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Effect of Heat Stress During Transport and Rest Before Slaughter, on the Metabolic Profile, Blood Gases and Meat Quality of Quail

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Abstract: Currently in Mexico there is no regulation ruling over transportation and the rest period before slaughtering quail. An experiment was carried out to evaluate transportation and rest period effects before sacrifice on the metabolic profile, blood gas, pH and meat quality of the Japanese quail carcass. Sixty quail (*Coturnix coturnix japonica*) were transported to slaughter, on arrival they were randomly divided in 2 groups: with (4 hours) rest and without rest (slaughtered right away). A blood sample was taken before and post sacrifice. After transportation, the rested quail had significantly lower lactate levels ($p < 0.05$), compared to the quail without rest (36.33 ± 6.17 vs. 21.64 ± 2.14 , respectively). The results showed that when quail are stressed, pCO_2 and lactate levels tend to diminish significantly ($p < 0.05$) compared to mammals. Rest showed a direct effect on temperature ($p < 0.05$), diminishing acidity, reflected through hot and cold carcass pH as compared to the group of quail without rest.

Key words: Stress, transport, meat quality, quail

Introduction

Due to an increase in quail meat consumption, it is necessary to get familiar with factors that influence the muscle metabolism which is reflected in meat quality, said factors can occur before and during sacrifice of the animal, as well as in the subsequent processing of the carcass. *Ante mortem* factors such as catching, caging, transport and immobilizing on the slaughter line, have great impact on the metabolic status of the animal at the moment of sacrifice and in development of a ridged cadaver (Schreurs, 2000; Woelfel *et al.*, 2002). Manual handling before sacrifice has been identified as a potential source for injury and quail stress (Kettlewell and Mitchell, 1994; Elrom, 2001), therefore repercussions from stress before sacrifice and quality deficient transport and stunning influence the quality of the carcass meat.

Health problems arise from poor handling and transportation from the farm to the slaughter house (Randall *et al.*, 1994). Transportation is an important activity of the farming industry. It is a topical controversial area of animal welfare. Several workers have confirmed that transportation for short or long periods can impose stress on animals (Warris, 1995; Knowles, 1998; Broom, 2003; Ali *et al.*, 2006). Signs of this stress have

been demonstrated in different animal species by Ali *et al.* (2006), for example, increased heart rate (Kent and Ewbank, 1986), increased adrenal-cortical activity (Ruiz-de-la-Torre *et al.*, 2001), decrease hormonal immunity (Machenzie *et al.*, 1997), increased morbidity and mortality (Chirase *et al.*, 2004).

We have known for a long time now, that stress causes changes in muscle metabolism in animals and therefore produces differences in meat quality (Remignon *et al.*, 1998). The main consequence of transport is metabolic fatigue and dehydration which is a result of survival metabolic rate, when evaporation and acid-base increase. The birds are metabolically exhausted after dealing with a variety of events such as beating their wings after unloading and muscle contraction due to vibration (Elrom, 2001). Vehicle vibration has a negative effect on the birds, when attempting to maintain balance they contract their muscles during transport. Randall *et al.* (1994), indicate there is evidence that the birds are stressed from the mechanical vibration and implicates physiological and physiological tension (Mitchell and Kettlewell, 1994). Knowles *et al.* (1996), indicates that calorific stress is an important problem for the bird before and during transport. While a bird is under calorific stress, a series of changes are mediated by the hormonal system

(Etches *et al.*, 1995). Pre-sacrifice stress results in muscle glucose exhaustion causing abnormal characteristics in the carcass meat. A well rested animal has sufficient glucose to develop normal glycolysis *post mortem* and pH diminishes correctly post sacrifice (Elrom, 2001). Several clinical, biochemical, hormonal and immunological effects of transportation stress have been documented in farm animals (Ali *et al.*, 2006).

Bird stress before and during the sacrifice causes acid base disequilibrium, as well as unbalanced energy metabolism, however there are no studies to date that evaluate blood gas, acid base disequilibrium, metabolic profile and biochemical indicator records of the hot and cold carcass meat quality of quail. The aim of the study was to evaluate the effect of stress produced by environmental temperature (calorific stress) during transport, as well as the effect of a rest period prior to sacrifice on metabolism, blood gas and hot and cold carcass meat quality of Japonica quail.

Materials and Methods

The study was performed at the Meat Center from the Livestock Teaching Center at the Faculty of Veterinary School in Cuahutitlán (National Autonomous university of Mexico, located in the State of Mexico), in accordance with the guidelines of the ethical use of animals in applied ethologic studies described elsewhere.

Animals and treatment: A total of 60 four month old quail (*Coturnix japonica*) were monitored. The birds were divided in two groups. Group 1 was not given rest after transport, Group 2 was made up of birds that had been given a rest period of 4 hours before sacrifice.

Blood sampling: Blood samples were obtained before sacrifice; a sample from the radial (medial) vein was taken for the quail baseline energy profile and blood gas levels. At sacrifice, the blood sample was taken by decapitation. Hematocrite (%), glucose (mg/dL), serum electrolytes [Na^+ , K^+ and Ca_2^+ (mmol/L)] and blood lactate (mg/dL) levels, partial pressure of carbon dioxide [PaCO_2 (mm Hg)] and oxygen [PaO_2 (mm Hg)], were obtained by means of an automatic blood gas and electrolyte analyzer (GEM Premier 3000, Instrumentation Laboratory Diagnostics S.A. de C.V. Mexico).

Transport: Both groups were transported from the same place; the duration of transportation was 95 min at 60 Km/h. The birds were transported under high noon climatic conditions (on a road 2165 meters above sea level) the average temperature inside the vehicle was 45°C with a wind speed of 3 mps). It is important to indicate that during the trip these conditions are normal in the Central Mexico area.

In order to measure average environmental conditions during transportation a Delta TRAK laser thermometer

(Model 15005) was used and an airspeed indicator to quantify wind speed, the light intensity was measured with a luxmeter, model LM-80.

Sacrifice: Immediately after arriving to the slaughter house, group 1 was weighed and sacrificed; whereas group 2 was given 4 hours rest with water provided *ad libitum*. The birds were sacrificed by decapitation.

Procedures: Post sacrifice, the birds were gutted. The weight of the head, feet, entrails and carcass meat was taken following the classifications of the bird type. The carcass yield was measured compared to the body weight. The pH was taken with a Hanna Instrument (penetration pH electrode, H18314, membrane pH meter, 115V/60Hz. Cod. 1.1176). The temperature was taken with a laser thermometer (Delta TRACK, model 15005), both measurements were taken from the *Pectoralis* and *Biceps femoris* before and post thermic shock on the hot carcass meat and after 24 hours of refrigeration.

Statistical analysis: The variables corresponding to the acid base equilibrium, energetic metabolism and blood gas were statistically analyzed using a random design, where the base values of birds with and without rest before sacrifice were compared. The following was the mathematical model used:

$$Y_{ij} = \mu + I_i + \xi_{ij}$$

$I = 1, 2, 3$ Treatments
 $j = 1, 2, 3...$ Repetition

Where:

Y_{ij} = Variable response (weight at birth)
 μ = General mean
 I_i = Effect of treatment
 ξ_{ij} = Random error

The Tukey test was used in order to determine significant differences between treatment of mean ($p < 0.05$). The "t" test was used for independent sampling of the variable response of the statistically analyzed carcass measurements.

The criteria for a t-student test was:

$$t = \frac{(\bar{X}_1 - \bar{X}_2) - (\mu_1 - \mu_2)}{S_{\bar{X}_1 - \bar{X}_2}} \approx t_{\text{de..student}}$$

Where:

$$\bar{X}_1 - \bar{X}_2 = \text{Differences in the simple mean.}$$

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Table 1: Mean and standard error of blood gases, metabolic profile and energy unbalance in quail subjected to caloric stress

Variables	Baseline levels n = 40	Group 1 Without rest n = 15	Group 2 Resting n = 30
	Mean±SEM	Mean±SEM	Mean±SEM
pH	7.41±0.008 ^B	7.51±0.01 ^A	7.52±0.008 ^A
pCO ₂ (mm/Hg)	47.27±1.08 ^A	29.6±1.18 ^B	32.25±0.54 ^B
pO ₂ (mm/Hg)	41.32±1.06 ^A	38.33±1.48 ^{AB}	35.75±1.13 ^B
Na ⁺ (mmol/L)	147.6±0.80 ^{AB}	149.71±0.78 ^A	145.5±1.04 ^B
K ⁺ (mmol/L)	7.08±0.33 ^A	6.36±0.21 ^A	6.76±0.19 ^A
Ca ⁺⁺ (mmol/L)	1.28±0.02 ^A	1.24±0.02 ^A	1.20±0.04 ^A
Glucose (mg/dL)	248.32±2.68 ^A	235.33±15.06 ^{AB}	216.37±6.42 ^B
Lactate (mg/dL)	58.69±2.98 ^A	36.33±6.17 ^B	21.64±2.14 ^C
Haematocrite (%)	42.21±1.02 ^A	43.92±1.79 ^A	45.44±1.14 ^A

Group 1: Birds were sacrificed immediately after transport. Group 2: birds given access to water and 4 hours rest before sacrifice. ^{A,B,C} Mean with different superscript on the same line are significantly different (p<0.05). In order to determine significant differences between the mean treatments the Turkey test was used. To establish the statistical differences between the pH values the Kruskal Wallis test was used

Table 2: Mean and standard error of weight and yield of carcass before and after sacrifice

Carcass variables	Group 1 Without rest n = 15	Group 2 Resting n = 30	P (α)
	Mean±SEM	Mean±ESM	
Before transport live weight (g)	235.27±5.87	225.93±3.46	0.1775
Live weight pre-sacrifice (g)	224.82±5.69	221.40±3.80	0.6191
Live weight without feathers (g)	210.08±5.31	204.09±3.36	0.3468
Head weight (g)	13.48±0.76	10.43±0.24	0.0006
Legs weight (g)	4.06±0.14	4.24±0.12	0.3747
Red viscera weight (g)	11.65±0.43	11.09±0.33	0.3051
Green viscera weight (g)	15.44±1.02	14.46±0.88	0.4691
Hot carcass weight (g)	159.48±5.00	154.33±3.71	0.4129
Cold carcass weight (g)	167.68±4.97	199.30±34.88	0.3768
Slaughter yield (%)	68.23±0.78	68.34±1.30	0.9455

Group 1: birds sacrificed immediately after transport. Group 2: birds with access to water and 4 hours rest before sacrifice. The “t” test was used to statistically analyze independent samples

$$\mu_1 - \mu_2 = 0, \text{ under } H_0.$$

$$S^2 \bar{X}_1 - \bar{X}_2 = S^2_p (1/n_1 + 1/n_2)$$

$$S^2_p = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{(n_1 - 1) + (n_2 - 1)}$$

n₁ y n₂ = Size of the samples.

S₁², S₂² = Observation variance of each simple.

A Kruskal Wallis test was run to establish statistical differences between pH values. The program SAS ver. 6.12 (1996) was used for all the statistical analysis.

Results

The results of the metabolic profile and blood gas between baseline levels and the variance of quail subjected to calorific stress (with and without rest) is shown in Table 1. It is important to point out that the birds in groups 1 and 2 had significantly higher pH levels (p<0.05) at sacrifice, compared to the baseline levels. The quail in group 2 showed mean with significantly lower pO₂ levels (p<0.05) compared to baseline levels. Regarding electrolyte results, Na⁺ was

significantly less (p<0.05) for group 2 birds compared to birds in group 1. With regard to energetic metabolism parameters, the birds that were given rest (group 2) had significantly diminished (p<0.05) glucose concentration levels, compared to the base levels and the same trend was observed regarding lactate levels.

Table 2 shows the effect of calorific stress that the quail were subjected to before sacrifice, regarding weight and carcass yield. The effect of calorific stress had on the pH and *Pectoralis* muscle temperature of the bird's carcass meat is shown in Table 3. The pH before thermic shock had a statistical significant effect (p<0.05) on the comparison groups, the quail from group 1 had lower pH. Temperature before and post thermic shock had a significant effect (p<0.01), since the quail from group 2 had on the average diminished temperatures compared to the quail from group 1.

Table 4 shows the effect of calorific stress on pH and the temperature of the *Biceps femoris* muscles. The pH before thermic shock and post refrigeration were highly significant (p<0.01) since the birds from group 2 (given rest) had lower pH levels. Regarding temperature before shock, post shock and post refrigeration, we also observed a highly significant effect (p<0.01), the quail that were given rest showed an average lower than the quail from group 1 (without rest).

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Table 3: Mean and standard error of pH variables of quail *Pectoralis* muscle, recorded at various times of the sacrifice

	Group 1 Without rest n = 15 Mean±SEM	Group 2 Resting N = 30 Mean±ESM	P (α)
Pre shock pH	5.84±0.04	6.04±0.06	0.0451
Pre shock temperature (°C)	28.27±0.43	24.63±0.30	0.0001
Post shock pH	5.95±0.04	6.11±0.06	0.0645
Post shock temperature (°C)	14.32±0.44	12.36±0.23	0.0007
Post refrigeration pH	5.86±0.05	5.86±0.03	0.7588
Post refrigeration temperature	9.62±0.29	10.06±0.14	0.3034

Group 1: birds sacrificed immediately after transport. Group 2: birds with access to water and 4 hours rest before sacrifice. The "t" test was used to statistically analyze independent samples

Table 4: Mean and standard error of pH variables of quail *Biceps femoris* muscle, recorded at various times of the sacrifice

	Group 1 Without rest n = 15 Mean±SEM	Group 2 Resting n = 30 Mean±ESM	P value
Pre shock pH	6.70±0.03	6.58±0.03	0.0085
Pre shock temperature (°C)	26.44±0.33	23.16±0.34	0.0001
Post shock pH	6.80±0.02	6.74±0.04	0.3119
Post shock temperature (°C)	14.67±0.48	12.66±0.28	0.0018
Post refrigeration pH	6.88±0.03	6.75±0.02	0.0001
Post refrigeration temperature	10.17±0.28	11.06±0.15	0.0178

Group 1: birds sacrificed immediately after transport. Group 2: birds with access to water and 4 hours rest before sacrifice. The "t" test was used to statistically analyze independent samples

Discussion

Critical blood variables: Birds are equipped to cope the pH in body fluids during metabolism. The resulting metabolites are acidic and unless regulated can alter the acid base balance of the normal status (Borges *et al.*, 2004). We observed in this study that the birds subjected to calorific stress had increased pH levels in their blood (7.52 for rested and 7.51 for birds without rest), compared to baseline levels (7.41 mmHg), showing a significant difference, which agrees with Durgun and Keskin (1998), who exposed quail to calorific stress at 42°C for 150 min and observed that 7.41 is the normal pH for non fasting quail and that the rise in temperature entails a rise in pH levels; this increase is evidenced in the decline of HCO₃⁻ since the acids in the blood (H⁺) combined with bicarbonate ions (HCO₃⁻) forms carbonic acid (H₂CO₃), while converting into CO₂ and H⁺ because of a carbonic anhydrase reaction. The CO₂ result of this reaction is removed by the lungs and the H⁺ ions are excreted by the kidneys together with the left over HCO₃⁻ in order to maintain acid base proportion (Borges *et al.*, 2004).

For blood pCO₂ differences were observed between the birds exposed to stress (32.25 mmHg for birds with rest and 29.6 mmHg for birds without rest) and baseline levels (47.27); this data agrees with information from Durgun and Keskin (1998), who obtained similar descending CO₂ values for non fasting quail without CO₂ stress (baseline level 33.30) and for thermo stressed quail, 19.1. However, the difference between baseline levels of these studies is mainly due to the altitude at which the experiment was carried out for which the CO₂ pressure would logically be different. Aside from this we agree with Durgun and Keskin (1998), in the fact that with higher stress and temperature, the birds CO₂

concentration diminishes.

Calorific stress reduces blood pCO₂. During calorific stress maintaining an adequate pCO₂ level is essential due to the fact that the panting process is renewed continually in the respiratory system of the birds, in an attempt to maintain them cool with the humidity in their lungs (Borges *et al.*, 2004). This panting increases CO₂ loss in the lungs which can lead to partial reduction of CO₂ pressure and therefore, loss of bicarbonate in the blood plasma (Etches *et al.*, 1995). At the same time the low concentration of hydrogen ions cause increase in plasmatic pH which results in respiratory alkalosis (Etches *et al.*, 1995; Durgun and Keskin, 1998; Olanrewaju *et al.*, 2006).

Some studies have reported a reduction in Na⁺ and K⁺ levels in plasma due to calorific stress which is probably a result of haemodilution after consuming water (Borges *et al.*, 2004), or body water loss as a result of diminishing extracellular fluid volume (Olanrewaju *et al.*, 2006). However, differences in the duration of calorific stress at the time of sampling can indicate some or no change, whether Na⁺ y K⁺ plasma increases or decreases (Borges *et al.*, 2004).

Regarding sodium, in this study we differ with what was reported by Borges *et al.* (2004), Olanrewaju *et al.* (2006) and in the results obtained by Durgun and Keskin (1998), who mention a decrease in plasma sodium levels found in birds subjected to calorific stress, since the birds without rest had higher plasmatic levels (149.71) compared to the baseline levels (147.6) and the birds with rest (145.5). However, we do agree with results from Knowles *et al.* (1996) who obtained elevated levels of plasma sodium which they attributed to a dehydration process caused by water evaporation. Dehydration is caused by body water loss that is

accompanied by solutes loss and causing physiopathological complications.

Hypertonic or hypernatraemia dehydration is the loss of solutes free water and is characterized by hypernatremia ($\text{Na}^+ > 145 \text{ mEq/L}$) and hyperosmolarity (plasma osmolality $> 295 \text{ mosmol/Kg}$). The main cause of non replenished water loss in skin and lungs, the birds respiratory speed is accelerated in high temperatures since water evaporation becomes an important recourse to dissipate heat (Wiernusz, 1999; Toyomizu *et al.*, 2005). Haemodilution produces diminished Na^+ concentrations and a fraction of K^+ evacuates from the blood flow apparently due to alteration of membrane permeability. When this K^+ translocation phenomena diminishes during or after acute stress or due to adaptation to chronic calorific stress and excess K^+ is excreted, K^+ concentrations return to their lowest normal status (Borges *et al.*, 2004) as can be seen in the K^+ values obtained in this study from the resting quail.

Increase in haematocrit and haemoglobin together with pO_2 reduction can be related to an increase in metabolic activity necessary to satisfy the energetic demands for maintenance under extreme stress conditions. Also, an increase in haematocrit and haemoglobin suggest an increase of erythropoiesis as a compensatory reaction to the loss of O_2 in tissue (Olanrewaju *et al.*, 2006) as was observed in this study.

We won't discuss glucose and lactate levels found in this study due to the fact that they are very low compared to other studies and would reflect as normal glucose and lactate levels under stressful conditions for which we agree with Riesenfeld *et al.* (1981), regarding the fact that the quail metabolism must be investigated with more detail, they mention that quail hepatocyte has a high metabolic rate and has certain interesting properties which show unusual interdependence in glucose regulation and lipid metabolism and that same must be evaluated more thoroughly.

Carcass pH and temperature: Regarding quail meat, we observed that the pH in the *Pectoralis* muscle of the rested quail pre shock, was 6.04 with an indicial temperature of 24.63°C , while the birds without rest were recorded at 5.84 with an initial temperature of 28.27°C . This affirms that there was a significant difference between the pH of both groups and high significance differences between temperatures, since the birds without rest had higher pH acid levels and temperatures; this could be explained by results from Lyon and Buhr (2001) and Sandercock *et al.* (2001), who indicate that the grade of animal stress of suffering at the moment of sacrifice, elevates the glycolysis level *post mortem* due to an increase in muscular temperature, causing rapid consumption of glycogen, lesser initial pH and earlier *rigor mortis*.

On the other hand, we observed that the final pH of the *Pectoralis* muscle in the rested bird group diminished to

5.86, with post refrigeration temperature at 10.06°C , the bird group without rest had an average of 5.86 pH and temperature of 9.62°C . These values indicate that there was no significant difference; however, Lyon and Buhr (2001), mention that muscle pH in the center of the breast could continue to diminish over a 24 h period (pH 5.6-5.4) when carcass are cooled rapidly; this mainly in poultry meat. The pH value of 5.86 corresponding to this work is slightly above this range; however, we need to consider that this range is identified for chicken, not quail. Quail *Pectoralis muscle* is composed of myofibres mixed with red- and white-type fibres, for which greater glycolytic tissue is present compared to other tissues (Remignon *et al.*, 1998).

Just as in the *Pectoralis*, the pre-thermal shock temperature of the *Biceps femoris* in the bird group without rest was higher than the group with rest, we observed a highly significant difference ($26.44 \text{ vs. } 23.16^\circ\text{C}$) as before mentioned because of the high grade of stress at sacrifice. However the pH of the *Biceps femoris* pre-thermal shock in both cases, was higher in the case of *Pectoralis* muscles (6.70 with rest and 6.58 without rest), this higher pH is due to the fact that the vascular system eliminated lactic acid from the muscle *ante mortem*.

Regarding final pH (post-thermal shock) both groups had an increase (6.88 vs. 6.75, respectively), evidencing a highly significant difference between groups, which was due to rest since the *Biceps* in birds with rest have a lower pH than the birds without rest. The red fiber leg muscles reach final pH between 2 and 3 hours *post mortem* (6.0 to 5.9 pH) in chicken. According to Warris *et al.* (1995), Remignon *et al.* (1998), Elrom (2001), this is due to the fact that transport progressively reduces hepatic glycogen and increases the final pH of the *Biceps*. Complete depletion of glycogen *ante mortem* gravely limits regeneration of ATP and inhibits contraction producing softer meat.

Conclusions: The effect of transport significantly reduced the lactate and pCO_2 levels for birds without rest in regards to the baseline samples. The effect of rest had a direct influence, showing a gradual decrease of pH and temperature of the hot and cold carcass. There was no significant effect on the weight of the carcass or organs because of transport with and without rest. Other relevant aspects that derive from this study are the importance of the rest period. It seems that the birds that had rest after transport significantly diminished gradually in temperature and acidity reflecting same through the pH of the warm carcass of the *Pectoralis* muscle, regarding the group of birds without rest. Submitting the warm carcasses to a rapid temperature change (ice water) caused decreasing *post mortem* enzymatic changes reflected in temperature and pH of the carcass. When characterizing the critical parameters of quail blood samples of this study considering birds with rest,

transported bird and birds with rest before sacrifice, we can establish that transport had a direct undesirable effect on the quality of quail meat; therefore it will be necessary to carry out future study with the intent of applying scientific numeric data to welfare and transport standards for quail in Mexico.

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