METABOLISM AND NUTRITION

Water-Holding Capacity in Chicken Breast Muscle Is Enhanced by Pyruvate and Reduced by Creatine Supplements¹

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ABSTRACT In commercial production, chickens are subjected to feed withdrawal prior to slaughter and exposed to stress during transport and handling of the animals at the slaughterhouse; this causes plasma glucose and glycogen stores in liver and muscle to decrease, which has a negative impact on meat quality. The aim of the present study was to investigate how supplementation of the energy complements creatine and pyruvate during the fasting period would affect postmortem pH decrease, water-holding capacity, and color of the meat. Female Ross 208 broilers were supplemented with glucose combined with either pyruvate or creatine via the drinking water for 18 or 42 h prior to slaughter, i.e., before and throughout the fasting period. Chickens were slaughtered

at 42 or 43 d of age. Temperature and pH were measured at 1, 10, 30, and 45 min and 1, 2, 4, 8, and 12 h postmortem. The results showed that the pyruvate and glucose supplementation increased the pH at 45 min postmortem by 0.25 units and decreased drip loss of musculus pectoralis major (PM) by 50 to 65% in chickens supplemented for 42 h. The creatine and glucose supplementation reduced pH at 3 and 4 h postmortem by 0.32 to 0.42 units, increased the lightness (L*) by 2.3 to 5.6 units, and increased drip loss by 51 to 137% in the PM of chickens supplemented for 18 and 42 h. Pyruvate and glucose supplementation thus appear beneficial but whether this is concomitant with an overall improvement in meat quality remains to be determined.

(Key words: chicken, creatine, meat quality, pyruvate, supplementation)

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INTRODUCTION

In conventional broiler production, birds are subjected to feed withdrawal for 4 to 10 h prior to slaughter to reduce fecal contamination of slaughter equipment and bacterial spoilage of carcasses. During fasting, plasma glucose (Warriss et al., 1993; Edwards et al., 1999) and glycogen stores in the liver decrease (Warriss et al., 1988, 1993; Edwards et al., 1999; van-der-Wal et al., 1999), whereas muscle glycogen reserves are not (Warriss et al., 1993) or are only marginally affected (Warriss et al., 1988; Edwards et al., 1999). Stress exposure during transport and lairage, on the other hand, may cause severe glycogen depletion in the muscles. By increasing various energy depots in the muscle prior to slaughter, the anaerobic production of lactate postmortem may be delayed, thereby delaying a decline in pH. Postmortem muscle pH development exerts a strong influence on a number of meat quality attributes including water-holding capacity (WHC) and color development. Creatine supplementation to pigs has indicated that the moisture loss may be reduced (Berg and Allee, 2001), because increased creatine phosphate availability delays lactate formation and pH decline postmortem or because increased creatine phosphate creates increased intracellular osmotic draw (Hultman et al., 1996).

Creatine supplementation to humans increased creatine concentrations in the muscle, which has been shown to delay the onset of fatigue as a result of increased availability of creatine phosphate (Casey et al., 1996). Creatine concentrations in the muscle may be further improved by co-administration of glucose (Green et al., 1996), because high insulin levels in the blood stimulate creatine accumulation in the muscle (Steenge et al., 1998).

Pyruvate supplementation may activate the pyruvate dehydrogenase complex. In the ischemic muscle, activation of this complex increases creatine phosphate and decreases lactate concentrations (Timmons et al., 1996; 1998), which may delay the pH decline postmortem.

The aim of the present study was to investigate the effects on primarily WHC by supplementing chickens with glucose combined with creatine or pyruvate via the

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Abbreviation Key: a* = redness; b* = yellowness; IL = musculus iliotibialis; L* = lightness; PM = musculus pectoralis major; WHC = water-holding capacity.

drinking water immediately before and throughout the fasting period prior to slaughter.

MATERIALS AND METHODS

Birds and Management

One-day-old female chickens (Ross 208) were obtained from a commercial hatchery and raised in floor pens at the local research facility at the Danish Institute of Agricultural Sciences under standard conditions (density 12.5 chickens/m²) with free access to water and feed. Room temperature was gradually reduced from 33°C to 21°C during the first 21 d and maintained at 21°C for the rest of the study period. The relative humidity was maintained at 70%, and a 20L:4D regimen was used.

A total of 252 chickens were included in the investigation. Chickens were divided into 9 groups of 2 pens each (2 pens per 4 times 2 treatments and 2 pens for control, i.e., 18 pens). Treatment chickens received drinking water including 50 g/L glucose combined with 1) 9 g/L pyruvate + 1.75 g/L CaCl₂, 2) 18 g/L pyruvate + 1.75 g/L CaCl₂, 3) 9 g/L creatine, or 4) 15 g/L creatine; control chickens received pure water. All supplements were given for periods of 42 and 18 h prior to slaughter, respectively. Water was supplied from nipple drinkers until 6 d before slaughter when round bell-shaped drinkers were used. The introduction of round bell-shaped drinkers 6 d before slaughter was to customize the birds before supplementation was included. Water intake by the chickens during the supplementation period was recorded by weighing the round bell-shaped drinkers including supplements at the beginning and at the end of the supplementation period. Feed was withdrawn 10 h before slaughter. Chickens were slaughtered at 42 or 43 d of age at the local research slaughter plant of the Danish Institute of Agricultural Sciences.

Slaughtering Procedure

All animals were slaughtered on 2 successive days. At the day of slaughter, half of the chickens were collected in boxes according to expected time of slaughter (supplementation was thus given according to the time of slaughter) and transferred to the slaughterhouse on site at 0730 h. At the slaughterhouse chickens were shackled by the legs and hung on the processing chain for 4 min before passing through an electrified water bath (94 V:0.1 A for 40 s), killed by cutting the carotid arteries, and subsequently bled for 5 min. Carcasses passed through a 58 to 59°C warm scalding vessel (3.5 min) and plucker (2 min) before evisceration. Evisceration took place 15 min after

chickens were killed, and the carcasses were maintained at slaughterhouse temperature (approximately 18°C) until 1 h after killing, after which they were stored at 4°C until the last measurements at 12 h postmortem. For practical reasons it was not possible to carry out all the measurements on the same chicken; therefore, 90 of the birds, 10 from each group, were shackled and killed from 0750 to 0810 h. These birds were used for measurements of WHC and color. The remaining 36 chickens were shackled and killed in groups of 4 for determination of pH and temperature. These procedures were repeated on the second day of slaughter.

Temperature and pH Measurements

Temperature and pH were measured 1 (after stunning and bleeding), 10 (after scalding and plucking), 30, and 45 min and 1, 3, 4, 8, and 12 h postmortem in the right musculus pectoralis major (PM). At time points 1 and 10 min post mortem, chickens were taken off the chain during measurement and put back on the chain again. Temperature was measured with a Testo 110 insertion thermometer,³ and pH was measured with a pH meter Model 704,⁴ equipped with a Hamilton Tiptrode⁵ insertion glass electrode calibrated in buffers at pH 4.01 and 7.00⁶ at ambient temperature. After 1 h the carcasses were placed in a chilling room at 4°C.

WHC

Percentage drip loss was determined in musculus iliotibialis (IL) and PM 4 h after slaughter. Drip loss was determined essentially as described by Rasmussen and Andersson (1996). Briefly, PM and IL were taken from the carcass and samples were cut using a 25-mm cork borer at a right angle to the muscle fiber direction. Samples were placed in a special container equipped with lid to avoid evaporation, left at 4 to 6°C for 48 h, and drip loss was determined by weighing. Muscle fiber direction of the samples was horizontal to gravity, not vertical, as described in the original method (Rasmussen and Andersson, 1996). This compromise was made to allow comparison of drip loss between IL and PM. Otherwise IL is too thin for determining drip loss by this method.

Color

Color was measured 4 h after slaughter using a Minolta reflectance colorimeter⁷ and reported in the CIE system values of lightness (L*), redness (a*), and yellowness (b*). Samples were bloomed for 1 h at 4°C prior to determination of color from 3 and 4 replicate measures on IL and PM, respectively.

Statistical Analysis

The data were analyzed by the methods of least squares using the MIXED procedure (SAS, Version 8.02⁸). For the statistical analyses of the variables pH and temperature

³Testo 110, Testo, Lenzkirch, Germany.

⁴Metrohn M. 704, Metrohn, Herisau, Switzerland.

⁵Hamilton Tiptrode, Hamilton, Bonaduz, Switzerland.

⁶Radiometer, Copenhagen, Denmark.

⁷Minolta Chroma Meter CR-300, Minolta, Osaka, Japan.

⁸SAS Institute Inc., Cary, NC.

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TABLE 1. Means and SE of water consumption (L/bird) from 2 pens per treatment

		Supplementation time						
Supplement	Concentration	18 h	SE	42 h	SE			
Control	2 (7	0.386	0	0.568	0.056			
Pyruvate ¹	9 g/L	0.284	0.007	0.771	0.040			
,	18 g/L 9 g/L	0.398 0.229	0.017 0.010	0.875 0.636	0.035 0.050			
Creatine ¹	15 g/L	0.264	0.040	0.714	0.010			

¹Included 50 g/L of glucose.

in PM, the statistical model included the fixed effects of supplement, supplement level, treatment period, time points postmortem, and the random effects of slaughter day and bird within supplement, supplement level, treatment period, and slaughter day.

For the statistical analyses of the variables WHC, measured as drip loss, and meat color (L*, a*, and b* values) in PM and IL, the statistical model included the fixed effects of supplement, supplement level, treatment period, muscle, and the random effects of slaughter day and bird within supplement, supplement level, treatment period, and slaughter day. In the analysis of WHC, the initial sample weight was included as a covariate in the model if significant. All 2-way interactions were tested and included if significant. Results are presented as least squares means \pm SEM (unless otherwise stated) and considered to be significantly different if P < 0.05.

RESULTS

Water and Supplement Consumption

The water (including supplementation) intake varied between groups (Table 1), in that chickens supplemented with the highest concentrations of pyruvate and creatine consumed the highest amounts of water including supplement. However, differences in water including supplement waste between groups cannot be excluded.

pН

The creatine supplements reduced pH in PM at 3 and 4 h postmortem, and supplementation for 42 h also reduced pH after 8 h compared with that in the control group (Table 2). The pH of PM from chickens supplemented with pyruvate was higher overall at 45 min and 1 h postmortem compared with that of the control (P < 0.05) and the creatine-supplemented chickens (P < 0.001). At these particular time points, chickens supplemented with pyruvate for 42 h gave a higher pH than those supplemented for only 18 h (P < 0.05).

Temperature

The temperature in the PM of chickens fed creatine and pyruvate differed from that of the control chickens at various time points. There was increased temperature in pyruvate-supplemented chickens at 30 min, 45 min and 1 h postmortem and reduced temperature at 3 h postmortem (Table 3). However, the temperature in Table 3 does not reflect that of commercially slaughtered chickens but is valid for comparisons among the experimental groups.

WHC

The WHC was reduced in PM from all birds receiving creatine supplement, i.e., higher drip loss, and WHC was

TABLE 2. Least square means of pH in musculus pectoralis major of chickens at various time points postmortem (n = 8)

			Creatine supplement ¹				Pyruvate supplement ¹			
Time			18 h		42 h		18 h		42 h	
postmortem	SEM	Control	9 g/L	15 g/L	9 g/L	15 g/L	9 g/L	18 g/L	9 g/L	18 g/L
1 min	0.02	6.29 ^{ab}	6.19 ^{bc}	6.29 ^{ab}	6.26 ^{abc}	6.35 ^a	6.04 ^d	6.13 ^{cd}	6.21 ^{abc}	6.23 ^{abc}
10 min	0.02	6.16^{a}	6.10^{a}	6.04^{a}	6.14^{a}	6.19 ^a	5.88^{b}	6.15^{a}	6.09^{a}	6.16^{a}
30 min	0.02	6.01 ^a	6.04^{a}	5.85 ^b	6.09 ^a	6.10^{ab}	5.99 ^a	6.10^{a}	6.13 ^a	6.10^{a}
45 min	0.02	5.92 ^b	5.92^{b}	5.89 ^b	5.89^{b}	5.93 ^b	6.00^{b}	6.03 ^{ab}	6.16^{a}	6.17^{a}
1 h	0.02	5.98 ^{abcd}	5.97 ^{cde}	5.86 ^e	5.85^{e}	5.92 ^{de}	6.08 ^{abc}	6.04^{abcd}	6.19^{a}	6.13 ^{ab}
3 h	0.02	6.30^{a}	5.88^{c}	5.89 ^c	5.92 ^c	5.87^{c}	6.35^{a}	6.09 ^b	6.34^{a}	6.22ab
4 h	0.02	6.26 ^a	5.92 ^c	5.91 ^c	5.92 ^c	5.94 ^c	6.31 ^a	6.10^{b}	6.21 ^{ab}	6.20^{ab}
8 h	0.02	6.04^{abc}	6.05 ^{abc}	5.92 ^{cde}	5.86 ^{de}	5.84^{e}	6.05 ^{abc}	6.00 ^{bcd}	6.08^{ab}	6.16^{a}
12 h	0.02	5.94 ^{bcd}	5.98 ^{bc}	5.95 ^{bcd}	5.85 ^{cd}	5.81 ^d	5.99 ^{bc}	5.98 ^{bc}	6.02 ^{ab}	6.14 ^a

^{a-e}Means within a row lacking a common superscript differ (P < 0.05).

¹Included 50 g/L of glucose.

TABLE 3. Least square means of temperature ($^{\circ}$ C) in musculus pectoralis major of chickens at various time points postmortem (n = 8)

			Creatine supplement ¹				Pyruvate supplement ¹			
Time			18	3 h	42	2 h	18 h		42 h	
postmortem	SEM	Control	9 g/L	15 g/L	9 g/L	15 g/L	9 g/L	18 g/L	9 g/L	18 g/L
1 min	0.1	41.1a	40.1 ^{bc}	40.0°	40.4 ^{abc}	39.3 ^d	40.8ab	40.9 ^{ab}	41.0a	40.7 ^{abc}
10 min	0.1	41.5a	41.0^{abc}	41.3^{ab}	40.9^{abc}	40.4^{c}	41.2^{abc}	40.6^{ab}	41.1^{abc}	$40.7^{\rm abc}$
30 min	0.1	34.9 ^{cd}	34.1 ^{de}	33.9e	35.6abc	34.1^{de}	35.7 ^{ab}	35.7 ^{abc}	36.3a	35.3 ^{bc}
45 min	0.1	31.3^{f}	31.7^{def}	32.5 ^{bc}	32.8 ^{bc}	31.6^{ef}	33.3 ^{ab}	$32.4^{\rm cde}$	33.7^{a}	32.5 ^{bcd}
1 h	0.1	29.8 ^{de}	30.2^{cde}	30.1^{cde}	31.3^{ab}	29.8 ^e	31.2ab	30.8 ^{bcd}	31.7^{a}	30.8^{bc}
3 h	0.1	10.2^{a}	9.8 ^{ab}	9.9 ^{ab}	10.4^{a}	9.2 ^{bc}	9.4^{bc}	9.2 ^{bc}	10.4^{a}	8.7^{c}
4 h	0.1	$7.7^{\rm abc}$	$7.7^{\rm abc}$	8.1 ^a	$7.7^{\rm abc}$	7.0^{bc}	7.3 ^{bc}	6.9 ^c	7.8^{ab}	$7.7^{\rm abc}$
8 h	0.1	$4.0^{\rm b}$	$4.0^{\rm b}$	4.8^{ab}	4.8^{ab}	4.4^{ab}	4.2^{ab}	4.1^{b}	4.6^{ab}	4.9^{a}
12 h	0.1	4.0^{a}	3.6 ^a	3.8 ^a	3.8 ^a	4.0^{a}	3.9 ^a	3.7 ^a	3.7 ^a	3.6 ^a

^{a-e}Means within a row lacking a common superscript differ (P < 0.05).

further reduced after 42 h of supplementation compared with 18 h of supplementation (P < 0.001) (Table 4).WHC was increased in PM from chickens receiving pyruvate supplement for 42 h but was not significantly affected when supplemented for only 18 h. No differences in WHC were observed from IL.

Color

Chickens receiving creatine supplement had lighter (higher L*) PM compared with control and pyruvate-supplemented chickens as a whole (P < 0.001), and PM from chickens supplemented for 42 h were lighter than those supplemented for only 18 h (P < 0.001) (Table 5). Also yellowness (b*) was increased in PM from chickens receiving creatine, except those receiving the low concentration for 18 h (P = 0.20). The color of IL was not affected by any of the treatments [means (SEM): L* = 51.1 (0.2), a* = 5.60 (0.12), b* = 4.76 (0.08)].

DISCUSSION

All chickens receiving a combination of creatine and glucose had reduced pH in PM at 3 and 4 h postmortem. This reduction might have been caused by increased glycogen synthesis induced by myotube swelling (Low et al., 1996) from the osmotic draw created by increased creatine phosphate (Hultman et al., 1996). Total muscle creatine (Balsom et al., 1995), especially creatine phos-

phate (Balsom et al., 1994; Casey et al., 1996), has been shown to increase in the muscle upon creatine supplementation and more so when co-administered with glucose (Green et al., 1996).

The WHC was reduced (higher drip loss) in PM from all animals receiving creatine-glucose supplement. This finding was contrary to the hypothesis of increased WHC of meat from creatine-supplemented pigs (Berg and Allee, 2001; Maddock et al., 2002); however, not all studies confirm this theory (O'Quinn et al., 2000). WHC was not affected in IL, possibly because the fiber type composition is different from that of PM (lower proportion of fiber type II) (Schreurs, 2000), which might have resulted in only minor increases in creatine phosphate (Balsom et al., 1994) and glycogen compared with that of PM from creatine-supplemented chickens.

The color of PM was lighter from chickens receiving creatine supplement, an attribute also observed in creatine-supplemented pigs (Stahl et al., 2001).

In accordance with other studies on broilers, the lower pH in PM was concomitant with a lighter color (higher L*) (Fletcher, 1999) and reduced WHC (Dransfield and Sosnicki, 1999). All of the meat quality attributes, e.g., decreased pH, reduced WHC, and increased lightness, were more pronounced after 42 h than after 18 h of creatine supplementation, indicating further incorporation of creatine after 18 h of supplementation.

Stahl et al. (2001) found improvements in the quality of fresh pork when supplementing pigs for 5 d but reduced

TABLE 4. Least square means of water-holding capacity expressed as percentage of drip loss from musculus pectoralis major (PM) and musculus iliotibialis (IL) (n = 20)

				Creatine supplement ¹				Pyruvate supplement ¹			
			18	3 h	42 h		18 h		42 h		
Muscle	SEM	Control	9 g/L	15 g/L	9 g/L	15 g/L	9 g/L	18 g/L	9 g/L	18 g/L	
PM IL	0.06 0.06	1.80 ^d 0.47	2.72 ^{c2} 0.61	3.09 ^{bc2} 0.52	4.27 ^a 0.43	3.52 ^{b2} 0.58	1.57 ^d 0.53	1.54 ^d 0.49	0.63 ^e 0.44	0.90 ^e 0.50	

 $^{^{\}mathrm{a-e}}\mathrm{Means}$ within a row lacking a common superscript differ (P < 0.05).

¹Included 50 g/L of glucose.

¹Included 50 g/L of glucose.

 $^{^{2}}P = 0.06$ for difference between means with superscript c.

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TABLE 5. Least square means of color determinations: L* (lightness), a* (redness) and b* (yellowness) of musculus pectoralis major (n = 20)

			Creatine supplement ¹				Pyruvate supplement ¹			
		18 h		18 h 42 h 18 h		h	42	h		
Color	SEM	Control	9 g/L	15 g/L	9 g/L	15 g/L	9 g/L	18 g/L	9 g/L	18 g/L
L* a* b*	0.7 0.25 0.24	50.2 ^a 4.00 ^{bc} 3.98 ^{ab}	52.5 ^{bc} 4.28 ^c 4.35 ^{bcd}	53.5° 3.80 ^{bc} 4.60 ^{cd}	55.5 ^d 4.31 ^c 5.31 ^e	55.8 ^d 3.60 ^{ab} 4.84 ^{de}	50.4 ^a 3.55 ^{ab} 4.15 ^{abc}	49.6 ^a 3.51 ^{ab} 3.72 ^a	51.1 ^{ab} 4.36 ^c 4.24 ^{abcd}	49.5 ^a 3.03 ^a 3.69 ^a

^{a-e}Means within a row lacking a common superscript differ (P < 0).05.

quality when supplementing for 10 or 15 d. In the present study broilers were supplemented with a combination of glucose and creatine, whereas pigs in the referred studies were supplemented with creatine alone, at a dose approximately 5 to 10 times lower when calculated as grams of creatine per kilogram of animal (chickens drank on average 0.33 L/d). Conversely, the duration of the period of supplementation was approximately 3 to 20 times longer for the pigs. Although these very different experimental designs make it difficult to compare results, it seems as if high concentrations or long supplementation periods (possibly different limits for different species) of creatine to meat-producing animals in the final days or hours before slaughter may have negative effects on meat-quality parameters.

Chickens supplemented with pyruvate showed increased pH and temperature at 45 min and 1 h postmortem, and chickens supplemented for 42 h also had increased WHC. Both increased pH and WHC were more pronounced after 42 h than after 18 h of pyruvate supplementation, indicating further incorporation of pyruvate after 18 h of supplementation.

There is no obvious explanation for the increased temperature of pyruvate supplemented chickens, and the concomitant increase in WHC is contrary to observations on pigs (Schäfer et al., 2002). However, at elevated pH, denaturation of proteins would be reduced which might result in the observed increase in WHC compared with that of control chickens.

In conclusion, a combined glucose and creatine supplement to broilers decreased pH, increased the lightness, and reduced WHC, all of which appear unfavorable in relation to meat quality. Conversely, a combined glucose and pyruvate supplement reduced the rate of pH decline and increased WHC in PM, which may appear as an improvement in meat quality; however, the high pH caused by pyruvate supplementation may negatively influence shelf life and processability, and the low pH by the creatine supplementation may be beneficial to shelf life and processability. Consequently, we consider it premature to draw any conclusions on the effect of the supplementations used on overall meat quality.

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