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Effects of Creatine Monohydrate on Growth Performance, Carcass Characteristics and Meat Quality of Yellow-Feathered Broilers

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Abstract: Nine hundred, 42 days old male yellow-feathered broilers were used to evaluate the effects of Creatine Monohydrate (CMH) on growth performance, carcass characteristics and meat quality. The birds received either a no-CMH diet (0 mg kg^{-1}) a low (250 mg kg^{-1}), a moderate (500 mg kg^{-1}) or a high-CMH diet (1000 mg kg^{-1}) for 21 days. CMH supplementation did not affect the average daily weight gain, average daily feed intake or feed efficiency. Additionally no significant differences were discovered in the dressing, eviscerated, breast muscle or thigh muscle percentage. However, there was a decreasing trend in the pH values of the pectoralis major at 24 h postmortem and of the thigh muscle at 45 min postmortem as the level of CMH supplementation increased. No significant difference was observed in cooking loss, shear force value, moisture or crude protein percentages. However, supplementing with CMH increased the slow-twitch red and the fast-twitch white fiber ratio in the gastrocnemius muscles. The results suggested that CMH supplementation has no effect on growth performance or carcass characteristics but it potentially triggered a transition from fast-twitch red muscle fibers to slow-twitch red and fast-twitch white muscle fibers.

Key words: Creatine, broiler, growth performance, carcass characteristics, meat quality

INTRODUCTION

Creatine is normally produced in the liver, kidneys and pancreas from glycine, arginine and methionine. For animals, phosphocreatine metabolism is an important energy buffering pool that is rapid, direct and efficient so that Adenosine Triphosphate (ATP) remains stable at the cellular level. However, creatine continues to be broken down in the body's metabolic processes. Thus, many animals such as growing broilers are not able to produce enough creatine in modern intensive farming conditions (Casey, 2011). Research in humans has revealed that dietary supplementation with Creatine Monohydrate (CMH) at 20 g day^{-1} for 5 days increased the intramuscular creatine load by 20% (Greenhaff, 1996). An increase in intramuscular creatine has also been reported to significantly improve the recovery rate of the knee extensor muscle function in centrifugal types of muscle damage (Cooke *et al*., 2009). The study of Niessen *et al*. (2008) showed that oral supplementation with CMH can repair mitochondrial damage. Additionally, Kley *et al*. (2007) used CMH to treat hypokinesia patients and found that their energy parameters and lean body mass were greatly improved.

Two possible mechanisms suggest that creatine may enhance muscle performance and protein synthesis. Due to an increase in the amount of energy stored as phosphocreatine, creatine-loaded muscle has the capacity to maintain normal physiological function and to delay the onset of muscle fatigue (Casey *et al*., 1996). Increased concentrations of intramuscular phosphocreatine attract water into the muscle cell and increase the cell volume (Hultman *et al*., 1996). Earlier research has suggested that creatine can help the body quickly provide ATP through the creatine-phosphocreatine Energy Shuttle System, improve the muscle aerobic metabolism enhance the biological oxidation of mitochondria, maintain the ATP concentration and buffer muscle lactic acid accumulation (Bessman and Carpenter, 1985). However, the results of creatine application in pigs have varied based on their experimental conditions, animal varieties, diet nutrition level and feeding environment. For example, in a study to evaluate the effect of meat quality of Duroc and Landrace pigs by supplementing with CMH in water at a level of 50 g day^{-1} for 5 days before slaughter, Lindahl *et al*. (2006) found that CMH can increase the storage of phosphocreatine in muscle, postpone the drop in pH value and give rise to less red and yellow color at 24 h

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postmortem. Subsequently, Rosenvold *et al.* (2007) attempted to replicate the findings in crossbred pigs but the results revealed that there was no effect on meat quality including pH, water-holding capacity, color, juiciness or tenderness. Therefore, the specific mechanism behind the initial result requires further study.

The study of creatine application in poultry production is rare in the literature. Henckel treated 208 Ross broilers with a creatine and glucose solution for 18 and 42 h before slaughter and the results showed that the pH value was increased at 45 min postmortem and decreased in the drip loss of the musculus pectoralis major after slaughter. Another study conducted by Nissen and Young (2006) obtained similar results. These studies implied that supplementing creatine with carbohydrates could moderate the ultimate postmortem pH decline and improve the water-binding capacity. Hence, the objective of this study is to determine whether supplementing with creatine in the corn-soybean meal broiler basal diet is a potential method to improve broilers growth performance, carcass characteristics and meat quality.

MATERIALS AND METHODS

Birds and management: Nine hundred, 42 days old male yellow-feathered broilers (Lingnan yellow broilers, a quality meat-type chicken that enters the market at 63 days of age) with a mean initial BW of 0.73±0.01 kg were randomly assigned to four treatments with 5 pens per treatment and 45 birds per pen. The birds received a no-CMH diet (0 mg kg^{-1}) or a low (250 mg kg^{-1}) a moderate (500 mg kg^{-1}) or a high-CMH diet (1000 mg kg^{-1}) for 21 days (i.e., 42 and 63 days old). All procedures were approved by the Committee on Animal Experiments of South China Agricultural University. The birds were reared in floor pens (2×3 m each) under 24 h light at a mean temperature of 25 and were fed standardized meals. The basal diet composition and nutritional contents are shown in Table 1. Feed and drinking water were available *ad libitum* during the study. Birds fasted overnight and were weighed before slaughter.

Slaughtering procedure: Four birds per pen were slaughtered on day 21 of the experiment (that is 63 days old birds) and 5 mL blood was taken from the vein axils before slaughter. Within 15 min postmortem, the carcass was plucked to determine carcass weight. The intestines, windpipe, reproductive organs, gall bladder, spleen, esophagus and the content and cuticle of the gizzard were then removed and the semi-eviscerated carcass weight was obtained. The semi-eviscerated carcass was further processed to remove the head, neck, legs, heart, liver, proventriculus, gizzard and abdominal fat and the eviscerated weight was obtained. The breast muscles (pectoralis major and minor) and the leg muscles (boneless drum and thigh) were weighed after separation. The entire left Pectoralis Major (PM) and Thigh Muscle (TM) of the broilers were sampled for the determination of meat quality. Then, muscle samples were stored at 4°C for 24 h and the pH value, meat color, water-holding capacity and tenderness characteristics were determined. Muscle samples for histological analysis were obtained from the right side of carcass, then cut into 0.5×0.5×1.0 cm specimens and quickly preserved in liquid nitrogen until needed for analysis. Fascia and fat were also removed from the right side of the carcass and stored at -30°C for analyzing the nutritional composition of the meat.

Table 1: Ingredients and nutrient contents of basal diet

Ingredients (%)	Values	Nutrient content[2] (%)	Values
Corn	71.73	Metabolisable energy (MJ kg^{-1})	13.17
Soybean meal	14.30	Crude protein (g kg^{-1})	170.00
Corn gluten meal	6.40	Calcium	0.80
Mixed grease	2.98	Total phosphorus	0.57
Dicalcium phosphate	1.62	Available phosphorus	0.38
Limestone	1.14	Salt	0.35
Salt	0.33	Lysine	0.85
DL-Methionine (98%)	0.02	Met+Cys	0.69
Lysine (65%)	0.42	Threonine	0.68
Threonine (98%)	0.06	Tryptophan	0.16
Premix[1]	1.00		
Total	100.00		

[1]Added per kg of diet: DL-Methionine, 1200 mg; retinylpalmitate, 5.5 mg; cholecalciferol, 0.05 mg; DL-α-tocopheryl acetate, 40 mg; menadione, 3 mg; thiamin, 3 mg; riboflavin, 4.5 mg; pyridoxine, 7 mg; cyanocobalamin, 0.03 mg; nicotinic acid, 50 mg; Ca-pantothenate, 8 mg; folic acid, 1.5 mg; choline chloride, 600 mg; Mn, 80 mg as $MnSO_4 \cdot H_2O$, Fe, 80 mg as $FeSO_4 \cdot H_2O$, Zn, 50 mg as ZnSO ·H Q; Cu, 10 mg as $CuSO_4 \cdot 5H_2O$; Co, 0.4 mg as $CoSO_4$, iodine 0.35 mg as KI, Se, 0.25 mg as $NaSeO_3$. [2]Calculation is according to Feed Composition and Nutritive Values in China. 2009. 20th Edn.

Growth performance measurements and carcass characteristics: Growth performance was determined on day 21 of the treatment (the birds were 63 days old). Prior to slaughter, body weight and feed consumption data were obtained from every pen to calculate the Average Daily Feed Intake (ADFI), Average Daily Gain (ADG) and feed efficiency (F:G). The carcasses were eviscerated and dissected manually and then the dressing-out percentage, semi-eviscerated percentage, eviscerated percentage, breast and leg muscle percentage were evaluated according to the procedure described by Wang (2002).

Meat quality measurement: Muscle pH was determined using an electronic pH meter (METTLER TOLEDO pH 310, BemPu Instruments Pte Ltd. Switzerland) at 45 min

($pH_{45\ min}$) and 24 h postmortem ($pH_{24\ h}$ also called ultimate pH). Each sample was measured 3 times and the average value was taken as the final result. At 45 min and 24 h postmortem, the meat color was measured in duplicate by an Opto-star Chroma Meter (OPTOSART Ltd. Japan) on the exposed cut surface of the PM and TM. The Opto-star Chroma Meter shone a small amount of infrared light on the surface of the meat and the color was determined by the reflected light. The moisture-binding property was assessed as the weight loss in cooking and the yield of the cooked product (Millet *et al*., 2005). For shear values at 24 h postmortem, approximately 20 g (fresh weight) of muscle was heated for 20 min in zip-sealed plastic bags in a water bath at 85°C. After cooling to the ambient temperature, the shear force value was measured in triplicate as described by Froningl and Uijttenboogaart (1988).

Meat nutritional composition measurement: The dry matter of meat was obtained by drying at 125°C to a constant weight (AOAC, 2002). Crude Protein (CP), referring to the international standard ISO 937:1978 Meat and meat products determination of nitrogen content was applied. Intramuscular Fat (IMF) was determined by ether extraction in a Soxhlet apparatus after acid hydrolysis (ISO 1443:1978).

Postmortem muscle fiber: The early steps of postmortem muscle fiber assessment include selection and dyeing where in serial transverse muscle sections (10 μm) were obtained from each frozen muscle sample using a cryostat microtome (Leica, Germany) at -21°C and mounted on glass slides. Myosin Adenosine Triphosphatase (ATPase) and Succinate Dehydrogenase (SDH) activity were detected after acid (pH 4.3) pre-incubation (Solomon and Dunn, 1988). Then, sample slides were examined with a Motic BA310 biological microscope (Motic China Group Co., Ltd. China) equipped with Motic Images Advanced 3.2 Software. Micrographs were obtained at 100x magnification. Six views were captured from each section for further analysis. Indices of fiber ratio (%), fiber area (μm^2) and fiber density (number per mm^2) were calculated for all types of muscle fibers.

Statistical analysis: Descriptive statistics were calculated using ANOVA by SAS (version 9.1, SAS Institute Inc., North Carolina-USA). The data were presented as means with SEM. The pen was used as the experimental unit. Student-Newman-Keuls multiple range tests were applied to compare treatment means. The linear and quadratic effects of CMH supplementation were analyzed using a contrast statement. A $p<0.05$ was considered statistically significant.

RESULTS

Growth performance: The effects of CMH on yellow-feathered broilers growth performance are shown in Table 2. Feeding CMH did not significantly affect the average final body weight, Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) or feed efficiency (F:G) of yellow-feathered broilers from days 42-63. However, CMH supplementation numerically reduced the ADFI and F:G compared to the control group. At the beginning of the feeding trial, there was no significant difference in the average initial body weight among the different levels of CMH-supplemented groups.

Carcass characteristics: Data on Dressing Out (DO), Semi-Eviscerated (SEV), Eviscerated (EV), Pectoralis Major (PM) and Thigh Muscle (TM) percentages are depicted as the carcass shown in Table 3. There was no significant difference in the dressing out, semi-eviscerated, eviscerated, breast muscle and leg muscle percentages among the different levels of CMH-supplemented groups. This finding revealed that supplementing with CMH for 21 days before slaughter has no effect on the carcass characteristics of yellow-feather broilers.

Meat quality: Table 4 shows the effects of CMH supplementation on the meat quality of yellow-feathered broilers. There was an obvious decreasing trend in pH

Table 2: Effect of Creatine Mono Hydrate (CMH) on yellow-feathered broilers growth performance

	CMH (mg kg^{-1})					p-value	
Parameters	0	250	500	1000	SEM	Linear	Quadratic
Initial BW (kg)	0.727	0.727	0.734	0.727	0.016	-	-
Final BW (kg)	1.560	1.569	1.550	1.565	0.014	0.937	0.977
ADG (g/birds)	39.600	40.10	38.80	39.90	0.300	0.882	0.898
ADFI (g/birds)	109.800	108.0	107.2	107.8	0.900	0.423	0.596
F:G	2.770	2.700	2.760	2.710	0.030	0.693	0.918

BW = Body Weight, ADG = Average Daily body weight Gain, ADFI = Average Daily Feed Intake, F:G = the ratio to ADFI and ADG. The results are expressed as means with SEM, n = 5. Means with different letters within a low are significantly different ($p<0.05$)

Table 3: Effect of Creatine Monohydrate (CMH) on yellow-feathered broilers carcass characteristics

	CMH (mg kg^{-1})					p-value	
Parameters (%)	0	250	500	1000	SEM	Linear	Quadratic
DO	91.08	91.10	90.35	89.97	0.29	0.112	0.272
SEV	91.78	92.16	92.01	91.63	0.16	0.676	0.482
EV	75.06	75.03	75.17	74.98	0.18	0.949	0.973
BM	14.10	13.65	14.49	14.29	0.20	0.446	0.714
LM	19.77	19.29	19.35	19.96	0.17	0.692	0.265

DO = Dressing Out, SEV = Semi-Eviscerated weight, EV = Eviscerated weight, BM = Breast Muscle weight, LM = Leg Muscle weight. The results are expressed as means with SEM, n = 5. Means with different letters within a low are significantly different ($p<0.05$)

Table 4: Effect of Creatine Mono Hydrate (CMH) on yellow-feathered broilers meat quality

	CMH (mg kg^{-1})					p-value	
Parameters	0	250	500	1000	SEM	Linear	Quadratic
Pectoralis major							
$pH_{45\ min}$	6.30	6.23	6.27	6.240	0.03	0.548	0.461
$pH_{24\ h}$	6.27^{a}	6.08^{bc}	6.13^{b}	6.050^{c}	0.01	<0.001	<0.001
Opto-$star_{45\ min}$	13.80	13.50	13.10	12.10	0.32	0.117	0.238
Opto-$star_{24\ h}$	14.80	14.50	15.50	16.30	0.33	0.079	0.197
$CL_{24\ h}$ (%)	15.10	16.95	16.19	16.68	0.28	0.071	0.112
$SFV_{24\ h}$ (N)	14.10	13.20	15.10	16.50	0.47	0.032	0.046
Thigh muscle							
$pH_{45\ min}$	6.69^{a}	6.71^{a}	6.67^{a}	6.510^{b}	0.02	0.004	0.014
$pH_{24\ h}$	6.56	6.48	6.55	6.480	0.02	0.285	0.566
Opto-$star_{45\ min}$	15.50	15.70	15.80	16.40	0.22	0.207	0.344
Opto-$star_{24\ h}$	16.90^{c}	17.6^{c}	20.00^{a}	20.90^{a}	0.39	<0.001	<0.001
$CL_{24\ h}$ (%)	20.07	19.31	20.17	20.17	0.40	0.754	0.855
$SFV_{24\ h}$ (N)	14.40	14.30	14.10	16.00	0.44	0.226	0.240

Opto-$star_{45\ min}$ = Color of muscle at 45 min postmortem, Opto-$star_{24\ h}$ = Color of muscle at 24 h postmortem, $CL_{24\ h}$ = Cooking loss of muscle at 24 h postmortem, $SFV_{24\ h}$ = Shear Force Value of muscle at 24 h postmortem. The results are expressed as means with SEM, n = 5. Different letters indicate significant differences (a<b<c, p<0.05)

Table 5: Effect of Creatine Monohydrate (CMH) on yellow-feathered broilers meat nutritional composition on fresh weight basis

	CMH (mg kg^{-1})					p-value	
Parameters (%)	0	250	500	1000	SEM	Linear	Quadratic
Pectoralis major							
Moisture	79.42	79.86	79.73	80.01	0.10	0.052	0.143
CP	18.77	18.33	18.36	17.98	0.11	0.011	0.044
IMF	0.38^{b}	0.36^{b}	0.44^{ab}	0.48^{a}	0.02	0.010	0.021
Thigh muscle							
Moisture	79.26	79.40	80.07	79.34	0.15	0.564	0.358
CP	17.35	16.79	16.75	16.89	0.19	0.550	0.659
IMF	1.96	1.69	1.55	1.34	0.16	0.166	0.394

CP = Crude Protein, IMF = Intra Muscular Fat of muscle. The results are expressed as means with SEM, n = 5. Different letters indicate significant differences (a<b, p<0.05)

value at 24 h postmortem in PM (linear, p<0.001, quadratic and p<0.001) and at 45 min postmortem in TM (linear, p = 0.004, quadratic and p = 0.014) as CMH supplementation was increased. However, there was no significant difference in the TM pH value at 24 h postmortem or in the PM value at 45 min postmortem. To some extent, CMH supplementation had different effects in different muscle tissues. Supplementation with 1000 mg kg^{-1} CMH suspended the pH value decline after 24 h postmortem in TM. Additionally, CMH supplementation at different levels presented an increasing trend (linear, p<0.001, quadratic and p<0.001) of TM color at 24 h postmortem as CMH supplementation increased. However, no significant differences were observed in the cooking loss and shear force values of both PM and TM at 45 min and 24 h postmortem.

Meat nutritional composition: The nutritional composition including moisture, CP and IMF of yellow-feathered broiler meat is presented in Table 5. There was no significant difference in the moisture percentage and CP of either PM or TM. However, a significant difference existed in the IMF of PM which showed an increasing trend (linear, p = 0.010, quadratic and p = 0.021) as CMH supplementation increased.

Table 6: Effect of Creatine Mono Hydrate (CMH) on fiber ratio, area and density in gastrocnemius muscle at early postmortem

	CMH (mg kg^{-1})					p-value	
Parameters	0	250	500	1000	SEM	Linear	Quadratic
Fiber ratio (%)							
βR	10.03^{c}	15.59^{a}	13.26^{b}	12.12^{b}	0.28	<0.001	<0.001
αR	46.22^{a}	36.80^{c}	33.46^{c}	36.73^{c}	0.56	<0.001	<0.001
αW	41.84^{c}	46.40^{b}	51.53^{a}	51.40^{a}	0.66	<0.001	<0.001
Area (μm^2)							
βR	1411.00^{b}	1716.00^{a}	1600.00^{ab}	1382.00^{b}	38.00	0.988	0.001
αR	1848.00^{ab}	1992.00^{a}	1972.00^{a}	1737.00^{b}	27.00	0.351	0.002
αW	2565.00^{a}	2317.00^{ab}	2484.00^{a}	2102.00^{b}	41.00	0.001	0.004
Density (No./mm^2)							
βR	38.00^{c}	63.00^{a}	63.00^{a}	46.00^{b}	1.00	0.052	<0.001
αR	168.00^{a}	136.00^{b}	127.00^{c}	137.00^{b}	2.00	<0.001	<0.001
αW	171.00^{c}	184.00^{b}	190.00^{ab}	200.00^{a}	3.00	<0.001	<0.001

βR = Slow-twitch red myofiber, αR = Fast-twitch red myofiber, αW: Fast-twitch white myofiber. The results are expressed as means with SEM, n = 5. Different letters indicate significant differences (a<b<c, p<0.05)

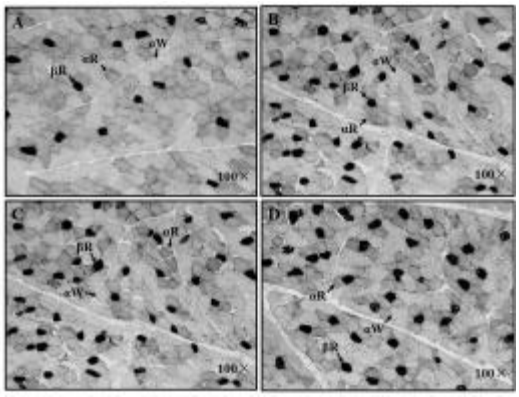

Fig. 1: Gastrocnemius muscle after staining with ATPase and SDH Enzymatic Histochemical Method

Characteristics of early postmortem of muscle fibers: Three types of gastrocnemius muscle fiber are shown in Fig. 1. The fiber ratio, cross-sectional area and density of different myofibers in the gastrocnemius muscle are shown in Table 6. The results showed that supplementing with CMH increased the slow-twitch red (βR) and the fast-twitch white (αW) fiber ratio with increasing trends (linear, p<0.001, quadratic and p<0.001) as the CMH supplementation increased. Meanwhile, CMH markedly reduced the fast-twitch red (αR) fiber ratio in the gastrocnemius muscle (linear, p<0.001, quadratic and p<0.001) the result of which was practically the same as reducing density. Interestingly, the concentration of

CMH supplement affected the cross-sectional area of the myofibers in the gastrocnemius muscle. The cross-sectional areas of βR and αR myofibers demonstrated a quadratic tendency (quadratic, p = 0.001 and p = 0.002 for βR and αR, respectively) as CMH supplementation increased.

DISCUSSION

This study has shown that CMH supplementation in corn-soybean meal broiler rations did not affect yellow-feathered broilers growth performance, meat nutritional composition or carcass characteristics but did lead to a decrease in the pH value of PM at 24 h and the ratio and density of αR myofibers in the gastrocnemius muscle at early postmortem. These findings were in agreement with the results of Nissen and Young (2006). Creatine and phosphocreatine swiftly replenish ATP in vertebrates which require a high and variable-Energy Buffer System. Thus, creatine supplementation is expected to reduce the intake of average daily feed as it can efficiently compensate for the energy requirements of the body with the creatine and phosphocreatine shuttle. Guzik *et al.* (2000) reported that creatine added to nursery pig diet for 14 days significantly increased ADFI and decreased F:G. However, James *et al.* (2002) found that feeding CMH did not affect ADG, ADFI or F:G in finishing pigs. Practically, a numerical decrease in ADFI in CMH treatments was observed in the study groups compared to the control group. Possible reasons for this effect include the duration of the treatment period, the offered dosage, the breed and age of the animal, the individual metabolism and the intrinsic state.

Kley *et al.* (2007) used CMH to treat hypokinesia patients and found that lean body mass was greatly improved. Both Casey and Greenhaff (2000) and Mihic *et al.* (2000) reported that CMH ingestion has significantly increased total body and fat-free mass in humans. It is inferred that lean body mass increased by CMH supplementation is linked with protein metabolism in the body. In this way, creatine supplementation is equal to energy intake for increasing the storage of phosphocreatine. This result implies that when energy intake increases, protein accretion also increases linearly until it reaches an upper limit. Pettigrew and Esnaola (2001) emphasized that a large number of growing pigs cannot consume enough energy to maximize the protein accretion rate and this situation can sometimes continue up to market weight. Additionally, Young *et al.* (2007) found that both IGF-I and myostatin mRNA expressions were decreased in M. longissimus dorsi of a Duroc pig fed with a basal diet supplemented with CMH at concentrations of 25 and 50 g day^{-1}. However, the present findings revealed that no significant differences were observed in the final body weight, ADG, carcass traits (i.e., DO, EV, BM and LM percentage) or the CP of TM and PM. This result is consistent with the research of Rosenvold *et al.* (2007). Consequently, further research on the mechanism of protein synthesis and degradation and the level of protein by CMH supplementation is needed.

In postmortem muscle, lactic acid is produced by the transformation of the substrates glycogen, glucose and glucose-6-phosphate through glycolysis. Lactic acid accumulation and the release of protons from ATP hydrolysis in postmortem muscle induce a pH decline (Bendall, 1979). Thus, the ultimate pH of meat is highly dependent upon the amount of glycogen present in the muscle (Allen *et al.*, 1997). In the study, a decreasing trend (linear, $p<0.001$, quadratic and $p<0.001$) in the pH values of PM at 24 h was presented as CMH supplementation increased indicating that CMH supplementation may augment the glycogen content of PM with a CMH supplementation dose-independent increase. From the corn-soybean meal nutrient contents (Table 1) a high glucose intake from carbohydrates is most likely preserved as glycogen in the liver and is used subsequently as a source of energy during transportation and metabolism, leaving glycogen stores in the muscles at a high level. The high glycogen level in the muscles at slaughter will lead to a high production of lactic acid and thus a low $pH_{24\ h}$. Therefore, researchers inferred that creatine supplementation may improve the absorption of glucose into muscle. The correlation between absorption of creatine and glucose in broilers is still needed. Further, $pH_{24\ h}$ in TM displayed no difference among the treatments, although CMH supplementation at the level of 1000 mg kg^{-1} gave rise to a significant decline in $pH_{45\ min}$. This result showed that a high level of CMH supplementation can postpone the pH value decline within 24 h postmortem in TM. Researchers speculate that a high level of CMH supplementation may reduce lactic acid accumulation for the decrease in glycogen storage in TM.

The lightness and yellowness of meat color were found to correlate negatively to pH (Allen *et al.*, 1997). Thus when the pH declined the meat color's lightness and yellowness increased. The findings demonstrate that the meat color of TM at 24 h postmortem was increasingly dark (linear, $p<0.001$, quadratic and $p<0.001$) as CMH supplementation increased. However, it was observed that the pH value of TM at 24 h postmortem was not significantly affected by CMH supplementation. One possibility is that CMH supplementation increases the concentration of intramuscular phosphocreatine in TM and the increased concentrations of intramuscular

phosphocreatine subsequently attract water into the muscle cells thus increasing the cell volume (Hultman *et al*., 1996). More water molecules in the muscle would cause more light to be absorbed by the muscle and the meat would thereby appear darker in color (Kauffman and Marsh, 1987; Cornforth, 1994).

Tenderness, as determined by shear force value is an important index for the evaluation of meat quality. A lower shear force value implies improved tenderness. However, muscle fiber characteristics may also affect meat tenderness. Lengerken *et al.* (1997) discovered that muscles having a low fiber density but with large cross-sectional fibers are prone to rapid postmortem pH value decline and high drip losses which is known to alter meat tenderness. Gondret *et al.* (2006) added that a lower tenderness of meat is partially due to a larger mean myofiber cross-sectional area. Thus, researchers also determined muscle fiber characteristics at early postmortem. Because only αW myofiber is exhibited in PM (Sams *et al*., 1992; Zhang *et al*., 2009) here we emphasize that muscle fibers in the gastrocnemius muscle displayed three myofiber types (Fig. 1) after staining with ATPase and SDH Enzymatic Histochemical Method (Solomon and Dunn, 1988). A significant increase in both the fiber ratio and the density of βR and αW myofibers was found, comparing CMH treatments to the control group as well as a decline in αR myofiber (Table 6). Moreover, different levels of CMH supplementation presented increasing trends (linear, $p<0.001$, quadratic and $p<0.001$) of βR and αW myofiber ratios as CMH supplementation increased which led to essentially the same result as density. Slow-twitch myofibers (also called βR myofibers) contain more mitochondria and myoglobin and utilize aerobic oxidation for their energy requirements whereas fast-twitch myofibers (including αR and αW myofibers) contain more glycogen and produce energy through anaerobic pathways (Gattenlohner *et al*., 2002). Thus, researchers inferred that CMH supplementation can alter glucose oxidation by increasing the oxidative (βR) and glycolysis (αW) muscle fiber ratio. The response of the cross-sectional area of βR and αR to CMH supplementation at different levels has been observed to demonstrate a quadratic tendency (quadratic, $p = 0.001$ and $p = 0.002$ for βR and αR, respectively) as CMH supplementation increased. A high level of CMH supplementation can potentially decrease the βR and αR cross-sectional area of the gastrocnemius muscle. However, in the present study, no significant differences were observed in the shear force value of TM after CMH supplementation. A potential reason is the difference in effects on muscle fiber ratio, density and cross-sectional area of TM in response to CMH supplementation at different levels.

CONCLUSION

CMH supplementation did not affect yellow-feathered broilers growth performance, carcass characteristics, meat quality attributes (water-holding capacity and color), edible quality (tenderness) or meat nutritional composition. However, it contributed to a significant decrease in pH value at 24 h postmortem of PM and a decline in buffering pH value within 24 h postmortem in TM at high levels of CMH supplementation. Meanwhile, it may lead to the transition from fast-twitch red muscle fibers to slow-twitch red and fast-twitch white muscle fibers in the gastrocnemius muscle. Consequently, researchers can only draw a speculative conclusion that CMH holds a potential application value in altering broilers meat quality to a certain extent. In future research, researchers intend to further investigate the mechanisms that act on the transition of muscle fiber types by CMH supplementation.

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