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Insensibilization of California Breed Rabbits and it's Effect on Sanguineous pH, Temperature, Glucose Levels, Creatine Kinase and Slaughter Performance

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Abstract: The aim of this research was to study the effect of cervical dislocation and electric stunning of the California breed rabbit with a rest period before sacrifice (at 70 days of age) as well as the effect of same on sanguineous pH, temperature, glucose levels, creatine kinase, and slaughter performance. Forty male and female rabbits of the California breed were used. The sacrificed animals had either cervical dislocation or electrical stunning performed on them, then sacrificed according to normal regulatory practices. Our study is based on two insensibilization methods, cervical dislocation and electric stunning. The results show that plasmatic glucose and temperature before sacrifice and after insensibilization were not affected significantly ($p>0.05$) in the rest period or by sacrificial method. The pH of the animals that were given a rest period previous to sacrifice was significantly different from the animals that were not given rest; no significant difference between the insensibilization methods was found. We found a significant difference for bleeding times ($p<0.05$) between cervical dislocation with rest compared to electrical stunning without rest. The rest period ($p>0.05$) did not significantly affect the volume of blood recovered during bleeding. However when comparing bleeding volume by insensibilization method significant statistical differences ($p<0.05$) were observed. There were no significant difference in the variables of carcass evaluation; however, the lack of a rest before sacrifice affected pH values and temperature of the rabbit meat significantly.

Key words: Pre-sacrifice rest period, insensibilization, rabbit meat, slaughter, glucose level

INTRODUCTION

Rabbits are mainly bred for meat, skin and hair production; they are also used as laboratory animals (Boorthakur *et al*., 2002).

Sacrifice of rabbits begins with unloading individual rabbits from transportation boxes, later cervical dislocation or electrical stunning is performed. The objective of this procedure is to daze the animal in order to carry out a humane sacrifice; complying with standard requirements. Recently critical reports have been promoting efficient processing of a quality rabbit meat product. Over the years the way a rabbit is dazed has changed and has been replaced by electric stunning for rabbit sacrifice. Today the use of gas to daze rabbits before sacrifice is not cost efficient. Electric stunning is usually carried out with electrical devices which shock the brain of the animal (Cavani *et al*., 2003). After the death of the animal (when oxygen no longer is supplied to muscle), structural and biochemical changes take place that constitute transformation from muscle to meat. Abnormalities in meat quality diminish aptitude for transformation into meat products; in this sense sausage product quality is related to the initial quality of the meat (Wirth and Leistner, 1992). There are a many factors that can alter the quality of the slaughter and meat quality as

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well, the objective of this work was to study the effect of cervical dislocation and electric stunning of the California breed rabbit and rest period before sacrifice at 70 days of age as well as the effect of same on sanguineous pH, temperature, glucose levels, creatine kinase, and slaughter performance.

MATERIALS AND METHODS

This study was carried out in the Meat Production Workshop at the Farming training center of the Cuatlitlan Faculty of Superior Studies (FESC 4) in Mexico State, Mexico. The experiment planning consisted in quality evaluation of handling techniques during reception, rabbit slaughter and meat product conservation in walk-in refrigerators.

Animals: Forty California breed (male and female) rabbits from the rabbit farm at FESC 4, were fatted with a commercial balanced feed. The study was carried out with 70 days old rabbits, distributed by sex randomly in the following treatments (Table 1).

Handing before sacrifice: Instant temperature readings were taken (1 sec) with a ThermoScan Braun thermometer. All the animals were weighed, using a PCL type "Tor Rey" digital scale with a 20 kg capacity in order to determine the weight of hot carcass and slaughter performance.

Handling at sacrifice: All the animals were stunned and sacrificed according to normal required standard procedures. Insensibilization was carried out based on two methods: Cervical dislocation described by Zoot (2003) and electrical stunning established by Dal Bosco *et al.* (1997). Cervical dislocation is performed on the rabbit by holding its head with one hand and the legs with the other, the cervical vertebrae are then stretched with rapid movement, separating the atlas and axis bones. Electrical stunning was carried out with low voltage (110 v, 60 Hz for 5 sec), alligator clips are attached to one ear and the other in the anal region while holding the animal by its hind legs; and sacrifice is carried out by hanging the rabbit upside-down on a hook by its rear leg and cutting the carotid artery.

Table 1: Experimental design

	With rest				Without rest			
Group	C. D.		E. S.		C. D.		E. S.	
Sex	F	M	F	M	F	M	F	M
Number	8	2	7	3	3	7	4	6
Total no. of animals	10		10		10		10	

C.D.: Cervical Dislocation, E.S.: Electrical Stunning

Once stunning or dislocation was performed temperature was taken once again when bleeding began. In the fist 10 sec a blood sample was taken to measure plasmatic glucose concentration, pH and blood temperature. Energetic profile of the rabbit was taken during bleeding with a digital BioSensor Optium MediSense® measuring glucose levels.

The pH measured during bleeding was taken with a digital potentiometer model KS-701 24003 with a CH701 electrode (Coprovet Corporation, Zapopan Jalisco, Mex.). The blood temperature was taken with a laser thermometer.

Another 5 mL blood sample was taken and placed in a test tube without anti-coagulant, the sample was then placed in a centrifuge at 2500 rpm for 15 min. Once the serum was obtained, it was placed in a freezer and sent to a laboratory to evaluate the lactate and creatine kinase.

During the slaughter weight of the red viscera (heart, trachea, lung, liver and spleen) and green viscera (stomach, intestines and caecum) were measured as well as the by-products (skin, front and hind legs), using a PCL type "Tor Rey" digital scale with a 20 kg capacity.

Carcass evaluation: Once the slaughter was complete the weight of the hot carcass was taken with the before mentioned scale and a Hanna Instruments (Penetration pH electrode, HI8314, membrane pH meter 115V-60Hz. Cod.1.1176) potentiometer was used to sample the pH of the carcass. The measurement was taken form the *Bicep femoris* muscle of the right leg, similar to the technique used by Blasco and Piles (1990) and Hernández *et al.* (1998). Later weights and measures were taken for cold carcass, after a period of 22 h in refrigeration at between 0 and 4°C, in order to calculate loss.

Blood analysis: Blood samples were collected in sterile test tubes without anti-coagulant (in order to analyze creatine kinase and lactic dehydrogenase enzymes), these were placed in a centrifuge at 3000 rpm for 10 min, once the serum was obtained it was divided into approximately 3 mL measurements for later enzyme evaluation. The serum was kept in refrigeration until it was sent to the laboratory.

Creatine Kinase (CK) *Horizon Diagnostic.* For a quantitative analysis of the CK in serum, a Horizon Diagnostic Spectrum Abbot automatic analyzer was used on the reagent. The Lactic Dehydrogenase activity (LDH) was quantified using the Spectrum Abbot automatic analyzer with reagents from STANBIO (Stanbio, 2000).

Statistical analysis: The response variables were analyzed statistically using a completely randomized design, with the following mathematical model:

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

Where:

Y_{ij} = Response variable
μ = General media
ι_I = Effect of the treatment
ξ_{ij} = Random error

In order to determine significant differences between medias the Tukey test ($p<0.05$) was used. Statistical analysis was elaborated using the SAS program (1997) version 6.12. The results were analyzed by the proposed model along with the following procedures:

The Kruskal-Wallis test was run for the pH variables and *Longissimus dorsi* and *Biceps femoral* muscle temperatures.

RESULTS

Table 2 shows the sacrificed rabbit's physiological indicators with and without rest periods after cervical dislocation or electric stunning. The plasmatic glucose, body temperatures before sacrifice and after insensibilization were not significantly affected ($p>0.05$) by the rest period. In the case of creatine kinase variables, significant differences ($p<0.05$) between insensibilization methods and between animals that had or had no rest period were observed, that is to say the animals that had a rest period and were then sacrificed by cervical dislocation produced significantly different results compared to the animals that were sacrificed by electric stunning with and without a rest period before the sacrifice.

Table 2: Sacrificed rabbit's physiological indicators with and without rest periods after cervical dislocation or electric stunning

	Rest		Without rest	
Physiological traits	Cervical dislocation	Electrical stunning	Cervical dislocation	Electrical stunning
Ear temperature at weight (°C)	35.70±0.310^{A}	35.07±0.355^{A}	35.65±0.260^{A}	35.81±0.108^{A}
Post-stunning ear temperature (°C)	36.96±0.519^{A}	36.49±0.431^{A}	35.91±0.368^{A}	36.02±0.386^{A}
Glucose (mg dL^{-1})	132.50±3.70^{A}	134.72±5.66^{A}	138.72±4.78^{A}	125.00±4.07^{A}
CK (IU L^{-1})	1592.88±33.63^{A}	842.40±53.29^{B}	1209.55±164.60^{AB}	772.08±46.83^{B}
DHL (U L^{-1})	160.19±50.65^{A}	307.60±29.14^{B}	706.32±46.76^{C}	417.53±28.16^{B}
pH blood	7.41±0.04^{AB}	7.31±0.03^{B}	7.47±0.04^{A}	7.47±0.03^{A}
Blood temperature (°C)	19.60±1.23^{A}	20.90±0.98^{A}	18.27±0.27^{A}	20.70±0.44^{A}
Bleeding time (min)	46.59±3.26^{B}	51.49±3.49^{AB}	55.60±2.54^{AB}	62.30±3.14^{A}
Blood volume (mL)	45.00±7.41^{A}	50.90±8.41^{B}	46.36±4.62^{A}	73.00±6.59^{C}

^{A,B,C}: Statistical differences, ANOVA

Table 3: Meat and viscera yield for rabbits sacrificed by cervical dislocation and electric stunning with and without a rest period.

	Rest		Without rest	
Slaughter traits (kg)	Cervical dislocation	Electrical stunning	Cervical dislocation	Electrical stunning
Live weight	2.86±0.08^{A}	2.85±0.07^{A}	2.76±0.07^{A}	2.90±0.10^{A}
Hot carcass weight	1.63±0.04^{AB}	1.65±0.04^{A}	1.55±0.04^{AB}	1.59±0.06^{B}
Slaughter performance	56.99±0.35^{A}	58.11±1.23^{A}	56.29±0.41^{A}	55.02±0.58^{A}
Green viscera weight	0.129±0.01^{A}	0.113±0.007^{A}	0.110±0.005^{A}	0.118±0.009^{A}
Red viscera weight	0.467±0.01^{A}	0.456±0.01^{A}	0.446±0.01^{A}	0.467±0.02^{A}
Offal weight	0.529±0.01^{A}	0.555±0.01^{A}	0.489±0.02^{A}	0.525±0.02^{A}
Cold carcass weight	1.72±0.04^{A}	1.70±0.05^{A}	1.63±0.04^{A}	1.64±0.06^{A}

^{A,B,C} Statistical differences, ANOVA

Table 4: Media and standard error of the media for the temperature variables and pH for the *Biceps femoralis* muscle taken at different times, for rabbits that had cervical dislocation and electric stunning with and without a rest period.

	Rest		Without rest		
pH and temperature values	Cervical dislocation	Electrical stunning	Cervical dislocation	Electrical stunning	P(α)
pH 0	6.41±0.050	6.23±0.072	6.7±0.061	6.31±0.043	0.0001
Temperature 0	35.6±0.819	36.0±1.070	38.54±0.365	38.1±0.314	0.0018
pH 1	6.41±0.082	6.02±0.055	6.4±0.044	5.99±0.060	0.0001
Temperature 1	22.3±0.615	27.27±1.087	23.54±0.492	25.4±0.541	0.0004
pH 4	6.14±0.042	5.90±0.063	6.12±0.052	5.86±0.030	0.0002
Temperature 4	9.5±0.341	9.63±0.278	12.72±0.821	11.4±1.166	0.0157
pH 24	5.7±0.163	5.81±0.018	5.85±0.031	5.85±0.022	0.7067
Temperature 24	3.66±0.166	3.72±0.140	2.90±0.342	4.0±0	0.0079

Kruskal-Wallis test

The DHL enzyme levels in serum showed significant differences ($p<0.05$) between insensibilization methods with and without a rest period. The animals that were sacrificed by cervical dislocation had differences between groups and were different from the animals sacrificed by electric stunning.

Regarding blood pH, the animals that had a rest period were significantly different from the animals that had no rest period, however no significant difference between cervical dislocation and electric stunning was observed.

The insensibilization method as well as the rest period significantly affect ($p<0.05$) the bleeding time variable, the animals with rest period and cervical dislocation bleed more rapidly than the animals without a rest period and electric stunning; however this last group expulsed a greater volume of blood, which was significantly different from the other treatments. We affirm that a rest period before sacrifice does not affect blood volume compared to insensibilization method; the animals that were stunned electrically bleed larger quantities of blood.

Table 3 shows meat and viscera yield for rabbits sacrificed by cervical dislocation and electric stunning with and without a rest period. There were no significant differences in any of the variables analyzed, with exception to weight of hot carcass, where a statistically significant effect with a rest period ($p<0.05$) is appreciated, that is to say that animals that rested yielded more hot carcass, although without yielding favorably cold. There were no significant differences between the variable for cervical dislocation and electric stunning.

Table 4 shows the media and standard error of the media for the temperature variables and pH for the *Biceps femoralis* muscles taken at different times, for animals that had cervical dislocation and electric stunning performed on them with and without a rest period. The Kruskal-Wallis test showed that the temperature results were similar to the results from the *Longissimus dorsi* muscles ($p<0.01$); in the case of the pH variable we observed highly significant differences ($p<0.01$) in all the values with the exception of that which was evaluated at the 24 h mark.

DISCUSSION

De la Fuente (1998) reported on transported rabbits at two different times of the year (summer and winter) and at two densities (high and low) glucose levels (mg dL^{-1}) of 119.61, 113.91, 101.40 and 78.84, respectively. Regarding LDH (U/L) he found 651.44, 838.78, 432.17 and 339.32, respectively. The creatine kinase enzyme, 2278.82, 2019.42, 1550.78 and 1221.97, respectively, finding significant differences for all three parameters only in the time of year, the levels being higher in summer than in winter. Rabbits exposed to heat and kept at high density have higher concentrations of creatine kinase enzymes. These higher levels of creatine kinase agree with what Andelatif and Modawi (1994) found, they reported that creatine kinase enzymes were higher when body temperature of the rabbits increased. According to these author's results, this is due to muscular damage produced in the skeletal and cardiac muscles. Higher levels of this enzyme in the blood are due to increased cellular membrane permeability caused by body temperature change. In our study regarding the creatine kinase enzymes we found significant differences between insensibilization methods and a rest period.

There was greater muscular activity in cervical dislocation with rest period, compared to electric stunning without a rest period. There was greater muscular activity and damage to the membrane with cervical dislocation.

Choudhury *et al.* (2003) mention that Chinchilla rabbits at 90 days old weighed 2.73±10 kg at the time of sacrifice, the hot carcass weighed 1.70±0.070 kg, with a meat yield of 62.26±1.08. When comparing said results with our investigation, we noted differences principally due to racial factor. Stahly (1992) reported that weight at sacrifice increases with age and that the greater the weight at sacrifice, the greater the meat yield.

Studies carried out by Blasco and Piles (1990) on pH at zero hours in two muscles: *Longissimus dorsi* and *Biceps femoralis* showed that there were no significant differences in sacrificed rabbits that did not suffer stress caused by transportation. On the contrary, in our work we found significant difference in all the values between cervical dislocation and electric stunning. The before mentioned is justified by the methodology used in this investigation. However, even short trips have been proven to diminish glycogen in muscle and increase muscular temperature (Agnes *et al*., 1990) although it is always reflected in the final pH of the meat (Fernandez *et al*., 1996; Lensink *et al*., 2001). The absence of effect on the pH can be due to low stress factor conditions. The relation between glycogen content in muscle and the final pH is lineal only when glycogen levels are very low. Therefore, reductions of moderate glycogen are not reflected in the final pH of the meat, although the well being of the animal is affected.

It is worth mentioning that the value of the final pH is highly related to the sacrificial method, which at the same time are dependent on the geographical region and center in charge of sacrifice, that is to say, there is no established method for sacrifice.

Other pH values published by various authors regarding the *Longissimus* muscle of the rabbit have been: 5.66-5.7 (Blasco and Piles, 1990), 5.45-5.63 (Ristic, 1986) and in the leg 5.87 and 6.03 (Lambertini *et al*., 1996) 5.98-6.08 (Niedzwiadek *et al*., 1996). To date no one has found a rabbit breed that has kinetic acidification or particularly abnormal pH values, as in the case of swine, where meat defects can be encountered, rabbit meat is not exudative. The muscles on the front section of rabbit meat have higher pH than the muscles on the rear portion (Renou *et al*., 1986).

After sacrifice the muscle looses oxygen and nutrients and attempt to maintain integrity dissipating its energy reserves, suffering property changes during *rigor mortis*. One of the consequences of this phenomena en rabbit meat is diminishing pH, that passes from a value of 7.0 to 7.2 in the muscle, to optimum pH depending on the muscle that oscillates between 5.6 (for muscles with glycolytic activity) to 6.4 (oxidative muscles). Postural muscles are slow contraction and oxidative muscles responsible for maintaining posture, carrying out slow and repetitive movements with the least amount of energy and are very resistant to fatigue. Dalle-Zotte (2002) points out that regardless of the insensibilization method used, extreme stress is proven by the release of catecholamines associated with diminished energy reserves and an increase in the grade of acidification.

Regarding cervical dislocation and electric stunning, electric insensibilization diminishes initial falling pH, compared to insensibilization by cervical dislocation. In studies carried out by La Fuente and Lopez (2000) they found that electric stunning accelerates early muscular acidism, when compared to insensibilization by cervical dislocation. Electric insensibilization favors exhaustion of muscular energy reserves (ATP and glycogen), and causes shortening of sarcomere and this is why there is not a large effect on the final pH, processing time or tenderness of the rabbit meat (Dalle-Zotte, 2002). It is possible that adrenaline accelerates the process of *rigor mortis* but without affecting the final pH.

Further studies are needed to evaluate the acid and mineral base, the energetic metabolism and the effect of the insensibilization method on blood-gas analysis in detail.

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