The effect on the number of attempts was analyzed using the chi-square distribution.

RESULTS

Table 1 shows latency time and the number of attempts needed to induce tonic immobility depending on each of the two densities analyzed. Latency time was significantly affected by density, with higher values for the birds at standard density. The difference among the number of attempts was marginally significant, with tonic immobility being successfully induced in the first attempt in a higher proportion of chickens at standard density.

Table 1. Latency time (median and interquartile range) and number of attempts to induce tonic immobility depending on the stocking density for broiler chickens in an industrial production system

Stocking density	Latency time (s)		Number of induction attempts. Absolute and relative frequencies			
	Median (IR)	p	1 attempt	2 attempts	+2 attempts	p
Standard	72 (38-143)	0.005	661 (91.8%)	56 (7.8%)	3 (0.4%)	0.052
Reduced	63 (32-127)		639 (88.8%)	71 (9.9%)	10 (1.3%)	

Non-significant differences between sexes were observed in any trait (Table 2).

Table 3 outlines the effects of stocking density on each sex. Median latency time, both for male and female samples, resulted higher in birds at standard density. No significant differences in the number of attempts were observed.

Table 2. Effect of sex on latency time (median and interquartile range) and number of attempts to induce tonic immobility in broiler chickens in an industrial production system.

Sex	Latency time (s)		Number of induction attempts. Absolute and relative frequencies			
	Median (IR)	p	1 attempt	2 attempts	+2 attempts	p
Male	67 (33-136)	0.19	645 (89.6%)	65 (9%)	10 (1.4%)	0.136
Female	70 (36-133)		656 (91.1%)	61 (8.5%)	3 (0.4%)	

Table 3. Effect of sex on latency time (median and interquartile range) and number of attempts to induce tonic immobility in broiler chickens reared at two different stocking densities in an industrial production system.

Sex	Density	Latency time (s)		Number of induction attempts Absolute and relative frequencies			
		Median	p	1 attempt	2 attempts	+2 attempts	p
Male	Standard	73 (38-146)	0.003	333 (92.5%)	24 (6.7%)	3 (0.8%)	0.079
	Reduced	55 (30-124)		315 (87.5%)	39 (10.8%)	6 (1.7%)	
Female	Standard	72 (37-140)	0.005	332 (92.2%)	28 (7.8%)	0 (0%)	0.173
	Reduced	68 (34-130)		324 (90%)	33 (9.2%)	3 (0.8%)	

Non-significant differences among the seasons of the year were observed in the value of the indicators related to tonic immobility (Table 4).

Table 5 shows the amount (kg) of poultry produced per house unit area at the end of husbandry, and the relative difference between both stocking densities depending on each season. These results show that a reduction in the stocking density meant a reduction in the kilograms produced per square meter. This way, more live weight individually obtained from the birds at a reduced density did not suffice to compensate for the overall bigger production from the house with standard density.

Table 4. Latency time (median and interquartile range) and number of attempts to induce tonic immobility depending on the season of the year for broiler chickens in an industrial production system.

Season of the year	Latency time (s)		Number of induction attempts. Absolute and relative frequencies			
	Median (IR)	p	1 attempt	2 attempts	+2 attempts	p
Summer	63 (35-110)	0.466	319 (88.6%)	34 (9.5%)	7 (1.9%)	0.063
Autumn	78 (37-144)		330 (91.7%)	29 (8.1%)	1 (0.2%)	
Winter	73 (33-144)		334 (92.8%)	23 (6.4%)	3 (0.8%)	
Spring	70.5 (33-147)		318 (88.3%)	40 (11.1%)	2 (0.6%)	

Table 5. Meat production and relative difference at the final stage of the broiler chicken cycle reared at two different stocking densities in each of the four seasons of the year in an industrial production system

Stocking density	Season of the year			
	Summer	Autumn	Winter	Spring
Standard (kg/m^2)	38.2	37.8	35.3	38.4
Reduced (kg/m^2)	35.2	34.0	34.8	35.5
Relative difference (%)	7.85	10.05	1.42	7.55

Figure 1 shows the effect of stocking densities in each season of the year. No significant differences were observed in terms of density in summer (p=0.169) or autumn (p=0.898). But the latency time in the birds stocked at standard density resulted statistically higher in winter (p=0.031) and, in this sense, showed a marginally significant difference in spring (p=0.057). Moreover, in winter and spring, differences in the upper values of the third quartile were observed, which were higher for the birds stocked at standard density. Birds

at a reduced density occupying this quartile present more consistent latency times. When analyzing the number of induction attempts in summer and autumn, it is observed that a higher number of chickens at standard density required one attempt in each season. Statistically, this is a significant difference only in summer (p=0.003), although it is a clear trend in autumn (p=0.065). Regarding the results in winter and spring, the number of induction attempts showed no differences (p=0.898 and p=0.870, respectively), and the values between one density and the other were nearly equal.

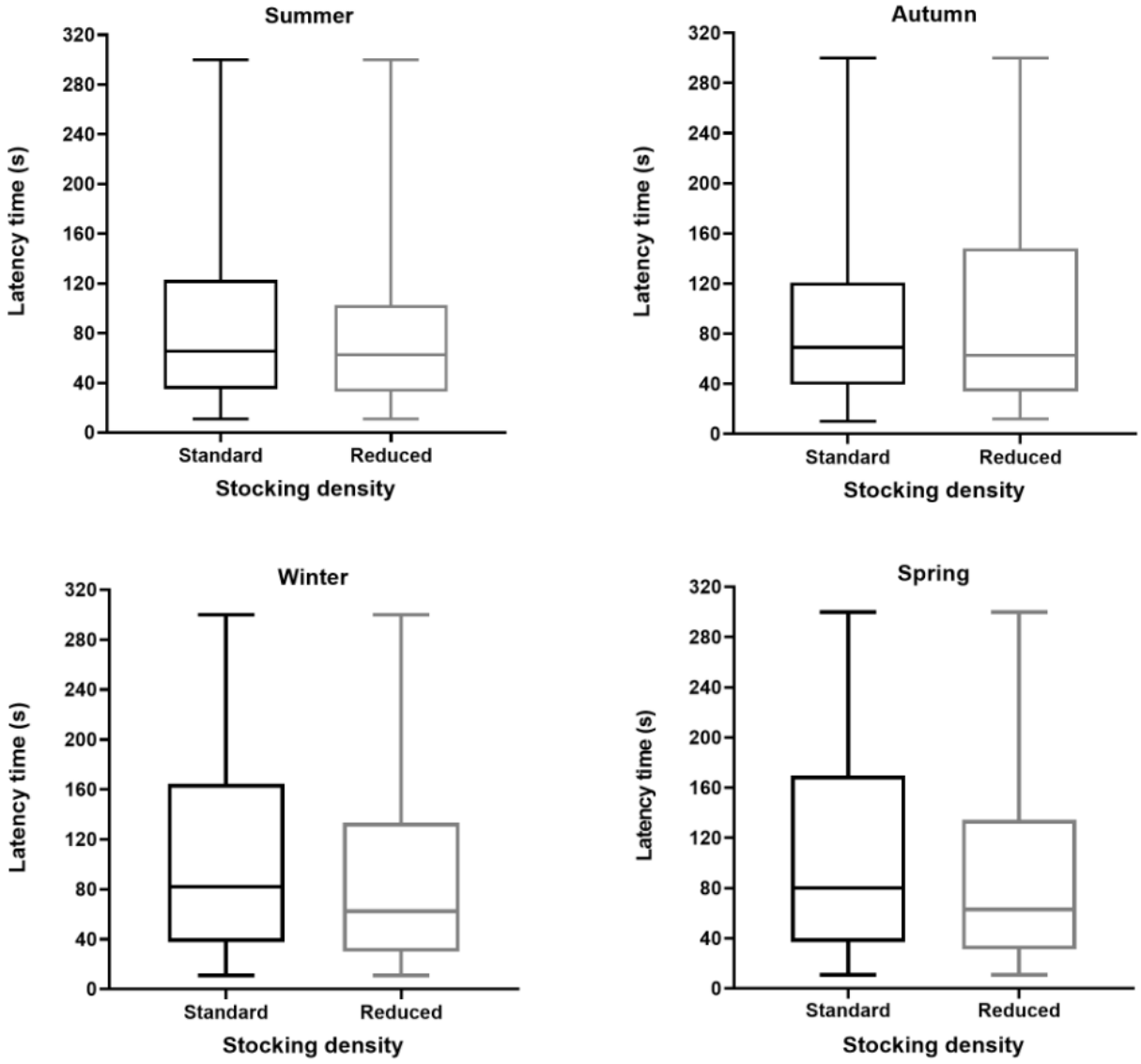

Figure 1. Latency time according the stocking density in each season

DISCUSSION

Tonic immobility is an unlearned behavior caused by different kinds of stimuli, and is characterized by a catatonic state in which the ability to respond is reduced for a varying period of time —it is considered the response by vertebrates to predators. As tonic immobility measures the level of fear in poultry, it is used to assess welfare in the poultry industry (9). Since birds' emotional health is affected by their sex, the season of the year and the stocking density, the hypothesis of this work studies how these factors may alter the latency period and the number of attempts to induce tonic immobility in broiler chickens kept in an industrial production system.

The growing need to enhance technical indexes for poultry meat production at industrial levels has led to develop management strategies that seek to improve the efficiency of the system. Among these, higher stocking densities has been prevalent due to its easy application without considerable higher costs of production. In this work, more availability of space meant a reduction in the latency time and a increase in the number of attempts to induce tonic immobility, suggesting that the stability of the mental health of chickens stocked with more space available is higher, and their reactivity to threat stimuli is lower. However, the tonic immobility test, in which fear is considered as a source of stress, showed no improvements in the resulting physical production. Increasing the stocking density in order to maximize profitability is a habitual and common management technique in feedlot, which aims at reducing costs of production. This assumption is underpinned by the results obtained in this study: the number of kilograms of chicken per square meter resulted higher when the stocking density was standard (around 7-10%), except during winter, in which case this difference was smaller. The values of tonic immobility reported in this study are consistent with the values reported by other authors for feedlot chickens in industrial systems of production (10). Uzum and Toplu (11) analyzed different alternatives to reduce the negative effects of heat stress; one of them is that reducing the stocking density resulted in a shorter latency time in the tonic immobility test. Tahamtani et al (12) showed that a reduced density and an enhanced environment with ramps and perches resulted in a shorter latency time. Meanwhile, Bach et al (13) compared a bigger distance to feeders and waterers and an increase in stocking density, with higher values for the tonic immobility test. An increase in stocking density causes fear reactions that can also be identified in other systems of production, chickens reared in cages with high stocking densities showed higher values of tonic immobility (14). In Das and Lacin (15), however, no higher values in the latency time of tonic immobility were observed, having analyzed two different densities (12 and 20 chickens per square meter) with higher values than those used in this work. Latency times for the chickens stocked at different densities were equal, but the chickens at low density spent more time standing, sitting or lying (16). Campbell et al (17) observed no differences in the groups of free-range birds at different densities, while Larsen et al (18) reported higher immobility values in chickens with less access to pasture versus chickens with more access. A reduced stocking density allows birds to move around and explore the surroundings, which impacts positively on their mental state.

Fear testing has proven contradictory between males and females, but it is assumed that the trend to develop fear reactions between each sex is different. The results in this study showed there is no sex-specific difference in the latency time and the number of attempts in the tonic immobility test, and coincide with Altan et al (10), who found no sex-specific differences in this test applied to 49 days of age feedlot chickens. Archer (19) studied how genetics and sex affect the response to fear in two different tests, and observed a higher latency time and a lower number of attempts in males, regardless of their genotype. Reaching results that are different from our findings, Agnvall et al (20) worked with the third-generation red junglefowl (an ancestor of the domestic chicken) and found higher values in females than in males for the immobility time and for the fear reaction to humans. Similarly, Nätt et al (21) found that females remained immobile for a longer time than males, vocalized longer and tried to escape more times in the predator test, indicating a higher level of fear. These authors also observed that, while females were more active, males showed more comfort when perching and jumping/flying. These differences in sex-specific behaviors are widely known, but their origins have not been deeply explained so far.

Genetic improvement of poultry has brought more efficient chickens in terms of productivity, but these are less resistant to changes in the environment, which can be reflected in emotionally related aspects that create more dependency to an appropriate environment. An intensive poultry system is usually regarded stressful, given the bird's confinement to houses that allow little comfort adjustments to adapt to the climate of the region. Modern lines of feedlot chicken production are adjusted to allow rapid growth, and often present difficulties to face environmental adversities, which is the reason why indoor houses become an important factor to provide favorable welfare conditions. In this study there is no report of significant differences for the tonic immobility test in relation with the season of the year, and it can be assumed that its effect on the chickens' emotional health was not relevant. Skomorucha et al (22) studied the

effects of heat stress during the second rearing stage of three hybrid chicken genotypes on tonic immobility, resulting in a higher latency time in one of the genotypes. This proves lower tolerance to reduced levels of welfare, related to high temperatures. On the assumption that lighting programs can be a cause of physiological stress for the birds, Fidan et al (23) studied the effect of photoperiods and light intensity on tonic immobility in standard hybrid chickens. The results showed no statistical differences in any of the variables. During a trial period using temperature values outside the thermoneutral zone for feedlot chickens, Egbuniwe et al (24) observed that the duration of tonic immobility was shorter in relation to a control group when ascorbic acid was administered.

In conclusion, the duration of tonic immobility is a useful tool to indicate fear in specific situations. By reducing density, the latency period is reduced and the number of induction attempts in chickens in tropical zones is increased, while the same effect is not obtained when it comes to the sex and season of the year.

Ethics committee

The methods used in the experiments were approved by the internal committee for the care and use of laboratory animals of Centro Regional INTA Santa Fe (protocol 18-001).

Conflict of interest

The authors have no conflicts of interest to declare.

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REFERENCES

1. Mellor DJ. Operational details of the five domains model and its key applications to the assessment and management of animal welfare. Animals. 2017; 7(8):60. https://doi.org/10.3390/ani7080060

2. Jones RB. Fear and adaptability in poultry: insights, implications and imperatives. Worlds Poult Sci J. 1996; 52(2):131-174. https://doi.org/10.1079/WPS19960013

3. Gilman TT, Marcuse FL. Animal hypnosis. Psychol. Bull. 1949; 46(2):151-165. https://doi.org/10.1037/h0060434

4. Nash RF, Gallup GG. Habituation and tonic immobility in domestic chickens. J. Comp. Physiol. Psychol. 1976; 90(9) 870-876. https://doi.org/10.1037/h0077265

5. Tong HB, Lu J, Zou JM, Wang Q, Shi SR. Effects of stocking density on growth performance, carcass yield, and immune status of a local chicken breed. Poult. Scie. 2012; 91(3):667-673. https://doi.org/10.3382/ps.2011-01597

6. Elfwing M, Nätt D, Goerlich-Jansson VC, Persson M, Hjelm J, Jensen P. Early stress causes sex-specific, life-long changes in behaviour, levels of gonadal hormones, and gene expression in chickens. PLoS One. 2015; 10(5):e0125808. https://doi.org/10.1371/journal.pone.0125808

7. Sinkalu VO. Melatonin modulates tonic immobility and vigilance behavioural responses of broiler chickens to lighting regimens during the hot-dry season. Physiol. Behav. 2016; 165:195-201. https://doi.org/10.1016/j.physbeh.2016.07.019

8. Jones RB, Faure JM. Sex and strain comparisons of tonic immobility ("righting time") in the domestic fowl and the effects of various methods of induction. Behav. Process. 1981; 6(1):47-55. https://doi.org/10.1016/0376-6357(81)90015-2

9. Wang S, Ni Y, Guo F, Fu W, Grossmann R, Zhao R. Effect of corticosterone on growth and welfare of broiler chickens showing long or short tonic immobility. Comp Biochem Phys A. 2013; 164(3), 537-543. https://doi.org/10.1016/j.cbpa.2012.12.014

10. Altan O, Seremet C, Bayraktar H. The effects of early environmental enrichment on performance, fear and physiological responses to acute stress of broiler. Arch. Geflügelkunde. 2013; 77(1):23-28. https://www.european-poultry-science.com/The-effects-of-early-environmental-enrichment-on-performance-fear-and-physiological-responses-to-acute-stress-of-broiler,QUlEPTQy

11. Uzum MH, Oral Toplu HD. Effects of stocking density and feed restriction on performance, carcass, meat quality characteristics and some stress parameters in broilers under heat stress. Rev Med Vet. 2013; 164:546-554. https://www.revmedvet.com/2013/RMV164_546_554.pdf

12. Tahamtani FM, Pedersen IJ, Toinon C, Riber AB. Effects of environmental complexity on fearfulness and learning ability in fast growing broiler chickens. Appl Anim Behav Sci. 2018; 207:49-56. https://doi.org/10.1016/j.applanim.2018.04.005

13. Bach MH, Tahamtani FM, Pedersen IJ, Riber AB. Effects of environmental complexity on behaviour in fast-growing broiler chickens. Appl Anim Behav Sci. 2019; 219:104840. https://doi.org/10.1016/j.applanim.2019.104840

14. Shields S, Greger, M. Animal welfare and food safety aspects of confining broiler chickens to cages. Animals. 2013; 3(2):386-400. https://doi.org/10.3390/ani3020386

15. Das H, Lacin E. The effect of different photoperiods and stocking densities on fattening performance, carcass and some stress parameters in broilers. ISR J VET MED. 2014; 69(4):211-220. http://www.ijvm.org.il/sites/default/files/different_photoperiods_and_stocking_densities.pdf

16. Son JH. The effect of stocking density on the behaviour and welfare indexes of broiler chickens. J Agric Sci Technol A. 2013; 3(4A):307. https://doi.org/10.5536/KJPS.2011.38.1.001

17. Campbell DL, Hinch GN, Downing JA, Lee C. Fear and coping styles of outdoor-preferring, moderate-outdoor and indoor-preferring free-range laying hens. Appl. Anim. Behav. Sci. 2016; 185:73-77. https://doi.org/10.1016/j.applanim.2016.09.004

18. Larsen H, Hemsworth PH, Cronin GM, Gebhardt-Henrich SG, Smith CL, Rault JL. Relationship between welfare and individual ranging behaviour in commercial free-range laying hens. Animal. 2018; 12(11):2356-2364. https://doi.org/10.1017/S1751731118000022

19. Archer G. Sex, genetics and test type affect the responses of chickens to fear testing. Int Poult Sci. 2018; 17(7):320-326. https://doi.org/10.3923/ijps.2018.320.326

20. Agnvall B, Jöngren M, Strandberg E, Jensen P. Heritability and genetic correlations of fear-related behaviour in red junglefowl–possible implications for early domestication. PloS One. 2012; 7(4):e35162. https://doi.org/10.1371/journal.pone.0035162

21. Nätt D, Agnvall B, Jensen, P. Large sex differences in chicken behavior and brain gene expression coincide with few differences in promoter DNA-methylation. PLoS One. 2014; 9(4):e96376. https://doi.org/10.1371/journal.pone.0096376

22. Skomorucha I, Sosnówka-Czajka E, Muchacka R. Effect of thermal conditions on welfare of broiler chickens of different origin. Ann Anim Sci. 2010; 10(4):489-497. http://www.izoo.krakow.pl/czasopisma/annals/2010/4/art16.pdf

23. Fidan ED, Nazlıgül A, Türkyılmaz MK, Aypak SÜ, Kilimci FS, Karaarslan S, Kaya M. Effect of photoperiod length and light intensity on some welfare criteria, carcass, and meat quality characteristics in broilers. R Bras Zootec. 2017; 46(3):202-210. http://doi.org/10.1590/S1806-92902017000300004

24. Egbuniwe IC, Ayo JO, Kawu MU, Mohammed A. Effects of betaine and ascorbic acid on tonic immobility, superoxide dismutase and glutathione peroxidase in broiler chickens during the hot-dry season. J Vet Behav. 2016; 12:60-65. https://doi.org/10.1016/j.jveb.2015.11.001