

ANIMAL WELFARE IN BROILERS: PHYSIOLOGICAL PROFILES IN RESPONSE TO TRANSPORT TO ABATTOIR, LAIRAGE AND EXSANGUINATION

Bienestar animal en pollos de engorde: perfiles fisiológicos en respuesta a los periodos de transporte, reposo y degüello

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ABSTRACT

The main objective of the present study was to evaluate the physiometabolic blood responses of broilers during arrival, post-lairage and exsanguinations. Post-handling in each period *ante-mortem* was blood sampled to determine metabolic changes, acid-base balance, and blood gas exchange: pH, hematocrit (%), glucose (mg/dL), electrolytes [Na⁺, K⁺ and Ca⁺⁺ (mmol/L)], and lactate levels (mg/dL), partial carbon dioxide [pCO₂ (mmHg)] and oxygen pressures [pO₂ (mmHg)], total carbon dioxide [TCO₂ mmol/L], excess base [BE(B) mmol/L], oxygen saturation [SO₂c (%)], and total hemoglobin [THbc g/dL]. Results showed that the post-transport group had differences (P<0.05) only for SO₂c, PO₂, K⁺ and Na⁺ compared to Control group. Glucose, Ca⁺⁺, PCO₂, and Htc values during post-lairage differed (P<0.05) from controls and the exsanguination values. Lactate concentrations were lower exsanguination compared to controls (29.39 mg/dL Vs. 41,11 mg/dL), respectively. In conclusion, transport generated physiological and metabolic imbalances; in contrast, the period of rest with food and water to be restored gas exchange and acid-base balance.

Key words: Stress; welfare; chicken; lactate.

RESUMEN

El objetivo del presente estudio fue evaluar las respuestas fisiometabólicas sanguíneas en pollos de engorde finalizados durante el transporte, arribo, post-reposo y degüello en planta faenadora. Al término de su manejo en cada periodo *ante mortem* se tomaron muestras sanguíneas para determinar cambios metabólicos, desequilibrio ácido-base e intercambio gaseoso sanguíneo: pH, hematocrito (%), glucosa (mg/dL), electrolitos [Na⁺, K⁺ y Ca⁺⁺ (mmol/L)], y niveles de lactato (mg/dL), presión parcial de dióxido de carbono [pCO₂ (mmHg)] y presión de oxígeno [pO₂ (mmHg)], total de dióxido de carbono [TCO₂ mmol/L], exceso de base [BE(B) mmol/L], saturación de oxígeno [SO₂c (%)], y total de hemoglobina [THbc g/dL]. Los resultados mostraron que el grupo post-transporte presentó diferencias (P>0,05) en SO₂c, PO₂, K⁺ y Na⁺ con respecto al grupo control. Las concentraciones de lactato fueron menores a la exsanguinación en comparación al grupo (29,39 mg dL Vs. 41,11, respectivamente) En conclusión, el transporte generó desbalances fisiológicos y metabólicos, en contraste, el periodo de descanso con alimento y agua pudo restablecer el intercambio gaseoso y balance ácido base.

Palabras clave: Estrés; bienestar; pollo; lactato.

INTRODUCTION

Currently, there is concern about the welfare of poultry during the period between leaving the production unit and slaughtering at a processing plant [30, 31, 39]. Though many researchers have studied stress in animals through different approaches, the stress mechanisms triggered during slaughtering in bird species have physiological repercussions on the animals' homeostasis that directly affect their welfare [16, 18, 21, 39]. This is important because the main objective of ensuring animal welfare is to eliminate fear and pain while they are alive, since the slaughtering procedure entails new experiences that inevitably cause fear [5, 26].

One of the major problems that the broiler industry faces today is, precisely, pre-slaughter stress, which is responsible for a substantial proportion of the losses that occur when birds arrive at an abattoir (Death on Arrival - DOA). Transport is characterized as a highly stressful process [13, 15] because the birds experience noise, vibration, motion, overcrowding, food and water deprivation, social disruption, [11] high temperatures and humidity variations, often combined—especially in tropical climates—with poor ventilation, all of which can result in significant losses during the pre-slaughter process. Additional problems, such as muscle hemorrhages and increased mortality, may also occur [9]. As a result of such factors some chickens do not survive the trip, often dying in route of infectious disease, heart and circulatory disorders, and the trauma they experience during capture and caging [19]. According to estimates, 40% of pre-slaughter losses are related to heat stress brought on by temperatures that are either too high or too low [25]. Therefore, one of the most crucial elements in the survival of the birds is lairage, which allows them adequate contact with the new environment and reduces the earlier temperature stress [8]. However, the poultry industry still lacks information on variations in the length of lairage and their implications for the survival and welfare of birds prior to reception at slaughterhouses [9, 25, 26, 39].

It is well known that animals' responses can be evaluated through mortality records and data on trauma, non-invasive assessment of physiological disorders [29] and behavioral observations, among other approaches, all of which have some validity as measures of welfare. Additional measurements include the effects of transport-induced stress, weight loss suffered by broilers, higher circulating levels of catecholamines, cortisol and creatine phosphokinase, increased heart rate and cell volume, and evidence of dehydration in several cases, all of which evidence clinical, biochemical, hormonal and/or immunological aspects of animal welfare [2]. Currently, the accumulated knowledge related to metabolic profiles and blood gas exchange in slaughter animals provides an understanding of the repercussions of ante-mortem stressors [17, 26]. In this context, the objective of the present study was to evaluate the physiological blood responses of broilers during arrival, post-lairage and exsanguination (at bleeding).

MATERIAL AND METHODS

Experimental procedure

This research was conducted after being approved by the Institutional Sub-Committee for the Care of Experimental Animals (SICUAE) of the M.Sc. and Ph.D. Programs in the Sciences of Animal Production and Welfare at the Universidad Nacional Autónoma de México (UNAM), using a routine form of transportation on an ostrich farm located in central Mexico in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All handling of the birds throughout the experiment was in full compliance with the Official Mexican Norm NOM-051-ZOO [37], and all experimental procedures were conducted in accordance with the guidelines for the ethical use of experimental animals Sherwin et al.[27].

The study was carried out at a private slaughterhouse located in the State of Mexico in central Mexico. A total of 105 fattened Ross broilers aged 47 ± 2 d and with an average weight of 3.2 kg were used, all from the same farm. Hence, three repetitions were performed for each sample period, 35 birds in each replicate for a total of 105 male birds sampled. Each bird was sampled 4 times for a total of 420 blood samples. During the fattening process, all birds were fed a diet with 18% gross protein and 3225 kcal of metabolizable energy. They were housed at a temperature of 20°C with 65% relative humidity. At the end of the fattening period and one d before transport, the birds were selected at random and identified. All the chickens chosen had a fasting period of 4 hours before transfer to the abattoir. They were transported and monitored during arrival and post-lairage, and exsanguination. The chickens were captured their wings against the body in one sole movement. They were then placed in cages (crates) and introduced into the transport vehicle by a mechanical forklift.

Transport of the birds took place at night. They were carried in conventional plastic cages at a loading density of 0.078 m²/bird; equivalent to 7 chickens per cage (crates). Total trip duration was 3 h at an average speed of 75 km/h. During this period they received no food or water. Average environmental conditions upon arrival were 15.5°C, 58% and 1 lux for temperature, relative humidity and luminosity, respectively.

Upon arrival at the slaughterhouse, the chickens were housed and allowed to rest in a holding pen for a period of 8 h with available space of 7-10 birds/m². They were given water throughout their time in the holding pen, but food (12% gross protein and 3000 kcal of metabolizable energy) was available *ad libitum* only during the first 4 h of lairage (to facilitate gastric emptying). Average temperature, relative humidity and luminosity in the holding pen were 21.6°C, 55.5% and 150 lux, respectively.

At the end of the lairage period, the chickens were transferred to the slaughter area using the capture method described above. There, they were rendered unconscious individually by cervical dislocation, in accordance with norm NOM-062-ZOO [38]. Climatic monitoring in the killing area indicated average

temperature, relative humidity and luminosity of 19.9°C, 67.1% and 2075 lux, respectively.

Sampling stages: Blood monitoring and physiological profiles

Prior identification of each chicken made it possible to evaluate their physiological conditions by establishing key points before slaughtering.

The first blood samples were taken 24 h before transporting the chickens to the slaughterhouse (Reference values), while they were still in the corrals that had housed them during the fattening process. The reference sample was taken at night to reduce stress on the birds. Blood samples were obtained by subjecting each bird so as to expose the radial vein of one wing and using a syringe (BD Plastipak™, New Jersey, United States) with a previously heparinized 22-mm needle (1000 UI lithium-heparin) to prevent any modification of the blood gas values. The average time required to obtain each sample was less than 10 seconds (s) per bird, timed from the moment of subjection until the end of the process. The birds' otic temperature was measured together with the first blood sampling (Braun ThermoScan® IRT 4520, Kronberg- Germany). The second sampling period was conducted immediately after the chickens arrived at the slaughterhouse (Post-transportation), in order to assess their stress responses due to the effects of transport. The third blood samples were taken at the end of the lairage period (8 h) (Post-lairage). On both of these occasions the sampling technique followed the parameters described above.

The fourth and final blood sample was obtained as the birds were sacrificed by the cervical dislocation method, followed by a transversal incision in the jugular vein to begin the process of exsanguinations. All personnel involved in killing and sampling were experienced and received previous training.

The physiological profile of each sample obtained was analyzed, taking into account the following critical blood variables: hematocrit (%), glucose (mg/dL), electrolytes [Na⁺, K⁺ and Ca⁺⁺ (mmol/L)], and lactate levels (mg/dL), partial carbon dioxide [pCO₂ (mmHg)] and oxygen pressures [pO₂ (mmHg)], total carbon dioxide [TCO₂ mmol/L], excess base [BE(B) mmol/L], oxygen saturation [SO₂c (%)], and total hemoglobin [THbc g/dL]. All samples were analyzed using an automatic blood gas and electrolyte analyzer (GEM Premier, Instrumentation Laboratory Company, Lexington, USA, and Instrumentation Laboratory SpA Milano, Italy).

Statistical analysis

Results were analyzed using a mixed model for repeated tests and the SAS 9.0 [36] statistical program. Normality assays were performed (PROC UNIVARIATE, SAS 9,0) [36] for all the variables examined and for the different ante-mortem stages considered.

The statistical model utilized was as follows:

$$Y_{ijk} = \mu + \delta_i + d_{ij} + t_k + (\delta t)_{ij} + \epsilon_{ijk}$$

Where:

Y_{ijk}= Response variable

μ= General mean

δ_i= Fixed effect of in the treatment

d_{ij}= Random effect associated with the chicken in the treatment

t_k= Fixed effect of the period

(δt)_{ik}= Fixed effect of the interaction of treatment and the period

ε_{ijk}= Random error associated with the chicken in the treatment

Study data from the 4 stages were compared by means of an analysis of variance followed, if applicable, by a Tukey test. For comparisons of pH blood values among the 4 stages a Kruskal-Wallis test was performed.

Lactate, glucose, hematocrit, electrolytes and blood gases were summarized as mean ± SD. Since pH blood levels correspond to log units, these data were summarized as medians (ranges).

The researchers who conducted the evaluation and collected the study outcomes were not aware of the treatment regimens and did not participate in the selection of the animals or data analysis. The researcher who conducted the analyses was not aware of the treatment regimens.

RESULTS AND DISCUSSION

Gas exchange

FIG. 1 and TABLE I present the results of the analyses of gas exchanges in the different periods evaluated. The blood pO₂ and pCO₂ concentrations decreased significantly in the post-transport and post-lairage periods with respect to reference values (P<0.05), though no differences were observed in comparison to the other sampling periods. SO₂ values decreased by approximately 16.66% with respect to reference values upon arrival at the abattoir (P<0.05), but then remained unchanged up to the moment of exsanguination. TCO₂ values, meanwhile, first declined (12.42%), but later increased (4.5%) significantly (P<0.05) during post-lairage and exsanguination, respectively, compared to reference values. In this regard, with respect to PO₂ concentrations during exsanguination, where no changes with respect to reference values were observed, Maldonado et al. [10] in contrast to this study, reported an increase in PO₂ in chickens that were decapitated but not allowed a rest period after transport. This suggests that the 4 h of rest before sacrificing provided in the present study allowed the chickens to recover from the hypoxic condition experienced during transport. The decrease in PCO₂ and TCO₂ concentrations after the rest period suggests a process

of respiratory alkalosis or hypocapnia, a phenomenon that has also been observed in hens subjected to caloric stress where the increase in respiration rates leads to a reduction in blood partial pressure of CO₂ (pCO₂) [6, 17, 32]. On the other hand, the effect of consuming and metabolizing the food offered during the rest period could negatively influence pCO₂ concentrations in the

birds, due to the complete oxidation of carbohydrates, lipids and proteins, such that CO₂ is transported at 80% by the erythrocytes, where it reacts with water in the presence of carbonic anhydrase to produce carbonic acid, a reaction that is reversed in the lungs to eliminate CO₂ and water [23].

TABLE I

MEAN ± STANDARD ERROR (SE) OF CRITICAL BLOOD VALUES IN BROILERS FOR OXYGEN CONCENTRATIONS AND BASE LEVELS IN THE DIFFERENT ANTE-MORTEM STAGES (N=105)

Variables	Reference values	Post-transport	Post-lairage	Exsanguination
pH*	7.39 ± 0.01	7.38 ± 0.01	7.41 ± 0.01	7.42 ± 0.03
TCO ₂ (mmol/L)	27.68 ± 0.40 ^a	27.59 ± 0.41 ^a	24.20 ± 0.59 ^b	28.93 ± 0.55 ^c
BE _{ecf} (mmol/L)	1.14 ± 0.48 ^a	1.11 ± 0.49 ^a	-1.49 ± 0.73 ^b	3.21 ± 6.98 ^c
BE (B) (mmol/L)	0.69 ± 0.32 ^a	0.85 ± 0.45 ^a	-1.25 ± 0.69 ^b	2.97 ± 0.93 ^c
SO _{2c} (%)	72.34 ± 3.21 ^a	59.57 ± 3.85 ^b	66.25 ± 4.27 ^{ab}	72.0 ± 5.01 ^{ab}
THbc (g/dL)	10.22 ± 0.20 ^a	10.0 ± 0.24 ^{ab}	9.38 ± 0.18 ^b	8.16 ± 0.46 ^c

^{a, b, c} Different literals in the same row indicate significant differences, Tukey (P<0.05). *Kruskal-Wallis analysis (P<0.05). n= 105 broilers sampled. Total carbon dioxide [TCO₂ mmol/L], excess base [BE (B) mmol/L], intracellular base [BE_{ecf}], oxygen saturation [SO_{2c} (%)], and total hemoglobin [THbc g/dL].

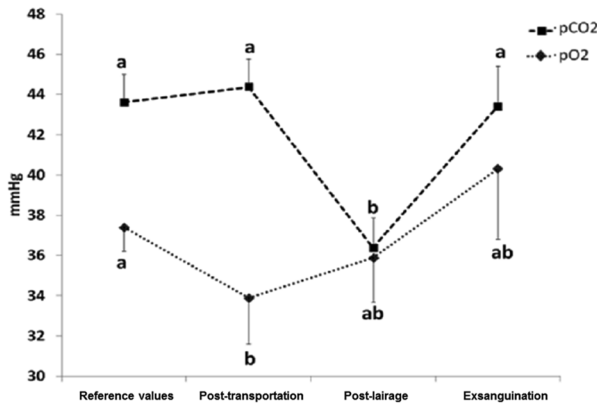


FIGURE 1. EVALUATION OF BLOOD GAS EXCHANGES IN BROILERS IN THE REPOSE (REFERENCE VALUES), ARRIVAL AT ABATTOIR (POST-TRANSPORT), POST-LAIRAGE, AND EXSANGUINATION PERIODS (N=105).

^{a, b, c}. Different literals in the same row indicate significant differences. Tukey test (P<0.05).

Hydric and mineral balance

FIG. 2 presents the behavior of the electrolytes during the 4 experimental phases evaluated. The concentrations of K⁺ reached their highest levels (P<0.5) during post-transport and exsanguination with respect to the reference and post-lairage values. In contrast, it was during the latter two stages that concentrations of Na⁺ reached their highest levels (P<0.05). Concentrations of Ca⁺⁺ decreased significantly (P<0.05) during post-lairage compared to the two previous stages. Afterwards, during exsanguination, these concentrations reached values 18.75 higher than those found in post-lairage. Finally, the percentage of

hematocrit (HTC) showed a significant decrease from post-lairage forward, reaching levels 19.7% and 13.12% below reference and post-lairage values during exsanguination, respectively. These changes in mineral metabolism triggered by an adjustment in the original hormonal status as a result of environmental stress factors brought about during animal transport affect mainly calcium, sodium and potassium levels [14]. Calcium increases in the extracellular fluids lead to a considerable intensification of the contractility of muscle cells, including the heart muscle cells [12]. In addition, the decrease in Na⁺ concentrations could be related to the Na⁺ that is lost in the water from the body that occurs when the extracellular fluid volume is reduced. Puvadolpirod and Thaxton [24] observed that chickens infused with Adrenocorticotrophic Hormone (ACTH) are known to exhibit polydipsia and polyuria throughout the stress and recovery periods, and that excess water consumption is required to clear away the metabolic uric acid and excess electrolytes [20]. The main cause of non-replenished water loss in the skin and lungs is that the birds' respiratory rate accelerates under high temperatures because water evaporation becomes an important recourse for dissipating heat [7]; however, the results show that both K⁺ and Na⁺ concentrations stabilized during lairage, suggesting that the 4 h of rest offered were sufficient to allow reestablishment of mineral levels and reduce the effects of transport-induced stress. Olanrewaju et al. [20] mentions that hematocrit values in chickens increase after administration of ACTH. Increases in hematocrit values were also observed with different trip times [22, 28], which may suggest dehydration. In contrast, in this study no changes in this indicator were seen after transport, suggesting that transport-induced stress was tolerable for the birds associated, as mentioned above, with increased loading density.

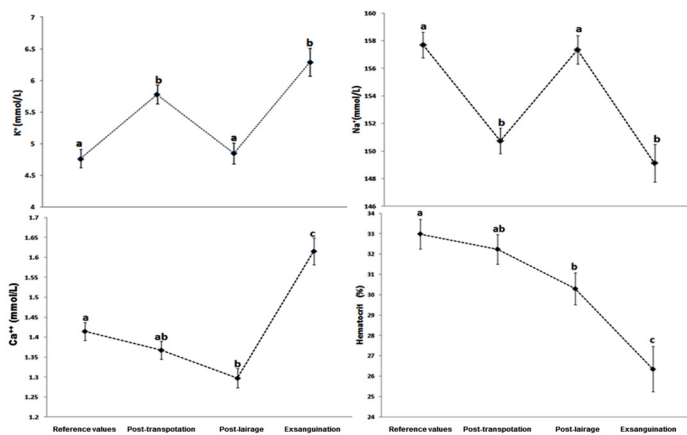


FIGURE 2. VARIABLES OF BLOOD BIOCHEMICAL PROFILES (K⁺, NA⁺, CA⁺⁺ AND HEMATOCRIT) OF FATTENED BROILERS PRIOR TO TRANSPORT (REFERENCE VALUES), UPON ARRIVAL AT THE ABATTOIR, POST-LAIRAGE AND EXSANGUINATION (N=105).

a, b, c. Different literals in the same row indicate significant differences. Tukey test (P<0.05).

Metabolic profile and acid-base balance

Glucose concentrations (FIG. 3) increased significantly (P<0.05) in the post-transport period compared to reference values; however, after the repose period they decreased with respect to those observed during the post-transport time (P<0.05), even reaching levels below the reference values. During exsanguination, they returned to levels similar to reference values and the post-transport measurements. With respect to lactate concentrations, they remained unchanged until just before exsanguination, when they decreased significantly (P<0.05) compared to the three previous stages. Zhang et al. [35] point out that plasma glucose is affected by transport time and that glucose decreased significantly as trip time increased. Yue et al. [34] observed that the limited quantities of glycogen available were unable to provide sufficient continuous plasma glucose as the fasting period associated with transport and recovery was extended. The 3-h fasting recovery period did not slow the depletion of plasma glucose but, rather, accelerated it.

Despite this, pH concentrations suffered no modifications in any of the stages evaluated. With regards to the BE_{efc} and BE indicators (B), values declined significantly (P<0.05) after the post-lairage period in relation to reference values. However, during exsanguination both indicators presented significant increases (P<0.05) compared to the three previous periods. Turning to THbc concentrations, they showed a decrease (P<0.05) of 0.85 g/dL during post-lairage compared to reference values; however, during exsanguination a second decrease was observed, which reached concentrations 11.3% and 20,16% below the post-lairage and reference values (P<0.05), respectively. With respect to the excess of base (BE) and intracellular base (BE_{efc}) only during post-lairage and exsanguination, both indicators presented significant changes. In these periods the concentrations of BE_{efc}

and BE decreased and increased, respectively, are attributed to the buffering action of the bicarbonate system that neutralizes increases in lactate levels [3]. In addition, Borges et al. [4] noted that the base excess value (BE_{efc}) was closest to zero (-0.30) in the heat stress room at a 240 mEq/kg Dietary Electrolyte Balance (DEB). A blood base excess value near zero is desirable, as it indicates conservation of the acid-base balance required for optimal performance [1]. The reduction of THbc observed during post-repose in this study could be associated with a phenomenon of hemodilution and an adaptive response that allows water loss through evaporation without compromising plasma volume [33].

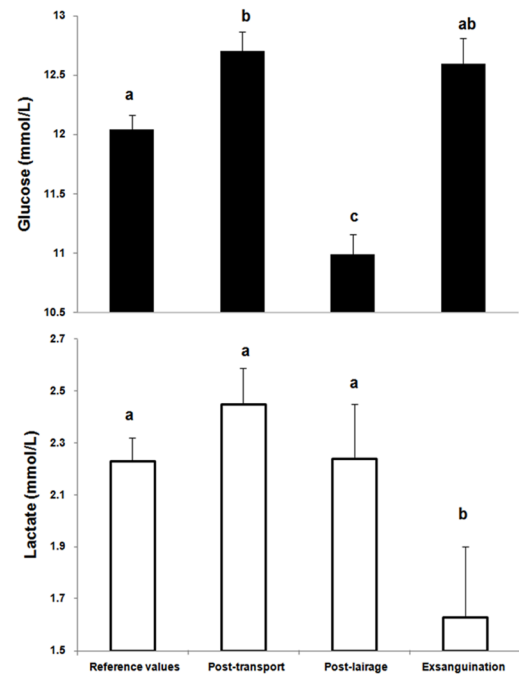


FIGURE 3. MEAN AND STANDARD ERROR OF ENERGY METABOLISM AND ACID-BASE BALANCE IN BROILERS ANTE-MORTEM FOR THE POST-TRANSPORT, POST-LAIRAGE AND EXSANGUINATION (N=105).

a, b, c. Different literals in the same row indicate significant differences. Tukey test (P<0.05).

CONCLUSIÓN

In conclusion, transport generated alterations in gas exchange, and mineral balance. The period of rest allowed the restoration of gas exchange and mineral balance. Glucose concentrations increased significantly in the post-transport period compared to reference values; however, after the repose period they decreased with respect to those observed during the post-transport time, even reaching levels below the reference values. With respect to lactate concentrations, they remained unchanged until just before exsanguination, when they decreased significantly. During exsanguination imbalances existed in gas exchange, acid-base balance and energy and mineral balance.

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