EFFECT OF OUTDOOR ACCESS ON ETHOLOGICAL BEHAVIOR, HEALTH AND PERFORMANCE OF BROILERS IN THE TROPICAL MEXICAN CONDITIONS †

[EFECTO DEL ACCESO AL EXTERIOR SOBRE EL COMPORTAMIENTO ETOLÓGICO, LA SALUD Y EL COMPORTAMIENTO PRODUCTIVO DE POLLOS DE ENGORDA EN CONDICIONES TROPICALES DE MÉXICO]

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SUMMARY

Background. There is a concern among poultry meat consumers due to broiler conditions during rearing period in commercial production systems. Consumers trust the organic and free-range poultry production systems because they suppose are more suitable and natural, giving high nutritive value and low-fat content to the meat, improving also the chicken welfare. **Objetive.** Two studies evaluated the effect of an outdoor access on ethological behavior, health indicators and productive performance of Hubbard broilers in the rearing period. **Methodology.** The first experiment evaluated outdoor access system in spring (april to may), and the second one was implemented in summer (june to august). In both studies included two groups: a) chickens with outdoot access (OA) and b) chickens at indoor rearing only (WOA). In the first experiment, ethological behaviors (EB) were described; forage intake (Fo), feed intake (FI), live weight gain (LWG) and carcass characteristics (Cc) were measured, blood components (BC), total immunoglobulins (Ig) and parasite eggs counts in excreta (Pc) were also determined, besides microscopic gut lesions in chickens (ML) were evaluated. In the second experiment, live weight gain (LWG), feed intake (FI) and forage intake (Fo) were measured, also carcass characteristics (Cc) were determinated. Analysis of variance by one way ANOVA was performed. **Results.** In first experiment, it was found that OA and WOA chickens stayed the most time resting. However, inside and outside, moving and foraging behaviors were observed in OA, while in WOA treatment foraging of chickens was the less frequent conduct. WOA showed higher final live weight and FI, but there were no differences in LWG and feed conversion in comparison with OA. Gizzard and caeca weights were heavier in OA treatment. No differences in both carcass yield and abdominal fat were found. At the last two weeks of age the forage intake amount per bird was 1.93 ± 0.97 g and 2.06 ± 0.87 g of DM/d of *Leucaena leucocephala* and *Pennisetum purpureum*, respectively. OA chickens had fewer leukocytes number (lymphocytes, eosinophils) and total inmmunoglobulins, but more heterophils and blood hemoglobulin. No differences between treatments in *Eimeria* oocysts in excreta were found. However, higher distribution and severity in microscopic gut lesions in birds WAO treatment were found. While in second experiment it was found that both OA and WAO broilers had similar final weight, feed intake, carcass yield and abdominal fat, but OA chickens had a trend to be higher in both weight gain and better feed convertion. Likewise, AO and WAO broilers had similar tibial ash content. Also, AO broilers consumed 0.50 ± 0.36; 0.49 ± 0.50 and 0.60 ± 0.32 g of DM/d of *Leucaena leucocephala*, *Brosimum alicastrum* and *Moringa oleifera*, respectivily. **Implications.** It is basic to have the knowledge regarding poultry production with outdoor access in tropical conditions. **Conclusions.** Outdoor access stimulated natural behaviours expression and did not affect productive performance. Also, AO did not produce hematological changes or severe microscopic lesions.
Keywords: broilers; outdoor access; behavior; health; performance.

RESUMEN

Antecedentes. Los consumidores de carne de pollo están preocupados por las condiciones de las aves durante su crianza en los sistemas de producción comercial. Desde el punto de vista del cliente, la producción orgánica y en libertad es más natural, con carne de alto valor nutritivo y bajo contenido de grasa, además de mejorar el bienestar del pollo. **Objetivo.** Se realizaron dos estudios para evaluar el efecto del acceso a extrerior sobre el comportamiento

† S*ubmitted March 3, 2021 – Accepted August 11, 2021.*
ISSN: 1870-0462.

etológico, la salud y el comportamiento productivo de pollos de engorda Hubbard durante su periodo de crianza. **Metodología.** En el primer experimento se evaluó el acceso a exerior durante primavera (abril a mayo), y en el segundo en verano (junio a agosto). En ambos experimentos se emplearon dos tratamientos: a) pollos de engorda con acceso a exterior (OA) y b) pollos de engorda con crianza en interior únicamente (WOA). En el primer experimento, se describió el comportamiento etológico (EB), se midió el consumo de forraje (Fo), consumo de de alimento (FI), ganancia de peso (LWG) y las características de la canal (Cc), también se determinaron los componentes sanguíneos (BC), inmunoglubulinas totales (Ig) y la cantidad de huevos de parásitos en las excretas, adicionalmente, se evaluaron las lesiones microscópicas en el intestino de las aves (ML). En el segundo experimento, se midió la ganancia de peso (LWG), consumo de alimento (FI) y consumo de forraje (Fo), también se determinaron las características de la canal (Cc). Los resultados se analizaron mediante análisis de varianza de una vía. **Resultados.** En el primer experimento, se encontró que los pollos de ambos tratamientos (OA y WOA) pasaron más tiempo en receso. Sin embargo, dentro y fuera del corral, los pollos del tratamiento OA manifestaron movimiento y forrajearon, en tanto que en las aves del tratamiento WOA el forrajeo fue la conducta menos frecuente. Las aves del tratamiento WOA resultaron con mayor peso final y consumo de alimento, pero no hubo diferencias en LWG y la conversión alimenticia, en comparación con las aves del tratamiento OA. La molleja y los ciegos fueron más pesados en el tratamiento OA. No se encontraron diferencias en el rendimiento de la canal y grasa abdominal. Las últimas dos semanas de edad, las aves consumieron 1.93 $\pm$ 0.97 g y 2.06 $\pm$ 0.87 g de MS/d de *Leucaena leucocephala* y *Pennisetum purpureum*, respectivamente. Los pollos del tratamiento OA tuvieron menor cantidad de leucocitos (linfocitos, eosinofilos) e inmunoglobulinas totales, pero mayor cantidad de heterofilos y hemoglobulina. No hubo diferencias en los ooquistes de *Eimeria* excretados. Sin embargo, se encontraron lesiones microscópicas más distribuidas y severas en el intestino de las aves del tratamiento WOA. En el segundo experimento, se encontró que los pollos OA y WAO tuvieron un peso final, consumo de alimento, rendimiento en canal y grasa abdominal similares, pero aquellos de OA tuvieron una tendencia de ganancia de peso más alta y una mejor conversión de alimento. Asimismo, los pollos AO y WAO tuvieron un semejante contenido de cenizas en la tibia. Los pollos AO consumieron 0.50 $\pm$ 0.36; 0.49 $\pm$ 0.50 y 0.60 $\pm$ 0.32 g de MS/d de *Leucaena leucocephala, Brosimum alicastrum* y *Moringa oleífera*, respectivamente. **Implicaciones.** Es fundamental tener conocimiento acerca de la producción de pollos de engorda con acceso al exterior en condiciones tropicales. **Conclusiones.** El acceso al exterior estimuló la expresión de comportamientos naturales, y no afectó el desempeño productivo; además, no produjo alteraciones hematológicas ni lesiones intestinales microscópicas graves.

Palabras clave: pollos de engorda; acceso al exterior; comportamiento etológico; salud; comportamiento productivo.

INTRODUCTION

Poultry meat sector is continually growing, especially for demand in developing countries (Mottet and Tempio, 2017). Consumers are concerned due to chicken condition regarding conventional production systems; this issue has arisen and, therefore, affinity for organic and free-range poultry production is growing (Souillard *et al*., 2019). Outdoor access represents an alternative for chicken production, because it promotes natural behaviors expression and from the consumer point of view is more natural and better for chicken welfare (de Jonge and van Trijp, 2013; Meseret, 2016). Outdoor access can provide a generous space in an open area, free range air and the opportunity to select forage and nutrients from the field (Fanatico *et al.*, 2016). On the other side, there is the posiblility of predation or diseases from wildlife besides sporadic feed (Sanchez-Casanova *et al*., 2019). Chicken's meat raised in production system with outdoor access has more added value because consumers associate it with better flavour, high nutritive value, low fat content and high vitamins and minerals in meat (Fanatico *et al*., 2005; Fanatico *et al*., 2007; Michalczuk *et al.*, 2014). Therefore, consumers prefer poultry products from chickens growing in alternative production systems (Fanatico *et al*., 2006). Nevertheless, several studies of outdoor access system effects have been carried out in laying hens (Petterson, Freire and Nicol, 2016; Yilmaz Dikmen *et al.*, 2016; Larsen *et al*., 2018). Studies in broilers suggest the use of slow-growing or local breeds chickens with outdoor access, because are more adaptable due to their rustic qualities and high foraging behavior being different of fast-growing broiler chickens (Moyle *et al*., 2014; Castellini *et al*., 2016). Although, in United States some producers use fast-growing broiler chickens in outdoor access systems (Moyle *et al*., 2014). The literature of outdoor access on poultry is scarce under tropical conditions. Because of this, there is a need to evaluate the effect of outdoor production system in broiler chickens in the tropics. Therefore, the aim of the present study was to describe the effect of an outdoor system on ethological behavior, some health indicators and performance of fast-growing chickens.

MATERIALS AND METHODS

In the present study, two experiments were performed. In the first one, the effect of outdoor access on the ethological behavior, forage intake, productive performance, carcass characteristics, blood components and immunoglobulins, parasitic counts and microscopic lesions of broiler chickens was evaluated. In the second, outdoor access effect on productive performance, forage intake and carcass characteristics of broilers in rearing period was examinated.

Study site

Both experiments were conducted at the experimental area of Animal Nutrition Department of Faculty of Veterinary Medicine and Animal Science of the Universidad Autónoma de Yucatán (FMVZ-UADY), located at 20°58'N and 89°37'W in the southeast of Mexico with warm sub-humid weather and an average temperature of 26.2 $\pm$ 2.2 °C (INEGI, 2017). The first experiment was carried out trough April to May (spring), while the second was in June to August (summer), with average temperatures of 29.8 $\pm$ 1.06 °C and 28.9 $\pm$ 2.01 °C, respectively.

Animals

In both experiments, mixed sex Hubbard chickens (1 male:1 female ratio) one-day old were used, they were housed in a reception pen, fenced with wire mesh and equipped with wood shavings as bed, heater, feeder and drinker. From eight-day old chickens were randomly distributed in definitive pens of 1.1 x 1.5 m, which had wood shavings as bed, feeder and drinker each one. The wood shavings bed in pens was turned every second day and was changed weekly. In both experiments, birds were vaccinated *in ovo* for Marek's disease. On both 7 and 21 days old Gumboro disease vaccine was applied and Newcasttle vaccine on 15 day old.

In first experiment, fifty chickens were randomly distributed in 6 pens with 8 or 9 birds each one, which had a density of 5-6 chickens/m^2, in such a way that were 3 pens (replicates) per treatment (outdoor vs indoor). In the second experiment, ninety birds were randomly distributed in 10 pens with 9 chicks each, which had a density of 6 chickens/m^2, in such a way that were 5 pens (replicates) per treatment.

For birds with outdoor access, the yard had 1.1 x 10 m, fenced with wire mesh, soil floor, herb and natural grass-grown vegetation, and availability of native trees as shade.

Experimental groups

In both experiments, two treatments were evaluated: a) experimental group, where birds had outdoor access during the day (08:00 to 17:00 h) throught pophole doors (40 x 40 cm) and confined inside pen during the night, from eight-day old (OA), and b) control group, where chickens stayed inside the pen 24 h without outdoor access (WOA).

Feeding

In both experiments, all birds received commercial feed, according to the initial (1 to 21 d), grower and finisher (22 to 42 d) (21 and 19% crude protein respectively and 3200 kcal/kg ME in both feeds), rearing stages diets. Feed and water were offered *ad libitum* inside pens. For chickens under OA treatment, additionally to natural vegetation in outdoor, grass and tropical trees forage were offered. In the first experiment, *Leucaena leucocephala* and *Pennisetum purpureum* were given. In the second, *Leucaena leucocephala*, *Brosimum alicastrum* and *Moringa oleifera* were granted. Fresh forage was offered daily to all birds inside pens from 8 day old.

Experiment 1

Ethological behavior

The chicken activity was recorded by an ethogram including a list of 10 behaviors, based on Fanatico *et al.* (2016) and Bergmann *et al.* (2017). Once a week, always in same day, chickens were observed (from 08:00 to 17:00 h) on intervals time of five minutes, on weeks 4, 5 and 6 of rearing period. The ethogram was used for the two experimental groups. Six behaviors when chickens inside pen were observed: feeding, drinking, foraging, moving (walking/running), resting and dust bathing, and four behaviors registered when birds stayed in outdoor area: foraging, moving (walking/running), resting and dust bathing. Behaviors were registred individually and recorded in tables, and the frequencies were estimated as a percentage of the total behaviour, data were pooled for obtain an average (Castellini *et al*., 2016).

Forage intake

On second week, *Leucaena leucocephala* and *Pennisetum purpureum* forages were offered to OA treatment chickens, inside pens each day as adaptation period. From weeks 5 and 6 forage intake was measured once a week. Forages intake was estimated with forage refusal method as the difference between offered and rejected on dry matter basis, divided by total number of chickens per pen, obtaining average grams intake per bird.

Productive performance

Chickens were individually weighted at one-day old and subsequently body weight were recorded weekly from 1 to 6 week-old to estimate live weight gain. Final weight gain was estimated as the difference between the initial and final weight (Medina *et al*., 2014). Feed intake average per bird was measured from 4 to 6 week-old (Holdsworth *et al*., 2004; Carranza *et al*., 2011; Habibi *et al*., 2014); twice a week, at beginning and end. Feed intake was calculated as the difference between feed offered and refused, divided by the total number of birds per pen (Holdsworth *et al*., 2004; Bozkurt *et al*., 2016).

Carcass evaluation

At slaughter (42-day old), 24 chickens of each treatment were randomly selected for carcass evaluation. Abdominal fat from around gizzard and viscera was dissected on each bird (24 OA and 22 WOA) and stored in polyethylene containers at -18 ºC. For each chicken selected, gizzard and caeca were weighed. Carcass yield was recorded after 2h chilling at 4 ºC (Marx *et al*., 2016; Woo-Ming *et al*., 2018).

Blood components and total immunoglobulins

Blood samples were obtained on 41 day old taken from 20 and 21 chickens from OA and WOA treatments, respectively, for determining type and amount components of erythrocytic and leucocitic series. Besides, another 24 and 23 chickens from OA and WOA treatments were selected for taken blood serum samples for determining immunoglobulin content. Blood samples were collected on each bird and divided in two parts that were kept on BD Vacutainer® tubes, one part was kept on tube with EDTA K2 as an anticoagulant, for haematological analysis, and the other part on tube without anticoagulant for serum analysis. Samples were taken by brachial veinpuncture. Samples were tempered before stored in refrigeration (Espín *et al.*, 2014; Colas *et al.*, 2016). White and red blood cells contents were determinated on the samples collected with EDTA K2 tube, besides the volume of the cellular packet (VPC) or hematocrit was determinated by microhematocrit method. Blood hemoglobin amount was estimated as one third of the microhematocrit (Avilez *et al*., 2015). Leukocyte differential count by blood smear was performed, which determined relative and absolute values of lymphocytes, heterophyles, eosinophyls, monocytes and basophyls (Maxine, 1984; García-González *et al*., 2012).

Blood serum was obtained for centrifugation at 3,000 revolutions per second for 5 minutes. Then immunoglobulin contents were determined as total serum proteins detected by Biuret method (Silva *et al.*, 2007; Nedeljkovic *et al*., 2013).

Parasite eggs counts in excreta

Excreta samples were obtained at slaughter on 42 day old, from 13 and 6 randomly chickens from OA and WOA treatments, respectively. Samples were collected directely from intestine, 4 g of excreta was taken from each bird, stored in polyethylene bags and transported immediately to laboratory for processing (Holdsworth *et al*., 2004; Naidoo *et al.*, 2008). Parasite eggs presence was determined by flotation technique; and oocyst counts from *Eimeria* spp. per gram by Mcmaster technique (Holdsworth *et al*., 2004; Abbas *et al*., 2017; Ahad *et al*., 2018).

Microscopic lesions gut in chicken

At slaughter day, chicken guts were examined by midline disection, from base of gizzard to the rectum (Orengo *et al*., 2012; Sing *et al*., 2015), presence or absence of macroscopic lesions were detected in this area. Small intestine with macroscopic lesions presence, gut segments were collected. Intestinal segments from 24 chickens of OA treatment were chosen, and segments were taken in 22 birds under WOA treatment; each segment was taken once from different chickens. Intestinal segments were kept in polypropylene bottles with 10% formalin for 24 hours, then embedded in paraffin and 5 µ thick sections were obtained, which were stained with hematoxylin-eosin. The sections were placed on slides with Canada balsam, and with optical microscope were observed (Geneser, 2003). Microscopic lesions were classified using criteria A and B proposed by Goodwin *et al.* (1997). Classification A estimates infection extent, and classification B evaluates infection severity. In classification A, 10 slide fields randomly were reviewed with 10x objective microscopic, to found microscopic lesions from *Eimeria* spp. When lesions were not found on field, this was classified as 0; if it was present in one field as 1; in two fields as 2; in three fields as 3, and in four or more fields as 4. For classification B, the field with most cellular damage caused by *Eimeria* was selected. In the selected field, with 40x objective microscopic, the damage percentage of enterocyte villus was estimated, then without any damage it was classified as 0; if affected villus was less than 25% as 1; if damage was 25 to 50% as 2; between 51 and 75% as 3; and more than 75% villus damage as 4.

Experiment 2

Productive performance

All chickens were weighted at one day old, and subsecuently once a week per treatment, and average values for each treatment were estimated. Final body weight gain was estimated as the difference between the initial and the final weight at week 6 (Medina *et al*., 2014). Feed intake average was estimated on weekly basis and calculated as difference between feed offered and refused, divided by the total number of birds per pen (Holdsworth *et al*., 2004; Bozkurt *et al*., 2016).

Forage intake

From second week, *Leucaena leucocephala*, *Brosimum alicastrum* and *Moringa oleifera* forages inside each pen were offered to chickens of OA treatment. From week 2 to 6 forage intake was measured once a week. Forage intake was estimated with forage refusal method, calculating difference between offered and rejected amount of each forage divided by total number of chickens per pen on dry basis; then, offered and refused dry matter of each forage was measured for calculated dry matter intake. Forage samples were dried at laboratory by electric oven at 60 ºC for 48 h.

Carcass characteristics

The slaughter day (42 day old), chickens of each treatment were randomly selected for carcass evaluation. Samples of abdominal fat from 15 bird were dissected and stored in polyethylene container, also carcass yield was recorded (Marx *et al*., 2016). Besides, tibial bone samples were collected from 6 randomly chosen chickens per treatment. From the right leg muscles and tendons around tibial bone were dissected, collected and stored in individual polyethylene bags. The ash content in tibial bone was estimated, by muffle oven incineration (Onyango *et al*., 2003; Walk *et al*., 2011).

Statistical analysis

In both experiments, a completely randomized desing were used. In the first experiment, each ethological behavior was shown as percentage for each experimental group. Forage intake data of the chickens in OA treatment was reported as g of DM/d per bird. Besides, live weight gain, feed intake, abdominal fat weight, gizzard and caeca weight were analyzed with analysis of variance by one way ANOVA (Minitab, v. 19). Normality and variance homogeneity were confirmed for each variable. Blood components and total immunoglobulins were also examined by one way analysis of variance (ANOVA). Parasite eggs counts were analysed by General Linear Model (GLM) (Statgraphics, 2010). Data from severity and distribution of microscopic lesions in gut were analizated with Chi square test (Minitab, v. 19). For second experiment, forage intake data was reported as g of DM/d per bird. Also, live weight gain, feed intake, abdominal fat weight and also ash tibia content were analyzed with analysis by one way ANOVA. Normality and variance homogeneity were confirmed for each variable. Significant differences were considered if $P \leq 0.05$.

RESULTS

Experiment 1

Ethological behaviours are observed in Table 1. Inside pens, the most frequent conduct was “resting” during the last four weeks of rearing period in both treatments (outdoor 38.66-61.10%; indoor 64.00-78.70%). Furthermore, in OA treatment, birds demostrated that “resting” was most frequent even in outdoor area. In addition, the behaviors "feeding" (OA until 13.33%; WOA until 20.44%) and "drinking" (OA until 5.78%; WOA until 7.11%) were the other two most comun in both treatments. However, it was observed a higher percentage of birds "moving" and "foraging", inside and outside the pen in OA treatment (until 5.78%; until 7.41%). On the contrary, in WOA treatment, the "foraging" behavior was the less frequently conduct (until 2.22%).

Chickens without outdoor area access (WOA) showed higher final live weight and feed intake compared to OA birds (outdoor LWG 2258g; FI 4316g vs indoor LWG 2567g; FI 5199g; P<0.05, Table 2). Nevertheless, no significant differences in the body weight gain and feed conversion were recorded between treatments (outdoor BG 1270g; Fc 3.63g/kg

Table 1. Broiler behaviours with and without outdoor access in rearing period. Experiment 1.

	With outdoor access				Without outdoor access			
Activities (%)[1]	4 Wk	5 Wk	6 Wk	7 Wk	4 Wk	5 Wk	6 Wk	7 Wk
Inside pen								
Feeding	13.33	10.67	13.89	9.72	20.44	14.22	15.74	10.19
Drinking	4.44	5.78	2.31	4.17	7.11	6.67	5.09	5.56
Foraging	4.89	6.66	7.41	3.70	2.22	0.89	1.39	0.93
Moving	2.67	5.78	2.31	3.70	2.22	3.56	3.24	4.17
Resting	57.78	38.66	61.10	55.56	64.00	71.56	71.30	78.70
Dust bathing	0.00	0.89	0.46	2.31	4.00	3.11	3.24	0.46
Outside pen								
Foraging	4.00	0.00	1.85	7.41	NA	NA	NA	NA
Moving	2.22	1.78	0.93	2.31	NA	NA	NA	NA
Resting	9.33	16.44	9.72	8.80	NA	NA	NA	NA
Dust bathing	1.33	13.33	0.00	2.31	NA	NA	NA	NA

[1]Frequencies estimated as a percentage of the total behaviour. Calculated percentage values of n = 25 birds observed per treatment. Wk: Week. NA: Not available.

Table 2. Productive performance and carcass characteristics of Hubbard chickens with and without outdoor access in rearing period. Experiment 1.

Traits	With outdoor access	Without outdoor access	SEM	*P* value
Final live weight (g) [1]	2258	2567	69.10	0.003
Total weight gain (g) [1]	1270	1388	72.10	0.254
Totak feed intake (g) [1]	4316	5199	40.32	0.000
Feed convertion ratio (g/kg) [a 1]	3.63	4.02	0.21	0.210
Carcass yield (%) [2]	69.99	72.52	0.89	0.051
Abdominal fat (g/kg carcass) [3]	2.42	2.74	0.27	0.397
Gizzard weigth (g/kg carcass) [3]	2.36	1.93	0.10	0.004
Caeca weight (g/kg carcass) [3]	0.91	0.77	0.04	0.011

[a] Average until week 4. Calculated mean values: [1] from n = 3 (OA and WOA) chickens; [2] n = 24 (OA and WOA) poultry and [3] n = 24; 22 (OA; WOA) birds SEM: standard error of the mean

vs indoor BG 1388g; Fc 4.02g/kg; P>0.05) during the last four rearing weeks (Table 2). In addition, table 2 shows that only gizzard and caeca weigths were different between treatments (OA gizzard 2.36g/kg; caeca 0.91g/kg vs WAO gizzard 1.93g/kg; caeca 0.77g/kg; P<0.05), being lower both organs in WAO treatment. Also, it is destacable that there was no difference in the carcass yield or the weight of abdominal fat between treatments (OA carcass: 69.99%; fat 2.42g/kg vs WAO carcass 72.52%; fat 2.74g/kg; P> 0.05).

Besides, chickens of AO treatment consumed an average of 1.93 $\pm$ 0.97 g of DM/d of *Leucaena leucocephala* and 2.06 $\pm$ 0.87 g of DM/d per bird of *Pennisetum purpureum*, during the last two weeks of rearing period.

Chickens in OA treatment had fewer white cells or leukocytes in blood than those in WOA treatment (outdoor 3990mm^3 vs indoor 4933mm^3; P<0.05). Specifically, regarding lymphocytes and eosinophils number, chickens in OA treatment had significant fewer in comparison with WOA treatment (OA lymp 46.5%; eosin 9% vs WAO lymp 54.5%; eosin 13.14%; P <0.05), the same trend occurred on total immunoglobulins (OA 50.26mg/ml vs WOA 60.80mg/ml; P<0.05). However, heterophils in OA chickens blood was higher than WAO birds (OA 39.4% vs WAO 26.4%; P <0.05). Moreover, on erythrocytic series, hemoglobin value observed in OA was significantly higher than WOA treatment (outdoor 8.27g/dl vs indoor 7.62g/dl; P<0.05) (table 3).

Regarding *Eimeria* egg counts, there were no differences between treatments (P: 0.962; SEM: 337.45), being 1284.62 $\pm$ 777.12 and 1308.33 $\pm$ 1358.46 oocysts per gram/excreta from OA and WOA respectively.

The microscopic lesions in gut, associated with presence of coccidia (*Eimeria* spp.), were 33.3% for OA treatment and 63.6% for WOA. Lesions were characterized by necrosis, intestinal villi atrophy, also lymphocytic, histiocytic and eosinophilic inflammation (Table 4). In chickens with outdoor access, 23.08 % had microscopic lesions, distribution with scores 1 to 4, in comparison with 50.55 % of

Table 3. Blood components and inmunoglobulins content in broiler chickens with and without outdoor access. Experiment 1.

Traits[1]	With outdoor access	Without outdoor access	SEM	*P* value
Hemoglobin g/dl	8.27	7.62	0.20	0.026
Hematocrit %	28.7	29.8	0.58	0.185
Leukocytes (mm^3)	3990	4945	297.46	0.029
Heterophils %	39.4	26.4	1.57	0.000
Lymphocytes %	46.5	54.4	1.95	0.006
Monocytes %	5.15	6.05	0.99	0.525
Eosinophils %	9.00	13.10	0.80	0.000
Total inmunoglobulins (mg/ml) [2]	50.26	60.80	3.53	0.040

Calculated mean values: [1] from n = 20; 21 (OA; WOA) chickens and [2] n = 24; 23 (OA; WOA) birds. The mean number of neutrophils in band, basophils and myelocytes was zero in both treatments. SEM: standard error of the mean.

Table 4. Microscopic lesions in gut broilers with and without outdoor access. Experiment 1.

Lesion form	Lesion presence (%)	
	With outdoor access[1]	Without outdoor access[2]
Atrophic, necrotic, lymphohistiocytic, and diffuse moderate eosinophilic enteritis associated with coccidia.	29.20	50
Lymphohistiocytic and eosinophilic enteritis.	20.80	22.70
Lymphohistiocytic enteritis.	20.80	13.64
Lymphohistiocytic and eosinophilic enteritis associated with coccidia.	NA	4.54
Atrophic lymphohistiocytic enteritis eosinophilic and hemorrhagic.	12.50	NA
Atrophic necrotic lymphohistiocytic, eosinophilic and neutrophilic enteritis associated with coccidia and bacteria.	NA	4.54
Necrotic lymphohistiocytic, eosinophilic, neutrophilic and hemorrhagic atrophic enteritis associated with bacteria and coccidia.	4.16	NA
Necrotic lymphohistiocytic and eosinophilic atrophic enteritis with necrotic cryptitis, associated with coccidia.	NA	4.54
Without apparent pathological changes.	8.30	NA

[1] Frecuency of calculated percentage values from n = 24 chickens. [2] Frecuency of calculated percentage values from n = 22 birds. NA: Not available

Tabla 5. Productive performance and carcass characteristics of Hubbard chickens with and without outdoor access in rearing period. Experiment 2.

Traits	With outdoor access	Without outdoor access	SEM	*P* value
Final live weight (g) [1]	2022	1917	38.19	0.056
Total weight gain (g) [a 1]	1755	1653	36.78	0.053
Total feed intake (g/bird) [a 1]	4155	4161	23.86	0.593
Feed convertion (g feed/ kg gain) [a 1]	2.40	2.58	0.06	0.022
Carcass yield (%) [2]	67.71	67.51	0.36	0.702
Abdominal fat (%) [3]	3.38	3.20	0.24	0.593
Tibia ash (%) [4]	32.01	33.46	1.02	0.340

[a] Data from week 4. Calculated mean values: [1] from n = 5 (OA and WOA) chickens; [2] n = 42; 45 (OA; WOA) birds; [3] n = 15 (OA and WOA) poultry; [2] n = 6 (OA and WOA) broilers. SEM: standard error of the mean

chickens without outdoor access (p: 0.0025). Besides, the severity of microscopic lesions in scores 1 to 4 were 8.68% in outdoor chickens and 54.95% of indoor birds (p = 0.0003).

Experiment 2

No significant differences between treatments were found neither for live weight nor for feed intake (outdoor LWG 2022g; FI 4155g vs indoor LWG 1917g; FI 4611g; P>0.05, Table 5). Nevertheless, chickens under OA treatment showed better feed conversion from week four; in addition, it was observed that carcass yield and abdominal fat percentage were not significantly different between OA and WAO (OA carcass: 67.71%; fat 2.38 % vs WAO carcass 67.51%; fat 3.20%; P>0.05). Also, tibial bone ash content was similar in both treatments (outdoor 32.01% vs indoor 33.46%; P>0.05) (Table 5).

Besides, chickens in AO treatment consumed *Leucaena leucocephala*, *Brosimum alicastrum* and *Moringa oleifera* forage, 0.50 $\pm$ 0.36; 0.49 $\pm$ 0.50 and 0.60 $\pm$ 0.32 g of DM/d per bird respectively, during of rearing period (2-7 weeks).

DISCUSSION

Experiment 1

Chickens with and without outdoor access stayed the most time resting at inside pens, even when OA treatment birds stayed in the outside area. Although they had access to field, some chickens at outdoor may wanted to stay close to pen because they could be afraid to open spaces or lack of suitable shelter (Sossidou *et al.*, 2011; Fanatico *et al.*, 2016). Besides, increased resting time could be related to increased live weigths during rearing period (Eriksson *et al.*, 2010). Even when chickens can get the outdoor area, the time of access and activities at outside depend on some factors such as age and genetics, flock size, time of the day and outdoor features (Almeida *et al.*, 2012). In slow-growing chickens with outdoor access, Fanatico *et al.* (2016) found that when adding enrichment in outdoor area, the most frequent behaviors were foraging and walking, in contrast when birds stayed inside the pen, it was observed that the more common behaviours of these birds were feeding, standing and sitting. Ponte *et al.* (2008) found a similar situation with fast-growing chickens, these tend to go outdoor for foraging and manifest more activity if outside area had an enriched environment. Meat chickens preffer areas with trees and tall grass, shader, dry areas for dust bathing and shelter from aerial predators (Dal Bosco *et al.*, 2014). Several studies reported that slow-growing meat chickens are more active and tend more to go outdoor in comparison than fast-growing birds (Almeida *et al.*, 2012; Castellini *et al*., 2016; Bergmann *et al.*, 2017). Nevertheless, in current study under tropical conditions, Hubbard chickens moving and foraging, inside and outside the pen, when birds had outdoor access. These results agreed with Eriksson *et al.* (2010), who found Ross chickens increased foraging and activity levels in an organic system with outdoor access, with low protein in feed. Besides, in the present experiment, birds had outdoor access from the second week of age; however, previous studies reported that chickens had access to outdoor area around 21 days old. An earlier exposure effect and familiarization may cause the major tendence to free-range access (Taylor *et al.*, 2017). However, when Hubbard chickens do not have outdoor access (on indoor birds) low activity levels, foraging specifically, were observed. That lower activity probably is due to either a poor stimulated environment or for ausence of free-range exposure; offering enrichements and complex enriched environments such as free-range, could increase natural behaviors such as foraging or dust bathing (Riber *et al.*, 2018).

Hubbard chickens are fast-growing genotype (Fanatico *et al*., 2007); although these birds may difficult be adapted to outdoor access, they use feed more efficiently in comparison than slow-growing chickens, have greater breast-meat yields and uniform carcass characteristics (Moyle *et al*., 2014; Woo-Ming *et al*., 2018). Further, some studies found that outdoor access did not affect growth and carcass yield in fast-growing breeds, in brief or prolongate free-range access period (Fanatico *et al*., 2009; Woo-Ming *et al*., 2018). In the current study, Hubbard chickens without outdoor access gained more weight and had more feed intake during rearing period, possibly caused by lower activity level inside pen where chickens stayed the most time feeding. But no difference in both feed conversion for the last four weeks and abdominal fat were found; in contrast, Fanatico *et al.* (2009) found that chickens at outdoor access had worse feed conversion and had lower fat. In addition, Hubbard chickens with and without outdoor access had similar carcass yield, like that found by Woo-Ming *et al*. (2018). These results suggest that fast-growing chickens can show their productive genetic potencial even when they have outdoor access, and they could be used also in free-range system production. Concerning gizzard and caeca weights changes between chickens with and without outdoor access, it is possibly related to forage intake of birds. Chickens fed dietary insoluble fiber develop heaviest gizzards, because need a muscular adaptation due to the greater demand for grinding (Sacranie *et al.*, 2012). In addition, caeca are site with microbial fibre fermentation and volatile fatty acids production. Caeca size is proportional to their digestion capacity, so dietary fibre causes their elongation (Savón, 2002; Savón, 2005; Svihus, Choct and Classen, 2013). Although literature declare that foraging habit is less common in fast-growing broilers compared to slow-growing ones (Yngvesson *et al.*, 2016), in the present study the forage intake per bird was 4 g of DM/d for *Leucaena leucocephala* and *Pennisetum purpureum* forages during last 2 weeks of age. Ponte *et al*. (2008) found that broilers ate 2.5 to 4.5% DM of forage during spring and autumm; this amount was near to forage intake estimated in the present study. Also, the findings were close to that reported by Almeida *et al.* (2012) for slow-growing genotype (5 to 8 g MS/d), estimated with crop content measurement method. Hence foraging habit is present in fast-growing broilers in tropical conditions, this can result in benefit due to pasture contains tocopherols, carotenoids, flavonoids and fiber, comparing to feed (Ponte *et al.*, 2008; Dal Bosco *et al.*, 2016). This aspect is important in tropical ecosystems, because the vast natural potencial for forage plants as a source of nutrients can be used (Sanchez-Casanova *et al*., 2019). Also, forage intake can contribute to nutrients and reduce feed intake so also diet cost (Ndelekwute *et al*., 2018).

Poultry health depens on well development of immune system; hematological analysis is useful for knowing circulating blood cells status, excreta samples can show presence or ausence of gastrointestinal parasites and gut inspection could exhibit lesions or damage, all this provides an overview of chicken’s health and their immune system. In the present study, the hemogram evaluated red and white blood cells, for knowing avian cellular response because in several diseases hematological changes are present (Avilez *et al*., 2015; Gutiérrez-Castro and Corredor-Matus, 2017). Indoor chickens had higher blood leukocytes, specifically lymphocytes and eosinophyls in comparison with outdoor chickens, similar hematological changes in *Eimeria* infection has been found (Adamu *et al*., 2013; Akhtar *et al*., 2015). These lymphocytes count increased in indoor chickens as result of an infection disease (Samour, 2010), and high eosinophils amount are associated with a parasitic infection and tisular damage (O´Malley, 2007). This increased peripheral blood leukocytes are part of immune response caused by *Eimeria* infection. Although *Eimeria* oocysts excretion between chickens with and without outdoor access did not differ. It is possible that, on sampling moment, indoor chickens had greater *Eimeria* parasites amount on merogony (or schizogony) stage, because it is known that sporozoites exposition cause a cellular and humoral response, specific and non-specific, for preventing parasite reproduction and development (Yun *et al.*, 2000), and eventually will be shedding oocysts. Otherwise, the low oocysts excretion in chickens with outdoor access, could be related to the higher number of heterophils, because they are both part of the first line defence and the major phagocytic leukocytes in birds (Scanes, 2015). Thus, chickens without outdoor access also had higher immunoglobulins amount as sign of increased antibody titer; it was required some time to development after primary infection, in response to repeated infections, and directed against extracellular stages of antigens (Gómez-Verduzco *et al.*, 2010; Wallach, 2010). Furthermore, the fewer hemoglobin of indoor chickens could be related to blood loss for hemorrhages in caeca and intestines (Akhtar *et al*., 2015; Melkamu, Chanie and Asrat, 2018), likely caused for epithelial invasion of intracellular stages (sporozoites, merozoites and microgametes) in merogony and gametogony phases during *Eimeria* infection. Possibly indoor pen condition caused chickens without outdoor access ingest available sporulated oocysts from bed, so reinfection process was mainteined. In contrast, outdoor chickens had extensive area for droppings deposition and this fact reduced reinfection probability or produced a mild parasitic infection with an immune cellular response, that could not be remarkably observed by the sampling moment, but that likely caused improvement in intestinal health, which was reflected in less gut damage. Hence, low percentages, distribution and severity in gut lesions from outdoor chicken reflected better intestinal health in comparison with indoor ones because *Eimeria* infection cause a high damage and microscopic lesions in gut (Sharman *et al.*, 2010; Quiroz-Castañeda and Dantán-González, 2015).

Experiment 2

In second experiment outdoor access did not affect weight gain in chickens; these results are consistent with those found in experiment one, and others studies (Fanatico *et al*., 2009; Woo-Ming *et al*., 2018), that confirms the possibility to use fast-growing chickens as alternative for outdoor system without negative impact on performance. Nevertheless, both outdoor and indoor chickens had similar final weigh when rearing in summer, in contrast with a higher weight gain of indoor chickens found when rearing in spring season from experiment one. Seasonal variations have influences on productive performance of broilers; it has been shown that broiler raised in summer had low both feed intake and weight gain (e.g. Koknaroglu and Atilgan, 2007; Sarma *et al.*, 2020). In the current experiment, it is shown a trend on feed intake and weight gain to be lower, but most notably in chickens without outdoor access, thus possible high temperatures produced thermal stress in chickens (Barlett and Smith, 2003). On the contrary, outdoor chickens where natural vegetation could improve environment leading to adequate comfort zone (Sarma *et al.*, 2020). However, chickens with outdoor access had better feed conversion since week four; also, carcass yield and abdominal fat were similar between outdoor and indoor chickens. Thus, in modern broiler production, both genotype and environment are the main factors to influence the performance (Sarma *et al.*, 2020). Hence, fast-growing chickens can have similar performance with and without outdoor access even in the summer season with tropical conditions.

Regarding skeletal health in fast-growing chickens, it is important an adequate skeletal development, because genetic selection has produced a greater body weight in combination with a body mass in skeletal muscles that do not correspond to bones growth rate and represent a challenge for bone mineralization (Suchý *et al*., 2009; Sanchez-Rodriguez *et al.*, 2019). Tibial bone is the one the most mineralized skeletal bone in chickens, so it represents a good indicator of overall skeletal mineralization (Applegate and Lilburn, 2002). Also, important features are total bone mineral and ash contents in bones and indicate hardness and strength (Faustin, Sarmiento and Sandoval, 2019; Sanchez-Rodriguez *et al.*, 2019), specifically calcium and phosphorus contents (Shim *et al.*, 2012). In this experiment, similar percentages of ash content in fast-growing chickens either with and without outdoor access was found, similar values were found by Shim *et al.* (2012) in slow- and fast-growing strains. Results suggest that the skeleton bears the same load and had similar bone breaking strength in fast-growing chickens with or without outdoor access.

Chickens with outdoor access ate around 1.5 g of DM/d per bird of *Leucaena leucocephala*, *Brosimum alicastrum* and *Moringa oleifera* forages during summer season. The forage intake could be affected by the high thermal sensation during summer season, because it is probable that chickens reduced the spend time at outdoor and also the behavior intake for both feed and forage in comparison with spring season. Even Dal Bosco *et al.* (2014) found that slow-growing chickens reduced their forage intake in summer when they did not have an environmental enriched with trees and shelter, probabily because of thermal stress. Hence, in this second experiment, forage intake was present in fast-growing chickens, so these results are consistent with those found in experiment one.

CONCLUSION

Since experiment one, it was found that outdoor access can be suitable alternative for fast-growing broiler chickens rearing, because stimulate natural behaviours expression (foraging, dust bathing) and enabling the moving on field. In addition, outdoor access chickens had less feed intake, probably related to forage intake, that did not affect their feed conversion, and so had similar carcass yield to birds without outdoor access on spring season. Also, hematological changes showed that chickens with outdoor access did not present severe infection and likewise, had too less score gut damage. On second experiment, fast-growing chickens had similar final weight, feed intake, carcass yield, abdominal fat and tibial ash content with and without outdoor access during summer season. Besides it is confirmed that when chickens had an outdoor area, forage intake behaviour is present in broilers.

Acknowledgements
Authors declare their acknowledment to the staff of the animal nutrition department at FMVZ-UADY.

Funding. This research was funded by the authors.

Conflict of interest. The authors declare that there is no conflict of interest.

Bioethics committee authorization. The present research had compliance with ethical standars according to the Reglamento de la Ley Federal Mexicana de Sanidad Animal, Titulo 3, Capítulo 1: Del bienestar de los animales.

Data availability. Data are available upon request with the corresponding author.

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