Effects of Feed Deprivation and Electrical, Gas, and Captive Needle Stunning on Early Postmortem Muscle Metabolism and Subsequent Meat Quality

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ABSTRACT The general method for stunning poultry before slaughter is by immersion of a chicken's head into an electrified waterbath. This method results in carcass and meat quality deficiencies. The major problems are hemorrhages and a delay in onset of rigor mortis, which increases the risk of cold shortening with early deboning. In two experiments, this study examines the early postmortem metabolism in the breast muscle and its effect on ultimate meat quality. The first experiment describes the effects of 5 h feed deprivation on the availability of glycogen from the liver and the breast muscle, of waterbath and head-only electrical stunning on pH and metabolite levels up to 6 h in unprocessed muscle, and the consequences on meat quality. The second experiment compares the same measurements after waterbath and head-only electrical stunning, $CO_2/O_2/N_2$ and Ar/CO_2 gases, and captive needle stunning. Metabolic degradation halted after 6 h without processing or after 4 h under conventional conditions after waterbath and $CO_2/O_2/N_2$ stunning. With other stunning methods, this occurrence is at a faster rate, largely depending on muscle activity. Muscle glycogen does not need to be exhausted for energy generation to cease. If glycogen is a limiting factor, as found with head-only stunning, pH drops too rapidly and affects water-holding capacity and color. Hemorrhage scores were higher with electrical stunning than with other stunning methods. Gas stunning affected color and, to a lesser extent, water-holding capacity. Captive needle stunning scored between gas and electrical stunning on most measurements.

(*Key words*: electrical stunning, gas stunning, captive needle stunning, early postmortem muscle metabolism, meat quality)

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INTRODUCTION

In the European Union it is a legal requirement that animals are stunned prior to killing. A good stun instantaneously induces a state of unconsciousness and insensibility to pain that lasts until the death of the animal, immobilizes the animal for easier killing by exsanguination, and does not have a negative effect on meat quality. Poultry are commonly stunned by means of an electrified waterbath. Electrical current runs from the submerged electrode through the head and body of the chickens, with the shackles or a metal bar against the legs functioning as the ground electrode (Bilgili, 1992). The electrical settings of a waterbath stunner are subject to a conflict of interests. A high amperage electrical stun is necessary to guarantee induction of immediate and lasting unconsciousness (Gregory and Wotton, 1987), whereas high currents also lead to muscle supercontractions and subsequent hemorrhaging in muscle tissue caused by rupture of blood vessels and damage to muscle fibers (Veerkamp et al., 1987; Gregory and Wilkins, 1989; Hillebrand et al., 1996a; Kranen et al., 1998).

Besides hemorrhages, electrical stunning has been shown to affect meat quality through its effects on the early postmortem muscle metabolism (Papinaho and Fletcher, 1996). Early deboning requires a rapid degradation of postmortem muscle metabolism to prevent irreversible contraction of the muscle at deboning, increasing the toughness of the meat. Metabolic degradation depends on the metabolic state at slaughter, which is affected by energetic exhaustion due to feed deprivation, stress, and muscle activity. Metabolic degradation also depends on the metabolic rate early postmortem, which is affected by struggling, stunning, and processing factors, such as plucking, electrostimulation, and chilling (Grey et al., 1974; Poole and Fletcher, 1998; Craig et al., 1999; Kang and Sams, 1999, Schreurs, 1999).

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Abbreviation Key: ATP = adenosine triphosphate.

Considerations of animal welfare and of carcass and meat quality have led to several studies on alternative methods of stunning and killing chickens. Gas stunning, also called controlled atmosphere stunning, has been accomplished by immersion of a bird in an environment with anaesthetic gas or an anoxic gas mixture (Raj et al., 1990, 1997; Poole and Fletcher, 1995). Alternative forms of electrical stunning (Hillebrand et al., 1996a) and captive bolt stunning (Lambooij et al., 1999) have also been explored.

All of the above mentioned studies investigated the impact of some stunning methods on early postmortem muscle metabolism or carcass or meat quality parameters. However, the physiological responses modulated by different stunning methods and preslaughter conditions are not yet fully understood, and there is a need for a comparative, integrating study on the effects of different stunning methods on postmortem muscle metabolism.

Postmortem Muscle Metabolism and Meat Quality

The maximum energy postmortem that can be used is that which is present in the muscle at the time of slaughter. Remaining glycogen will be turned into glucose. The muscle will quickly become anaerobic, and glucose will only go through glycolysis to supply adenosine triphosphate (ATP). This effect will result in the accumulation of lactate and H^+ and a concomitant decrease in pH. ATP and other high-energy molecules, such as creatin phosphate, will be used to maintain the energy supply. Eventually no more ATP can be produced and the energy supply will be compromised. When 80% of the ATP is depleted, rigor mortis sets in (DeFremery, 1966).

The time at which rigor mortis sets in depends on the energy stores in the muscle at slaughter and the rate of metabolic degradation. Furthermore, the metabolic rate determines the rate of decrease in pH and the ultimate pH, which can affect meat color and water-holding capacity through protein denaturation (Warris and Brown, 1987). Metabolic degradation is reported to happen early postmortem, within 6 h after slaughter in unprocessed muscle (Grey et al., 1974; Schreurs, 1999; Savenije, unpublished).

Stunning and Killing

Electrical stunning and hemorrhaging have a major effect on hemodynamics and the levels of plasma metabolites in chickens, partly due to an increase in plasma catecholamine concentrations, which are further sustained by cerebral ischemia (Hillman and Lundvall, 1981; Ploucha et al., 1981), but these effects are unknown in a terminal situation such as slaughter. Glucose concentrations in blood samples taken during exsanguination after electrical stunning at 100 V were about 12.7 m*M* (Wal et al., 1999).

Stunning methods have a large impact on carcass quality (hemorrhages, broken bones) and on meat quality (pH, carbohydrate metabolites, protein integrity, color, shear value, and water-holding capacity) (Raj et al., 1990, 1997; Lambooij et al., 1999). Electrical stunning probably influences the postmortem metabolic state, most likely through induction of muscle activity (e.g., convulsions) in the animal. Tonic convulsions as observed with waterbath stunning deplete the energy store in the muscle less than clonic convulsions as observed with head-only stunning or death struggle (Grey et al., 1974; Fletcher, 1991; Papinaho and Fletcher, 1996; Ali et al., 1999). Muscle pH and R-values are the parameters most studied to determine the effect of stunning methods on postmortem muscle metabolism. Papinaho and Fletcher (1996) reported a higher pH and lower R-value in breast muscle immediately after stunning with 50 or 125 mA (pH 6.16 and 6.29, R-values of 0.85 and 0.84, respectively) compared to no stunning (pH 5.86, R-value of 1.04), lasting up to 6 h postmortem. Similar results are reported by Craig et al. (1999) for 125 mA stunning compared to 11 V/500 Hz and no stunning. Göksoy et al. (1999) and Lambooij et al. (1999) reported higher pH in the breast muscle immediately after 80 and 110 mA electrical stunning (pH 6.48 and 6.52, respectively) compared to captive bolt concussion and captive bolt with air-pressure stunning (pH 6.15 and 6.39, respectively).

The current study describes two experiments that examine the effects of feed withdrawal and alternative stunning methods to the conventional waterbath on early postmortem muscle metabolism and the consequences for meat quality. In the first experiment, natural energy degradation in unprocessed, early postmortem breast muscle was studied after feed deprivation and two different electrical stunning methods. The second experiment compared the effect of two electrical, two gas, and one mechanical method of stunning on the early postmortem muscle metabolism in normally processed breast muscle. Given this insight in the early postmortem carbohydrate metabolism, conclusions will be drawn about the effects of energy status and processing on meat quality parameters and the consequences of early deboning.

MATERIALS AND METHODS

Animals and Housing

Experiment 1. At the poultry facility of the Institute for Animal Science and Health (ID-Lelystad BV)[3] Ross-208 broiler chickens (n = 160) were reared in two pens from 1 d of age until slaughter. Room temperature was gradually decreased from 33 C at 1 d of age to 18 C at 5 wk of age. Standard broiler feed (3,106 kcal metabolizable energy/kg, 21% crude protein) and water were available ad libitum. A light regimen of 1 h light:3 h darkness was applied.

[3]Institute for Animal Science and Health (ID-Lelystad BV), P.O. Box 65, 8200 AB Lelystad, The Netherlands.

Experiment 2. Five-week-old Ross broiler chickens (n = 480) were obtained from a local broiler farm and housed in the poultry facility of ID-Lelystad BV. The chickens were housed for 1 wk in floor pens under the same conditions as in Experiment 1. Approval for carrying out these experiments was obtained from the ethical committee of ID-Lelystad BV.

Treatment Factors

Experiment 1. On the same day in 2 consecutive wk, 80 chickens of the same flock (n = 160), at 5 and 6 wk of age, respectively, were slaughtered. Chickens of the same flock that were 1 wk older were used as the replication. The chickens were randomly divided among two feeding treatments and two stunning methods before slaughter.

Feeding Method. Feed was withheld from the chickens 5 h before transport, or feed was available until transportation. Chickens were then cooped with eight chickens per crate and transported to the slaughter facility.

Stunning Method. Chickens were removed from their crates, hung by the legs from shackles, and then electrically stunned with a conventional waterbath (100 V, 50 Hz, 4 s) with only one chicken present in the waterbath at a time, or electrically stunned across the head only (100 V, 50 Hz, 4 s) using a pair of scissors-like tongs to connect the stunning electrodes to both sides of the head.

Chickens were slaughtered in batches of four chickens from the same treatment group. Batches of four chickens were slaughtered 5 min apart with the treatment factors of the batch determined randomly. After being stunned, chickens were exsanguinated by cutting one jugular vein. Bleeding time was 2 min. Immediately after cutting the neck, a 5-mL blood sample was collected into a heparinized tube and stored on slushy ice. The chickens were then skinned and eviscerated manually with no scalding or plucking. Immediately after evisceration, the liver was separated for pH measurement. The legs, wings, and head were removed, and the chest was labeled with an identification number and stored in a cooling cell at 0 to 4 C.

Experiment 2. On 4 consecutive d, 120 chickens of the same flock (n = 480) were deprived of access to feed and water 4 h before slaughter. Subsequently, the chickens were cooped and transported to the slaughter facility. Time between catching and slaughter never exceeded 2 h. Prior to slaughter the birds were stunned by one of five different methods:

1. Electrically, using a conventional waterbath (100 V, 50 Hz, 10 s). This method is commonly used in slaughterhouses in northwestern Europe.
2. Electrically, across the head only (100 V, 300 Hz, 1 s), with the bird hanging in a funnel for fixation as described by Hillebrand (1996a).
3. Anoxia, by immersion in a box (75 × 75 × 75 cm) containing a gas mixture of 70% argon, 30% CO_2 for 3 min.
4. Anesthesia followed by anoxia, by immersion in a commercial gas stunning tunnel manufactured by STORK PMT using a gas mixture of 40% CO_2, 30% O_2, and 30% N_2 for anesthesia, and a gas mixture of 80% CO_2 and 20% N_2 for killing.
5. Mechanically, using a captive hollow needle stunning device, which after a shot in the head delivers a pressurized air discharge (2 bar, 0.5 s) into the brain (Hillebrand et al., 1996b). Birds were hanged in a funnel for fixation.

Slaughter was in batches of four chickens of the same treatment group. The batches of four chickens were slaughtered 5 min apart with the treatment factors of the batch determined randomly. After being stunned, all chickens were exsanguinated by cutting one jugular vein. Bleeding time was 2 min. The chickens were then scalded, plucked, and eviscerated mechanically, and carcasses were stored in a chilling tunnel at 0 to 4 C.

Liver pH Measurements (Experiment 1)

Liver pH was measured with a combined glass/reference Ingold electrode pH meter[4] in the right liver lobe immediately after evisceration.

Sampling Procedures

Experiment 1. At 0, 1, 2, 4 and 6 h after slaughter a 4.5-g sample was cut from the M. pectoralis superficialis of one carcass out of each batch of four. The first three samples were taken from the right muscle, the other two were from the left muscle. From this sample, 2.5 g was cut off, frozen in liquid nitrogen, and then stored in aluminum cups at –80 C until analysis of R-values and metabolite levels. The remaining 2.0 g was used for pH measurement as described by Jeacocke (1977). After the 6 h sample was taken, the breast muscle of the other three carcasses from the same batch of four was removed from the carcass and stored individually in plastic bags at 4 C for meat quality measurement at 96 h.

Experiment 2. At 1, 2, 4, 8, 24, and 48 h after slaughter, 16 carcasses per stunning treatment were retrieved from the chilling tunnel, deboned, and the M. pectoralis superficialis was collected. The breast muscle was scored for hemorrhages, color was measured, and one-half was individually stored in a plastic bag at –20 C for later analysis of water-holding capacity and shear force. A 4.5-g muscle sample was taken from the other breast half. From this sample, 2.5 g was frozen in liquid nitrogen and stored in aluminum cups at –80 C until analysis of R-values and metabolite levels, and 2.0 g was used for pH measurement as described by Jeacocke (1977).

Carcass and Meat Quality Measurements

Hemorrhage Scores (Experiment 2). The part of the breast muscle that had been attached to the carcass was

[4]Schott-Gerate GmbH, Hattenbergstrasse 10, 55122 Mainz, Germany.

scored by three persons for hemorrhages immediately after deboning. The scale used was previously described by Kranen et al. (1998). Final score was calculated as the mean of the three individual scores.

Color Measurements. Color measurements were carried out using a Minolta Chroma Meter.[5] Data were recorded in the instrument memory and subsequently downloaded into a personal computer for further analysis. Duplicate measurements were taken.

Water-Holding Capacity. The water-holding capacity of the breast meat was measured by the filter paper method, as previously described by Kauffman et al. (1986). Single measurements per muscle were taken.

Shear Force Measurements. The half-breast muscle was heated in its plastic bag in a water bath at 96 C for 10 min; then shear force was measured in triplicate as described by Froning and Uijttenboogaart (1988).

Blood Metabolite Measurements (Experiment 1)

After all chickens were slaughtered, blood samples held on the slushy ice were taken to the lab and analyzed for glucose and lactate concentrations by means of a blood analyzer.[6]

Measurements of Postmortem Muscle Metabolism

Preparation for R-value and Metabolite Measurements. From the frozen muscle samples 1.0 g was cut, homogenized in 12.5 mL perchloric acid solution (0.85 *M* $HClO_3$) for 40 to 60 s, and put on slushy ice. The homogenates were centrifuged at 3,000 × *g* for 10 min, and the supernatant was put in separate tubes containing phosphate buffer.

R-value Measurements. Analysis was according to Honikel and Fischer (1977). From the supernatants, 100 μL was pipetted into 2.5 mL of 100 m*M* sodium phosphate buffer (pH = 7.0); absorbance was read at 250 nm (A_{250}) and 260 nm (A_{260}). The R-value was calculated as A_{250}/A_{260}. Measurements were made in duplicate.

Glucose Measurements. Free glucose measurements were made with a diagnostics kit (no. 115)[7] with modifications to the original kit protocol. All volumes of reagents and samples were reduced to fit into the wells of a microtiter plate (approximately 200 μL). Volumes used were as follows: 10 μL of neutralized sample, blank, or standard and 25 μL of enzyme and color reagent. The standard curve ranged from 0 to 250 μg/mL.

Glycogen Measurements. Glycogen measurements were carried out as free glucose measurements on samples previously hydrolyzed with amyloglucosidase (Passoneau and Lowry, 1993).

ATP Measurements. ATP was measured with a ATP diagnostic kit (no. 366-UV)[7] with modifications to the original kit protocol. All volumes of reagents and samples were reduced to fit into the wells of a microtiter plate (approximately 200 μL). Volumes used were 25 μL neutralized sample, blank, or standard; 10 μL of GAPD/PGK enzyme solution, previously diluted with four parts of PGA buffer; and 125 μL NADH solution. The standard curve ranged from 0 to 500 μg/mL.

Lactate Measurements. Lactate was measured using a lactate kit (no. 826B)[7] with modifications to the original kit protocol. All volumes of reagents and samples were reduced to fit into the wells of a microtiter plate (approximately 200 μL). One part of the sample was diluted with two parts water prior to analysis. Volumes used were 10 μL diluted sample, blank, or standard and 200 μL of enzyme solution. The standard curve ranged from 0 to 400 μg/mL.

Concentrations of the metabolites were measured in microtiter plates in a microplate reader[8] equipped with a 340-nm optical filter and were evaluated with Softmax microplate analysis software.[8]

Presentation of Results and Statistics

All single measurements and repeated measurements per sampling time were analyzed on treatment effects with regression analysis and ANOVA in the Genstat statistical software package (Genstat 5, 1993), with the following model:

$$y_{ijk} = \mu + F_i + S_j + R_k + F{*}S_{ij} + F{*}R_{ik} + T{*}R_{jk} + F{*}S{*}R_{ijk} + e_{ijk} \quad \text{(Experiment 1)}$$

or

$$y_i = \mu + S_i + R_j + S{*}R_{ij} + e_{ij} \quad \text{(Experiment 2)}$$

where y = response variable, μ = population mean, F = feed withdrawal (no or yes), S = stunning method (Experiment 1: waterbath, head only; Experiment 2: $CO_2/O_2/N_2$, Ar/CO_2, or captive needle), R = replicate (slaughter day, Experiment 1: Days 1 to 2; Experiment 2: Days 1 to 4), and e = residual error. Pairwise differences between measurements at different sampling times were analyzed with Student's *t*-test.

RESULTS

Liver pH and Blood Metabolite Measurements (Experiment 1)

For each treatment group data on liver pH and glucose and lactate concentrations in blood sampled during exsanguination are given in Table 1. Immediately after evisceration, pH in the liver was higher ($P \leq 0.05$) in chickens

[5]CM525i; Minolta Camera, Benelux BV, 3600 HA Maarssen, The Netherlands.
[6]ABL 605; Radiometer, Nederland BV, 2718 RR Zoetermeer, The Netherlands.
[7]Sigma-Aldrich Chemie BV, 3331 II Zwijndrecht, The Netherlands.
[8]Molecular Devices, Ltd., Winnersh Wokingham, RG41 5RB, UK.

TABLE 1. Liver pH and blood metabolites, Experiment 1

	Feed withdrawn		Fed until transport		Significance		
Item	Waterbath	Head only	Waterbath	Head only	Feed × stun	Feed	Stun
Liver pH[1]	6.6 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	NS	***	NS
Blood glucose (m*M*)	12.2 ± 0.9	12.9 ± 1.1	13.0 ± 0.9	14.2 ± 0.8	NS	**	**
Blood lactate (m*M*)	4.9 ± 1.1	4.8 ± 1.3	5.0 ± 1.6	5.3 ± 1.6	NS	NS	NS

[1]Means ± standard deviations in each treatment group (n = 5) for liver pH, and glucose, and lactate in blood sampled during exsanguination from broilers on Slaughter Day 1 and Slaughter Day 2.

$^{**}P < 0.01$; $^{***}P < 0.001$.

that had feed withdrawn (pH 6.6) than in chickens that were fed until transport (pH 6.4). Stunning method did not affect liver pH. Glucose concentrations in blood were lower ($P \leq 0.001$) in chickens that had their feed withdrawn (12.6 m*M*) than in chickens that were fed until transport (13.6 m*M*). Additionally, head-only stunning resulted in increased blood glucose levels (13.3 m*M*) compared to waterbath stunning (12.4 m*M*). These effects were stronger on the first slaughter day than on the second ($P \leq 0.05$). Neither feed withdrawal nor stunning method or replicate had any effects on blood lactate concentrations.

Measurements of Postmortem Muscle Metabolism

Experiment 1. The data on breast muscle pH, R-value, and metabolite concentrations are shown in Table 2. For each parameter, treatment effects are described first, followed by the effects measured in time post mortem.

Feed withdrawal did not affect the pH in the breast muscle. Breast muscle pH was significantly ($P \leq 0.01$) higher (0.2 units) on the second slaughter day compared to the first. This change was consistent throughout all sampling times. Additionally, the pH in head-only stunned chickens was significantly ($P \leq 0.001$) lower than the pH in waterbath-stunned chickens. Up to 4 h postmortem, this difference was consistently 0.2 units and decreased to 0.1 units at 6 h. With both stunning methods, pH decreased significantly ($P \leq 0.01$) between 0 and 1 h postmortem, leveled between 1 and 2 h, and then decreased ($P \leq 0.001$) further after 4 and 6 h in waterbath-stunned chickens and decreased until 4 h in head-only stunned chickens.

Neither feed withdrawal nor slaughter day had a significant effect on the R-value, the ratio between inosine and adenosine components, in the breast muscle. Head-only stunning resulted in a higher ($P \leq 0.001$) R-value (0.04 units at 0 h postmortem and 0.14 units thereafter) than waterbath stunning. In waterbath-stunned chickens the R-value remained stable until 2 h postmortem and increased significantly ($P \leq 0.001$) at 4 and 6 h. In chickens that were head-only stunned, the R-value increased ($P \leq 0.001$) after 1 h postmortem, leveled until 2 h, and then increased further at 4 h ($P \leq 0.001$) and 6 h ($P \leq 0.05$).

Initial glycogen levels were higher ($P \leq 0.05$) on the second slaughter day (14.5 μmol/g) than on the first (6.1 μmol/g). Waterbath stunning resulted in higher ($P \leq 0.001$) glycogen levels up to 4 h postmortem than head-only electrical stunning. At 6 h postmortem, glycogen levels were not significantly different between treatment groups. Within treatment groups, glycogen levels did not change significantly up to 2 h postmortem, after which a rapid decrease set in. At 6 h postmortem, glycogen levels were not significantly different from 0.0 μmol/g.

No effects of feeding or stunning treatment on muscle glucose were found. Glucose profiles were similar on both slaughter days but occurred an hour earlier on the second day. A significant ($P \leq 0.05$) glucose peak was found after 1 h postmortem on the second slaughter day (6.1 μmol/g; 5 wk old: 1.0 μmol/g) and after 2 h on the first slaughter day (8.2 μmol/g; 6 wk old: 0.8 μmol/g). Both peaks were followed again by a significant ($P \leq 0.001$) decrease. At 6 h postmortem, chickens slaughtered on the second day had higher ($P \leq 0.05$) glucose levels (9.8 μmol/g) compared chickens slaughter on the first day (4.4 μmol/g).

ATP levels were not affected by feeding treatment or replicate. Initial postmortem ATP levels did not differ between treatments. ATP levels were significantly ($P \leq 0.05$) higher in waterbath-stunned chickens at 1, 2 and 6 h postmortem, but not at 4 h, compared to chickens that were head-only stunned. Within treatment groups, ATP did not change up to 2 h postmortem and decreased significantly ($P \leq 0.05$) to very low levels at 4 and 6 h postmortem.

Postmortem muscle lactate concentrations were not affected by replicate or feeding treatment. Through all sampling times, lactate levels were higher ($P \leq 0.001$) in chickens that were head-only stunned compared to waterbath-stunned chickens. In waterbath-stunned chickens lactate remained stable in the first hour postmortem, increased significantly ($P \leq 0.05$) up to 4 h, and then leveled off. In head-only-stunned chickens lactate had already increased at 1 h, stabilized until 2 h, increased again at 4 h, and then leveled off.

Experiment 2. The data on breast muscle pH, R-value, and metabolite concentrations are shown in Table 3. For each parameter treatment effects are described first, followed by the effects measured in time postmortem. No significant effects of slaughter day were found.

The breast muscle pH was highest ($P \leq 0.001$) in waterbath- and $CO_2/O_2/N_2$-stunned chickens, lower in Ar/CO_2- and captive-needle-stunned chickens, and lowest in head-only-stunned chickens at 1, 2, and 4 h postmortem.

TABLE 2. Muscle pH, R-value, and metabolites, Experiment 1

	Feed withdrawn		Fed until transport		Significance		
Item	Waterbath	Head only	Waterbath	Head only	Feed × stun	Feed	Stun
Muscle pH[1]							
0 h	6.7 ± 0.2[a]	6.4 ± 0.1[a]	6.7 ± 0.2[a]	6.4 ± 0.1[a]	NS	NS	***
1 h	6.6 ± 0.2[ab]	6.3 ± 0.1[b]	6.6 ± 0.2[a]	6.3 ± 0.1[b]	NS	NS	***
2 h	6.5 ± 0.1[b]	6.2 ± 0.1[b]	6.5 ± 0.2[a]	6.2 ± 0.2[b]	NS	NS	***
4 h	6.2 ± 0.2[c]	6.0 ± 0.1[c]	6.2 ± 0.2[b]	5.9 ± 0.2[c]	NS	NS	***
6 h	6.0 ± 0.1[d]	5.9 ± 0.1[c]	5.9 ± 0.2[c]	5.8 ± 0.2[c]	NS	NS	*
R-value							
0 h	0.81 ± 0.02[c]	0.85 ± 0.04[c]	0.82 ± 0.02[c]	0.86 ± 0.06[d]	NS	NS	***
1 h	0.81 ± 0.01[c]	0.99 ± 0.18[b]	0.85 ± 0.07[c]	0.97 ± 0.11[c]	NS	NS	***
2 h	0.85 ± 0.08[c]	1.00 ± 0.17[b]	0.86 ± 0.06[c]	1.01 ± 0.12[c]	NS	NS	***
4 h	1.06 ± 0.14[b]	1.25 ± 0.13[a]	1.08 ± 0.16[b]	1.26 ± 0.09[b]	NS	NS	***
6 h	1.22 ± 0.12[a]	1.32 ± 0.09[a]	1.26 ± 0.11[a]	1.36 ± 0.04[a]	NS	NS	***
Glycogen (μmol/g)							
0 h	12.3 ± 7.8[a]	7.3 ± 3.9[a]	14.7 ± 9.6[a]	7.0 ± 5.4[a]	NS	NS	**
1 h	10.8 ± 5.8[a]	4.3 ± 4.1[ab]	12.6 ± 7.4[a]	5.4 ± 3.9[a]	NS	NS	***
2 h	12.4 ± 7.8[a]	7.1 ± 6.6[a]	15.5 ± 10.2[a]	6.3 ± 5.0[a]	NS	NS	**
4 h	4.5 ± 3.2[b]	2.5 ± 2.9[bc]	5.7 ± 4.8[b]	1.4 ± 0.9[b]	NS	NS	**
6 h	0.4 ± 1.0[b]	0.3 ± 0.6[c]	0.7 ± 1.7[b]	0.1 ± 0.4[b]	NS	NS	NS
Glucose (μmol/g)							
0 h	2.4 ± 1.4[b]	2.9 ± 2.3[bc]	3.9 ± 4.7[b]	2.9 ± 1.4[b]	NS	NS	NS
1 h	3.0 ± 3.1[b]	3.9 ± 3.1[bc]	3.0 ± 2.5[b]	4.5 ± 4.1[ab]	NS	NS	NS
2 h	4.3 ± 4.6[ab]	4.5 ± 4.5[ab]	4.5 ± 4.2[b]	4.8 ± 5.1[ab]	NS	NS	NS
4 h	1.6 ± 2.1[b]	1.1 ± 0.7[c]	1.6 ± 1.1[b]	2.5 ± 2.1[b]	NS	NS	NS
6 h	6.0 ± 3.9[a]	7.2 ± 3.4[a]	7.8 ± 4.3[a]	7.2 ± 4.6[a]	NS	NS	NS
ATP[2] (μmol/g)							
0 h	13.0 ± 5.1[a]	8.6 ± 5.3[a]	6.9 ± 3.8[b]	10.8 ± 4.6[a]	NS	NS	NS
1 h	9.6 ± 5.4[a]	7.5 ± 5.3[a]	12.1 ± 6.1[a]	6.9 ± 6.6[ab]	NS	NS	*
2 h	10.0 ± 4.8[a]	7.5 ± 5.4[a]	12.4 ± 4.7[a]	6.6 ± 4.5[b]	NS	NS	**
4 h	2.9 ± 2.6[b]	1.6 ± 2.6[b]	3.6 ± 3.5[bc]	2.2 ± 4.7[c]	NS	NS	NS
6 h	2.8 ± 3.0[b]	1.0 ± 1.1[b]	1.7 ± 1.6[c]	0.8 ± 1.0[c]	NS	NS	*
Lactate (μmol/g)							
0 h	38.5 ± 9.6[c]	58.4 ± 7.9[c]	40.0 ± 14.8[c]	58.7 ± 5.5[c]	NS	NS	***
1 h	44.8 ± 9.3[c]	69.9 ± 14.3[b]	48.9 ± 16.3[bc]	73.4 ± 15.5[b]	NS	NS	***
2 h	55.0 ± 12.9[b]	71.7 ± 13.6[b]	53.9 ± 13.9[b]	77.3 ± 13.4[b]	NS	NS	***
4 h	80.0 ± 13.0[a]	89.4 ± 7.2[a]	76.6 ± 12.3[a]	92.2 ± 10.6[a]	NS	NS	***
6 h	82.3 ± 10.2[a]	85.8 ± 8.9[a]	83.0 ± 7.6[a]	93.1 ± 8.6[a]	NS	NS	*

[a-c]Different superscripts indicate significant ($P < 0.05$) differences in time.

[1]Means ± standard deviations in each treatment group (n = 5) for pH and R-value measured at 0, 1, 2, 4, and 6 h postmortem in the M. pectoralis superficialis of broilers from Slaughter Day 1 and Slaughter Day 2.

[2]Adenosine triphosphate.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

From 8 h onward, no treatment effects on breast muscle pH were found. Head-only stunning resulted in a low pH by 1 h postmortem, which did not change over time. The pH in chickens stunned by $CO_2/O_2/N_2$, Ar/CO_2 or captive needle decreased until 4 h postmortem and remained stable at least until 48 h. The pH decrease after waterbath stunning continued up to 8 h postmortem, after which it changed no further.

The R-values at 1 and 2 h postmortem were highest ($P \leq 0.001$) in head-only stunned chickens, lower in Ar/CO_2- and captive-needle-stunned chickens, and lowest in waterbath- and $CO_2/O_2/N_2$-stunned chickens. At 4 h postmortem, waterbath- and $CO_2/O_2/N_2$-stunned chickens had a lower R-value ($P \leq 0.001$) than chickens from the other stunning groups. At 8 h, head-only stunning resulted in a higher R-value than the other stunning treatments. At 24 and 48 h, no treatment effects were found. In all stunning groups, the R-value increased from 1 h postmortem onward. With head only stunning the R-value reached its maximum plateau at 4 h. With the other stunning treatments, this plateau was reached after 8 h.

At 1 and 2 h postmortem, glycogen levels were highest ($P \leq 0.001$) in the breast muscles of waterbath- and $CO_2/O_2/N_2$-stunned chickens, lower in Ar/CO_2- and captive-needle-stunned chickens, and lowest in head-only electrically stunned chickens. After 2 h no differences between stunning treatments were found. In all stunning treatments, except head-only stunning, glycogen levels decreased significantly ($P \leq 0.001$) from 1 h to nearly 0.0 μmol/g by 4 h postmortem and after. Head-only stunning resulted in nearly 0.0 μmol/g at 1 h postmortem.

Muscle glucose levels at 1 and 2 h postmortem were higher ($P \leq 0.001$) in chickens that were head-only stunned than in chickens in all other stunning treatments. From 4 h onward, no effects of stunning treatment on glucose levels were found. In all stunning treatments except head-only stunning, glucose levels remained low until 2 h postmortem, then increased, and leveled off after 8 h. In head-only-stunned chickens, muscle glucose was already high at 1 h postmortem and did not change over time.

ATP levels in head-only-stunned chickens were lower ($P \leq 0.001$) than in all other stunning methods at 1 and

TABLE 3. Muscle pH, R-value, and metabolites, Experiment 2

Item	Waterbath	Head only	$CO_2/O_2/N_2$	Ar/CO_2	Captive needle	Significance
Muscle pH[1]						
1 h	6.4 ± 0.2[a]	6.0 ± 0.2	6.4 ± 0.1[a]	6.3 ± 0.2[a]	6.3 ± 0.2[a]	***
2 h	6.3 ± 0.2[b]	5.9 ± 0.2	6.3 ± 0.2[a]	6.1 ± 0.2[b]	6.3 ± 0.2[a]	***
4 h	6.1 ± 0.2[c]	5.8 ± 0.2	6.0 ± 0.2[b]	5.9 ± 0.2[c]	5.9 ± 0.1[b]	**
8 h	5.9 ± 0.1[d]	6.0 ± 0.2	5.9 ± 0.1[b]	5.9 ± 0.2[c]	5.9 ± 0.1[b]	NS
24 h	5.9 ± 0.1[d]	5.9 ± 0.1	5.9 ± 0.1[b]	5.9 ± 0.1[c]	5.9 ± 0.1[b]	NS
48 h	5.8 ± 0.1[d]	5.9 ± 0.1	5.9 ± 0.1[b]	5.9 ± 0.1[c]	5.9 ± 0.1[b]	NS
R-value						
1 h	0.87 ± 0.06[d]	1.14 ± 0.08[c]	0.88 ± 0.06[d]	0.94 ± 0.09[d]	0.94 ± 0.13[c]	***
2 h	0.94 ± 0.07[c]	1.27 ± 0.11[b]	0.93 ± 0.07[c]	1.09 ± 0.15[c]	0.99 ± 0.11[c]	***
4 h	1.20 ± 0.13[b]	1.35 ± 0.05[a]	1.17 ± 0.14[b]	1.29 ± 0.09[b]	1.29 ± 0.10[b]	***
8 h	1.35 ± 0.04[a]	1.38 ± 0.02[a]	1.35 ± 0.03[a]	1.36 ± 0.02[a]	1.36 ± 0.02[a]	**
24 h	1.37 ± 0.02[a]	1.38 ± 0.02[a]	1.38 ± 0.02[a]	1.37 ± 0.01[a]	1.38 ± 0.02[a]	NS
48 h	1.38 ± 0.02[a]	1.38 ± 0.02[a]	1.38 ± 0.03[a]	1.38 ± 0.02[a]	1.38 ± 0.02[a]	NS
Glycogen (μmol/g)						
1 h	12.7 ± 8.0[a]	0.4 ± 0.5	9.4 ± 6.9[a]	5.6 ± 6.0[a]	6.7 ± 6.1[a]	***
2 h	6.0 ± 5.7[b]	0.3 ± 0.6	6.9 ± 7.0[a]	2.5 ± 4.8[b]	4.9 ± 5.8[a]	**
4 h	0.4 ± 1.0[c]	0.2 ± 0.3	0.5 ± 1.2[b]	0.1 ± 0.4[c]	0.4 ± 0.7[b]	NS
8 h	0.3 ± 0.5[c]	0.2 ± 0.6	0.3 ± 0.5[b]	0.2 ± 0.5[c]	0.1 ± 0.2[b]	NS
24 h	0.1 ± 0.2[c]	0.1 ± 0.3	0.1 ± 0.2[b]	0.0 ± 0.0[c]	0.3 ± 0.7[b]	NS
48 h	0.1 ± 0.2[c]	0.0 ± 0.0	0.0 ± 0.1[b]	0.1 ± 0.2[c]	0.1 ± 0.3[b]	NS
Glucose (μmol/g)						
1 h	0.8 ± 0.8[b]	2.5 ± 1.5	0.3 ± 0.3[d]	1.0 ± 1.2[b]	0.5 ± 0.8[b]	***
2 h	0.5 ± 0.5[b]	3.2 ± 1.7	1.0 ± 1.0[cd]	1.4 ± 1.5[b]	1.0 ± 1.1[b]	***
4 h	1.7 ± 1.6[b]	3.0 ± 2.9	2.1 ± 2.0[bc]	3.8 ± 2.9[a]	3.2 ± 2.0[a]	NS
8 h	3.1 ± 2.3[a]	2.8 ± 2.1	3.8 ± 2.6[a]	3.3 ± 3.1[a]	3.1 ± 1.6[a]	NS
24 h	3.2 ± 2.2[a]	2.5 ± 2.5	3.2 ± 2.4[ab]	2.6 ± 1.9[ab]	3.3 ± 2.1[a]	NS
48 h	4.2 ± 3.4[a]	3.0 ± 2.0	3.9 ± 2.4[a]	3.3 ± 3.0[a]	2.8 ± 2.0[a]	NS
ATP[2] (μmol/g)						
1 h	5.4 ± 2.6[a]	1.5 ± 1.2[a]	5.5 ± 2.0[a]	4.6 ± 1.9[a]	4.4 ± 2.4[a]	***
2 h	3.1 ± 1.9[b]	0.9 ± 0.9[b]	4.4 ± 3.6[b]	2.0 ± 1.7[b]	4.1 ± 2.8[a]	***
4 h	1.0 ± 1.0[c]	0.0 ± 0.1[c]	1.3 ± 1.2[c]	0.3 ± 0.5[c]	0.5 ± 0.9[b]	***
8 h	0.4 ± 0.5[cd]	0.0 ± 0.1[c]	0.4 ± 0.5[cd]	0.4 ± 0.5[c]	0.1 ± 0.2[b]	**
24 h	0.0 ± 0.0[d]	0.0 ± 0.0[c]	0.0 ± 0.0[d]	0.0 ± 0.0[c]	0.0 ± 0.0[b]	NS
48 h	0.0 ± 0.0[d]	0.0 ± 0.0[c]	0.0 ± 0.0[d]	0.0 ± 0.0[c]	0.0 ± 0.0[b]	NS
Lactate (μmol/g)						
1 h	49.3 ± 10.7[d]	79.0 ± 7.4	46.9 ± 11.2[c]	58.7 ± 9.6[c]	55.8 ± 11.1[b]	***
2 h	59.5 ± 13.2[c]	82.0 ± 10.4	58.7 ± 9.3[b]	70.3 ± 10.5[b]	61.4 ± 10.5[b]	***
4 h	75.8 ± 9.8[b]	79.0 ± 10.4	78.7 ± 9.6[a]	84.2 ± 9.1[a]	81.2 ± 7.0[a]	NS
8 h	84.4 ± 7.5[a]	79.5 ± 11.2	80.6 ± 11.1[a]	77.4 ± 11.2[ab]	78.7 ± 5.4[a]	NS
24 h	83.1 ± 8.6[ab]	80.4 ± 9.3	81.4 ± 6.6[a]	81.2 ± 7.9[a]	79.4 ± 8.6[a]	NS
48 h	79.3 ± 11.5[ab]	77.2 ± 7.7	80.9 ± 10.3[a]	76.2 ± 12.9[ab]	80.9 ± 9.1[a]	NS

[a-d]Different superscripts indicate significant ($P < 0.05$) differences in time.

[1]Means ± standard deviations in each treatment group (n = 5) for pH and R-value measured at 0, 1, 2, 4, and 6 h postmortem in the M. pectoralis superficialis of broilers from Slaughter day 1 and Slaughter Day 2.

[2]Adenosine triphosphate.

**$P < 0.01$; ***$P < 0.001$.

2 h postmortem. At 2 h postmortem, ATP levels in the Ar/CO_2 treatment group were reduced. At 4 and 8 h postmortem, low levels of ATP were still found in the waterbath- and $CO_2/O_2/N_2$-stunned groups, whereas ATP in the other treatment groups was reduced to nearly 0.0 μmol/g. After 24 h, no ATP was found in any of the treatment groups.

At 1 h postmortem, lactate was highest ($P \leq 0.001$) in chickens that were head-only stunned, lower in chickens that were Ar/CO_2 and captive needle stunned, and lowest chickens that were stunned by waterbath or $CO_2/O_2/N_2$. At 2 h, lactate was highest ($P \leq 0.001$) in head-only-stunned chickens, followed by Ar/CO_2-stunned chickens, and was lowest in the other stunning treatments. From 4 h onward, no effect of stunning treatment on lactate was found. After head-only stunning, lactate started high and did not differ over time. With the other stunning methods, lactate increased significantly ($P \leq 0.05$) up to 4 h postmortem and stayed at that level until 48 h.

Carcass and Meat Quality Measurements

Experiment 1. Data on shear force, water-holding capacity, and color measurements per treatment group are shown in Table 4. Shear force data were not equally distributed between the two stunning groups. This unequal distribution was corrected for in the analysis by taking up residual variance as a random factor in the model. Shear force at 96 h postmortem was significantly ($P \leq 0.001$) higher in waterbath-stunned chickens (24.9 N) than in head-only stunned chickens (14.4 N). Shear force was not affected by feed withdrawal or replicate.

Water-holding capacity and color were measured in chickens slaughtered on the second day only. No treat-

TABLE 4. Meat quality, Experiment 1

Item	Feed withdrawn		Fed until transport		Significance		
	Waterbath	Head only	Waterbath	Head only	Feed × stun	Feed	Stun
Shear force (N)[1]	22.9 ± 15.6	13.5 ± 3.3	26.9 ± 19.9	15.3 ± 3.6	NS	NS	***
Water-holding capacity (mg)	41 ± 5	43 ± 5	42 ± 6	41 ± 4			
Color							
L*	53.9 ± 3.1	53.9 ± 2.4	53.1 ± .9	53.5 ± 2.3	NS	NS	NS
a*	8.3 ± 1.2	8.7 ± 0.9	9.0 ± 1.2	9.0 ± 1.0	NS	NS	NS
b*	16.5 ± 1.8	16.9 ± 1.6	17.0 ± 1.9	16.0 ± 0.9	NS	NS	NS

[1]Means ± standard deviations (n = 15) in each treatment group for shear force of the M. pectoralis superficialis in broilers on both slaughter days; the L*, a* and b* color measurements; and water-holding capacity of broilers at 96 h postmortem from Slaughter Day 2 only.

***$P < 0.001$.

ment effects were found on water-holding capacity or on the L*, a*, and b* values for color at 96 h.

Experiment 2. Data on hemorrhage scores, shear force, water-holding capacity, and color measurements per treatment group are shown in Table 5. No effects of slaughter day were found.

The highest ($P \leq 0.001$) hemorrhage score was found with waterbath stunning (score of 3.6). Hemorrhage score was lower after head-only electrical stunning (score of 3.1) and was lowest after captive needle and both gas stunning treatments (score of 1.8).

Shear force was not affected by stunning treatment. In all treatment groups, a significant ($P \leq 0.05$) reduction in shear force of about 50% was found between 4 and 8 h postmortem.

Up to 8 h postmortem, water-holding capacity was lower (higher filter paper measurements; $P \leq 0.001$) in head-only stunned chickens than in any of the other treatment groups. From 24 h onward, stunning treatment was not found to have any effect on water-holding capacity. Except after head-only stunning, in which water-holding capacity was low from 1 h postmortem and did not change over time, water-holding capacity after the other stunning treatments decreased significantly ($P \leq 0.05$) between 8 and 24 h. No changes in water-holding capacity were found after 24 h.

The L*, a*, and b* color parameters did not change over time. The L* value was highest ($P \leq 0.001$) after the gas stunning treatments (60.5), lower following captive needle stunning (59.7), and lowest after electrical stunning (58.7). The a* value was higher ($P \leq 0.001$) after electrical stunning (4.4) than after captive needle or gas stunning (3.5). The b* value was higher after $CO_2/O_2/N_2$ stunning (11.7) than after any of the other stunning treatments (11.1).

DISCUSSION

Feed Withdrawal

Feed withdrawal for 5 h before transport was found to deplete the glycogen store in the liver, which is mobilized to maintain the energy supply during this period. This result was also reflected by a lower glucose concentration in the exsanguinated blood after feed deprivation. Even though central glycogen stores were largely depleted, energy demand apparently was not so high that local energy stores like muscle glycogen were affected. Prolonged feed deprivation can cause a reduction in yields, which has been suggested to be caused by muscle breakdown for energy supply (Veerkamp, 1986). The periods of feed deprivation used in this study were too short for such effects to occur.

TABLE 5. Meat quality, Experiment 2

Item	Waterbath	Head only	$CO_2/O_2/N_2$	Ar/CO_2	Captive needle	Significance
Hemorrhage score[1]	3.6 ± 1.1	3.1 ± 1.1	1.6 ± 0.9	1.7 ± 0.8	2.0 ± 0.8	***
Shear force (N)						
≤ 2 h	27.7 ± 10.4[a]	33.2 ± 12.7[a]	26.2 ± 7.1[a]	32.2 ± 19.0[a]	29.0 ± 15.1[a]	NS
4 h	22.9 ± 10.6[b]	23.8 ± 13.1[b]	25.5 ± 13.9[a]	22.7 ± 12.6[b]	22.4 ± 9.5[b]	NS
≥ 8 h	15.1 ± 5.6[c]	15.5 ± 4.4[c]	15.2 ± 7.4[b]	13.4 ± 3.3[c]	14.6 ± 4.3[c]	NS
Water-holding capacity (mg)						
≤ 8 h	48 ± 15[b]	65 ± 24	44 ± 12[b]	49 ± 16[b]	50 ± 15[b]	***
≥ 24 h	64 ± 17[a]	71 ± 19	61 ± 20[a]	68 ± 18[a]	67 ± 20[a]	NS
Color						
L*	58.3 ± 3.2	59.1 ± 3.0	60.8 ± 3.8	60.2 ± 3.7	59.7 ± 4.0	***
a*	4.5 ± 1.7	4.3 ± 1.4	3.2 ± 1.1	3.5 ± 1.1	3.8 ± 1.4	***
b*	11.1 ± 1.6	11.1 ± 1.6	11.7 ± 1.6	10.9 ± 1.3	11.3 ± 1.7	**

[a-c]Different superscripts indicate significant ($P < 0.05$) differences in time.

[1]Means ± standard deviations in each treatment group (n = 5) for pH and R-value measured at 0, 1, 2, 4, and 6 h postmortem in the M. pectoralis superficialis of broilers from Slaughter Day 1 and Slaughter Day 2.

**$P < 0.01$; ***$P < 0.001$.

Stunning Method

After head-only electrical stunning, blood glucose levels were 8% higher than blood glucose concentrations after waterbath stunning. Possibly this effect can be attributed to an increased demand caused by a high incidence of clonic convulsions, including wing flapping, as observed after head-only stunning (Hillebrand et al., 1996a). The exact pathway that stimulates this glucose production is not clear (Kjaer, 1999). This conclusion was supported by low glycogen and high lactate concentrations in the breast muscle of head-only stunned chickens immediately after slaughter. With tonic convulsions, as found with waterbath stunning, the breast muscle only contracts and relaxes once, thereby consuming little energy (Lambooij et al., 1999). The high metabolic rate in the breast muscle found after head-only stunning was consistent in all metabolic parameters measured (low pH, glycogen, and ATP; high R-value and lactate; in Experiment 2, high glucose also).

Intermediate metabolic rates were found after Ar/CO_2 and captive needle stunning, with Ar/CO_2 having a slightly higher rate (faster decrease in muscle pH, glycogen, and ATP and increases in R-value and lactate after 2 h). Here also, convulsions might have played a key role. Convulsions were reported to occur at 19 to 41 s after immersion in a Ar/CO_2 gas mixture (Raj et al., 1998). The number of convulsions after captive bolt stunning was dependent on the area of the brain that was damaged (Hillebrand et al., 1996a). With captive needle/air pressure stunning, convulsions are mostly mild to moderate and consist of muscle fibrillation rather than wing flapping (Lambooij et al., 1999).

The lowest metabolic rates (slowest decrease in muscle pH, glycogen, and ATP and increases in R-value and lactate) were found after waterbath and $CO_2/O_2/N_2$ stunning. These stunning methods are reported to induce very little muscular activity (Lambooij and Pieterse, 1997; Gerritzen et al., 2000). Similar relationships between muscle activity, metabolic rate as indicated by pH and R-value, and electrical stunning have been reported in normally processed muscle (Papinaho and Fletcher, 1996). The metabolic rates found after waterbath and $CO_2/O_2/N_2$ stunning in normally processed muscles were still faster than the naturally occurring postmortem metabolism, indicating that processing treatments, most notably plucking, also stimulate muscle metabolism.

In unprocessed muscles, differences in glycolytic metabolism between stunning methods had not disappeared after 6 h postmortem, indicating that metabolites might not have reached their ultimate levels in all carcasses. Fletcher and Papinaho (1996) reported ultimate pH and R-value were reached at 6 h postmortem after waterbath stunning at 50 and 125 mA and plucking. Schreurs (1999) showed significant changes between 6 and 24 h postmortem in R-values but not in metabolite levels or pH of unprocessed breast muscle of waterbath-stunned (100 V, 50 Hz) chickens. Under normal processing conditions, differences between stunning methods in glycolytic metabolism have mostly disappeared by 4 h postmortem.

Early Postmortem Muscle Metabolism

The pH, R-value, and glycogen, glucose, ATP, and lactate concentrations were recorded up to 6 h postmortem in unprocessed muscle and up to 48 h in normally processed muscle. We hoped to gain a better understanding of how energy balance in the muscle is maintained, i.e., the rate at which energy stores are depleted naturally and after normal processing. The naturally occurring processes of metabolic degradation in broiler chickens stopped after 6 h postmortem. Schreurs (1999) reported no differences in glycolytic metabolites in unprocessed muscle between 6 and 48 h, although the R-value was found to increase slightly between 6 and 24 h.

In normally processed carcasses, most glycolytic metabolite levels did not change after 4 h postmortem, whereas no further changes in the R-value were found after 8 h. The exceptions were waterbath stunning, after which changes in muscle pH, ATP, and lactate were observed up to 8 h postmortem, and $CO_2/O_2/N_2$ stunning, after which changes in ATP levels were also found up to 8 h postmortem. Compared to the ATP levels immediately after slaughter in unprocessed muscle (about 10 μmol/g), after normal processing, an ATP decrease of about 50% was established after 1 h. ATP levels decreased below 20 to 30%, which is generally accepted as the threshold at which rigor mortis sets in, at 4 h postmortem regardless processing and most stunning methods. Only after head-only stunning was this threshold crossed after 2 h. For a rigor mortis induction faster than 4 h, additional treatments, e.g., electrostimulation, must be applied (Sams, 1999).

In unprocessed muscle, glycogen concentrations remained stable for the first 2 h postmortem and became exhausted after 4 to 6 h postmortem. Except after head-only stunning, when glycogen stores were depleted at 1 and at 2 h after Ar/CO_2 stunning, glycogen levels in normally processed muscle remained stable also for 2 h but were exhausted at 4 h postmortem. Glucose concentrations are or become elevated after 4 h and remain high until 48 h. In unprocessed muscle, elevated glucose levels were found at 6 h postmortem. This result indicates that it is not necessary to exhaust the total available energy in the muscle at the time of slaughter to prevent cold shortening at early deboning but rather to disable ATP-generating steps in glycolysis, probably through pH decrease or enzymatic denaturation, which is independent of substrate availability.

Meat Quality

Hemorrhage scores of the breast muscle were significantly higher in electrically stunned chickens than in captive-needle- or gas-stunned chickens. Similar results between electrical and captive bolt stunning were found by Hillebrand et al. (1996a). Although hemorrhage score did

not differ between head-only and waterbath stunning, they noted a difference in the type of hemorrhages and attributed this difference to clonic or tonic convulsions, respectively. With captive needle stunning convulsions are mild, and with Ar/CO_2 stunning a bird is not restricted in its movement so hemorrhages are less likely to occur (Lambooij and Pieterse, 1997). With $CO_2/O_2/N_2$ stunning, convulsions are rarely observed (Gerritzen et al., 2000), resulting in very low hemorrhage scores (Uijttenboogaart, 1997).

In unprocessed muscle, a higher shear force was found at 96 h after waterbath stunning than after head-only stunning. This finding may be attributed to insufficiently reduced levels of ATP in some carcasses when they were deboned at 6 h postmortem, resulting in cold shortening. Rigor mortis was induced in all carcasses of head-only-stunned chickens at 6 h, preventing cold shortening. In normally processed carcasses, no stunning differences on shear force were found among stunning methods. A significant decrease in shear force occurred between 2 and 8 h postmortem in normally processed muscles. Although this decrease started within the period of metabolic degradation, it continued after glycolytic metabolism had ended at 4 h. Schreurs (1999) and Papinaho and Fletcher (1996) also showed a reduction in shear force when no more metabolic activity was observed in unprocessed and processed muscles, respectively.

Water-holding capacity did not differ among stunning treatments in unprocessed muscle but was lower up to 8 h postmortem in head-only stunned, normally processed chickens. Presumably, convulsions combined with processing factors caused the pH to decrease too rapidly, causing protein denaturation and leading to this result.

No effects of stunning method on color measurements were found in unprocessed muscle. In normally processed carcasses, gas-stunned chickens had a higher L* value and a lower a* value than electrically stunned chickens, with captive needle stunning being in the middle for both parameters. Chickens stunned with a $CO_2/O_2/N_2$ gas mixture had a higher b* value than the rest. Although these differences are in accordance with those found by Uijttenboogaart (1997), they are hard to explain, as they do not seem related to pH decrease causing protein denaturation or to heme content.

If industry is to gain from early deboning, the risk of cold shortening must be minimized by rapid degradation of muscle metabolism. It has been shown that feed deprivation quickly decreased the central energy supplies of chickens but, for periods up to 5 h before transport, did not affect muscle glycogen stores. The natural period in which glycolytic metabolism occurs in chicken breast muscle is 6 h. Metabolization of adenosine and inosine compounds continues at least 8 h postmortem. Stunning that causes wing flapping and clonic convulsions and processing will increase the initial rate of glycogenolysis and glycolysis. A complete exhaustion of available glucose is not necessary for induction of rigor mortis. Rather, disabling of ATP generation by other processes, e.g., pH decrease, is crucial. If meat is deboned after rigor has set in, shear force, water-holding capacity, and color correlate very poorly with parameters from the glycolytic metabolism.

Stunning with $CO_2/O_2/N_2$ increases the time before rigor mortis starts, and Ar/CO_2 and head-only stunning decrease the time to rigor mortis induction. The length of time also depends on the occurrence of clonic convulsions and wing flapping during stunning. A pH decline that is too rapid and caused by muscle activity and processing, as observed with head-only electrical stunning, will induce an early decrease in water-holding capacity. This effect should be taken into account when developing treatments to further reduce the time to rigor mortis induction. Otherwise, stunning methods do not affect meat quality through metabolic degradation. Electrical stunning results in more hemorrhaging, partly due to restraining. Although these problems are less with gas stunning, it causes a reduction in pluckability and affects meat color (Uijttenboogaart, 1997). Further research is required to address these issues.

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