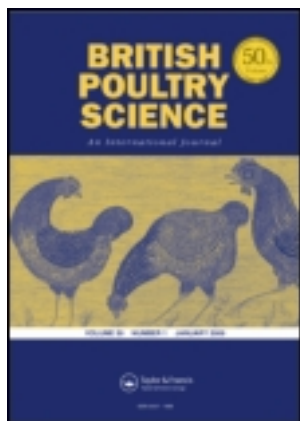


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Gas composition in controlled atmosphere stunning affects turkey meat quality traits

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Abstract 1. Investigations were made into the breast and leg muscle energy metabolism, and the quality of breast meat of turkeys after controlled atmosphere stunning or stun-killing (CAS) with various gas mixtures. In addition, the effect on meat quality of an increase in the chilling rate of turkey breast meat after hypercapnic or anoxic stun-killing was studied.

2. A total of 35 turkey toms within two replicate pens were individually stunned during consecutive weeks using one of 4 CAS methods. The stunning gases tested were high CO₂ concentration (60% CO₂ in air), high N₂ concentration (98% N₂, <2% O₂), a mixture of 76% N₂ and 24% CO₂, and a biphasic method (first minute in mixture containing 40% CO₂, 30% N₂, and 30% O₂; followed by two minutes in a mixture containing 60% CO₂ in air).

3. The birds stunned with N₂ displayed the highest initial reduction in muscle pH, but after 4 h *post mortem* there were no differences in pH values associated with the various CAS methods.

4. The CAS method alone had no statistically significant effect on the quality of turkey breast muscle when the chilling speed was rapid (0°C for 4 h, followed by storage at 4°C). When the chilling rate was slowed (20°C for 4 h followed by storage at 4°C), a significant decrease in cooking loss and in Warner-Bratzler shear force was recorded for birds stun-killed with CO₂.

5. This study shows that anoxic stun-killing with N₂ had no adverse effects on meat quality despite the rapid *post mortem* pH decrease. The CAS with N₂ allows rapid cooling of carcasses without the risk of cold shortening, whereas with CO₂-stun-killing of turkeys, the rate of chilling should be slower. Concerning meat quality, all the CAS methods tested were suitable for stunning turkeys.

INTRODUCTION

The most common method used to stun turkeys is the application of an electric current in a water-bath stunner. However, some welfare concerns exist with this method, including stress due to uncrating and shackling of live birds prior to stunning, and inadvertent pre-stun electric shocks on entering the water-bath stunner (FAWC, 2009). Electrical stunning has also been criticised on the grounds of reduced meat and carcase quality (Raj *et al.*, 1992, 1997). In electrical stunning, however, the settings of

a water-bath stunner are influenced by the conflicting interest between animal welfare and the quality of the end product. High amperage electrical stunning is necessary to guarantee induction of immediate and lasting unconsciousness (Gregory and Wotton, 1987), but high currents also lead to muscle supercontractions and subsequent haemorrhages in muscle tissue caused by rupture of blood vessels and damage to muscle fibres (Sams, 2001). Controlled atmosphere (gas) stunning or stun-killing (CAS) has been introduced as a solution to the problems associated with electrical stunning. The CAS

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systems are becoming more common in poultry stunning (FAWC, 2009), and CAS provides a solution to many animal welfare issues linked with electrical stunning. The major advantage of gaseous over electrical stunning is that CAS can be carried out without shackling and uncrating live birds. Furthermore, fewer carcass appearance defects, such as breast haemorrhages and broken bones, have been reported for poultry stunned with gas than for those stunned with high electrical current (Raj *et al.*, 1990a; Raj *et al.*, 1992, 1997; Savenije *et al.*, 2002).

The different gases used in CAS systems influence the welfare of birds during stunning. CO₂, in particular, is a problematic gas in relation to welfare. Its positive aspects are that unconsciousness is induced rapidly (Raj and Gregory, 1993, 1994) through hypercapnia (excess carbon dioxide in blood), it is of relatively low cost, and is easy and safe to use in the poultry industry as it is a heavier gas than air. The negative aspects are that it has a distressing respiratory effect and it is aversive when inhaled at high concentrations (Raj and Gregory, 1993; Raj, 1996; Lambooij *et al.*, 1999; McKeegan, 2004; McKeegan *et al.*, 2006). It is still, however, widely used in various concentrations in CAS systems. In poultry stunning, anoxia (deficiency of oxygen supply to tissues such as the brain) induced by inert gases such as Ar or N₂, is also applied. Anoxic stunning induces only a slight or no respiratory distress (Raj, 1996; Abeyesinghe *et al.*, 2007), although fierce muscle convulsions (wing flapping) frequently occur (Gerritzen *et al.*, 2000; Abeyesinghe *et al.*, 2007). The addition of CO₂, or CO₂ and O₂, to the gas mixtures reduces the intensity and duration of the convulsions (Poole and Fletcher, 1995; Gerritzen *et al.*, 2000; McKeegan *et al.*, 2007a).

In anoxic stunning, the rate of pH decrease is faster than in hypercapnic (CO₂) stunning, and in both CAS methods the early pH decrease is more rapid than with electrical stunning (Raj *et al.*, 1990b; Fleming *et al.*, 1991; Raj, 1994). The acceleration in pH decrease has been considered to be an advantage, enabling early deboning of breast meat without adversely affecting its tenderness (Raj *et al.*, 1990b; Raj, 1994). Without proper and rapid chilling of carcasses, the fast pH decrease may, however, become a problem. If early *post mortem* glycolysis is very fast and the pH decreases to a low level when the carcass temperature is still high, muscle proteins are denatured (Offer, 1991). This frequently leads to a low water-holding capacity and pale, soft, exudative (PSE) meat. Turkey PSE has been studied intensively (e.g. van Hoof, 1979; McKee and Sams, 1998; Wynveen *et al.*, 1999; Barbut *et al.*, 2008), and similarities in the development of the PSE defect have been found in turkey

and pork meat (Solomon *et al.*, 1998; Sosnicki *et al.*, 1998).

Due to the distressing respiratory effect of CO₂, several attempts have been made to substitute the use of high concentrations in poultry stunning. Nitrogen is a potential alternative for use in turkey stunning, but little information is available on its effects on meat quality. The aim of this work was to investigate the meat quality aspects associated with different gas mixtures currently used, or which could be used, in commercial turkey stunning or stun-killing. In addition, the effect of the rate of chilling on breast meat quality after anoxic or hypercapnic stunning was evaluated.

MATERIALS AND METHODS

Animals and housing

In total, 35 turkey toms (Nicolas 300 × BUT8) aged 95 d were bought from a commercial farm. On arrival, the birds were divided randomly into two groups and housed in adjacent wood-shaving littered pens. Light intensity (Gossen, Mavolux Digital, Germany) ranged between 3–8 lux under the lamps to 0–4 lux in the corners of the pens at turkey head height. The light:dark period was 16:8 h. Room temperature was maintained at 19 ± 1°C, and the relative humidity varied between 20% and 25%. Food and water were supplied *ad libitum* from two tower feeders and two bell drinkers per pen. The diet consisted of 80% commercial turkey complete feed (Iso-Punahelhta 4, Suomen Rehu PLC) and 20% whole-grain wheat (82.7 g/hectolitre, 130 g protein/kg).

As part of another study, half of the birds were administered pain medication (meloxicam, 0.5–1 mg/kg *i.v.*) at 103–113 d (group 1) or 110–120 d (group 2). During the statistical analyses in the present study, by comparing the results of medicated birds with those of non-medicated birds, it was ascertained that the medication had no effect on meat quality parameters. The birds were stun-killed during two 3-day periods: at the age of 116–119 d (group 1) and at the age of 123–125 d (group 2). The birds were gently handled, and in harvesting were held and carried from their home pen to the processing room and not raised or hung from their feet. The mean live weight of the birds at slaughter was 13.6 kg (SD 1.3 kg).

Gas stunning

The turkeys were individually subjected to CAS with a plastic paediatric mask equipped with an elastic rubber collar (Figure 1). The gas mixtures were delivered at a stream velocity of 15 l/min to the mask, directly from a gas bottle that had been

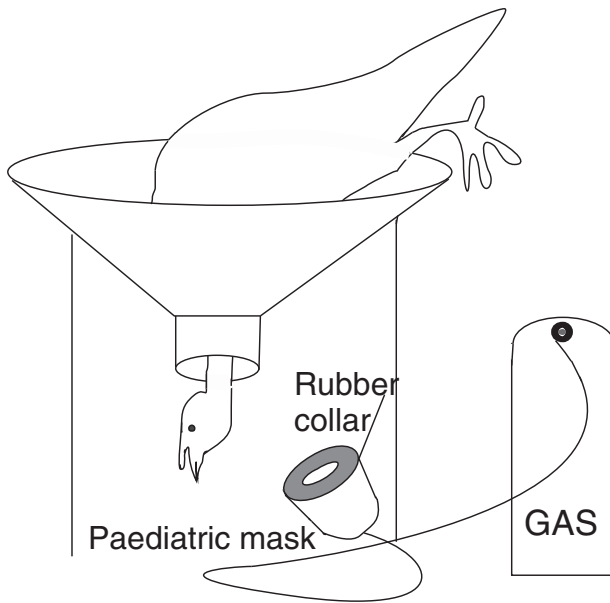


Figure 1. Schematic overview of gas stunning. Turkeys were subjected to the stunning gas mixtures, prepared beforehand, via a plastic paediatric mask equipped with an elastic rubber collar. The paediatric mask was placed on the turkeys' heads after a constant gas stream velocity of 15 l/min to the mask was attained.

prepared with the correct gas concentration (Oy Woikoski Ab, Finland), and the bird's head was placed in the mask. The gases used in turkey stun-killing were high CO₂ concentration (60% CO₂ in air), high N₂ concentration (98% N₂, <2% O₂), a mixture of 76% N₂ and 24% CO₂, and a biphasic method (first minute in a mixture containing 40% CO₂, 30% N₂, and 30% O₂, followed by two minutes in a mixture containing 60% CO₂ in air). The order of the gases was altered daily. The total exposure time was three minutes for each tested CAS process, resulting in the death of the birds. The birds were randomly distributed to the different stunning groups, and prior to stunning the birds were immobilised with a bleeding cone.

Bilateral neck cutting, aimed at severing both carotid arteries and jugular veins, was manually performed within one minute of the end of the stunning, and bleeding lasted for three min. The carcasses were not processed further.

Muscle sampling

At 20 min *post mortem*, a lengthwise incision was made in the skin covering the breast and leg muscles, prior to sampling muscle tissue. Muscle tissue samples (1 g) for glycogen and lactate analyses were cut from the central third of the left *M. pectoralis superficialis* and from the right *M. gastrocnemius*, frozen immediately in liquid nitrogen and stored at -80°C until analysed. Additional samples of 0.5 g from these muscles were obtained at 20 min, 1 h, 2 h and 4 h *post mortem*

for pH analyses. Each sample was taken at a depth of 2.5 cm and at least 1 cm from the previous sample area. Temperatures of the breast and leg muscles were monitored at the time of sample collection.

The right breast muscle was excised from the carcasses 20 min *post mortem* and 3 tissue samples of around 80 g were taken from the cranial third of the muscle, weighed and placed in plastic bags, then aged on ice. Three additional slices were excised from CO₂- and N₂-stun-killed birds, placed in plastic bags and kept at room temperature (18.6 ± 0.9°C) for 4 h, and thereafter kept at 0°C. After 6–8 h, all samples were stored at 4°C and treated as described in more detail below. The temperature declines of the muscle slices are shown in Table 1. One of the slices was used in temperature and pH analyses, and the other two in the analyses of drip loss, cooking loss and tenderness.

Measurements of pH

Before measuring the pH, a meat extract was prepared by homogenising 0.5 g of muscle in 5 ml of ice-cold sodium iodoacetic acid solution (5 mmol/l). The pH values were measured at room temperature with a Knick Portamess 752 pH meter (Berlin, Germany) equipped with a Mettler-Toledo Inlab 427 electrode (Columbus, OH, USA).

Determination of glycogen and lactate

For lactate and glycogen determinations, the samples were homogenised in ice-cold 0.1 mol/l phosphate buffer (pH 7.0) with a Polytron homogeniser (Thomas Scientific, USA), and the same extract was used for both lactate and glycogen determinations. The lactate content was determined spectrophotometrically (365 nm) using a commercial kit (Boeinger-Mannheim no. 139084; R-Biopharm AG, Darmstadt, Germany). Glycogen content was determined as the amount of total glucose by the method of Passoneau and Lowry (1993) using a Roche diagnostic kit no. 1447521 (Mannheim, Germany). The analysis method does not distinguish the origin of the glucose molecules, thus glycogen, glucose and glucose-6-phosphate all contribute to the total value for glucose. The glycolytic potential (GP), an estimate of the glycogen content present at the moment of slaughter, was calculated according to Monin and Sellier (1985):

$$GP = 2([\text{glycogen}] + [\text{glucose}] + [\text{glucose} - 6 - \text{phosphate}]) + [\text{lactate}].$$

Table 1. Temperature decrease in muscle slices initially chilled at 0°C or 20°C for 4 hours followed by storage at 4°C.

Time (h) <i>post mortem</i>	Chilling at 0°C		Chilling at 20°C	
	Temperature ¹	SEM	Temperature ¹	SEM
0.33	36.7 ^c	0.6	36.7 ^d	0.6
1	9.0 ^b	0.7	25.4 ^c	0.3
4	3.5 ^a	0.3	20.3 ^b	0.3
24	4.6 ^a	0.3	4.6 ^a	0.3

^{a,b,c,d}Values in the same column not sharing a common superscript differ significantly ($P < 0.05$).

¹ $n = 18$, except for 0.33 h *post mortem*, where $n = 16$.

The contents of glycogen, glucose and glucose-6-phosphate were determined simultaneously. Thus, regarding the present study, the formula can be written:

$$\text{glycolytic potential} = 2[\text{total glucose content}] + [\text{lactate}].$$

The reproducibility of the glycogen and lactate assays was determined as follows: a large muscle sample was ground in liquid nitrogen, divided into doses and stored at -80°C . This sample was analysed each time the samples were analysed. The coefficients of variation (CVs) were 10% for lactate content and 25% for glycogen content. Glycogen content, lactate content and pH were measured in duplicate.

Drip loss

To determine drip loss, the duplicate muscle slices cooled at 0°C and those initially cooled at 20°C were sewn into bags and hung freely, keeping the meat away from the drip. After storage at 4°C for 48 h *post mortem*, the samples were weighed again and the drip loss was expressed as a percentage of initial weight.

Cooking loss

After the drip loss measurements, the breast muscle slices were vacuum-packed (Multivac A 300/42, Sepp Haggemüller KG, Wolfertschwenden, Germany) in polyethylene bags and cooked in a water-bath at 85°C to a core temperature of 80°C. After cooking, the samples were cooled, drained and reweighed. Cooking loss was expressed as the percentage of pre-cooked weight.

Tenderness

Three pieces ($3 \times 1 \times 1$ cm) were cut from the cooked turkey slices parallel to the fibre axis and weighed. To determine shear force, an Allo-Kramer cutting device mounted in an Instron 4465 (Stockholm, Sweden) was used. Measurements with texturometer were

performed at room temperature. Samples were compressed perpendicular to the fibre axis, and the shear force was expressed in N/g.

Statistical analysis

The effects of different CAS methods on the turkey meat quality elements (drip loss, cooking loss, shear force, glycogen content, lactate content, glycolytic potential) were tested with a mixed model. The model included the stun-killing method as a fixed effect and the live weight of the animal at stunning as a covariate. The random element contained the day of slaughter. The effect of chilling temperature was tested with a similar mixed model, but taking repeated measurements into account. The effect of the CAS method on pH at different points was compared for the mixed model including the stun-killing method, as well as the interaction of stun-killing method and time *post mortem* as fixed effects, and the live weight of the animal was used as a covariate. The time *post mortem* was included as a repeated factor with the animal as the subject. The mean values for glycogen and lactate contents and glycolytic potential between different muscles of an animal were tested with paired sample *t*-tests. One-way ANOVA was used when the muscle slice temperature decrease during chilling was investigated. In all comparisons, appropriate conversions were applied to the variables to get a normal distribution of regression residuals when needed. The data from the two replicates were pooled since no statistically significant differences existed between the two groups of turkeys. The level of significance was set at $P < 0.05$. All statistical analyses were carried out using SPSS 15.0 for Windows (SPSS Inc., 2006).

Ethical aspects of the experiment were approved by the National Animal Ethics Committee in Finland.

RESULTS

Energy status of muscles

The values for breast and leg muscle glycogen content, lactate content and glycolytic potential of turkeys stun-killed using different CAS methods are presented in Table 2. The glycogen content was determined as the amount of total glucose, and thus the glycogen content also includes the glucose and glucose-6-phosphate contents. At 20 min *post mortem*, the glycogen content of the *pectoralis major* was higher in CO₂-stun-killed turkeys and tended to be higher in turkeys stun-killed using the biphasic

Table 2. Glycogen content (mM/kg), lactate content (mM/kg) and glycolytic potential (mM lactate equivalent/kg muscle) of turkey muscles (*M. pectoralis superficialis* and *M. gastrocnemius*). $n = 9$, except for biphasic stunning and leg muscle of N_2 -stunned birds, where $n = 8$.

	CO ₂			Biphasic			N ₂ /CO ₂			N ₂		
	95% CI			95% CI			95% CI			95% CI		
	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper	Lower
<i>M. pectoralis superficialis</i>												
Glycogen	37.7 ^b	32.7	43.0	37.8	32.5	43.4	32.5	27.9	37.4	30.7 ^a	26.3	35.5
Lactate	64.7	55.8	73.5	64.0	56.0	72.1	63.5	54.9	72.0	70.6	61.0	80.1
Glycolytic potential	139.9	129.8	150.8	138.0	127.5	149.3	129.4	120.2	139.5	132.2	122.7	142.5
<i>M. gastrocnemius</i>												
Glycogen	63.1	49.3	78.7	60.0	47.5	73.9	62.7	49.3	77.8	74.6	58.2	92.9
Lactate	22.0	16.0	27.9	20.2 ^a	15.1	25.3	22.0	16.3	27.6	26.4 ^b	20.2	32.5
Glycolytic potential	148.4	122.6	179.6	140.5 ^a	118.3	166.8	142.5	118.6	171.1	173.1 ^b	140.8	212.9

^{a,b}Values in the same row not sharing a common superscript differ significantly ($P < 0.05$).

method ($P = 0.05$) than in turkeys stunned with N_2 . The breast muscle lactate content was, however, similar regardless of the stunning method. By contrast, in the leg muscle (*M. gastrocnemius*) both glycolytic potential and lactate contents were higher in N_2 -stunned birds than in birds stunned with the biphasic method (Table 2). No statistically significant correlation was determined between breast muscle glycogen content and ultimate pH, or between glycolytic potential and ultimate pH.

Irrespective of the stunning method, the glycogen contents were higher and the lactate contents were lower ($P < 0.01$, for both) in the leg muscles than in the breast muscles. The glycolytic potential was significantly ($P < 0.05$) lower in the breast muscles than in the leg muscles of N_2 -stunned birds, while with other stunning methods, the glycolytic potentials in the leg and breast muscles were similar.

Change in muscle pH

The pH decrease was followed both in unloosened muscles (Figure 2, *M. pectoralis superficialis*; Figure 3, *M. gastrocnemius*) and in muscle slices chilled at different rates (Figure 4). Anoxic (98% N_2) stunned birds (Figures 2 and 3) displayed the lowest muscle pH values at one and two hours *post mortem*. At one hour *post mortem*, the breast muscle pH value of N_2 -stunned birds was significantly ($P < 0.05$) lower than that of birds stunned by any other method. In the leg muscle, the pH value was significantly ($P < 0.05$) higher in biphasic-stunned birds, and tended ($P = 0.06$) to be higher in CO_2 -stunned birds than in the N_2 -stunned birds. At two hours *post mortem*, the difference in breast muscle pH between N_2 -stunned and CO_2 -stunned birds, and between N_2 -stunned birds and

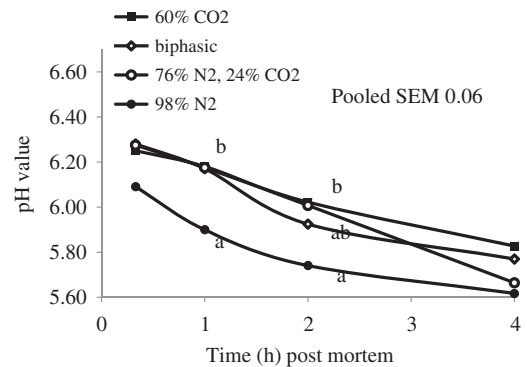


Figure 2. Decrease in pH of breast muscle of turkeys stunned with different gas mixtures. In different CAS methods, $n = 9$, except for the biphasic method, where $n = 8$. Values in the same time *post mortem* with different letters (a, b) indicate a significant ($P < 0.05$) difference in pH values.

those stunned with a mixture of 76% N_2 and 24% CO_2 , remained significant and tended ($P = 0.07$) to be significant between N_2 -stunned and biphasic-stunned birds. In the leg muscle at two hours *post mortem* the only statistically significant difference found was between N_2 -stunned and biphasic-stunned birds. At 4 h *post mortem*, there were no differences in pH values associated with different stunning methods (Figures 2 and 3), remaining so until 24 h *post mortem* (Table 3).

Besides the pH measurements of unloosened muscle, the effect of chilling temperature on the decrease in muscle pH was investigated. Tissue slices excised from breast muscles of N_2 - and CO_2 -stunned birds were chilled rapidly ($0^\circ C$) or slowly ($20^\circ C$). The pH decrease during chilling is presented in Figure 4. As in unloosened breast muscle, the difference in muscle pH between N_2 - and CO_2 -stunned birds became significant ($P = 0.029$) at 1 h *post mortem*. In CO_2 -stunned birds, the fast chilling increased

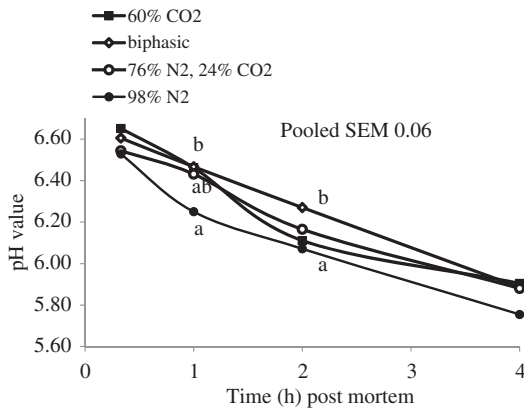


Figure 3. Decrease in pH of leg muscle of turkeys stunned with different gas mixtures. In different CAS methods, $n=9$, except for the biphasic method, where $n=8$. Values in the same time post mortem with different letters (a, b) indicate a significant ($P<0.05$) difference in pH values.

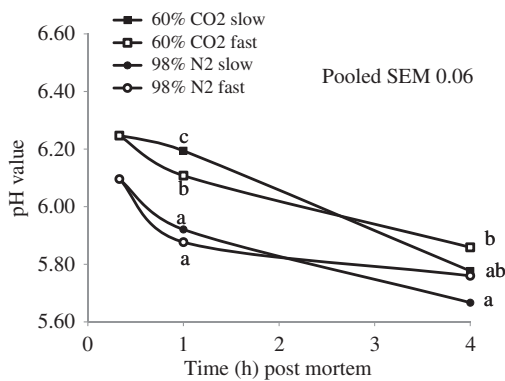


Figure 4. Decrease in pH of breast muscle of turkeys stunned with CO_2 or N_2 and chilled at different rates. For different stunning-chilling combinations, $n=9$. Values in the same time post mortem with different letters (a, b) indicate a significant ($P<0.05$) difference in pH values.

the early rate of pH decrease (1 h post mortem), but at 4 h post mortem the pH value of CO_2 -stun-killed fast-chilled birds was higher than the muscle pH value in N_2 -stun-killed slowly chilled birds, while other differences disappeared. In N_2 -stun-killed birds, the ultimate pH was attained 4 h post mortem irrespective of chilling rate, and no significant pH decrease occurred between 4 and 24 h post mortem. The change in pH was slower in birds stun-killed with a high concentration of CO_2 than in birds stun-killed with N_2 . CO_2 -stun-killed birds had not attained their ultimate pH by 4 h post mortem, since the fall in pH value was significant between 4 and 24 h post mortem in both slowly chilled ($P=0.026$) and rapidly chilled ($P<0.001$) muscles (Table 3, Figure 4). Neither the method of stun-killing, nor the chilling rate affected the ultimate pH of turkey breast muscle (Table 3).

Breast muscle quality

The stun-killing method alone had no significant effect on the quality of turkey breast muscle chilled at 0°C , but differences emerged when the rate of chilling was reduced (Table 3).

A lower chilling rate had an advantageous effect on muscle of birds stun-killed with CO_2 ; both the cooking loss and the Warner-Bratzler shear force were lower than in the corresponding fast-chilled muscles (Table 3). The breast muscle cooking loss of slower chilled CO_2 -stun-killed birds was significantly lower than that of any other stunning/chilling combination studied. A slower rate of chilling did, however, increase ($P=0.001$) breast muscle drip loss in both N_2 - and CO_2 -stun-killed birds relative to fast-chilled muscles (Table 3).

DISCUSSION

Effect of stun-killing gas on muscle post mortem energy metabolism

Anoxic stun-killing with N_2 was responsible for the lowest pH values during the first two hours post mortem, although the effect was not significant at every sampling time. The faster carbohydrate metabolism in N_2 -stun-killed birds was already evident 20 min post mortem as lower breast muscle glycogen content than in CO_2 -stun-killed birds, or in birds stun-killed with the biphasic method. However, the availability of muscle glycogen was not a limiting factor in muscle pH decrease for either stun-killing method, since no correlation existed between glycogen content and ultimate pH of muscle, and the method of stun-killing had no significant effect on ultimate pH.

The higher early post mortem muscle pH values after stun-killing with CO_2 -containing gas mixtures, compared with those after anoxic stunning, are consistent with earlier reports (Raj *et al.*, 1990b; Poole and Fletcher, 1995). Raj *et al.* (1992) reported that increasing the volume of CO_2 (10%, 20% or 30%) in argon mixtures resulted in an increase in early post mortem pH values (6.38, 6.60 and 6.61, respectively). A similar effect was recorded here, when a gas mixture of 76% N_2 and 24% CO_2 was used. The precise mechanism by which CO_2 retards the initial rate of pH decrease and rigor development post mortem is unknown. In CAS, increasing the amount of inert gas at the expense of CO_2 has been shown to cause more severe muscle convulsions during stunning and bleeding (Gerritzen *et al.*, 2000; McKeegan *et al.*, 2007b), as was also the case in the present study (unpublished results). A connection between severe wing flapping during anoxic stunning and rapid pH

Table 3. Meat quality factors of turkey breast muscle (*M. pectoralis superficialis*) after different stunning/chilling combinations. The muscle samples were initially chilled at 0°C or at 20°C for 4 hours, followed by storage at 4°C. n = 9, except for the biphasic method, where n = 8.

	CO ₂			Biphasic			N ₂ /CO ₂			N ₂		
	Mean	95% CI		Mean	95% CI		Mean	95% CI		Mean	95% CI	
		Upper	Lower		Upper	Lower		Upper	Lower		Upper	Lower
Ultimate pH												
(0°C)	5.69	5.60	5.78	5.71	5.62	5.81	5.74	5.65	5.83	5.75	5.65	5.84
(20°C)	5.67	5.58	5.76							5.68	5.59	5.77
Drip loss (%)												
(0°C)	0.6 ^a	0.4	0.9	0.6 ^a	0.4	0.8	0.5 ^a	0.3	0.7	0.6 ^a	0.4	0.9
(20°C)	1.3 ^b	0.9	1.9							1.6 ^b	1.0	2.5
Cooking loss (%)												
(0°C)	18.2 ^b	15.6	21.3	19.4 ^b	16.3	23.1	20.7 ^b	16.4	26.2	20.1 ^b	16.7	24.3
(20°C)	15.7 ^a	13.6	18.0							19.5 ^b	16.3	23.2
Warner-Bratzler shear (N/kg)												
(0°C)	184.0 ^b	110.5	306.1	188.7 ^b	114.8	310.4	126.3 ^{ab}	72.4	220.7	150.7 ^{ab}	85.0	267.2
(20°C)	97.6 ^a	55.0	173.3							130.6 ^{ab}	75.3	226.3

^{a,b}Values in the same quality element (row and column) not sharing a common superscript differ significantly ($P < 0.05$).

decrease has been proposed in many (Raj *et al.*, 1990b; Poole and Fletcher, 1995) but not all (Gault *et al.*, 2000) studies. Furthermore, the results of Papinaho *et al.* (1995) and Northcutt *et al.* (1998) suggest that the delay to early *post mortem* pH decrease caused by electrical stunning or CO₂ stunning is primarily a function of the elimination of *peri-mortem* struggle. However, this might not be the only reason for differences in the rate of pH decrease associated with different CAS methods. Sams and Dzuik (1995) found that the electrical stimulation-induced acceleration of glycolysis in breast muscles was not evident in broilers stunned with a minimum of 75% CO₂ in air prior to electrical stimulation.

The breast muscle glycogen contents recorded in this study correspond well with the results of Fernandez *et al.* (2001) and also with the results of Sante *et al.* (2000), who measured an average glycogen content of 53 mol/g in turkey breast muscle prior to stunning, and observed that glycogen stores were reduced by 40–50% of the resting value after high-frequency electrical stunning (150 mA, 480–600 Hz) and bleeding; while the reduction was 15–25% after low-frequency electrical stunning (150 mA, 50–300 Hz) and bleeding. Wing flapping during stunning or during bleeding was seen when high-frequency stunning was used (Sante *et al.*, 2000) and was probably the cause of the larger decrease in glycogen content. In the present study, there was no difference in glycolytic potential or lactate content of breast muscle associated with stunning method. The glycogen contents in turkey leg muscle (*M. gastrocnemius*) 20 min *post mortem* were

almost double those in *M. pectoralis superficialis*. Fernandez *et al.* (2001) also reported higher glycogen content in a muscle from a turkey leg (*M. iliotibialis*) than in the turkey's breast muscle. However, the initial lactate content of leg muscle was lower, and the rate of pH decrease was lower, than that in breast muscle in the present study, as well as in the study of Fernandez *et al.* (2001). In contrast to the breast muscle, no differences in the glycogen content of leg muscle were associated with the various CAS methods. This could be a consequence of the observation here of seemingly lighter convulsions in leg muscles compared with breast muscles during stunning (unpublished results). The present experiment was carried out under laboratory conditions and the birds were gently handled compared with commercial conditions. The birds were neither transported before harvesting nor raised or hung from their feet. This probably explains the high content of leg muscle glycogen.

Effects of CAS and rate of cooling on turkey breast muscle quality

Despite the low early *post mortem* pH values in N₂-stun-killed turkeys, the stun-killing method had no significant effect on fast-chilled turkey breast muscle quality (drip loss, cooking loss, tenderness). This is in contrast to the findings of others; increased drip loss (Wynveen *et al.*, 1999) and cooking loss (Northcutt *et al.*, 1998; Sante *et al.*, 2000) were reported for carcasses with rapid pH decrease. However, Raj (1994) found a decrease in cooking loss in anoxic stunned

birds with a higher rate of pH decrease compared with other stunning methods. A more rapid *post mortem* muscle pH decline was shown to occur after exposing poultry to the stress of crating, transport, heat and struggling (Froning *et al.*, 1978; Kannan *et al.*, 1997; McKee and Sams, 1997), all of which were absent from the present study. The rates of pH decrease were, however, in accordance with those of earlier gas stunning studies (Raj, 1994; Northcutt *et al.*, 1998) as well as with studies where electrical stunning was used (Sante *et al.*, 2000; Fernandez *et al.*, 2001).

The rate of breast muscle pH decrease in N₂-stun-killed birds was similar in meat chilled at 0°C to meat chilled at 20°C, and this was also the case with CO₂-stun-killed birds. However, McKee and Sams (1998) reported that unstunned but bled turkey carcasses kept at 20°C had slightly lower breast muscle pH at 2 h, and significantly lower breast muscle pH at 4 h *post mortem*, than carcasses kept at 0°C. They described similar breast muscle drip loss, cooking loss and shear force values after cooling at different temperatures. In the present study, by contrast, the rate of chilling had an effect on turkey breast meat quality, and slower chilling was particularly advantageous in CO₂-stun-killed birds, producing a decrease in cooking loss and shear force. The high shear force in fast-chilled CO₂-stun-killed birds could be a consequence of cold shortening, although both chilling regimes used in this study resulted in fairly rapid cooling. The small effect of chilling rate on the quality of meat from anoxic stun-killed birds is in agreement with the results of Raj (1994), who showed that the faster pH decrease in CAS (anoxia or hypercapnic anoxia) stunned turkeys compared with that for electrically stunned birds, had no adverse effect on meat quality (cooking loss or texture of cooked breast fillets), irrespective of carcass cooling rate (chilling at 3°C or 16°C). In the present study, slower chilling slightly increased the drip losses relative to faster chilling in both the N₂- and the CO₂-stun-killed birds.

In their review, Sosnicki *et al.* (1998) stated that a low pH (<5.8) combined with a high breast muscle temperature (>35°C) typically caused protein denaturation, leading to soft, watery and discoloured PSE meat. Despite the faster pH decrease in anoxic (N₂) stun-killed turkeys, none of the samples in this study entered this pH-temperature area, probably due to a rather high rate of cooling, even in the slower chilling group. At the same time, anoxic stunning seems to allow efficient chilling without an increase in toughness due to cold shortening (Raj *et al.*, 1990b), as was also the case in this study.

In the present study, it was found that N₂ is suitable for poultry stunning or stun-killing when

meat quality is of concern. Besides not having a negative effect on meat quality, a good stunning method instantaneously induces a state of unconsciousness and insensibility to pain that lasts until the death of the animal. Since the gas stunning or stun-killing methods do not induce instantaneous loss of consciousness, animal welfare during stunning is a matter of concern. CO₂ is widely used in CAS systems, although it is an acidic gas, pungent in high concentrations (Raj and Gregory, 1993; Raj, 1996; Lambooj *et al.*, 1999; McKeegan, 2004; McKeegan *et al.*, 2006), and is a potent respiratory stimulant that can cause breathlessness before inducing a loss of consciousness (Gregory *et al.*, 1990; Raj, 1996). Unlike CO₂, N₂ induces unconsciousness with only slight if any respiratory disruption (Raj, 1996; Webster and Fletcher, 2001; McKeegan *et al.*, 2007b). However, fierce muscle convulsions (wing flapping) frequently occur during anoxic stunning (Gerritzen *et al.*, 2000; McKeegan *et al.*, 2007a, b) and are associated with an increase in injuries like hemorrhaging during stunning (Raj *et al.*, 1997). This raises a welfare concern, since there is disagreement as to whether the bird is conscious when the convulsions start (McKeegan *et al.*, 2007b; Coenen *et al.*, 2009) or not (Raj *et al.*, 1998).

It has been suggested that some respiratory discomfort may be preferable to the risk of vigorous wing flapping and associated injury (Abeyesinghe *et al.*, 2007; McKeegan *et al.*, 2007a,b). Furthermore, CO₂ concentrations below 40% may induce only mild to moderate aversion in hens and broilers (McKeegan *et al.*, 2006) and it has been shown that biphasic CO₂-stunning results in less convulsing, fewer fractured wings and reduced breast meat hemorrhaging than anoxic stunning methods (McKeegan *et al.*, 2007a). Thus, biphasic CO₂-stunning and a moderate rate of chilling may represent the best compromise between meat quality and animal welfare during stunning with the various CAS methods currently available.

It can be concluded that anoxic stun-killing with N₂ has no adverse influence on meat quality. It is thus suitable for turkey stunning from a meat quality point of view, but may not be ideal from an animal welfare point of view. All of the CAS methods (N₂, mixture of 76% N₂ and 24% CO₂, CO₂, biphasic method) used in this study are suitable for turkey stun-killing when breast muscle quality is important. The rate of chilling has an impact on meat quality; carcasses after CO₂-stun-killing benefit from a lower rate of chilling, leading to reduced cooking loss and shear force, while carcasses after N₂-stun-killing may benefit from faster chilling.

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