# CREATINE AND GLUCOSE IN DRINKING WATER REDUCE $PH_{(3-4H)}$ AND WATER-HOLDING CAPACITY IN CHICKEN BREAST MUSCLE

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## Background

In conventional broiler production, animals are subjected to feed withdrawal for 4-10 h prior to slaughter, in order to reduce faecal contamination of slaughter equipment and bacterial spoilage of carcasses. During fasting, plasma glucose and glycogen stores in the liver decrease, whereas muscle glycogen reserves supposedly are only marginally affected. Decreased glucose/glycogen is expected to delay and reduce the production of lactate *post mortem*, and consequently delay the pH drop *post mortem* and increase the ultimate pH. *Post mortem* pH development, which is critical for a number of meat quality parameters, may be improved by energy-supplements via the drinking water. Increased intake of glucose as readily available substrate for the glycolysis, may sustain ATP production to some extend delaying lactic acid formation in the muscles *post mortem*. Furthermore, high insulin levels in the blood in response to glucose intake stimulate creatine accumulation in the muscle [1]. Creatine supplementation to humans increased creatine concentrations in the muscle, which has been shown to delay the onset of fatigue, possibly due to improvements in the ability to sustain ATP rephosphorylation from ADP as a result of increased creatine phosphate availability [2]. Additionally, creatine supplementation to pigs has indicated that moisture loss may be reduced [3]. Creatine phosphate availability was increased primarily in the fast twitch muscle fibres [2], which are the main fibre type in the breast muscle of domestic chickens. Creatine supplements to chickens are thus expected to also affect creatine concentrations particularly in the breast muscles.

## Objectives

The objectives of the present study were to delay the decrease in pH *post mortem*, decrease the ultimate pH and increase water-holding capacity, by supplementing chickens with glucose and creatine via the drinking water, immediately before and throughout the fasting period prior to slaughter.

## Methods

Chickens (Ross 208) were raised at our local research facility under standard conditions with free access to water and feed. Chickens were given drinking water including 50 g/l glucose combined with either 9 g/l creatine (low) or 15 g/l creatine (high), or control chickens receiving pure water. The supplements were given for periods of 18 or 42 h prior to slaughter respectively. The day before slaughter six control chickens were injected intravenously with an overdose of pentobarbital, and biopsies for determination of resting levels of metabolites were taken after 10 minutes. Chickens were slaughtered at 42 days of age, and feed was withdrawn 10 h before slaughter. The pH was measured 1, 10, 30, 45 min and 1, 3, 4, 8 and 12 h *post mortem* in *M. pectoralis major* (PM), and biopsies were sampled at the same time points, frozen immediately in liquid nitrogen and stored at – 80 °C. After one hour the carcasses were placed in a chilling room at 4 °C. Colour (L*) was determined by four replicate measurements four hours after slaughter on samples bloomed for one hour at 4 °C (only L* is shown), using a Minolta Croma Meter CR-300 (Osaka, Japan). Water holding capacity (WHC) was determined in PM four hours after slaughter as drip-loss, essentially as described by Rasmussen and Andersson (1996) [4]. The concentrations of muscle metabolites (glycogen and lactate) were determined as described elsewhere [5]. The data was statistically analysed using Proc. Mixed (mixed models ANOVA) in the SAS package, version 8.02.

## Results and discussions

Chickens were supplemented with a mixture of creatine and glucose on the basis of a pilot study showing a reduction in breast muscle pH in chickens supplemented with a combination of creatine and glucose, whereas creatine and glucose alone did not affect pH *post mortem* (data not shown). In humans, the ingestion of creatine in combination with carbohydrate increased the muscle content of total creatine approximately 60% more than when creatine was ingested alone [6]. The beneficial effect of combined administration of creatine and carbohydrate was ascribed partly to insulin-induced stimulation of creatine accumulation in the muscle [1]. Although the insulin concentrations in human blood necessary to trigger the increased creatine accumulation was deemed to be physiologically high, this mechanism may be at least partly responsible for the effect in chickens of co-administration of creatine and glucose.

This theory seemed to be confirmed in the present study where chickens supplemented with the high dose of creatine had increased glycogen levels up to 60 min *post mortem* compared to those supplemented with the low dose. However, the control chickens had glycogen levels comparable to chickens supplemented with the high creatine dose. This apparent difference may be categorised as a chance finding, but also large variations between individuals, as has also been described for creatine-supplemented humans [7], have also affected the results. When comparing glycogen and lactate contents in breast muscle from chicken with *M. longissimus dorsi* (LD) contents from pigs, it seems as if chickens are more sensitive towards stress and/or subjected to more stress full conditions prior to slaughter i.e. through transportation and electrical stunning, compared to conditions for pigs. At rest, assuming pentobarbital does not affect the metabolism, the levels of glycogen and lactate in chicken breast muscle was 50.7 µmol/g (SEM: 2.4, n=6), and 13.3 µmol/g (SEM: 0.7, n=5) respectively. One minute after slaughter the glycogen levels had decreased to an average of 19.5 µmol/g (SEM: 1.8, n=36), and lactate increased to 57.1 µmol/g (SEM: 2.4, n=36). In resting chickens the glycogen content in the breast muscle was approximately 35 % lower than that in LD from resting pigs [5], whereas lactate contents are comparable in the respective muscles from resting animals. One minute *post mortem* glycogen levels decreased by approximately 20% and 60% and lactate increased by 80% and 330% in pig [5] and chicken muscle respectively.

The low glycogen content in breast muscle from chickens supplemented with the low concentration of creatine most likely was caused by a temporary increased sensitivity to stress. The initial glycogen concentrations in the live animals were assumed to be similar for all groups as they displayed similar final concentrations of lactate and glucose. Despite differences in glycogen and lactate production *post mortem*, all chicken groups receiving creatine supplements had reduced pH in the breast muscle at 3 and 4 h *post mortem*, and both groups supplemented for 42 h also had reduced pH 8 h *post mortem* when compared to that of control chickens (Table 1). In accordance with other studies on broilers, the lower pH in the breast muscle was concomitant with a lighter colour [8] (higher L*) and increased drip-loss [9] (Table 2). These results are contrary to those reported for pigs supplemented with 25 g creatine monohydrate (CMH)/day for 5 and 10 days prior to slaughter, in which *M. semimembranosus*, but not loin, had a higher ultimate pH (24 h *post mortem*) and tendencies towards lower lightness and greater myofibre hydration [3]. Others found no effects of meat quality parameters following CMH supplementation to pigs for 10 days [10], or obtained higher shear force values and, as in line with our results, increased lightness after 10 and 15 days of CMH supplementation to pigs

[11]. Stahl *et al.* (2001) found improvements in the quality of fresh pork when supplementing pigs for 5 days, but reduced quality when supplementing for 10 or 15 days. In the present study broilers are supplemented with a combination of glucose and creatine whereas the referred studies on pigs are supplemented with creatine alone, at a dose approximately 5-10 times lower when calculated as g creatine/kg animal (chickens drank in average 0.33 l/d). On the other hand, the pigs were supplemented for approximately 3-20 times as long. These very different experimental set ups make it difficult to compare results, however, it seems clear that creatine supplement to meat producing animals in the final days/hours before slaughter may have negative results on meat quality parameters.

**Conclusions**

Chickens supplemented with a combination of glucose and creatine showed a lower pH in breast muscle at 3 and 4 h *post mortem*, increased lightness and increased drip-loss compared to that of control animals. The decreased pH was maintained for longer, lightness was higher and the increase in drip-loss was greater in chickens supplemented for 42 hours compared to those supplemented for 18 hours. In conclusion, a combined glucose and creatine supplement to broilers showed a negative effect on the meat quality parameters analysed in the present study.

**Table 1**. Least square means of pH (n=8), glycogen (µmol glucose/g muscle; n=4) and lactate (µmol glucose/g muscle; n=4) of *M. pectoralis major* at various time points *post mortem* Chickens were supplemented for 18 or 42 h with 50 g/l glucose combined with either 9 g/l creatine (low), 15 g/l creatine (high) or controls receiving water.

| Creatine | | Time *post mortem* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Supplement | | 1 min | 10 min | 30 min | 45 min | 1 h | 3 h | 4 h | 8 h | 12 h | SE |
| Low, 18 h | pH | 6.16 | 6.07 | 6.02 | 5.90 | 5.96 | 5.93*** | 5.98*** | 6.13 | 6.06 | 0.06 |
| | Glycogen | 4.9*** | 5.8*** | 4.6*** | 5.0 | 2.0** | 3.8 | 2.6 | 3.8 | 3.0 | 3.1 |
| | Lactate | 57.7 | 74.6 | 76.2 | 81.2 | 86.5 | 97.2* | 85.5*** | 106.3 | 100.6 | 5.7 |
| Low, 42 h | pH | 6.23 | 6.11 | 6.06 | 5.87 | 5.83 | 5.97*** | 5.98*** | 5.94* | 5.93 | 0.06 |
| | Glycogen | 10.9*** | 17.5** | 9.4* | 8.5 | 5.1* | 5.9 | 9.0 | 6.9 | 8.9 | 3.1 |
| | Lactate | 68.3* | 76.4 | 96.6** | 96.2 | 100.7 | 106.4 | 114.3 | 108.0 | 110.6 | 5.7 |
| High, 18 h | pH | 6.26 | 6.01 | 5.83* | 5.87 | 5.85 | 5.94*** | 5.97*** | 6.00 | 6.03 | 0.06 |
| | Glycogen | 25.0 | 27.1 | 17.3 | 14.7 | 11.5 | 7.0 | 4.6 | 6.5 | 5.3 | 3.1 |
| | Lactate | 58.6 | 64.7 | 84.6 | 94.6 | 100.4 | 107.3 | 107.8 | 113.6 | 110.1 | 5.7 |
| High, 42 h | pH | 6.32 | 6.16 | 8.08 | 5.91 | 5.91 | 5.93*** | 6.01*** | 5.92* | 5.89 | 0.06 |
| | Glycogen | 21.5 | 20.6* | 23.6 | 16.6 | 14.1 | ND | 9.6 | 6.4 | 7.0 | 3.1 |
| | Lactate | 60.2 | 64.7 | 80.9 | 84.3 | 98.3 | ND | 111.1 | 113.1 | 114.7 | 5.7 |
| Control | pH | 6.26 | 6.13 | 5.99 | 5.90 | 5.97 | 6.35 | 6.33 | 6.12 | 6.02 | 0.06 |
| | Glycogen | 28.9 | 29.7 | 20.6 | 12.5 | 15.4 | 9.8 | 8.1 | 4.6 | 5.6 | 3.1 |
| | Lactate | 52.4 | 66.2 | 73.4 | 89.6 | 92.1 | 116.5 | 120.4 | 111.8 | 108.2 | 5.7 |

*Different from control values at specific measurements within a column (*p<0.05, **p<0.01, ***p<0.001). ND: not determined.

**Table 2**. Least square means of drip-loss (%) and L* (lightness) of M. *pectoralis major*. Chickens were supplemented for 18 or 42 h with 50 g/l glucose combined with either 9 g/l creatine (low), 15 g/l creatine (high) or controls receiving water.

| | Control | Low, 18 h | Low, 42 h | High, 18 h | High, 42 h | SE |
|---|---|---|---|---|---|---|
| Drip-loss (%) | 1.80 | 2.72*** | 4.27*** | 3.09*** | 3.52*** | 0.16 |
| L* (lightness) | 50.4 | 51.8* | 53.0*** | 52.2* | 52.6** | 0.5 |

*Different from control values within rows (*p<0.05, **p<0.01, ***p<0.001).

**Pertinent literature**


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