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THE INFLUENCE OF DIFFERENT SOUNDS ON THE FEEDING BEHAVIOR OF BROILER CHICKENS AND THEIR IMPACT ON BLOOD PHYSIOLOGY AND CONDITIONING PLACE PREFERENCE (CPP)

Salwan M. Abdulateef*[1], Ziyad T. M. Aldhanki[1] and Saman A. Rashid[2]

[1]Department of Animal Production, College of Agriculture, University of Al-Anbar, Ramadi, Iraq.
[2]Department of Animal Production, College of Agriculture, University of Sulaimani, Sulaimani, Iraq-Kurdistan.

Abstract

Ten sounds Movement of Chicken Feet (1), Hens with Chicks (2), Clucking (3), Regular Soft Timid Hens (4), Chick to Chick (5), Chicks Peeping (6), Chicks Care (7), Chick Sounds (8), Movement in Hen House (9) and Movement of Chicks (10) were experimentally tested to determine the which best attracted chicks to feed and water. This study was conducted from 01/April/2015 to 19/April/2015at the experimental field of the Department of Animal Resources, College of Agriculture, Al-Anbar University, Iraq. Chicks (n=150) were randomly distributed among 10 treatments, which , corresponded to the 10 sounds mentioned above, with three replicates per treatment and 5 chicks per replicate (15 chicks/treatment). Rectangular wooden boxes were designed to test condition place preference (CPP) for sounds, and the study was carried out in four steps: habituation training, pre-conditioning, conditioning, and post-condition. Each day, blood was collected before and after each experimental sessions and analyzed for concentration of total protein, glucose, uric acid and cholesterol and behavioral traits were quantified. We developed a novel equation to calculate the CPP factor, and the results indicated that sounds 1, 4 and 7 yielded significant ($P < 0.05$) changes in the blood parameters, and best attracted chicks to food and increased in CPP factor.

Key words : Broiler chickens, Sound Stimuli, CPP Factor, feeding behavior, blood trai.

Introduction

Birds are like other animals in that they, reproduce and hatch new chicks to ensure the survival of species. Chicks exhibit two types of behavioral adaptation; they are either nidicolousornidifugous (Collias, 1952). Nidicolous birds are those that stay in their nest for a long time after hatching, due to their dependence on the parents for feeding, protection and learning survival skills. Chicks of these birds are altracial; they are helpless, blind, and without feathers when they hatch, such as pigeons. In contrast, nidifugous birds, whose chicks are called precocial are those that leave the nest shortly after hatching, and they have feathers and are relatively mature and mobile from the moment of hatching, such as chickens, turkeys and ducks. In the se birds (precocial), the parents teach the chicks to find food in their environment and to discriminate between potentially harmful items and those that are safe to consume. This is achieved through vocalization and visual displays, such as pecking, by the hen, that enable chicks to find feed and water that will also make them safer, reduce the exploratory behavior, and thus reduce the amount of energy used for foraging, and shift to growth (Starck and Ricklefs, 1998; Starck, 1998). In modern breeding operations, chicks are hatched and bred apart from hens, so they cannot hear the vocalizations. Furthermore, the bond between parents and progeny is interrupted, researchers have attempted to simulate these relationships by exposing chicks to some acoustic stimuli to minimize the time spent searching the brooding area and to create comfortable zone for the chicks. Storage energy is often lost as a results of these actions. Furthermore, Gottlieb (1963) said that auditory stimuli play an important role in the development of cognitive learning in birds and also improve the formation of the

Author for correspondence : Email: ag.salwan.mahmood@uoanbar.edu.iqo

brain and senses (Gottlieb, 1965). Johnston and Gottlieb (1981) showed that sound stimulation before hatching improved the biochemical and morphological changes of chicks (Wadhwa *et al*., 1999; Panicker *et al*., 2002; Alladi *et al*., 2005). Auditory signals strongly stimulate the attraction of chicks to feed and improve parental recognition, especially if the sounds are heard pre- and post-hatching (Gottlieb, 1966; Brown *et al*., 1967; Lickliter *et al*., 2002). Pre- and post-hatching and sound stimulation to promotes the development of perceptual and cognitive behavior and improves learning in chicks (Harshaw and Lickliter, 2011). Some authors investigated the presence of a hen and its influence on chick's behavior, especially feed preference. Tolman (1964) concluded that of a hen could play an important role in increasing feeding and inducing other behaviors in chicks through social facilitation (Aline *et al*., 2002). Campo *et al*. (2005) found that exposure to sound resulted in some physiological alterations annoying sounds or noises to have negative effects, while comfortable and quiet sounds have positive effects. This was confirmed by Chloupek *et al*. (2009), who suggested that exposure to high levels of sound for 10 min (at 80 and 100 dB) at the slaughterhouse will increase the plasma corticosterone level in chickens. Additionally, increased ratios of heterophil to lymphocyte within the blood were observed in chickens exposed to the sounds of vehicles (90 dB) (Brouček, 2014). The hearing range of birds from 1 to 4 kHz and they cannot hear ultrasonic sounds, frequencies above 20 kHz, nor infra sound, which is below 20 kHz (Necker, 2000).

Conditioned place preference (CPP) is a form of Pavlovian conditioning that is used to measure the motivational effects of objects (animals) or experiences, and this paradigm is a standard preclinical behavioral model for the study of rewarding and aversive effects on a subject (animal) (Tzschentke, 1998). One of the benefits of the CPP procedure is that it can be used to assess the conditioning effects of both positive and negative stimuli in a way that requires little training, but it also avoids undesired interactions, such as excluding high sounds or noises during testing (Tzschentke, 2007). Studies have shown that food produces a CPP in bird subjects, Jones *et al*. (2012) attempted to establish whether CPP could serve as a method for assessing the preferences of birds for places that had been previously paired with food or sound, The authors concluded that it was possible to use the CPP procedure to assess the effects of sounds and that it has potential for use in the assessment of other environmental stimuli and the quantitative or qualitative effects of dietary restriction (Buckley *et al*., 2012).

Poultry house environments lack the auditory stimuli that chicks may use to find feed and water, so chicks may lose hours searching the brooding area especially under mass production. Sometimes, they may search for many hours at the beginning of post-hatch life and lose their initial first-week weight. Some researchers have showed that hen calls can attract chicks to the brooding area and minimize the time spent searching for feed, but to our knowledge no previous study has tested the ability of different types of sounds to attract chicks to the brooding area during the first days post-hatching by using 10 different sounds and measure their effects on some blood biochemical traits.

Materials and Methods

This experiment was carried out at the Animal Resources Field of the College of Agriculture, Al-Anbar University, Iraq from 01/April/2015 to 19/April/2015. One hundred and fifty one day old broiler chicks (straight run, ROSS 308) were randomly distributed among 10 treatments with three replicates per treatment and 5 chicks per replicate (15 chicks/treatment). All chicks were treated in accordance with the animal ethics policies and procedures of the University of Al-Anbar, Animal Ethics Committee and the Veterinary Directorate in Anbar Province. All treatments included exposure to white noise and the hen vocalizations because these sounds contain all sound frequencies, which enable the chicks to distinguish vocalizations (Jones *et al*., 2012). The procedure was as follows:

First stage (Collection and analysis of sound)

Ten different sounds, which represent all ages and different conditions were collected as MP3 files and played a frequency of 200 -400 Hz with the Movement of Chicken Feet sound as the 1st treatment, the Hen with Chicks sound as the 2nd treat., the Clucking sound as the 3rd treat., Regular Soft Timid Hens sound as the 4th treat., the Chick to Chick sound as the 5th treat., the Chicks Peeping sound as the 6th treat., the Chick Care sound as the 7th treat., the Chicks sound as the 8th treat., Movement in Hen House sound as the 9th treat. and the Movement of Chicks sound as the 10th treat. These sounds were collected from websites or recorded, and they can be heard by visiting the following website: https://www.youtube.com/watch?v=zcJygc7DfYA.

The sounds used in this study were analyzed with a portable spectrograph and the program Audacity 2.0.6. Sound intensity, wavelength, and frequency were measured and the acoustic wave spectrum was drawn for each sound.

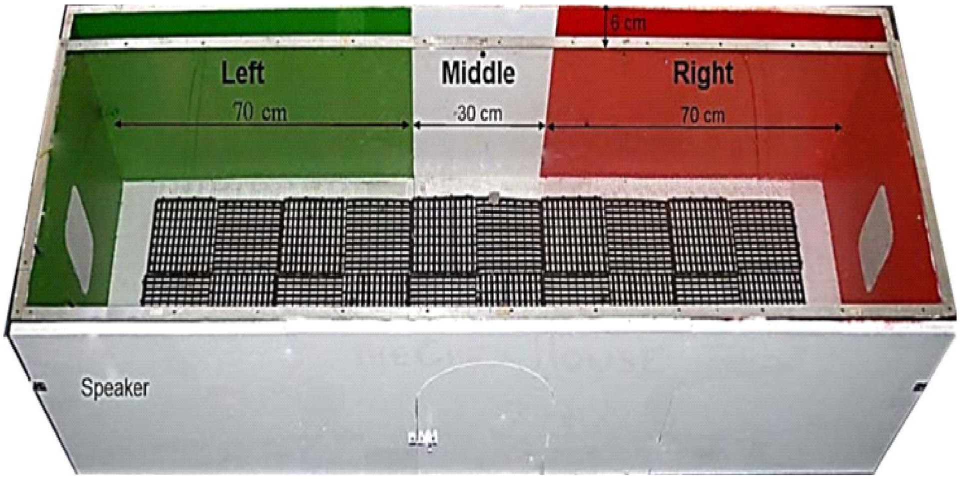

Plate 1 : The experimental Conditioning Place Preference (CPP) apparatus.

Second Stage (Conditioned Place Preference CPP Apparatus Design)

Plate 1 shows the experimental CPP box with its measurements as referred to by Jones *et al*. (2012). The rectangular box was made of 2 cm-thick wood sheets, and its dimensions were 56 cm (width) × 170 cm (length) × 43 cm (height). Its surface was covered with a transparent plastic slide except for, 6 cm from one of the longitudinal edges (170 cm in length) to provide ventilation for the chicks. The metal floor of the box was colored matte white, and a clathrate black rubber layer was placed on the white floor. The walls of the box were painted different colors. The center section, with a length of 30 cm (out of 170 cm) was painted a white metal color, and the right section was colored red and had a length of 70 cm. The left section was colored green and had a length of 70 cm (70 cm right +30 cm East +70 cm left = 170 cm). The box contained three hinged doors; each section had a door for the chicks to enter, and movable articulated walls divided the section. The box also contained two speakers with dimensions of 12 cm (width) × 18 cm (length), which yielded a sound intensity of 70 Hz to 20 000 Hz and were placed on the two 56-cm walls.

Third Stage (method for testing sound)

This stage was carried out in four steps: habituation training, pre-conditioning, conditioning, and post-conditioning as follows:

Habituation training

This phase lasted for the first 5 days post-hatch. A chick was placed in the center of the box with access to all three sections, and the section doors were closed. Each experimental session lasted for one minute, and the experimental sessions were conducted from 8:00 am to 4:00 pm on each day.

Pre-conditioning

This phase lasted for the three days following the previous phase (6th to 8th days post-hatch). The same chick tested for each replicate was placed in the center of the box with access to all three of the sections, and the section doors were closed. On the 6th day post-hatch two experimental sessions were carried out. The first session included the sound treatment (white noise treatment), and the second experimental session included exposing chicks to hen vocalizations. The originated from both sides of the box in each experimental sessions, which lasted for 5 minutes each and were conducted from 8:00 am to 5:00 pm. Each session began from the moment that the chick was placed in the middle of box. The 7th post-hatch day was used as a resting period for the experimental chicks.

On the 8th day post-hatch, two experimental sessions were conducted. The first session involved the sound treatment, and the second experimental session involved exposing the experimental chicks to white noise. The sound originated from both sides of the box in each of the experimental sessions, which lasted for 5 minutes each and were conducted from 8:00 am to 5:00 pm. Each session began from the moment that the chick was placed in the middle of box. Note that, treatments were reversed; the sounds that were heard in afternoon of the 6th day, were heard in the morning on the 8th day.

Conditioning

This phase lasted for the 8 days following the previous phase (from the 9th to 16th days post-hatch). The 9th, 11th, 12th and 16th post-hatch days were resting periods, but two experimental sessions were conducted on the 10th, 13th and 15th days. The first session included placing the chicks in the right section of the box, and continuously exposing them to the sound for 10 minutes. The second experimental session began one hour after the first; the chicks were not exposed to the tested sound but remained in the middle part of the box for 10 minutes. The treatments were reversed on day 10, to avoid biasing the chicks toward one section of the box.

Post-Conditioning

This phase lasted for 3 days, the 17th, 18th and 19th days post-hatching, and included the same actions as the preconditioning phase. However, the experimental sound was released from one side of the box to examine any sound preference on the 17th and 19th day post-hatch (sound was only used in the treatments), with a resting period at 18th day. The treatments on the 19th day were reverse of those on the 17th day.

Blood biochemistry parameters

Blood samples were collected from three chicks from each replicate (9 samples/treatment) from jugular vein at the age of 1 day and from the brachial vein thereafter. The blood was collected and analyzed before and after each experimental session on all of the days of the experiment by placing the blood samples in a coagulant tube and centrifuging for 5 minutes at approximately 6000 rpm/min. The separated blood plasma was then used to measure the levels of total protein, glucose, uric acid and cholesterol with kit supplied by the Spanish company Linear and the tests were conducted according to the manual (Wooton and Freeman, 1982).

Behavioral traits

The conditioning place preference factor (CPPF) was derived as follows :

- The total visits (TV) were estimated by multiplying the percentage of the number of visits (PNV) to the right or left section of the box × departure time (DT).
- The duration of stay (DS) of the chicks to the right or left section was estimated by calculated time of stay to chicks in the part of CPP apparatus.
- The total visits (TV) of the chicks to the right or left section was estimated by calculated by the percentage of chicks to visit one of the parts divided on number of total chicks.
- The duration of stay (DS) of the chicks to the right or left section was divided by the total visits (TV) to obtain the conditioning place preference factor (CPPF) as follows:

$$CPPF = \frac{(DS)\ Sec.}{(PNV)\ \% \times (DT)\ Sec.} \times 100$$

Statistical analysis

The biochemical parameter data were reported as means ± SEM and subjected to two-way, using a GLM model in the SAS system (SAS, 9.2) (SAS, 2004), followed by Duncan's multiple-range tests to analyze the differences among all treatments when the F-value was statistically significant ($P < 0.05$).

Results

Glucose concentration

Table 1 shows the impact of the main factors, the sounds and exposure time, on the blood biochemical traits during the habituation training portion of the CPP test. The results revealed significant differences ($P<0.05$) in glucose concentration among the main factors (sound treatments), especially in the plasma of chicks exposed to the Chick to Chick sound (4th treat.) and Chick Peeping (5th treat.). Glucose was significantly lower in these treatment compared with the other treatments (6th to 10th treat.), but there were no significant differences with the 1st, 2nd and 3rd treatments. On the other hand, there were no significant differences in the plasma glucose concentrations of chicks according to the other main factor, the time of exposure to the sound treatments (table 1). However the interactions of the main factors, shown in table (1) revealed that the differences in glucose concentrations were not primarily due to the treatments (sounds and exposing time); chicks that exhibited increased in glucose concentrations prior to treatment continued exhibit high concentrations.

The effects of the main factors in this study, the sounds and exposure time, on blood biochemical traits during the CPP preconditioning phase are illustrated in table 2, the results revealed that the 7th treatment, the Chick Sound, significantly decreased glucose concentrations in the plasma of chicks. There were insignificant differences between the other sound treatments (table 2) and the different exposure times. During the conditioning phase of the CPP, the glucose concentrations in chicks treated with different sounds did not differ significant with the time of exposure to the sounds (table 3), but chicks treated with the Regular Soft Timid Hens sound (4th treat.) had significantly higher plasma glucose concentrations compared with other treatments which were not significantly different from each other. A significant decrease was observed in plasma of chicks treated with the 5th treatment, the Chick to chick sounds (table 3). No significant differences in glucose concentration were observed among the main factors (sounds and exposure time) during the post-

Table 1 : Impact of different sounds on some biochemical blood traits of broiler chicks before and after habituation training[1] of the conditioning place preference (CPP).

Blood traits	**Time**[2]	**Sounds used**[3]									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Glucose(mg /100 ml)	Before	276.93ab	276.87ab	276.80ab	252.36 b	265.13 ab	313.27 a	312.67 a	313.80 a	312.53 a	313.47 a
	After	277.33ab	276.40ab	276.53ab	273.71ab	264.93 ab	312.07 a	311.53 a	312.20 a	311.53 a	313.00 a
	X	*277.13ab*	*276.63ab*	*276.67ab*	*263.04b*	*265.03b*	*312.67 a*	*312.10 a*	*313.00 a*	*312.03 a*	*313.23a*
Uric acid(mg/100 ml)	Before	3.07d	3.00d	3.13d	3.14 d	3.00d	3.00 d	3.00 d	3.07 d	3.00 d	3.00 d
	After	3.85b	3.58c	3.82b	3.86 b	3.87b	3.85 b	4.27 a	3.88 b	4.00 b	3.79 bc
	X	*3.46bc*	*3.29c*	*3.48ab*	*3.50 ab*	*3.43bc*	*3.43 bc*	*3.63a*	*3.47 ab*	*3.50 ab*	*3.39 bc*
Total Protein(g /100 ml)	Before	3.33bc	3.10bc	3.33bc	3.50 b	3.30 bc	3.37 bc	3.07 c	3.30 bc	3.37 bc	3.19 bc
	After	4.17a	3.99a	4.16a	4.06a	4.21 a	4.25 a	3.91a	4.15 a	4.14 a	4.09 a
	X	*3.75ab*	*3.55ab*	*3.75ab*	*3.78ab*	*3.76 ab*	*3.81 a*	*3.47b*	*3.72 ab*	*3.75 ab*	*3.64 ab*
Cholesterol(mg /100 ml)	Before	165.80b	165.80b	165.27 b	168.43 b	165.60 b	165.00 b	165.13b	165.47 b	165.27 b	165.33 b
	After	167.80b	167.27 b	166.67 b	167.79 b	167.33 b	167.13 b	167.07b	167.47 b	167.33 b	197.07 a
	X	*166.80*	*166.53*	*165.97*	*168.11*	*166.47*	*166.07*	*166.10*	*166.47*	*166.30*	*181.20*

Time of exposure		**Glucose (mg /100 ml)**	**Uric acid (mg/100 ml)**	**Total Protein(g /100 ml)**	**Cholesterol (mg /100 ml)**
	before	*291.64*	*3.04 b*	*3.28 b*	*165.69*
	after	*293.05*	*3.88 a*	*4.11 a*	*170.31*
Total mean		*292.35*	*3.46*	*3.70*	*168.00*
Prob.	*Times*	*NS*	*0.0001*	*0.0001*	*NS*
	Sounds	*0.0002*	*0.0089*	*NS*	*0.05*
	*Time*sounds*	*0.0193*	*0.0001*	*0.0001*	*0.05*
Pooled SEM		*3.6730*	*0.0304*	*0.0375*	*1.522*

[a–b] Means with different superscripts in each row are significantly different. *NS*= not significant.

[1]Habituation training was carried out from the first day to the fifth day post-hatch, and the numbers in the table represent 3chicks (out of 5) per treatment for 5 days (15 repl./ treat.).

[2] The blood traits measurements occurred, before chicks were exposed to the sounds in the box during habituation training and immediately after exposure.

[3] During habituation training sounds were played one minute on all of the days (days 1-5), and experimental sessions were conducted from 8:00 am to 4:00 pm.

1 = Movement of Chicken Feet, 2 = Hen with Chicks , 3 = Cluck, 4 = Hens Regular Soft Timid, 5 = Chick to Chick, 6 = Chicks Peeps, 7 = Care Chicks, 8 = Chicks Sound, 9 = Hen House Movement and 10 = Movement Chicks.

Table 2 : Impact of different sounds on some biochemical blood traits of broiler chicks before and after the preconditioning phase [1] of conditioning place preference (CPP) test.

Blood traits	Time[2]	Sounds used[3]									
		1	2	3	4	5	6	7	8	9	10
Glucose(mg /100 ml)	Before	335.00 ab	335.33 ab	335.00 ab	337.50 a	335.33 ab	334.67 ab	310.00 ab	334.67 ab	333.83 ab	335.67 ab
	After	337.17 a	335.33 ab	335.17 ab	337.17 a	335.00 ab	338.33 a	301.67 b	334.50 ab	333.17 ab	335.17 ab
	X	*336.08 a*	*335.00 a*	*335.0 a*	*337.33 a*	*335.17 a*	*335.92 a*	*305.83 b*	*334.58 a*	*333.50 a*	*335.4 a*
Uric acid(mg/100 ml)	Before	3.52 ab	3.50 ab	3.67 ab	3.33 ab	3.50 ab	3.50 ab	3.17 b	3.50 ab	3.50 ab	3.50 ab
	After	3.68 ab	3.50 ab	3.67 ab	3.82 ab	4.00 a	3.80 ab	3.68 ab	3.68 ab	3.73 ab	3.65 ab
	X	*3.60*	*3.50*	*3.67*	*3.58*	*3.75*	*3.65*	*3.43*	*3.59*	*3.62*	*3.58*
Total Protein(g /100 ml)	Before	3.43	3.58	3.67	3.57	3.58	3.58	3.33	3.58	3.58	3.60
	After	3.75	3.58	4.10	3.75	4.03	3.75	3.55	3.75	3.77	3.73
	X	*3.59*	*3.58*	*3.88*	*3.66*	*3.81*	*3.67*	*3.44*	*3.67*	*3.68*	*3.67*
Cholesterol(mg /100 ml)	Before	165.83	166.83	168.00	167.83	166.17	162.83	163.83	163.83	164.67	164.50
	After	166.00	165.83	167.00	168.17	166.83	163.67	164.33	163.17	164.00	163.83
	X	*165.92abc*	*166.33abc*	*167.50ab*	*168.00 a*	*166.50abc*	*163.25 c*	*164.08 bc*	*163.50 c*	*164.33abc*	*164.17 bc*

Time of exposure		**Glucose (mg /100 ml)**	**Uric acid (mg/100 ml)**	**Total Protein(g /100 ml)**	**Cholesterol (mg /100 ml)**
	before	*332.58*	*3.47 b*	*3.55 b*	*165.43*
	after	*332.20*	*3.72 a*	*3.78 a*	*165.28*
Total mean	*332.39*	*3.59*	*3.66*	*165.36*	
Prob.	*Times*	*NS*	*0.0044*	*0.0332*	*NS*
	Sounds	*0.0931*	*NS*	*NS*	*0.0348*
	*Time*sounds*	*0.05*	*0.05*	*NS*	*NS*
Pooled SEM		*2.2313*	*0.0430*	*0.0503*	*0.3717*

[a–b] Means with different superscripts in each row are significantly different. *NS*= not significant.

[1]the preconditioning phase was carried out on the 6th and 8th days post-hatch, and the numbers in table represent 3chicks (out of 5) per treatment for 2 days (10 repl./treat.).

[2] The blood trait measurements:occurred before chicks were exposed to sounds in the box during the preconditioning phase and immediately after exposure.

[3]Sounds in the preconditioning phase were palyed on the 6th and 8th days post-hatch.

1 =Movement of Chicken Feet , 2= Hen With Chicks , 3= Cluck , 4= Hens Regular Soft Timid, 5 = Chick to Chick, 6 = Chicks Peeps, 7 = Care Chicks, 8 = Chicks Sound , 9 = Hen House Movement and 10 = Movement Chicks.

conditioning phase of the CPP (table 4) except for the a significant decrease in the plasma of chicks treated with the Movement of Chicken Feet sound (1st treat.).

Uric Acid Concentration

A different trend was observed in the plasma uric acid levels of chicks treated with different sounds and different exposure times (table 1). For example, exposing chicks to the Clucking sound, the 2nd treatment, significantly decreased ($P < 0.05$) the uric acid concentration compared with the other treatments, but exposing chicks to Chick sound (7th treat.) significantly increased the uric acid levels. No significant differences appeared between the rest of the treatments, and there were also no significant differences between treatments in terms of the interactions of the main factors, especially after exposure to sounds such as the 2nd and 7th treatments. The preconditioning phase of the CPP presented in table 2 and no significant differences in uric acid concentration were found between the main factor (sounds). However, concentrations differed significantly with the time of exposure to sounds, especially after exposure to the 5th sound treatment (Chick to Chick sound). The same trend in uric acid concentration was observed in the conditioning phase of the CPP (table 3), and similar to the previous phase (the pre-conditioning phase), the same treatment (5thtreat.) significantly increased uric acid concentration.

In the post-conditioning phase of the CPP, uric acid concentration significantly increased under the 1st treatment, but there were no significant differences among the other treatments.

Total protein concentration

Total protein concentration. appeared to increase significantly in the plasma of chicks after exposure to all of the sound treatments (table 2) during the habituation training phase of the CPP, except for a significant decrease under the 7th treatment, the chicks care sound. However, the total plasma protein significantly increased in chicks subjected to the Chicks Peeps sound (6th treat.). The reverse trend was observed during the preconditioning phase of the CPP (table 2; there were no significant differences in total protein concentration between treatments. The Movement of Chicken Feet sound (1st treat.) in the conditioning phase of the CPP (table 3) significantly decreased total protein in the plasma of chicks, but the opposite effect was observed in chicks subjected to the 3rd treatment (Clucking sound). Exposure time significantly affected total protein concentration (table 3), especially under the 3rd treatment which differed significantly from the other treatments.

The main factor (the sounds and time of exposure to the sounds) did not have any significant influences during the post conditioning phase of the CPP which is clearly illustrated in table 4. However, significant decreases occurred under 1st treatment before exposure to the Movement of Chicken Feet sound (1st treat.) (table 4).

Cholesterol concentration

During the habituation training portion of the CPP test, the plasma cholesterol concentration only decreased significantly in the 10th treatment when chicks were exposed to the sound of Movement of Chicks, but the main factors did not differ significantly between treatments (table 1). However, significant increases were observed in the 3rd (Clucking sound) and 4th treatments (Regular Soft Timid Hens) (table 1) and a significant decrease occurred in the rest of the treatments. No significant differences in the time of exposure (main factor) were observed during the preconditioning phase of the CPP test (table 1). The same trend in plasma cholesterol occurred under the 3rd and 4th treatments during the conditioning phase of the CPP test (table 3), while, significant decreases occurred in the other treatments. The levels differed significantly with time of exposure especially after exposure to sounds (table 3).

The chick sound (8th treatment) significantly increased the cholesterol concentration in the plasma of chicks (table 4) during the post-conditioning phase of the CPP test, whereas, significant decrease were occurred in reset treatments, time of exposure was not significantly different between treatments.

Behavioral traits

Conditioning Place Preference factor

As shown in fig. 1, there are differences in the CPP factor values as the 4th treatment (Regular Soft Timid Hens sound) yielded a higher value followed by the 7th treatment (Chicks Care sound) and 1st treatment (Movement of Chicken Feet sound). It appears from the figure that of all the experimental treatments increased in the CPP factor after sound exposure compared to before, except the 2nd treatment (Hens With Chicks sound), where CPP factor values decreased after exposure to the sound.

The duration of stay

As shown in fig. 2, there are differences in the duration of stay the 4th treatment (Regular Soft Timid Hens sound) yielded a higher value followed by the 7th treatment (Chicks Care sound) and 1st treatment (Movement of Chicken Feet sound). It appears from the

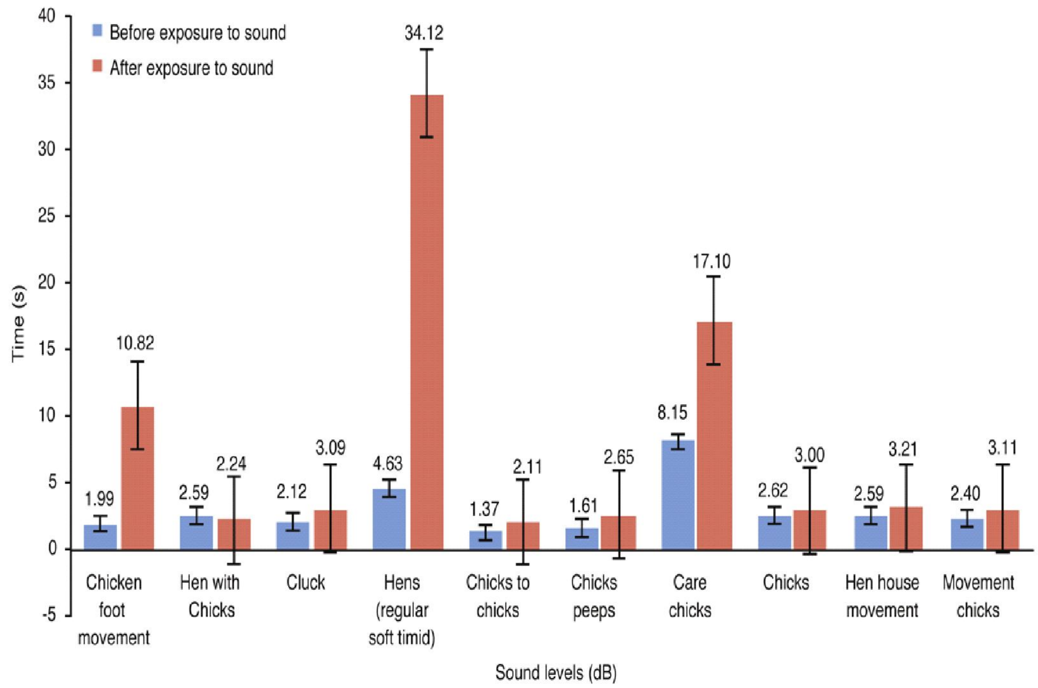

Fig. 1 : Value of the Conditioning Place Preference (CPP) factor for each experimental treatments (sounds) before and after exposure.

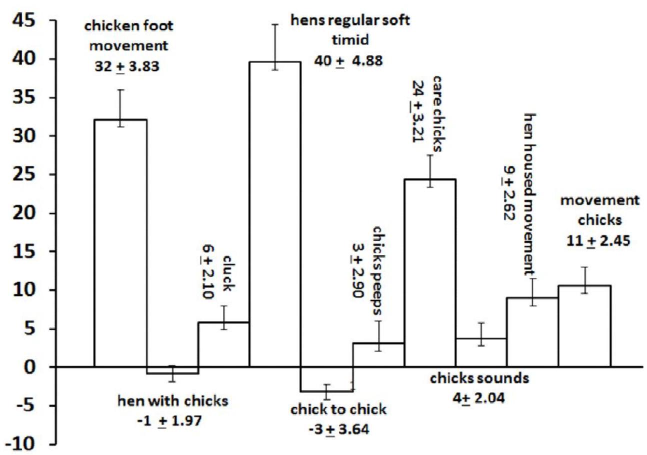

Fig. 2 : Shows the average difference in the duration of stay chicks (seconds) for treatments experiment using a test CPP.

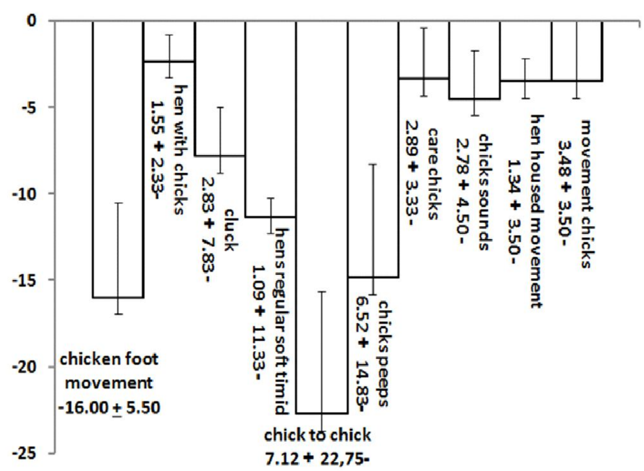

Fig. 3 : Shows the average difference in the total visits of chicks (seconds) for treatments experiment using a test CPP.

figure that of all the experimental treatments increased in the duration of stay after sound exposure compared to before, except the 2nd treatment (Hens With Chicks sound) and 5th (chicks to chicks) where values decreased after exposure to the sound

The total visits

As shown in fig. 3, in the total visits of the 5th treatment (chick to chick) yielded a higher value followed by the 1st treatment (Movement of Chicken Feet sound) and 6th treatment (Chicks Peeping), in the total visits of the 4th treatment (Regular Soft Timid Hens) was a higher value than the 3th treatment (Clucking) and followed by the 8th treatment (Chick Sounds). There was no difference between 9th (Movement in Hen House) and 10th (Movement of Chicks) which was higher from 7th (Chick Care) which is increased in the total visits about 2nd (Hens with Chicks).

Discussion

In this study, we observed the effects of sounds on broiler chicks and their behavior, and these effects were positive or negative depending on the type of sound and the degree of habituation to the sound. We tested ten

Table 3 : Impact of different sounds on some biochemical blood traits of broiler chicks before and after the conditioning phase[1] of the conditioning place preference (CPP) test.

Blood traits	Time[2]	Sounds used[3]									
		1	2	3	4	5	6	7	8	9	10
Glucose (mg /100 ml)	Before	333.78 a	333.78 a	334.00 a	336.00 a	334.88 a	334.11 a	337.78 a	334.11 a	333.00 a	334.22 a
	After	333.78 a	331.89 a	332.44 a	334.00 a	313.33 b	335.33 a	331.22 a	332.56 a	331.78 a	333.67 a
	X	*333.50 ab*	*332.83 ab*	*333.22ab*	*335.00 a*	*323.47 b*	*334.72 ab*	*334.50 ab*	*333.33 ab*	*332.39 ab*	*333.94ab*
Uric acid (mg/100 ml)	Before	3.28 ed	3.22 e	3.44 bcde	3.33 cde	3.33 cde	3.32 cde	3.33 cde	3.33 cde	3.33 cde	3.33 cde
	After	3.77 abc	3.58 abcd	3.72 abcd	3.82 ab	4.00 a	3.83 ab	4.00 a	3.88 ab	3.83 ab	3.73abcd
	X	*3.52*	*3.40*	*3.58*	*3.58*	*3.67*	*3.58*	*3.67*	*3.61*	*3.58*	*3.53*
Total Protein (g/100 ml)	Before	3.22 e	3.28 de	3.56 cde	3.38 cde	3.44 cde	3.43 cde	3.33 cde	3.44 cde	3.44 cde	3.47 cde
	After	3.76 abcd	3.79abcd	4.18 a	3.79abcd	4.03 ab	3.87 abc	3.81 abcd	3.81 abcd	3.76 abcd	3.79abcd
	X	*3.49 b*	*3.53 ab*	*3.87 a*	*3.58 ab*	*3.74 ab*	*3.65 ab*	*3.57 ab*	*3.63 ab*	*3.60 ab*	*3.63 ab*
Cholesterol (mg/100 ml)	Before	165.22abc	164.78abc	166.11ab	164.33abc	165.89 ab	162.67 bc	161.89 c	162.89 bc	163.33 bc	162.67bc
	After	166.33 ab	165.78 ab	166.22ab	167.11 a	165.67 ab	163.44abc	163.44abc	164.22abc	164.00abc	163.7abc
	X	*165.78 ab*	*165.28abc*	*166.17 a*	*166.50 a*	*165.0abcd*	*163.01 cd*	*162.67 d*	*163.56bcd*	*163.67bcd*	*163.22cd*

Time of exposure		**Glucose (mg /100 ml)**	**Uric acid (mg/100 ml)**	**Total Protein(g /100 ml)**	**Cholesterol (mg /100 ml)**
	Before	*334.56*	*3.32 b*	*3.40 b*	*164.0 b*
	After	*330.94*	*3.82 a*	*3.86 a*	*165.0 a*
Total mean	*332.74*	*3.57*	*3.63*	*164.48*	
Prob.	*Times*	*NS*	*0.0001*	*0.0001*	*0.0370*
	Sounds	*0.05*	*NS*	*0.05*	*0.0008*
	*Time*sounds*	*0.05*	*0.0001*	*0.0002*	*0.0200*
Pooled SEM		*1.0857*	*0.0356*	*0.0383*	*0.2538*

[a–b] Means with different superscripts in each row are significantly different. *NS*= not significant.

[1]The preconditioning phase was carried out on the 10[th] to 15[th] days post-hatch, and the numbers in the table represent 3chicks (out of 5) per treatment for 2 days (10 repl./treat.).

[2] The blood trait measurements: occurred before chicks were exposed to sounds in the box during the preconditioning phase and immediately after exposure.

[3]Sounds in the conditioning phase were played on the 10[th] to 15[th] days post-hatch.

1 =Movement of Chicken Feet , 2= Hen With Chicks , 3= Cluck , 4= Hens Regular Soft Timid, 5 = Chick to Chick, 6 = Chicks Peeps, 7 = Care Chicks, 8 = Chicks Sound , 9 = Hen House Movement and 10 = Movement Chicks.

Table 4 : Impact of different sounds on some biochemical blood traits of broiler chicks before and after the post-conditioning phase[1] of the conditioning place preference (CPP) test.

Blood traits	Time[2]	Sounds used[3]									
		1	2	3	4	5	6	7	8	9	10
Glucose(mg /100 ml)	Before	327.33	332.67	333.67	333.67	335.33	334.33	333.00	332.67	334.33	335.67
	After	332.33	332.67	335.00	332.67	334.33	334.00	332.00	333.33	333.67	334.67
	X	*329.83 b*	*332.67 a*	*334.33 a*	*333.17 a*	*334.83 a*	*334.17 a*	*332.50 a*	*333.00 a*	*334.00 a*	*335.17 a*
Uric acid(mg/100 ml)	Before	3.67 b	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a
	After	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a
	X	*3.83*	*4.00*	*4.00*	*4.00*	*4.00*	*4.00*	*4.00*	*4.00*	*4.00*	*4.00*
Total Protein(g /100 ml)	Before	3.83 b	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a
	After	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a	4.00 a
	X	*3.92*	*4.00*	*4.00*	*4.00*	*4.00*	*4.00*	*4.00*	*4.00*	*4.00*	*4.00*
Cholesterol(mg /100 ml)	Before	164.67 ab	165.33 ab	162.33 b	165.6 ab	163.33 ab	162.67 ab	163.00 ab	166.33 a	164.00 ab	162.67 ab
	After	164.67 ab	165.00 ab	163.00 ab	164.00 ab	163.67 ab	162.33 b	164.67 ab	166.00 ab	164.33 ab	164.33 ab
	X	*164.6abc*	*165.17 ab*	*162.67 c*	*164.8abc*	*163.50 bc*	*162.50 c*	*163.8abc*	*166.17 a*	*164.1abc*	*163.50 bc*

Time of exposure		Glucose (mg /100 ml)	Uric acid (mg/100 ml)	Total Protein(g /100 ml)	Cholesterol (mg /100 ml)
	Before	*333.27*	*3.97*	*3.98*	*164.00*
	After	*333.47*	*4.00*	*4.00*	*164.20*
Total mean		*333.37*	*3.98*	*3.99*	*164.10*
Prob.	*Times*	*NS*	*NS*	*NS*	*NS*
	Sounds	*0.0078*	*NS*	*NS*	*0.0344*
	*Time*sounds*	*NS*	*0.05*	*0.05*	*0.05*
Pooled SEM		*0.3206*	*0.0166*	*0.0083*	*0.2489*

[a–b] Means with different superscripts in each row are significantly different. *NS*= not significant.

[1]the post-conditioning phase was carried out on the 17th to 19th days post-hatch, and the numbers in the table represent 3chicks (out of 5) per treatment for 2 days (10 repl./treat.).

[2] The blood trait measurements: occurred before chicks were exposed sounds in the box during the preconditioning phase and immediately after exposure.

[3]Sounds in the post-conditioning phase were played on the 17th to 19th days post-hatch.

1=Movement of Chicken Feet , 2= Hen With Chicks , 3= Cluck , 4= Hens Regular Soft Timid, 5 = Chick to Chick, 6 = Chicks Peeps, 7 = Care Chicks, 8 = Chicks Sound , 9 = Hen House Movement and 10 = Movement Chicks.

different sounds and exposed chicks to them in the following stages: habituation training, pre-conditioning, conditioning, and post-conditioning. During the habituation training stage, we observed differences in blood biochemistry that may have been due to disturbance or anxiety, resulting from the bird encountering a strange place (CPP apparatus) with different sounds. When heard for the first time, new noises can induce a feeling of fear in chicks, as noted by Barnard (1983), which raises the concentration of the stress hormone known adrenocorticotropic hormone (ACTH). Next, the hormone corticosterone is secreted, and its main function is to assemble proteins, fat and glucose during difficult situations to make energy available to the brain, heart, nervous system and skeletal muscles (Mary, 1986). Therefore, we observed differences in the biochemical traits of blood due to the stress and fear experienced by the birds during the habituation training stage (Michael and Janice, 2012). In addition to the effects mentioned above, an imprinting phenomenon, which was discovered by Konrad Lorenz in his classic study of the development of social behavior in newly hatched chicks, occurs during "critical periods" that are restricted to the very early life of a chick (Minne and Decuypere, 1984). The learning process is an emotional test that happends at an early age and leads to the development of a pattern of synaptic contacts in the brain (Bock and Braun, 1998). A lack of learning, which may result from social deprivation, leads to long-lasting consequences for the formation of synapses and the function of neuronal networks (Bock and Braun, 1998, Bock *et al*., 2005). Therefore, this imprinting phenomenon can be used to determine the ability of chicks to effectively distinguish between different sounds.

During the pre-conditioning stage, chicks began learning in the strange place (CPP apparatus) and began of fell safet, which also occurs when chicks are exposed to familiar sounds, so during this stage, the chicks began to distinguish between sounds and exhibit a preference. However, the habituation stage produced the best results due to the reduction in fear and anxiety, which in turn reduced the secretion of stress hormones that affect blood traits (Möstl and Palme, 2002). This reduction in fear and anxiety did not only result from habituation but from hen vocalizations, which play an important role in quieting chicks and making them less fearful. Greenless (1993) noted that chicks become more active and relaxed if exposed to hen vocalizations or the sound of feeding, which led to improved feed consumption (Woodcock *et al*., 2004). In addition, hen vocalizations may decrease exploratory behavior, which reduces the amount of energy expanded to search for food and leaves more for growth. Druing the conditioning stage, the chicks had become adapted and habituated to the CPP**,** and as mentioned Jones *et al*. (2012), it was possible to assess whether the effects of the sounds were negative or positive (Bardo *et al*., 1995). The results show improved blood traits, which are tied to the chicks feeling comfortable and acclimated to the situation. Acclimation to the CPP might be explained by the secretion of the cocaine and amphetamine regulated transcript (CART) hormone, which is found in more than place including ventral tegmental area (VTA) of the brain, that play roles in rewards, stress and feeding (Hoffman, 1989). It also has an important role in increasing locomotor activity; animals that are injected with this hormone tend to return to where they received their dose (Kimmel *et al*., 2000). CART also functions in the regulation of energy homeostasis, as well as regulation of appetite, in which it has a synergistic relationship with other hormones including: leptin (Murphy, 2005), cholecystokinin and ghrelin (de Lartigue *et al*., 2007, Maletínská *et al*., 2008). CART also influences the activity of neurons, and its production is regulated by the cAMP response element-binding protein (CREB), which is involved in the formation of long-term memories and the development of cognition in animals (Rogge *et al*., 2009).

In the last stage (post-conditioning), the chicks were ready to choose the most preferred sound after training on the CPP, so this was an especially important stage in this study. During this stage, chicks were exposed to sound from one direction, which is in contrast with the previous stages where the sound came from two directions in the box, so the chick could express a performance. The blood traits measured in this experiment serve as indicators of biological characteristics, and the physiology and health of the chicks, and sounds 1, 4 and 7 yielded the best results based on those charactersistics.

As mentioned above, CART is synergistically involved with the hormone cholecystokinin (de Lartigue *et al*., 2007), stimulates the release of glucagon from pancreatic A-cells (McMurtry *et al*., 1996). Glucagon works to convert stored glycogen in the liver to glucose, which is released into the bloodstream, and this explaine the changes in the concentrations of glucose in the blood observed in the this study (Reece and Campbell, 2002).

In terms of cholesterol levels Jaskula et al. (a2009) observed changes in the levels of ghrelin, which is also syngeristically involved with CART that alterd the concentration of cholesterol in the blood because there is a positive correlation between ghrelin and cholesterol

(Jaskula *et al*., b2009).

The change in the total protein concentration might be due to a change in corticosterone activity because corticosterone has catabolic effects on muscles, skin, lymphatic tissue and bone (Eiler, 2004). Additionally, a change in uric acid levels can reflect a change in protein metabolism (Khazali, 2009). On the other hand, ghrelin administration can alter insulin and T4 levels and change serum concentration of uric acid (Ovais and Mahapatra, 2013).

Figs. 1, 2 and 3 shows that the treatments that produced the best blood parameters values are the same ones, that increased the CPP factor values, this which indicates the sounds that led to improved blood and behavioral traits, so the increased CPP factor values in response to the treatments are the result of the preference of the chicks for the different sounds. The chicks follow the track that leads to comfort, stability and safety (Aline *et al*., 2002) and increasing lengths of stay on the right or left sides of the box lead to increased CPP factors. This increase in duration is due to the chicks feeling comfortable and safe, because the acoustic stimulus improves the social emotions that stimulate the brain cells to secrete opioids, which work to numb the body and create a sense of comfort and safety (Cheng and Durand, 2004; Baldauf *et al*., 2005).

Acoustic stimulation with CPP enhances the secretion of CART in the brain which is a neuropeptide considered responsible for increasing locomotors activity, animals tend to return to the places where they secreted this neuropeptide (Hoffman, 1989; Douglass *et al*., 1995; Kimmel *et al*., 2000). Therefore, chicks prefer places that they remember as being comfortable, and they will stay as long as possible in the preferred part of the box during the test. This is due to the attractiveness of the sound, so chicks prefer the sounds that they associate with being comfortable and safe (Tachibana *et al*., 2003).

As the sound effect in chicks through Imprinting process, chicks are learning distinguish the sound and follow-up hen (Nakamori *et al*., 2013). Thus the sound of parents, whether hen sound will work to increase the size and area of the nerve synapsesand increases the differentiation of neurons and an increase in the length and size of the cores in nerve cells and nuclei of glial cells and also increases the expression of c-fos protein which participates in the development and differentiation of cells and increases the blood vessels and this was confirmed by Wadhwa *et al*. (1999), this agreed with Alladi *et al*. (2002), which reported that the sound of the parents play an important role in the development of synapses and the development of the spinal cord and brain and this agreed with Chaudhury *et al*. (2009, 2010 and 2013), who said that the sound play an important role in the evolution and the formation of the brain and nerves.

Acoustic stimulus on the secretion of the hormone norepinephrine, which helps excretion to facilitate information, which contribute to the formation of sensory functions as attention and memory processing, thus could chicks that characterize the best sound and are to remain part which emits favorite sound has a longer and this was confirmed by (Gibbs and Summers, 2005; Toukhsati *et al*., 2005).

But in the absence of the hen, they learn to distinguish the sound through the trial and error method are approaching sound. When their sense safety and comfort and relaxation for a long time will remain near the sound or vice versa, when feeling chicks fear, they will alienate, not stay for a long period of sound and this explains the visit chicks sound and length of duration of stay for a particular sound compared with another sound, this agreed with Nicol (2004) and Edgar *et al.* (2016), which reported that the sound and caring hen a significant impact in development and growth of the calm and comfort to the chicks.

Conclusion

In conclusion, the CPP procedure is a valid and reliable method to determine the sounds that chicks prefer to hear to feel safe and thus consume more feed. We conclude that exposing chicks to their favorite sound will improve blood, parameters and consequently, improve performance in broiler chicks. We also provided inconclusive evidence that early feeding will improve the health of chicks and help them overcome problems later in life and therefore performance.

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