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The effect of noise and music on young meat chickens' behaviour and stress state

Abstract

The effect of 21-day-long noise and music exposure on open-field (OF) behaviour and stress state of young meat chicks has been investigated. The noise decreased the final body weight of cocks by 10.56%, but the noise and music did not influence weight gain of pullets ($p>0.05$). Necropsy and histopathology did not reveal any stress-related changes. Immobility time and number of siting up were inconsistent. The fluctuating asymmetry in the growth of 3rd toes was significantly higher, while corticosterone level was significantly lower in the control group than is the noise- and music-treated ones (2.83±0.51 vs. 6.39±1.23 and 6.90±0.94 ng/ml). There were differences in the OF behaviour (eating, chirping, gasping), too. There is no real difference between music and noise treatment; chicks respond to music endocrinologically and ethologically the same way as they respond to noise. These results indicate, that the three-week-long noise and music exposure does not mean a substantial stress of pathoclinical signs. The noise and music conditioning decreased, but not eliminated the stress. The serum corticosterone values showed a mild stress state of noise- and music-exposed chickens, but these treatments seemed to decrease the noxious effects of loud noise challenge, establishing a state of eustress.

Keywords: noise, music, stress, chick, behaviour, corticosterone

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Introduction

It is known that noise exerts negative effect on the animals and it could be a severe source of stress,[1] while music has a positive effect on some species and could help reduce stress levels.[2] Gvaryahu et al.,[3] have found that chicks exposed to environmental enrichment and classical music were less afraid during challenging and ate more. In the study of Brantsaeter et al.,[4] chicks, raired in complex environment showed less anxiety on weeks 19 and 23 and used better all the dimensions of space. Roy et al.,[5] have found an improvement of postnatal perceptual behaviour and synaptic plasticity in chicks, received complex rhythmical musical stimuli before and during hatching. Freshly hatched chicks showed a spontaneous preference to consonant sounds over discordant ones.[6] The biological function of the fear is to keep animals away from the source of danger. The fearfullness expresses how an animal react to frightful stimuli. The fearfullnes is developed by multiple genetic and epigenetic factors. The role of the environmental effects, especially in young age is decisive.[7] In generally the fear has a useful function, but under some circumstances it may be damaging for the birds, if they cannot run away from the dreadful stimulus.[8,9] The keeping conditions of the production and laboratory animals cannot totally eliminate environmental noise. The developed fearfulness depends greatly from the external stimuli of the young age. The raising in a complex environment did decrease the degree of fearfulness in poultry,[10] mice,[11] and in pig.[12] Besides the appropriate environment, the conditioning of the animals may decrease the general fearfulness. The systematic desensitization has long been used for the treatment of phobias in human.[13] This technique may work in case of animals, too and not only for the pathological phobias but also for natural fears. Some chicken strains are notably sensitive to noise, in these strains some noises (like thunderstorm) may cause heavy losses due to acute panic reaction. In the present study the authors tested the effect of 20-day-long noise and music exposure on the behaviour and stress state of young meat chicks. The aim of this work was to condition young birds by using soft noises and music in order to expect a positive effect on the acoustic sensitivity for the time being and in later ages.

Materials and methods

Animals, treatments, procedures

Fifty one-day-old chicks (Ross-308 meat hybrid) were assigned into three treatments. The first group (n=17) has been treated daily with a looped 47-minute-long mix of different noises (hammer, female and male laughter, office machines, scream, sneezing, bell, helicopter, airplane, buzz/ring of telephone, sprinkling equipment, saw, siren, thunderstorm, tempest, sewing machine, alarm system etc.) from 8 a.m. to 6 p.m. during 1hours (Noise group, „N") in a separate room. The second group (n=16) has been exposed to musical stimuli (the Goldberg Variations of Bach, BW988, Music group, „M") for the same time in a different room and the control group (n=17) has been housed in a silent room („C"), with a background noise of 40-50dB. The orientation and the microclimate of the three rooms were the same. The birds were put in a cage of 0.5x2.0x0.8 m and a 12:12 h dark: light period was set. The environmental temperature was 34°C at the arrival of the birds, and it decreased by 1°C every day, until reaching the 25°C. Tap water and chicken feed (12.45 MJ ME/kg, 21% crude protein - Boly24522) were available 24hours *ad libitum*. The noise and music was displayed by a Mac Book Air and Logitech speakers, with a maximum sound pressure level (SPL) of 70dB. On the second day a study of the basic ethograms were registered with each animal. The birds were investigated in an empty room, where they have been placed on the open-field (OF) vivarium (60x27.5x30cm) for 5minutes. The evaluation of the behaviour elements in units of 6seconds was carried out by The Noldus Observer XT system (http://www.noldus.com/). During the OF-test the usual feed was available in a dish. The following elements of action catalogue were registered: eating, chirping, gasping, head-shaking, defecation. The other birds waited in a neighbouring, calm and quiet room. The weight of the animals has been measured after the OF-tests. On day 7, 14 and 20 another

ethograms have been taken, but during these OFs a 3-minute-long uncomfortable and loud noise-mix was played: "noise-challenged" (airplane take-off, tempest with thunderstorm, hammer, helicopter, ring of telephone, whistle, siren) displayed at 90 SPL. After each OF-test the tonic immobility time of the birds were measured, which is an indicator of the stress state of birds.[14,15] Time spent in supine position (second) and the number of attempts (1, 2 or 3) needed to release immobility has been measured. On the last day of the study (21st day) blood samples have been collected from each animal to measure the serum corticosterone level according to Csernus.[16] A necropsy was performed to find stress-related changes and to determine the sex of the animals. For a histopathological examination samples were collected from the jejunum, liver, tibia and spleen. During the necropsy the weight of the liver, spleen and thymus were measured. After the necropsy the 3rd toes were measured on both legs to determine the fluctuating asymmetry, which is the absolute value of difference between the length of the right and the left third toes.[17–19]

Statistical evaluation ethical issue

To compare the number of individuals of a behaviour element the Fisher's exact test and for the comparison of the groups' means the one-way ANOVA with post-hoc Tukey-test were used.[20] The experiment was approved by the Institutional Ethical Committee (SZIE ÁOTK MÁB 62/2000-22.1/2877/2011).

Results

At the end of the experiment (day 21) the weight of M group was the highest (M: 850±11, N: 806±36, C: 836±24 grams) but the difference was not significant (p=0.363, one-way ANOVA). Table 1 summarizes the final body weight according to the sex at the necropsy.

The number of animals with successfully induced immobility and the number of necessary attempts can be seen in Table 2.

Table 1 Weight of the animals measured on day 21 during the necropsy, gram, mean±SE.

	Cock	%C	n	Pullet	%C	n	Weighed mean	%C	n
Noise	788±81	89.44	6	811±32	98.07	11	806±36	96.41	17
Music	891±15	101.14	8	809±17	97.82	8	850±11	101.67	16
Control	881±48	100	5	827±25	100	11	836±24	100	16

Table 2 Number of attempts (mean±SE) needed to induce immobility, immobility time (s) and the number of animals showed immobility during the test, mean±SE.

Treatment	Day 7			Day 14			Day 20		
	# of attempts	Immobility time, s	# of immobile birds/total	# of attempts	Immobility time, s	# of immobile birds/total	# of attempts	Immobility time, s	# of immobile birds/total
Noise	1.86±0.23	24±8.2	14 /17	1.19±0.10	32±1.6	16 /17	1.29±0.12	44±3.1	15 /17
Music	1.38±0.18	56±4.5	16 /16	1.06±0.06	46±4.2	16 /16	1.50±0.21	89±6.4	16 /16
Control	1.88±0.18	52±3.7	16 /17	1.29±0.11	54±2.7	17 /17	1.31±0.12	95±6.1	16 /17

The longest immobility time (helplessless) was measured in the N group on day 7, in the C group on day 14 and in the C group on day 20. The difference is not statistically significant (p>0.05). On the basis of the histological and histometrical investigations it can be stated that there is no group-specific light microscopic tendentious alterations. The length of the gut villi in each group corresponded to the species and age, 150-180µm in the jejunum and 120-130µm in the ileum. The characteristic diameter of the bursae folliculi was 400-700µm and the measure of the thymic cortex was 300-800µm. The width and tissue structure of the epiphysis-diaphysis zones in the bone samples was intact, characteristic to the normal bone formation. The relative weigh (related to the body weight) of the liver, spleen and thymus of the three groups did not differ significantly.

The fluctuating asymmetry was significantly higher (one-way ANOVA, p<0.05) in the C group (mean±SE), N: 1.59±0.47, M: 1.50±0.36, C: 3.38±0.58mm) (Figure 1).

The serum concentration of corticosterone was significantly lower (p<0.05) in the C group (N=6.39±1.23, M=6.90±0.94 and C= 2.83±0.51ng/ml) (Figure 2).

In the basic behaviour on the day 2 the individuals of the group N ate significantly (p=0.0003) longer. There was no difference in the other actions and no bird shook its head. The data of the OF on day 7 (with noise challenge) is given in Table 3.

The members of the control group spent the longest time standing (p=0.136), while the standing time of the groups N and M was near the same. During the noise-challenging OF of day 14 this was against the group C that spent much the longest time standing, but the difference is not significant (p=0.0746). The birds of the group N spent significantly (p=0.0002) more time with eating, followed by the group M and C. The sitting time was the lowest at the group N (p=0.0965), (Table 4).

In the noise-challenging OF of day 20 the birds of the group C spent again the longest time standing. The control birds chirped again the longest, while the eating duration was the highest at the group N (Table 5). In comparison of the succeeding OFs, in the group N the incidence of eating decreased from OF of day 2 to the noise-challenging OF of day 7, but after that the number of individuals (8-4-17-12) as well as the eating time increased from OF to OF and the

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difference of the OF of day 7 (group N and C vs. M) and 14 (group N vs. M and C) is significant (p=0.001). In group N both the number of eating individuals and the eating time increased from OF to OF (8-4-17-12). In the group M the birds ate significantly (p=0.0253) more during the OF of day 20, than during the basic one. With the time more and more individuals ate (0-1-7-7). In the control group the birds ate significantly more during the OF of day 20 than during the OF of day 7 or 14 (p=0.01) and the number of acting animals increased, too (1-6-4-9) (Table 5).

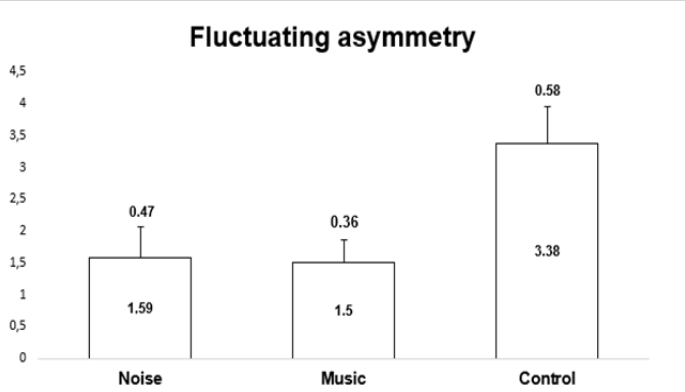

Figure 1 Mean and standard error of the fluctuating asymmetry (mm, the absolute value of the difference between the length of the right and left 3rd toes) in the different groups (mm).

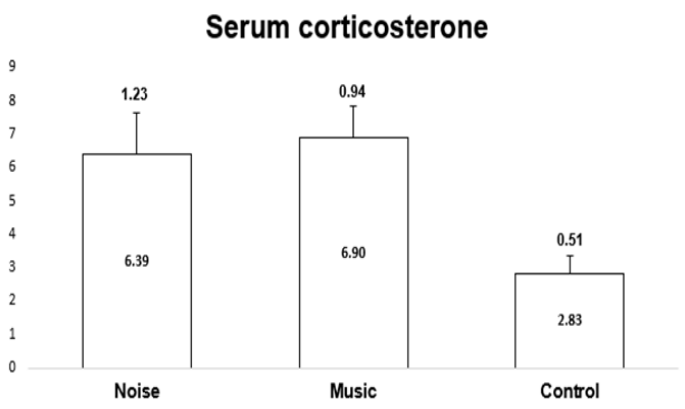

Figure 2 Mean and standard error of the concentration of serum corticosterone in the different groups (ng/ml).

Table 3 OF on day 7: time spent with the different behavioural elements, second or occasion, means±SE. Different uppercase letter means a significant difference (p<.05) in the same column. Numbers of birds: N=17, M=16, C=17, eating: N=4, M=1, C=6.

Treatment	Standing, s	Sitting, s	Eating, s	
Noise	102±14ab	67±15ab	12±3	
Music	86±14a	94±14a	1±3	
Control	138±9b	32±10b	12±9	
	Chirping, #	Gasping, #	Head shaking, #	Defecation, #
Noise	9±2ab	4.0±1.6	0.5±0.4	0.2±0.1
Music	4±2a	1.6±1.2	3.5±2.3	0.2±0.1
Control	14±2b	0.2±0.1	1.4±0.6	0.3±0.1

Table 4 OF on day 14: time spent with the different behavioural elements, second or occasion, means±SE. Different uppercase letter means a significant difference (p<.05) in the same column. Numbers of birds: N=17, M=16, C=17, eating: N=17, M=7, C=4.

Treatment	Standing, s	Sitting, s	Eating, s	
Noise	80±13	22±8	79±16b	
Music	75±12	54±15	29±16a	
Control	114±13	56±14	9±11a	
	Chirping, #	Gasping, #	Head shaking, #	Defecation, #
Noise	1.0±0.4	1.5±0.8	3.4±1.2	0.1±0.1
Music	0.4±0.4	1.5±1.1	4.5±1.2	0.1±0.1
Control	1.8±1.8	1.0±0.2	6.8±1.7	0.1±0.1

Table 5 OF on day 20: time spent with the different behavioural elements, second or occasion, means±SE. Different uppercase letter means a significant difference (p<.05) in the same column. Numbers of birds: N=17, M=16, C=17, eating: N=12, M=7, C=9.

Treatment	Standing, s	Sitting, s	Eating, s	
Noise	64±11	59±13	58±18	
Music	46±13	90±18	44±25	
Control	71±12	69±13	40±19	
	Chirping, #	Gasping, #	Head shaking, #	Defecation (#)
Noise	3.5±2.0	1.1±0.6	7.4±2.4	0.2±0.1
Music	2.1±0.9	0.6±0.2	7.3±2.0	0.2±0.1
Control	4.1±1.1	1.2±1.1	8.9±1.9	0.5±0.1

The number of chirping birds did not show a tendency in the control group (13-14-1-10), the frequency of chirping significantly decreased for the noise-challenging OF of day 14 and 20. In the group N the highest incidence of gasping occurred during the noise-challenging OF of day 7, followed by the day 14, 20 and the basic OF of day 2. The number of gasping birds was 2-8-5-4 from OF to OF. During the noise-challenging OF of day 14 significantly more birds gasped than during the other OFs (0-2-11-2). During the basic OF of day 2 no bird practised gasping. With the time the number of head shaking individuals increased (0-2-8-12); this behaviour element became more and more frequent and the value of the OF of the day 20 was significantly different (0.000673) from the basic value or the OF of day 7. In the basic behaviour, during the OF of day 2 there was no head shaking in any of the treatment groups. The number of the acting birds and the incidence of defecation did not differ significantly between the treatments.

Discussion

The final weight of cocks and pullets did not differ significantly, but the cocks' body weight shows a clear tendency: under the noise a decrease of 10.56%, compared to the control. The pullets seem to be less sensitive to the noise stress, than the cocks, which was the case in the mouse experiment of Korsós et al.[21] Based on serum corticosterone concentration and central benzodiazepine receptor density, Marin et al.,[22] found broiler cocks of day 15 more stress-susceptible, than pullets. The music had no effect on weight gain.

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The very early publication of Christensen et al.,[23] reports about the final weight increasing effect of dinner music at 70dB and a weight decreasing effect of the rock and roll at 85dB, but the differences are not significant. Gvaryahu et al.,[3] used a combined effect of filial imprinting, environmental enrichment by colours and classical music exposure at broiler chicken. This treatment significantly improved the final body weight by 60grams, but the acting factor cannot be differentiated. Voslarova et al.,[24] used intermittent noise exposure of 70 and 80dB for growing broiler chicken. Both treatments decreased the body weight gain. Cabaral et al.,[25] found no effect of different musical treatments (random, classical, metallic, reggae) at 60dB SPL on weight gain of growing quails. Nevertheless, the random and the classical music improved the egg production and feed conversion. The number of attempts needed to induce immobility was inconsistent and no significant differences have been found. However on day 7 the birds of the group N were the longest motionless (77±8.2 s) and had the lowest number of attempt to sit up, while on day 14 and 20 the control (54±2.7 and 95±6.1 s) produced this. Based on the tonic immobility data, on day 7 the highest stress state was in the noise-treated group. On the contrary, on day 14 and 20 the control showed the highest helplessless, which has a similar tendency to the data of fluctuating asymmetry. The differences are not significant, nevertheless they reflect a tendency.

Besides, the lower values of days 14 and 20, compared to those of day 20 show the habituation to this test. After only a noise-challenge of 100dB for 10 minutes, Chloupek et al.,[26] has found a significant decrease in the number of attempts to induce tonic immobility, but the duration of tonic immobility did not differ significantly between noise-treated (80 and 100dB) and control broilers. Hjelm[27] found only a tendency, whereas the early life stress did not influence tonic immobility in Leghorn chicks. In the trial of Campo et al.,[28] the classical music of 75dB, like a selection of different noises of 90dB are stressors for laying hens, based on the longer immobility time. On the contrary, in the experiment of the same team,[29] the tonic immobility duration did not reflect the decreased stress state of music-exposed laying hens. The predictive value of the tonic immobility test seems to be uncertain, partly because it is very breed specific.[29] The necropsy and the histological examination did not reveal sign of stress, there was no difference in the relative weight of the organs, either. Iyasere et al.,[30] found that besides the elevated corticosterone level, the decreased spleen weight is a reliable signal of the chronic stress in broiler. Therefore it can be stated that the noise- or music-treatment did not mean a substantial stress for the birds, it is rather a state of eustress.[31,32] Iyasere et al.,[30] emphasised, that it is the decreased spleen weight that is a reliable sign of the broiler chicken stress. In the present trial not only the weight of the spleens did not differ, but also the diameter of the periarteriolar lymphocyte sheets (40-60µm) and that of the germinal centres (70-90µm) in the spleens were similar in the three treatment groups. The fluctuating asymmetry basically was found to reflect stressful environment, the more a stressor influences the growing birds' wellbeing, the more asymmetric is the bone development.[33] The fluctuating asymmetry used to considered as a marker of animal welfare. Moreover, the developmental instability has been found positively correlated with the tonic immobility.[34] Dávila et al.,[29] has found that the auditory enrichment by classical music decreased the stress state of layer chicks, measured by fluctuating asymmetry and heterophil to lymphocyte ratio. In the present study the data of the fluctuating asymmetry was the highest in group C, while in the group N and M lower. Similar data to the present findings were published by Van Poucke et al.[35] They found no increase in the fluctuating asymmetry of broiler chickens at slaughter age after experimentally induced stress. The explanation is that there was no energy restraint (*ad libitum* feeding) and the genotype was of a very intensively growing one, both similarly to the present trial. It cannot be excluded, that the data of the present trial show that there was rather a state of eustress in the groups N and M, than real stress.

The blood corticosterone concentrations in unstressed chicken's fall in two ranges: 0.3 to 1.0 and 1 to 5ng/ml.[36] In the present experiment the corticosterone concentration was higher in group N and M, than in the group C, but the values are within the physiological ranges ($p<.05$) (N: 6.39±1.23, M: 6.90±0.94, C: 2.83±0.51ng/ml, Figure 2). In case of laying hens the normal value is 0.028ng/ml, while that of the slight/mild stress 0.035ng/ml.[37] In broilers Thaxton et al.,[38] found the concentration of 5-7ng/ml as physiological and that of 30-35ng/ml as a sign of (over)crowdedness. In a healthy commercial broiler stock Thaxton et al.,[39] found only an average of 0.907±10 (0.34-1.07)ng/ml, de Jong et al.,[40] 0.75-1.5ng/ml in *ad libitum* fed broiler. Handling increased corticosterone concentration of tuffed titmouse (*Baeolophus bicolor*) from 1-2 up to 5-44ng/ml.[41] Pál et al.,[42] measured 2.1-5ng/ml in plasma of broiler, while Türkylmaz[43] did 3.78-3.81ng/ml as physiological. Chloupek et al.,[26] has found a significant increase of the blood plasma of 80 and 100dB SPL noise-treated broilers, from 0.41-1.32 up to 3.2-4.74ng/ml. The blood collection was executed just after the noise challenge. Considered the corticosterone concentrations of the present experiment (Figure 2) there is no real differences between the noise- and the music-treated birds (6.38, 6.9ng/ml). This elevation (see control 2.83ng/ml) cannot be considered as a sign of distress, but rather as a sign of eustress.[31,32] The birds of the group C were standing and chirping longer than the group N and M. The tendency of changes in standing time was similar in the three group, showing, that it was not caused by the treatments. There was no regularity in case of the sitting incidence and duration. The eating time is generally shorter during the noise-challenging OF of day 7, than during the basic OF of day 2. After that it grows in each group, showing a habituation to the OF-procedure. The noise and music conditioning decreased,[44] but not eliminated the stress state during the noise challenge. It means that more eating and less tonic immobility show the decreased fearfullness. In group N both the number of eating individuals and the eating time increased from OF to OF (8-4-17-12). In the group M the birds ate significantly ($p=0.0253$) more during the OF of day 20, than during the basic one. With the time more and more individuals ate (0-1-7-7). In the control group the birds ate significantly more during the OF of day 20 than during the OF of day 7 or 14 ($p=0.01$) and the number of acting animals increased, too (1-6-4-9). The differences between the number of eating birds are significant (Fisher's exact test) of the OFs of the day 2 and 7, compared to the OFs of the day 14 and 20, in case of each treatment. The differences of the groups N and C are also significant on the OFs of the day 2 and 14. These show a general adaptation to the experimental conditions and the OF-procedure, a certain habituation. Nevertheless, the noise-treatment stimulated the eating behaviour. The Table 6 summarised the number of eating chickens, as well as the total eating time per group and OF.

The group N shows the highest values on every day. It means that the background noise treatment first drastically decreased the eating behaviour during the noise-challenged OF (see changes from OF of the day 2 to the day 7 of the group N), but after that the habituation increased the eating time (see changes from the day 7 to the day 14 and 20). Chirping number is practically the same in all groups during the OFs of the day 2. On the day 7, the chirping number of the group M is significantly lower, than in the group C. The tendency that the

chirping decreased after the first week in each group, showing also a habituation to the OF-procedure. However, the number of chirping birds did not show a tendency in the control group (13-14-1-10), the frequency of chirping significantly decreased for the noise-challenging OF of day 14 and 20, demonstrating that the habituation to the OF-device in itself mitigated the stress. The gasping is an important signal of stress in birds, it means a condition of being lost. The highest value was registered at the group N on day 7. The incidence of gasping increased during the noise-challenge in OF similarly in each group. It shows that the noise-challenge of 90dB meant a substantial stress to the birds, independently from the treatment. The head shaking shows that a bird is nervous. There was no head shaking at all during the basic OF of day 2, but later on its incidence continuously increased, similarly in each group, showing, that this is rather caused by the OF-procedure itself, than the noise or musical treatments. The head shaking is a reliable sign of the stress in birds. The modification in this behaviour element demonstrates that the noise-treatment meant a long-lasting stressor. The incidence of defecation and the number of acting birds did not differ between the treatment groups or the OF-measurements. To sum up, based on the behaviour analysis, the noise-challenge of the OF-measurement changed the action catalogue very similarly in case of the three treatment groups. The musical and noise treatment did not cause substantial alterations after a week-long habituation. It means, that the chosen background SPL was appropriate, because it did not mean a real stress for the animals. The repeated OF itself had a habituation effect.

Table 6 The total eating time (seconds) during the separate of's and the number of animals showed eating behaviour during the different open field test. Comparison of animal numbers is given after the Fisher's exact test.

Treatment	OF of day 2	OF of day 7	OF of day 14	OF of day 20	Fisher-test, p
Noise	594 (8)	198 (4)	1338 (17)	990 (12)	0.00001
Music	0 (0)	12 (1)	546 (7)	708 (7)	0.00091
Control	18 (1)	210 (6)	156 (4)	672 (9)	0.01843
Fisher-test, p	0.00082	0.1134	0.00001	0.3186	

Conclusions

From the point of view of the blood corticosterone concentration, the effect of music and noise is similar in the growing chickens. However, considering the eating behaviour and the tendency of weight gain changes, the music must have caused eustress and the noise must have been a slight, but real stress. However, owing to the great standard deviation, the differences in the final weight are not significant, a clear tendency can be found. The noise-treated cocks showed 10.56% lower final weight than the control. The blood corticosterone was significantly the lowest and the fluctuating asymmetry the highest in the control. Despite the fact, that the scientific committee of the European Union[45] stated, that the environmental enrichment, included the background music represents a good potential, in the present trial the music had no effect on final weight. In the future the effect of music on the production parameters should be tested using higher number of homogenous birds. The effect of noise-habituation may be tested by a higher than 90dB challenge. The behavioural and endocrinological effect of noise and music were similar to each other, without histopathological signs of stress, but from the point of view of labour safety, the background music has advantages. The state of the birds of the present trial may be evaluated as eustress.

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Conflicts of interest

The author declares that there are no conflicts of interest.

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