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Concentrations of creatine, creatinine, carnosine, and anserine in bovine longissimus muscle and their correlations with carcass and palatability traits

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Concentrations of creatine, creatinine, carnosine, and anserine in bovine longissimus muscle and their correlations with carcass and palatability traits

by

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AD: Alzheimer's disease

ALS: amyotrophic lateral sclerosis

AGAT: arginine: glycine amidinotransferase

BBB: blood brain barrier

B-CK: brain cytosolic creatine kinase

CK: creatine kinase

CN1: serum carnosinase

CN2: tissue carnosinase

CNS: central nervous system

Cr: creatine

Crn: creatinine

Fat thickness: fat thickness measured at 12^{th} rib

GAA: guanidino acetate

GAMT: S-adenosyl-L-methionine: N-guanidinoacetate methyltransferase

HCW: hot carcass weight

HD: Huntington's disease

KPH fat percentage: percentage of kidney, pelvic, and heart fat

M-CK: muscle cytosolic creatine kinase

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

mtPTP: mitochondrial permeability transition pores

OAT: δ-aminotransferase

PCr: phosphocreatine

PD: Parkinson's disease

ROS: reactive oxygen species

sMtCK: sarcomeric mitochondrial creatine kinase

uMtCK: ubiquitous mitochondrial creatine kinase

WBSF: Warner-Bratzler shear force

TBARS: Thiobabituric acid reactive substances

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Last, but not least, my deepest gratitude goes to my parents for their endless love and continuous support.

ABSTRACT

Creatine and carnosine have been considered as valuable nutraceuticals in meat products for a long time. Creatine as energy buffer plays an important role in cellular metabolism and shows protective effects in neurodegenerative diseases such as Parkinson's disease and Huntington's disease. Carnosine has multiple physiological functions associated with anti-oxidation and anti-aging. Although creatine and carnosine can be synthesized endogenously in human, studies show health benefits of creatine and carnosine supplementation. Because creatine and carnosine exist naturally only in animal muscle and brain tissues, meat is an important source of dietary creatine and carnosine. Few studies, however, have focused on beef creatine and carnosine contents and the factors influencing their concentrations.

The objective of this study was to investigate the variation of creatine and carnosine contents in longissimus muscle of Angus cattle and their correlations with carcass and palatability traits. Longissimus muscle samples were collected from 2,342 Angus cattle fed in Iowa (n=1,114), Texas (n=455), Colorado (n=391), and California (n=382). Creatine, creatinine, carnosine, and anserine were extracted from beef and quantified by high-performance liquid chromatography. The concentrations of these four compounds then were correlated with carcass and palatability traits including hot carcass weight (HCW), kidney pelvic heart (KPH) fat percentage, ribeye area, fat thickness, marbling score, calculated yield grade, sensory panel scores, Warner Bratzler shear force (WBSF), and thiobabituric acid reactive substances (TBARS).

Statistical analysis showed that the gender of cattle significantly ($P < 0.05$) affected the

concentrations of creatine, creatinine, carnosine and anserine. Bulls contained greater creatine content than did steers ($P < 0.05$) but produced lower amount of carnosine than did heifers ($P < 0.05$). Significant variation from feeding location was also observed. The correlations with carcass quality and sensory scores were significant but weak, which suggested that beef quality, especially tenderness, juiciness, and flavor, would not be strongly affected by selecting for higher creatine and carnosine contents.

THESIS ORGANIZATION

This thesis is presented in the form of one complete manuscript for submission to the Journal of Animal Science as a partial fulfillment of the requirements for a Master of Science degree. The title of the paper is "Concentrations of creatine, creatinine, carnosine, and anserine in bovine longissimus muscle and their correlations with carcass and palatability traits" It is formatted according to the requirement of the journal, with an abstract, introduction, materials and methods, results and discussion, implication, and references. The manuscript is preceded by a literature review section and followed by a general summary section. The general literature review introduces background information of the topics discussed in the manuscript. The summary section provides discussion about general conclusions and future research.

CHAPTER 1

GENERAL INTRODUCTION

Creatine and creatinine

I. Structure information

Creatine (Cr) (2-(1-methylcarbamimidamido) acetic acid) is a nitrogenous organic acid that exists naturally in vertebrates and plays an important role in cellular energy metabolism by interconversion to its high-energy phosphorylated analogue phosphocreatine (PCr). Creatine is in equilibrium with its spontaneously formed cyclic derivative creatinine (Crn) (2-amino-1-methyl-1*H*-imidazol-4-ol) in solution. In vivo, the conversion of Cr to Crn is irreversible and at an almost constant rate. Creatinine is excreted through urine. The chemical structures of Cr, PCr and Crn are shown at Figure 1.

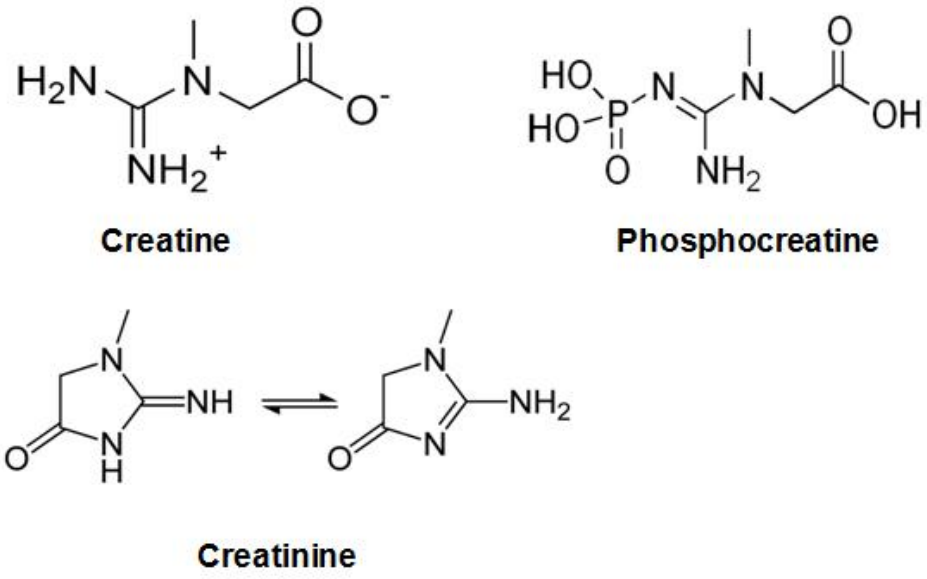

Figure 1. Chemical structures of creatine, phosphocreatine, and creatinine. (Adopted from Wyss et al, 2000)

II. Creatine kinase reaction

Cr/PCr/CK system functions as a shuttle of high-energy phosphate between sites of ATP production and ATP consumption in tissues with high and fluctuating energy demand such as muscle, heart and brain (Wallimann et al., 1992; Wyss and Kaddurah-Daouk, 2000). In higher vertebrates, four creatine kinase (CK) isoforms with specific functions have been found. They are named on the basis of tissue expression and subcellular distribution: two in muscle, muscle cytosolic isoform (M-CK) and sarcomeric mitochondrial isoform (sMtCK); two in brain, brain cytosolic isoform (B-CK) and ubiquitous mitochondrial isoform (uMtCK) (Beard and Braissant, 2010; Wyss and Schulze, 2002). As shown in Figure 2, the mitochondria creatine kinases consume ATP to convert Cr to its high-energy counterpart PCr for export to cytosol. The cytosolic creatine kinases dephosphorylate PCr and convert ADP to ATP for energy consumption.

Tissues with Cr/PCr/CK system (such as muscle, heart, and brain) have several advantages compared with tissues relying solely on ATP/ADP system, such as liver. First, PCr and Cr, relative to ATP and ADP, are smaller and less negatively charged. They can accumulate to much higher intracellular concentrations and have a faster diffuse coefficients than ATP and ADP (Wyss and Kaddurah-Daouk, 2000). Meanwhile, the free energy change ($\Delta G^{\circ\prime}$) (pH 7.0) of PCr hydrolysis is −45.0 kJ/mol compared with −31.8 kJ/mol for ATP, allowing Cr/PCr/CK system with a higher level of phosphorylation buffering ability (Schlattner, 2003). Finally, by stabilizing cellular ATP/ADP ratio, the Cr/PCr/CK system

also may protect the cell from unnecessary trigger of multiple metabolic responses (Schlattner, 2003; Wyss and Kaddurah-Daouk, 2000).

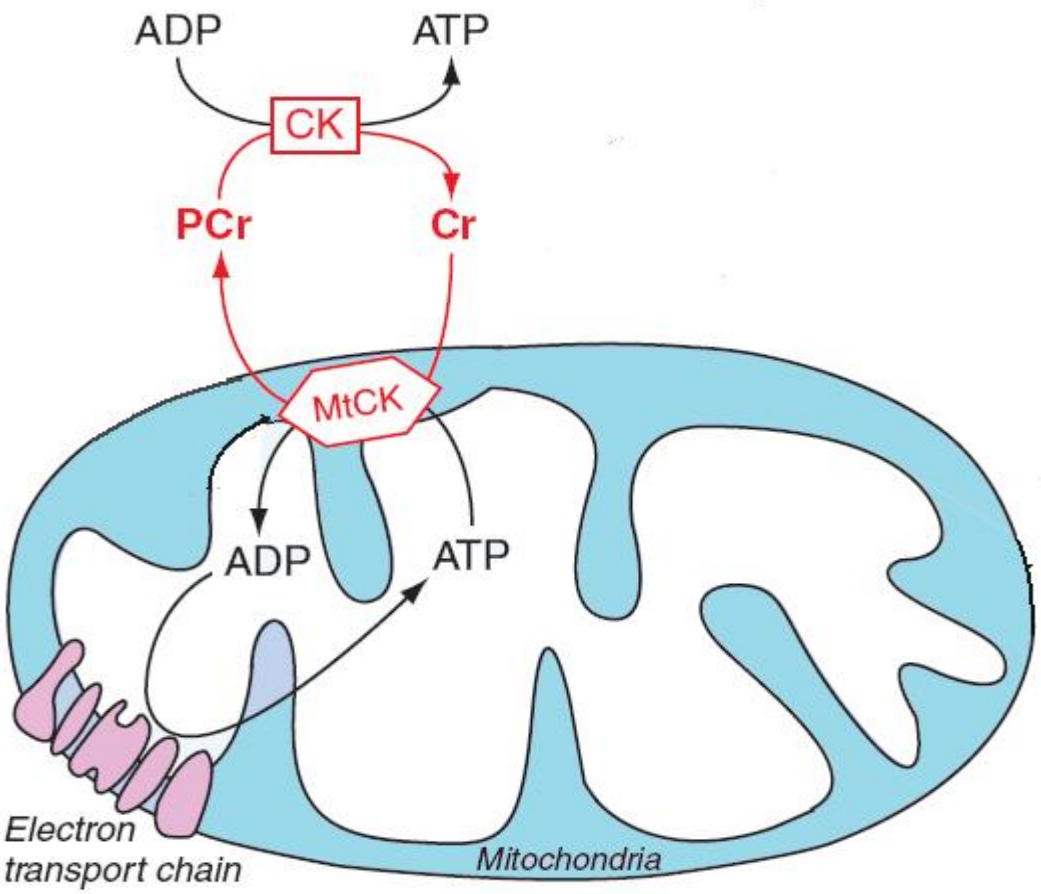

Figure 2. Creatine kinase reaction. The mitochondria creatine kinases (MtCK) consume ATP to convert Cr to its high-energy counterpart PCr for export to cytosol. The cytosolic creatine kinase (CK) dephosphorylates PCr and converts ADP to ATP for energy consumption. (Adopted from Beard and Braissant, 2010)

III. Creatine metabolism

A 70 kg young man contains approximately 120 g total Cr (Cr+PCr) (Schulze, 2003). More than 90% of Cr is distributed in muscle tissue. In human, Cr can be absorbed by small intestine, mainly from food such as fresh meat, fish, and dairy products. Beef is a good source of dietary Cr compared with other meat products. In the study of Pais et at. (1999) who investigated Cr contents in beef, chicken breast, chicken thigh, turkey breast, and fish, the highest Cr concentration (6.33 mg/g) was observed in beef top round steak.

Creatine also can be synthesized endogenously, primarily in kidney, pancreas, and liver (Schlattner, 2003; Wyss and Schulze, 2002). Two enzymes are essential for Cr biosynthesis, arginine: glycine amidinotransferase (AGAT) and S-adenosyl-L-methionine: N-guanidinoacetate methyltransferase (GAMT). The biosynthesis pathway is shown in Figure 3. AGAT transfers amidino group of arginine (limiting factor) to glycine and produces ornithine and guanidino acetate (GAA) in kidney. This reaction is regulated by Cr through a negative feedback loop in AGAT gene transcription. GAA then is methylated at the amidino group by GMAT using S-adenosyl-L-methionine to yield Cr and S-adenosyl-homocysteine in liver (Beard and Braissant, 2010; Schulze, 2003; Wyss and Kaddurah-Daouk, 2000). Growth hormone, thyroid hormone, and sex hormones were reported to modulate Cr endogenous synthesis through increasing expression of AGAT and GAMT (Guthmiller et al., 1994; Lee et al., 1994). Creatine is in equilibrium with its spontaneously formed cyclic derivative creatinine (Crn) in solution. Creatinine can be excreted through urine. In vivo, the conversion

of Cr to Crn is irreversible and at constant rate (Wyss and Kaddurah-Daouk, 2000).

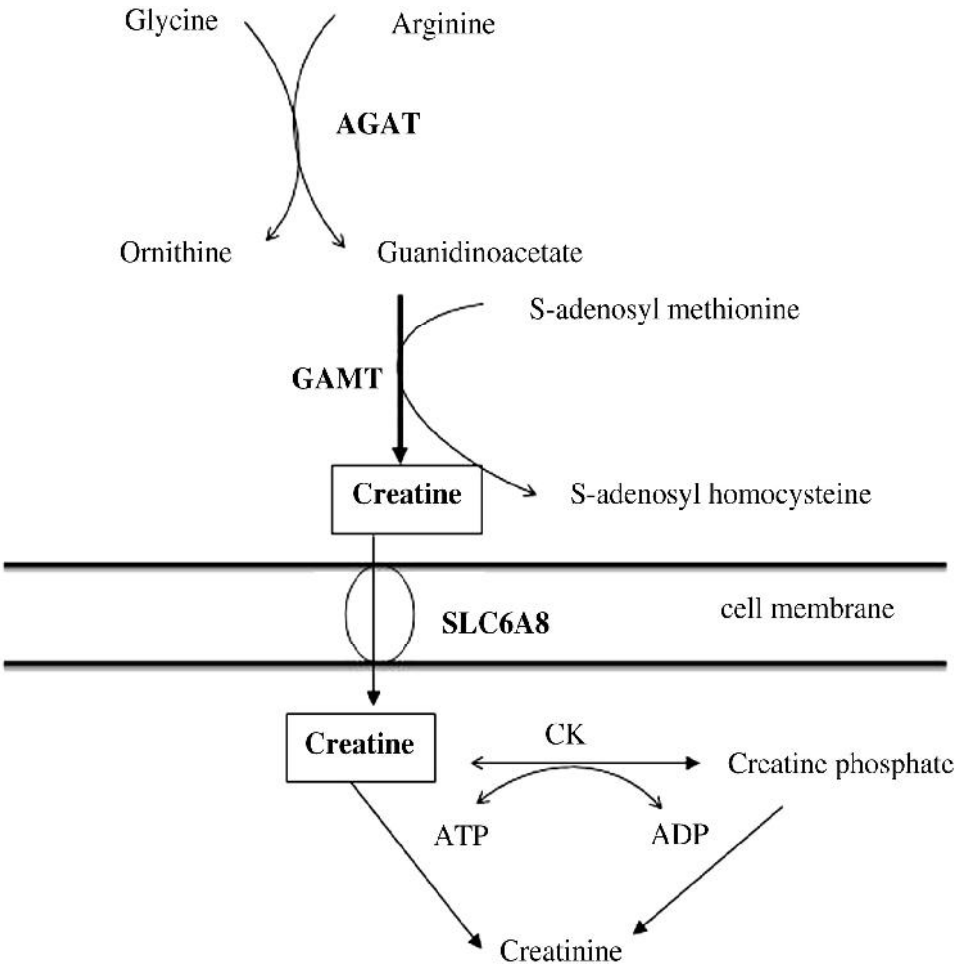

Figure 3. Creatine metabolism. Arginine: glycine amidinotransferase (AGAT) transfers amidino group of arginine (limiting factor) to glycine and produces ornithine and guanidino acetate (GAA) in kidney. GAA is then methylated at the amidino group by N-guanidinoacetate methyltransferase (GAMT) using S-adenosyl methionine to yield Cr and S-adenosyl homocysteine in liver. Creatine is transported by a member of the solute carrier family 6, SLC6A8. Creatine is in equilibrium with its spontaneously formed cyclic derivative creatinine. Creatinine can be excreted through urine. (Adopted from Schulze, 2003)

Creatine transport through blood to high energy demanding tissues involves a member of the solute carrier family 6, SLC6A8. SLC6A8 can transport Cr against a large concentration gradient (plasma [Cr] ~50 uM and intracellular [Cr+PCr] up to 40 mM) with co-transport of two Na^+ and one Cl^- (Guimbal and Kilimann, 1993). The transport is driven by sodium gradient established by Na^+/K^+ -ATPase. SLC6A8 expression is found in microcapillary endothelial cells at blood-brain barrier (BBB), which suggests that the BBB has at least limited permeability for peripheral Cr (Braissant, 2010).

IV. Creatine deficiency syndromes

AGAT, GAMT, and SLC6A8 genes are mapped at chromosome 15q11.2, 19p13.3 and Xq28, respectively (Schulze, 2003). Mutations in AGAT, GAMT, and SLC6A8 genes cause inborn error of metabolism, called Cr deficiency syndromes (Braissant, 2010). AGAT and GAMT deficiencies are autosomal recessive disorders, whereas SLC6A8 deficiency is an X-linked disease. Creatine deficiency syndromes are considered among the most common inborn errors of metabolism with the prevalence of all three Cr deficiencies being estimated at 2.7% of all mental retardation (Schulze, 2003). The prevalence of SLC6A8 deficiency was estimated at 2% of all X-linked mental retardations and 1% of mental retardation with unknown etiology in males (Schulze, 2003). Compared with SLC6A8, AGAT and GAMT deficiencies happen relatively rarer. The common phenotypes in all three disorders are almost complete lack of Cr in brain and neurological symptoms such as mental retardation,

developmental arrest, and delays in speech acquisition or epilepsy. GAMT deficiency also shows accumulation of the neurotoxic product GAA because of the lack of GAMT enzymatic activity in brain and body fluids. The combined impact of Cr deficiency and GAA accumulation might be responsible for the most severe phenotype.

Oral administration of Cr (0.35-2.0g/kg daily) is in part successful in treating AGAT and GAMT deficiencies (Braissant, 2010). For GAMT deficiency patients, combined arginine restriction and ornithine substitution helps decrease GAA concentration and improve clinical outcome. Supplementation with high does of Cr, however, failed to normalize brain Cr concentration. Even after treatment for months, total Cr concentrations in patients' brains stay significantly under normal level. Studies also show that early detection and pre-symptomatic treatment might prevent the phenotypic expression of diseases in AGAT and GAMT deficiencies (Braissant, 2010). On the other side, SLC6A8 deficiency can not be treated by oral Cr supplement. In fact, no effective therapies are available for patients suffering from SLC6A8 deficiency currently.

Although >90% of Cr is found in muscle tissue, the central nervous system (CNS) seems to be the main organ affected in patients with Cr deficiency syndromes. A potential explanation is that in muscle alternative phosphagen kinase systems could be developed under the Cr deficiency conditions (Schlattner, 2003), whereas certain Cr functions in brain can not be substituted by other systems. In recent years, evidences indicating that Cr may act as neuromodulator, co-transmitter, or even true regulator of appetite and weight (Almeida et

al., 2006; Cupello et al., 2008) were discovered. Studies in rat brain slices showed that Cr is released from neurons in an action potential-dependent manner, requiring the presence of Ca^{2+}, inhibited by Na^{+}-channel blocker tetrodotoxin, and enhanced by K^{+}-channels blockade 4-aminopyridine (4-AP) (Almeida et al., 2006). Furthermore, Cr and GAA were reported to act on post-synaptic $GABA_A$ receptors as a partial agonist (Cupello et al., 2008; De Deyn et al., 1991; Neu et al., 2002). The supporting evidence also included the expression of transport SLC6A8 in rat brain synaptosomes, which may suggest a Cr recapture mechanism in axon terminal membrane (Peral et al., 2010).

Besides the inborn Cr deficiency syndromes because of gene mutation of key enzymes and transporter in Cr metabolism, the secondary Cr deficiency in brain cells can be caused by other CNS pathologies, including hyperammonemia, stroke and gyrate atrophy of the choroid and retina (Beard and Braissant, 2010).

Hyperammonemia, which is usually caused by liver failure in adults, can lead to irreversible damage to brain. Studies show that pathogenic mechanisms of hyperammonemia may involve alterations in cerebral energy metabolism, neurotransmitter systems, nitric oxide synthesis, and several amino acid metabolism pathways (Cagnon and Braissant, 2007). Especially, excess of ammonium (NH_4^{+}) provokes a secondary Cr deficiency by inhibiting AGAT, GAMT, and SLC6A8 gene expression and activity (Braissant, 2010). Data from Braissant's group also show co-treatment with Cr exhibits protective effects on some pathological syndromes of hyperammonemia, especially axonal growth impairment.

Stroke is the second leading cause of human death worldwide. It is caused by blockage (ischemic stroke) or leakage (hemorrhagic stroke) of brain blood, that results in insufficient oxygen and glucose supply, leading to rapid loss of brain function (Feigin, 2005). Under the circumstances of stroke, PCr is depleted rapidly to regenerate ATP. Also significantly lower Cr concentrations were observed both in ischemic animal models and patients (Gideon et al., 1992; Lei et al., 2009; Mathews et al., 1995). This lower Cr level is believed to contribute to the failure of brain energy homeostasis and eventually induce neuronal death.

Gyrate atrophy of the choroid and retina is an inborn error of metabolism that is caused by a mutation of ornithine δ-aminotransferase (OAT) gene (Valle et al., 1981). OAT deficiency can increase concentration of plasma ornithine to 0.65-1.35 mM, which is 10-20 fold of normal level. Accumulated ornithine inhibits AGAT activity (Ki for ornithine is 0.25 mM) and results in lower concentration of GAA which is the precursor for Cr biosynthesis (Valayannopoulos et al., 2009). Gyrate atrophy patients often suffer from a progressive loss of vision, with total blindness usually occurring between the ages of 40 and 60. Gyrate atrophy usually does not affect intelligence; however, unspecific neurological impairment and premature degenerative changes may be observed in gyrate atrophy patients (Nanto-Salonen et al., 1999. Some researchers believe that neurological impairment in gyrate atrophy may be related to the secondary Cr deficiency in the CNS (Valayannopoulos et al., 2009).

V. Creatine Supplement

Cr is one of the most common dietary supplements for athletes and bodybuilders. At least three beneficial effects of Cr supplementation were observed in healthy people: increasing muscle mass, enhancing performance in high-intensity short-duration exercise and regulating carbohydrate metabolism (Branch, 2003).

Creatine supplementation was reported to increase muscle mass in an experiment conducted by Francaux and Poortmans (1999). The daily supplement of 21 g creatine monohydrate increased body mass by 2 kg on average compared with placebo group during 9 weeks strength-training program. In another double-blind experiment by Volek et al. (1999), Cr supplement subjects showed significantly greater increases in Type I (35% vs 11%), IIA (36% vs 15%), and IIAB (35% vs 6%) muscle fiber cross-sectional areas besides higher body mass, compared with placebo subjects. Willoughby and Rosene (2003) investigated the effects of oral creatine intake with resistance training on myogenic regulatory factor expression and found significant increase of myogenin and MRF-4 at both mRNA and protein concentrations. Simultaneous exercise, however, seems crucial for Cr supplement benefits. Ferreira et al., (2005) reported that in the rat model Cr supplement alone did not increase lean body mass, whereas Cr supplement in combination with exercise had larger effects on lean body mass than exercise alone. In the study of Louis et al.(2003), who used isotopic [1-(13)C] leucine to study human muscle protein turnover, no effect of Cr supplementation was observed for subjects who did not exercise. Therefore, some researchers

argued that increase in muscle mass with creatine supplementation must be associated with increased physical activity.

The Cr/PCr/CK system functions as a shuttle of high-energy phosphate and therefore enhances the ability to rapidly replenishing ATP during strenuous exercise. There is much evidence supporting enhanced performance by Cr supplementation in short-duration, high-density sports. A meta-analysis of 96 studies about Cr supplement by Branch (2003) summarized that Cr supplement improved performance on high-intensity exercise of short duration (< 30 s) but did not show significant effects on exercises lasting longer than 150 seconds. A study conducted by Vandenberghe et al. (1996) found that the ergogenic effect of Cr could be completely eliminated by caffeine intake. Therefore, attention needs to be paid because both Cr and caffeine are available in some common supplements. Cr is widely consumed by athletes. One of most popular supplement schedules is a 5-6 day loading period of 20 g per day, and sometimes followed by a maintaining period of 2-5 g per day.

Cr supplement also has been reported to affect muscle carbohydrate metabolism. Glycogen content increases simultaneously with Cr content in muscle during the loading period of 20 g Cr per day (Derave et al., 2003). Data about skeletal muscle-specific glucose transporter GLUT-4 mRNA and protein concentrations, however, were not consistent among different human experiments. Van Loon et al. (2004) reported no change in GLUT-4 mRNA or protein level during Cr supplement of 6 weeks, whereas in a similarly scheduled study (also 20 g Cr per day for 6 weeks) by Derave et al. (2003) GLUT-4 expression is increased

by Cr supplementation. A rat study by Ju et al. (2005) found increased expression of GLUT4 and its transcription factor myocyte enhancer factor 2 isoforms by Cr supplementation. The mechanism about how Cr affects muscle metabolism is not well understood yet. But, one hypothesis involves the Cr/PCr ratio, which is increased by Cr supplementation. Cr/PCr ratio was proved to modulate AMP kinase activity, which is one of key regulators of muscle metabolism (Ponticos et al., 1998).

VI. Neuroprotective effects of creatine

The brain represents only 2% of body weight but consumes up to 20% of the energy intake. Given the critical roles of Cr in cellular energy metabolism, the therapeutic potential of Cr for neurological diseases has been studied extensively in both animal/cellular models and human clinical trials. In these studies, Cr supplementation shows potential therapeutic values for targeting cellular energy impairment in neurodegenerative diseases (Adhihetty and Beal, 2008). In summary, the literature suggests that Cr supplementation exerts neuroprotective effects on Parkinson's disease (PD) and Huntington's disease (HD) but seems less effective in Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) (Adhihetty and Beal, 2008). Cr also may be used to modulate the neurological symptoms in stroke or hyperammonemia that cause secondary Cr deficiency during disease process (Braissant, 2010).

Parkinson's disease (PD) is a neurodegenerative disease caused by death of

dopaminergic neuron located in the substantia nigra in the midbrain. Early symptoms are movement-related, including shaking, rigidity, slowness, and abnormality in gait. Cognitive and behavioral problem may develop in later stages (Jankovic, 2008). Impaired activity of complex I in the mitochondrial electron transport chain has been proved as the key factor in the disease process. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was found to inhibit complex I activity and lead to PD-like syndromes in human and animal models (Langston et al., 1983). It is commonly used in PD studies. Matthews' group (1999) showed that oral Cr supplementation resulted in significant protection against MPTP-induced dopamine depletion and neuron death in mice. In a randomized, double-blind phase II clinical trial, 4 g/d of Cr was administrated for a period of two years to 60 PD patients. The results indicated that long-term Cr supplementation was safe and thus was recommended for Phase III clinical trials (Bender et al., 2006; Bender et al., 2008). According to Adhihetty and Beal's report in 2008, a double-blind, placebo-controlled, phase III clinical study involving 1,720 early-stage PD patients and lasting for 5-7 years was underway. This study is one of the largest PD clinical trials to date. The final report from this study, however, is not available yet.

Huntington's disease (HD) is caused by an autosomal dominant mutation on huntingtin gene that results in an abnormal polyglutamine expansion in the huntingtin protein. Normal huntingtin protein is expressed extensively in vertebrates with unclear function. Mutant huntingtin protein is reported to be toxic to neural cell by inducing transcriptional

dysregulation, oxidative injury, proapoptotic signaling, inflammatory reaction, and mitochondria dysfuntion (Beal and Ferrante, 2004). Cr supplementation in transgenic mice models of HD was shown to improve motor performance, increase life span, and delay the formation of mutant huntingtin aggregates (Andreassen et al., 2001; Dedeoglu et al., 2003; Ferrante et al., 2000). A 16-week, randomized, double-blind, placebo-controlled phase II clinical trial was conducted by (Hersch et al., 2006) to test the safety, tolerability, and efficacy of 8 g/day Cr in 64 subjects with HD. This study reported that Cr dosage was well tolerable and Cr treatment successfully suppressed serum 8-hydroxy-2'-deoxyguanosine, an indicator of oxidative injury to DNA. Because of the neuroproctective effects illustrated in both transgenic mice models and phase II clinical trials, a large scale phase III clinical trial has been approved and is currently ongoing.

Alzheimer's disease (AD) is one of the most common forms of dementia, which is characterized by loss of neurons in cerebral cortex and specific subcortical regions (Wenk, 2003). Late stage of AD shows inactive CK and Cr deposition, which implies Cr supplementation at late stage may not exert any improvement (Adhihetty and Beal, 2008). The benefits, however, remain possible with Cr supplementation in earlier stage.

Amyotrophic lateral sclerosis (ALS) is a progressive, usually fatal, neurodegenerative disease, caused by loss of motor neurons in CNS. Creatine administration in an ALS animal model (G93A mice) improved motor performance, protected against neurons loss, and decreased oxidative damage (Klivenyi et al., 1999). No beneficial effects of Cr

supplementation, however, were observed in two independent clinical trials with ALS patients (Groeneveld et al., 2003; Shefner et al., 2004). Further studies are needed to explain the differences in the efficacy of Cr treatment between ALS mice model and patients.

One common characteristic of neurological diseases is neuron death or loss of function in distinct areas of the brain. Mitochondria are not only the "powerhouse of the cell" but also regulate cell apoptosis because they produce the majority of damaging reactive oxygen species (ROS) by electron transport chain and can induce cell death by releasing pro-apoptotic factors to the cytosol (McBride et al., 2006). A continuum between inner and outer mitochondrial membrane, called mitochondrial permeability transition pores (mtPTP), is required for the release of pro-apoptotic proteins. Substantial evidence indicates that mitochondrial dysfunction is involved in pathogenesis of numerous neurodegenerative diseases, including HD (Browne et al., 1997), PD(Thomas and Beal, 2007), ALS (Beal, 2000) and AD (Ferreira et al., 2010). Although the mechanisms differ slightly in specific diseases, Cr supplementation has been proposed and/or proven to improve the overall bioenergetics and mitochondria function. Detailed information about the mechanism is shown in Figure 4.

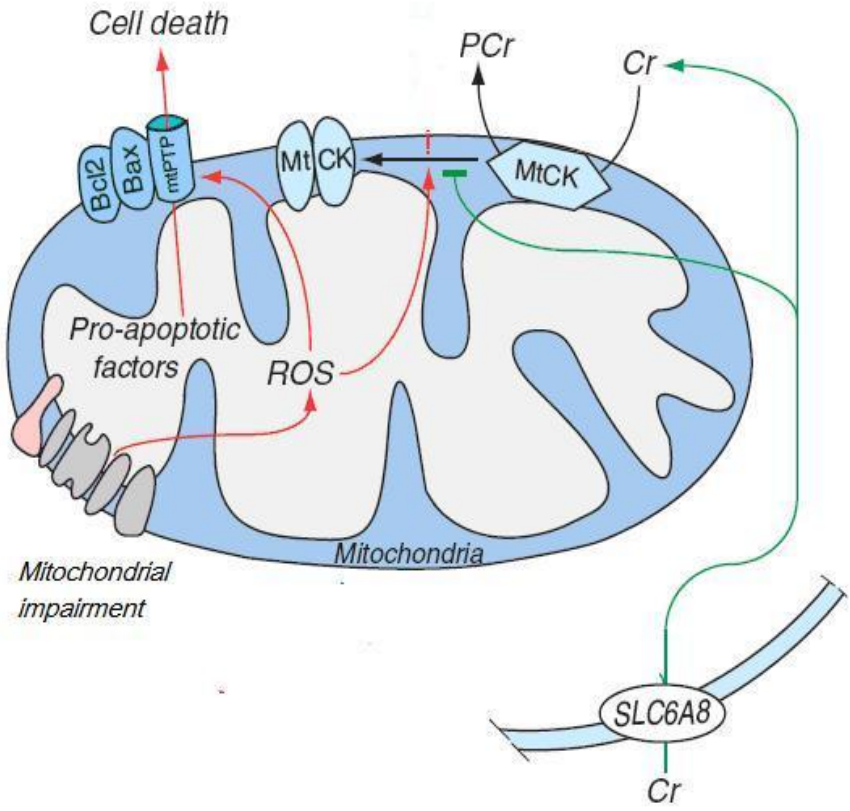

Figure 4. During the pathological process of neurodegeneration, mitochondrial impairment causes accumulation of ROS, which can change the octameric conformation of mitochondrial creatine kinase (MtCK) to its dimeric form. Octameric MtCK can suppress mtPTP opening by interacting with both the outer membrane component voltage-dependent anion channel (VDAC) and the inner membrane component adenine nucleotide translocase (ANT) whereas the inactivated dimeric form of MtCK can't. The shift of the octameric form to the dimeric form has also been shown to affect Ca^{2+} homeostasis, which is another factor contributing to the opening of mitochondrial permeability transition pores (mtPTP). Cr supplementation stabilizes the octameric form of MtCK, and therefore decrease mitochondrial apoptotic

susceptibility (Adopted from Adhihetty and Beal, 2008; Beard and Braissant, 2010).

Carnosine and anserine

I. Structure information

L-Carnosine (ß-alanyl-L-histidine) is a dipeptide composed of ß-alanine and L-histidine, which is highly concentrated in muscle and brain tissues of mammals. Carnosine performs multiple biological functions including pH buffering, anti-oxidation, anti-glycation, ant-aging, and chelation of divalent metal cations. Anserine (ß-alanyl-N-methylhistidine) is an N-methylated analogue of carnosine found mainly in fish and birds. The structures of carnosine and anserine are shown in Figure 5. Anserine has similar properties with carnosine in many aspects but is mainly found in non-mammalian species. In this review, the discussion is focused on carnosine.

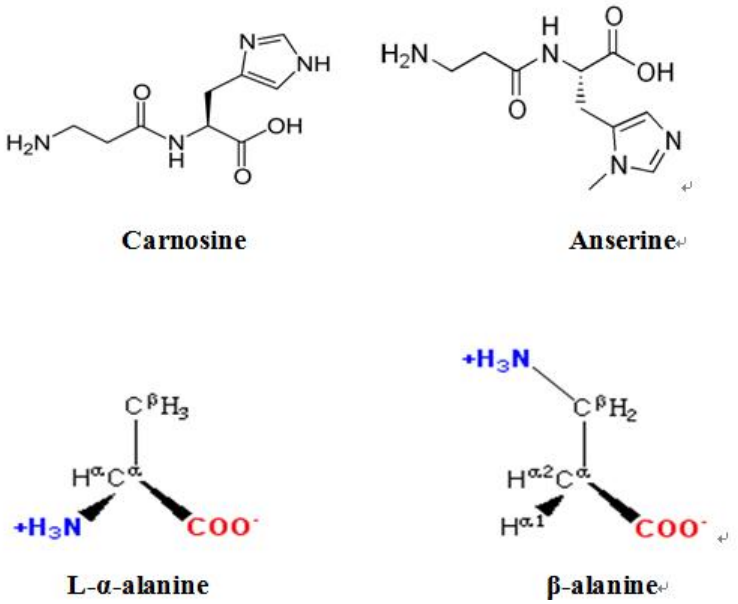

Figure 5. Structures of carnosine, anserine, L-α-alanine, and β-alanine.

II. Carnosine metabolism

As a dipeptide, carnosine has a relatively simple synthetic pathway compared with creatine. Carnosine is synthesized by carnosine synthase from its constituent amino acids ß-alanine and L-histidine. L-Histidine is an essential amino acid that can only be obtained from the diets, whereas ß-alanine is a non-essential amino acid and the rate-limiting precursor. In some cases, ß-alanine supplementation is used to increase muscle carnosine concentration. Differing from L-α-alanine that is used in major protein synthesis, the amino group of ß-alanine is located on ß carbon rather than α carbon. The structural difference between L-α-alanine and ß-alanine is also shown in Figure 5. ß-Alanine can be produced via the following three pathways: the decarboxylation of aspartate by gut microbes, the interchangeable reaction of pyruvate to L-α-alanine, and the deamination and carboxylation of the pyrimidine uracil (Tiedje et al., 2010). Recently, carnosine synthase was identified as the ATP-grasp domain-containing protein 1(ATP-GD 1) (Drozak et al., 2010). The structure and function details of this enzyme, however, remain unclear.

Carnosine hydrolysis is catalyzed by carnosinase. In human, two forms of carnosinase were discovered: serum carnosinase (CN1) and tissue carnosinase (CN2). CN1 was identified as a homodimeric dipeptidase with narrow substrate specificity whereas CN2 could exert dipeptidase activity on a wider range of substrates. Because of the existence of CN1 in human serum, the blood carnosine concentration is very low. On the contrary, rodents do not have carnosinase in serum, and their blood does contain much higher concentrations of

carnosine (Teufel et al., 2003).

Regulation of carnosine homeostasis is understood poorly. Nagai et al. (2003) suggested that muscle contraction or exercise influenced all aspects of carnosine metabolism, including synthesis, degradation, and transportation. According to the homology modeling by Vistoli et al. (2006), the activity of serum carnosinase is stimulated by citrate. Schulz et al. (1989), on the other hand, observed that cAMP inhibited the activity of carnosine synthase. These studies suggest that carnosine concentration is under metabolic regulation. Further studies, however, are required to reveal detailed mechanisms.

III. Physiological functions of carnosine

Carnosine performs multiple biological functions, including pH buffering, metal ion chelation, and anti-glycation. Carnosine exerts beneficial health effects, such as antioxidation, antiaging, and preventing hangovers (Hipkiss, 2009a).

The pKa of carnosine is 6.8, which fall into the physiological pH range (6.5-7.1) of myocytes. It can dynamically accept protons produced during muscle contraction. Therefore, the pH buffering ability of carnosine may explain its abundance in muscle tissue and also the protective role in ischaemia acidosis.

Carnosine can chelate divalent metal ions with its imidazole ring. It is considered as a protective agent against heavy metal-induced toxicity (Trombley et al., 2000). The chelation property with ferrous ions and other transition metals also contributes to the antioxidative

capacity. It is well known that transition metals promote the production of ROS through the Fenton reaction (Bondy et al., 1998).

Glycation is uncontrolled reaction of sugar aldehyde with amino groups of protein. It causes protein cross-linking and other harmful advanced glycation end products that are involved in the process of aging. *In vitro* studies showed that carnosine could react with multiple glycation reagents and the product was non-mutagenic. Therefore, carnosine could inhibit glycation and prevent protein cross-linking (Hipkiss et al., 1995). After ethanol consumption, serum acetaldehyde concentration increases to cause "hangover". Carnosine could react with acetaldehyde and be considered as an effective way to prevent "hangovers" and other alcohol-induced damages (Hipkiss et al., 1998). By decreasing the production of ROS and deleterious aldehydes, carnosine was proposed to suppress the process of aging at both cellular and whole organism levels (Hipkiss, 2009a).

IV. Carnosine supplement

Given the favorable physiological properties, such as pH buffering, anti-oxidation, and anti-glycation, carnosine has been proposed as a potential therapeutical adjuvant in various diseases, including cataract, diabetic complications, ischemia and Alzheimer's disease (Hipkiss, 2009b; Janssen et al., 2005; Kawahara et al., 2007; Quinn et al., 1992).

Because of the existence of serum carnosinase in human, the efficacy of carnosine supplementation is of concern. In the experiment by Gardner et al. (1991), administration of

4 g of carnosine increased plasma carnosine concentration up to 180 mg/ml within 0.5 hour after intake. The plasma carnosine, however, returned to undetectable level after 2 hours. In another study by Park et al. (2005), the absorption kinetics were studied by consuming cooked ground beef containing 248 mg of carnosine. Carnosine was detected in plasma 15 min after beef consumption, continued to increase up to 32.7 mg/L 2.5 hours after consumption, and then decreased to undetectable level at 5.5 hours. The studies suggest that dietary carnosine is absorbed intact and causes at least temporarily raised serum carnosine concentration. Antonini et al. (2002) showed that serum total antioxidant activity was increased by 11% 1 hour after intake of 450 mg of carnosine. This study indicates that, despite the presence of serum carnosinase, dietary carnosine supplement still has the potential to exert its health benefits on tissues.

Carnosine analogues were designed to resist carnosinase attack. N-acetyl-carnosine is one example. After entering cells, of N-acetyl-carnosine undergoes removal of its acetyl group and is transformed into carnosine. In fact, the ophthalmic drugs with N-acetyl-carnosine formula are currently available for patients with age-related cataracts (Babizhayev, 2004).

The ideal daily supplement dose for carnosine has not been decided yet. Currently, the commercial capsule for carnosine supplement contains 200-500 mg of carnosine. Carnosine naturally exists only in animal tissues, and therefore vegetarian diets may be deficient in carnosine. Carnosine concentration varies according to animal species and tissues (Hipkiss,

2009a). Muscle type, gender and age also were reported to affect carnosine content in tissue of humans and rodents. In humans, fast twitch muscle fibers have significantly higher amount of carnosine than do slow twitch fibers (Derave et al., 2010). Penafiel et al. (2004) observed the marked sexual dimorphism of carnosine and anserine in skeletal muscles of CD1 mice. They found that male mice have 3-4 fold higher carnosine contents than do females, and testosterone administration could increase female muscle carnosine content to a similar level of males. In humans, the same trend but smaller gender differences were observed. Mannion et al. (1992) studied carnosine concentrations in the lateral portion of the quadriceps femoris muscle of 50 healthy human subjects and reported that on average men had 20-25% higher carnosine concentration than did women. Besides, muscle carnosine content remarkably declines with age in both rodents and humans (Johnson and Hammer, 1992; Tallon et al., 2007).

CHAPTER 2

CONCENTRATIONS OF CREATINE, CREATININE, CARNOSINE, AND ANSERINE IN BOVINE LONGISSIMUS MUSCLE AND THEIR CORRELATIONS WITH CARCASS AND PALATABILITY TRAITS[1]

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Abstract

The objective of this study was to investigate the variation of creatine, creatinine, carnosine, and anserine contents in longissimus muscle of Angus cattle and their correlations with carcass and palatability traits. Longissimus muscle samples were collected from 2,342 Angus cattle fed in Iowa (n=1,114), Texas (n=455), Colorado (n=391), and California (n=382). Creatine, creatinine, carnosine, and anserine were extracted from beef and quantified by high-performance liquid chromatography. The concentrations of these compounds then were correlated with carcass and palatability traits including hot carcass

weight (HCW), kidney pelvic heart fat percentage (KPH), ribeye area, fat thickness, marbling score, calculated yield grade, sensory panel scores, Warner Bratzler shear force (WBSF), and thiobarbituric acid reactive substances (TBARS). Statistical analysis showed that the gender of cattle significantly ($P < 0.05$) affected the concentrations of creatine, creatinine, carnosine, and anserine. Bulls contained greater creatine content than did steers ($P < 0.05$) but produced lower amount of carnosine than did heifers ($P < 0.05$). Significant variation from feeding location was also observed. The correlations with carcass quality and sensory scores were significant but weak, which suggested that beef quality, especially tenderness, juiciness, and flavor, would not be strongly affected by selecting for higher creatine and carnosine contents.

Keywords: beef, creatine, creatinine, carnosine, anserine, palatability, carcass, correlations

Introduction

Creatine and carnosine have been considered as valuable nutraceuticals in meat products for a long time. Creatine as energy buffer plays an important role in cellular metabolism and shows protective effects in neurodegenerative diseases such as Parkinson's disease and Huntington's disease (Adhihetty and Beal, 2008; Beard and Braissant, 2010; Bender et al., 2006; Dedeoglu et al., 2003; Hipkiss, 2009b; Wyss and Kaddurah-Daouk, 2000). Carnosine has multiple physiological functions associated with anti-oxidation and

anti-aging (Antonini et al., 2002; Derave et al., 2010; Hipkiss, 2009b; Janssen et al., 2005; Trombley et al., 2000). Creatinine is the spontaneously formed cyclic derivative of creatine. Anserine is an N-methylated analogue of carnosine found mainly in fish and birds. Because creatine and carnosine exist naturally only in animal muscle and brain tissues, meat is an important source of dietary creatine and carnosine. Few studies, however, have focused on creatine and carnosine concentrations in beef and the factors influencing their concentrations.

Health concerns have arisen about beef intake mainly because of its high amount of saturated fatty acids. To improve the nutritional value of beef, creatine and carnosine, as beneficial nutraceuticals, could be potential targets for selection. In the meantime, beef producers and consumers highly value meat quality and palatability traits, especially tenderness. Ideally, producers would like to select beef for optimal health values without compromising production efficiency and consumer satisfaction. To fulfill this goal, the relationship among beef creatine and carnosine contents with meat quality and sensory characteristics must be understood first. Therefore, the objective of the present study was to investigate (1) the variation of creatine and carnosine along with creatinine and anserine contents in longissimus muscle of Angus cattle and (2) their correlations with carcass and palatability traits. The potential influences of gender and feeding location also were evaluated in our study.

Materials and Methods

Animal Resources and Sample Collection

Angus cattle (n=2,342) in this study had related genetic background, but were raised in different feedlots in Iowa (n=1,114), Texas (n=455), Colorado (n=391), and California (n=382). Cattle from Iowa included three gender types: bulls (n=558), steers (n=316), and heifers (n=240). Both steers (n=192) and heifers (n=199) were available in Colorado. Cattle from Texas and California were all steers. Cattle were harvested and processed by contemporary groups (n=33) on the basis of feeding location, gender, birth season, and harvest date.

Carcass and Palatability Data Collection

Carcass data, including hot carcass weight (HCW), kidney pelvic heart fat percentage (KPH), ribeye area, fat thickness, marbling score, and calculated yield grade were obtained by trained personnel. Rib sections were collected from each carcass at commercial facilities at Iowa, Texas, Colorado, and California. More detailed information for animal management, sample collection, and processing can be found in Garmyn et al. (2011).

Assessment of palatability traits also was described in Garmyn et al. (2011).. In summary, frozen steaks were thawed at 4°C for 24 h and then cooked to a final internal temperature of 68 °C. Six cores of 1.27 cm diameter were removed from each cooked steak for Warner Bratzler shear force (WBSF) measurement. The mean value of six measurements was used in further analysis of each sample. Trained sensory panel measured each sample on initial tenderness, overall tenderness, initial juiciness, sustained juiciness, and amount of

connective tissue with an 8-point scale (higher value for more favorable quality). Beef flavor, painty/fishy flavor, and livery/metallic flavor were evaluated with a 3-point scale (1=not detectable, 2=slightly detectable, and 3=strong). Thiobarbituric acid reactive substances (TBARS) were used to evaluate lipid oxidation, and the results were expressed as mg of malonaldehyde per kg of sample.

Creatine, Creatinine, Carnosine, and Anserine Analysis

Creatine, creatinine, carnosine, and anserine were extracted from beef and analyzed by high-performance liquid chromatography by using methods adapted from Mora et al. (2007). Briefly, 6 mL of 0.01 *N* HCl was added to 2 g of ground, homogenized beef and vortexed for 15 sec. These samples were placed on an orbital shaker for 15 min and centrifuged for 20 min at 12,000 × *g* and 4° C. The supernatant was filtered through glass wool and 250 µL were deproteinized by mixing with 750 µL of acetonitrile. These samples were held at 4° C for 20 min before centrifugation at 10,000 × *g* for 10 min.

Chromatography was performed by using a Hewlett-Packard 1050 HPLC equipped with a quaternary pump, autosampler, and variable wavelength detector. An injection volume of 20 µL of deproteinized supernatant was separated on an Atlantis HILIC silica column (4.6 × 150 mm, 3 µm; Waters Corporation, Milford MD). Solvent A consisted of 0.65 mM ammonium acetate in water/acetonitrile (25:75, v: v) with pH at 5.5. Solvent B consisted of 4.55 mM ammonium acetate in water/acetonitrile (70:30, v: v) with the same pH. The solvent

gradient was linear from 0% solvent B to 100% solvent B in 13 min and a return to 0% solvent B in 2 min followed by re-equilibration at initial conditions for 5 min. Solvent flow rate was set at 1.4 mL/min. The variable wavelength detector was initially set at 236 nm and was changed to 214 nm after 4 min. Sample peak areas were correlated to standard curves of creatinine, creatine, carnosine, and anserine for quantification.

Statistical Analysis

Statistical analyses were performed with SAS (SAS Inst. Inc., Cary, NC). The average, minimal, and maximal values for each trait were calculated by using PROC UNIVARIATE.

Because not all genders were present or equally represented at each feeding location (e.g., Iowa was the only location from which samples included all three gender types, whereas heifer and bull samples were missing at California and Texas), gender effects were evaluated on samples from Iowa by a mixed model with gender and birth season as fixed effects, dam and sire as random effects, and harvest age as covariate. Similarly, only steer samples were available from four locations. A mixed model with feeding location as fixed effect, dam and sire as random effects was applied to evaluate the effect of feeding location using only steer samples. Harvest age was not included in this model because it was only recorded in the production system from Iowa. Birth season was dropped because of a lack of degrees of freedom. The P-value was obtained from analysis of variance and considered to be significant if it was less than 0.05.

The simple correlation coefficients were calculated by using PROC CORR of SAS. Partial correlation coefficients were obtained from MANOVA/PRINTE option in PROC GLM. The Spearman correlation coefficients were reported for traits with ranked scores, including marbling scores and sensory scores. For other traits, Pearson correlation coefficients were used. The correlation was considered significant when the P-value was less than 0.05.

Results and Discussion

Variation in beef creatine, creatinine, carnosine, and anserine concentrations

The simple statistics of beef creatine, creatinine, carnosine, and anserine concentrations in longissimus muscle of Angus cattle are shown in Table 1. The concentration of creatine ranged from 2.58 to 6.86 mg/g of ground beef, with an average at 5.26 ± 0.01 mg/g. We observed highest carnosine content at 5.72 mg/g and lowest content at 2.28 mg/g, with an average at 3.72 ± 0.01 mg/g of beef. Our data fell into similar ranges reported by Purchas et al. (2005) in the study of Angus-cross heifers raised in United States and New Zealand. They, however, reported a lower average creatine concentration (3.82 mg/g) but a higher average carnosine concentration (4.27 mg/g).

Beef is a good source of dietary creatine and carnosine, when compared with other meat products, especially red meat. In the study of Pais et at. (1999) who investigated creatine contents in beef, chicken breast, chicken thigh, turkey breast, and fish, the highest

creatine concentration (6.33 mg/g) was observed in beef top round steak. Hipkiss (2009) also reported higher carnosine concentration in beef than in pork or lamb. According to our study, the amount of creatine present in a typical serving (100 g) of beef is generally lower than that recommended for supplementation (e.g., a typical creatine supplement protocol asks for 5 g of creatine per day for four days and then a maintenance dose of 2 g per day) (Wyss and Kaddurah-Daouk, 2000). Although the ideal daily supplement dose for carnosine has not been decided yet; the commercial capsule usually contains 200-500 mg of carnosine, which is similar with the amount in one serving of beef (100 g).

Effects of gender and feeding location on beef creatine, creatinine, carnosine, and anserine concentrations

The results of gender effect based on samples from Iowa are shown in Table 2. The gender of cattle significantly affected the concentrations of creatine, creatinine, carnosine, and anserine in longissimus muscle. Bulls contained greater creatine content than did steers ($P < 0.05$), which could be attributed to the effects of sex hormones on endogenous creatine synthesis (Krisko and Walker, 1966; Wyss and Kaddurah-Daouk, 2000). In contrast, heifers had 9.6% higher amounts of carnosine than did bulls ($P < 0.05$), which indicated an opposite gender effect with rodents and humans (Mannion et al., 1992; Penafiel et al., 2004). Their studies showed males tended to have greater muscle carnosine content than do females in both mice and humans. We also observed that heifers had significantly higher ($P < 0.05$)

creatinine concentration than did steers and bulls, whereas steers had higher anserine content than did bulls and heifers ($P < 0.05$).

The same gender effect model was applied to carcass and palatability traits. As shown in Table 3, gender was a significant source of variation on each reported carcass trait. Bulls produced the heaviest hot carcass weight and the largest ribeye area ($P < 0.0001$), which was followed by steers; and heifers received the lowest scores on both traits. In contrast, bulls received significantly lower marbling scores than did steers ($P < 0.001$). These results were expected because testosterone promotes rapid lean muscle growth but decreases fat deposition (Fritsche and Steinhart, 1998; Guillemin et al., 2009; Schreurs et al., 2008). They were also in agreement with previous studies of Choat et al. (2006), which suggested that steers had higher rate of gain than did heifers across a feeding period. Our study, however, found that steers had highest yield grade, KPH fat percentage, and 12^{th} rib fat thickness ($P < 0.05$), which were not observed in the study of Choat et al. (2006).

Table 4 shows gender effect on beef palatability traits, including both trained sensory panel scores on tenderness, connective tissue amount, juiciness and flavor, and other measurements, including Warner-Bratzler Shear Force (WBSF) and thiobarbituric acid reactive substances (TBARS). Steers received more favorable ratings from the sensory panel on initial tenderness, overall tenderness, and connective tissue amount compared with bulls. Although similar gender effects on beef tenderness were reported in other studies (Peachey, 2002; Purchas et al., 2002) and could be explained by the positive association between

intramuscular fat content and tenderness (Wood J.D., 2008), no significant difference between steers and bulls on WBSF ($P > 0.05$) were observed. On the other hand, heifer was reported to have the lowest WBSF score ($P < 0.05$) and the highest TBARS value ($P < 0.05$) in this study. Steers received significantly higher scores on beef flavor and significantly lower painty/fishy flavor than other two groups. No significant differences among groups were observed on sensory scores regarding initial juiciness, sustained juiciness, and livery/metallic flavor in our study ($P > 0.05$).

Effects of feeding location on beef creatine, creatinine, carnosine, and anserine are reported in Table 5 based on data of steers. No significant differences among four feeding locations were found on creatinine ($P > 0.05$). In contrast, feeding location is a significant source of variation for creatine, carnosine, and anserine. Steers fed in California had significantly lower amount of creatine than did those fed in both Texas and Iowa. Steers fed in Colorado had the highest carnosine concentration, followed by steers fed in Iowa and California, whereas steers fed in Texas had the lowest carnosine concentration in longissimus muscle. With limited information about harvest age for cattle from Colorado and Texas, we could not rule out the possibility that the difference was actually attributed to cattle age. It is well accepted that skeletal muscle carnosine concentration declines with age in humans and rodents (Johnson and Hammer, 1992; Tallon et al., 2007). In the study of Purchas R.W. (2005), who compared the production systems of US and New Zealand, beef samples from two countries showed significant difference on both creatine and carnosine contents. The

genetic differences between US and New Zealand cattle in their study, however, need to be considered to interpret results properly.

Correlations among beef creatine, creatinine, carnosine, and anserine with carcass and palatability traits

The simple correlation coefficients with carcass and palatability traits are present in Table 6 and Table 7, respectively. Creatine concentration was correlated positively with ribeye area (r=0.085), TBARS (r=0.099), and connective tissue amount (r=0.096) but negatively with fat thickness (r=-0.133), marbling (r=-0.235), calculated yield grade (r=-0.127), juiciness (r=-0.135 for initial juiciness and r=-0.102 for sustained juiciness), and flavor (r=-0.066 for beef flavor and r=-0.134 for painty/fishy flavor). Positive correlations were found among creatinine with hot carcass weight (r=0.085), fat thickness (r=0.284), marbling (r=0.082), calculated yield grade (r=0.271), tenderness (r=0.095 for initial tenderness and r=0.056 for overall tenderness), juiciness (r=0.191 for initial juiciness and r=0.133 for sustained juiciness), and flavor (r=0.149 for beef flavor, r=0.124 for painty/fishy flavor and r=0.066 for livery/metallic flavor). We observed weak negative correlations between creatinine with KPH fat percentage (r=-0.091) and ribeye area (r=-0.083) but relatively strong negative correlation with TBARS (r=-0.419). Similarly, carnosine was correlated negatively with fat thickness (r=-0.239), marbling (r=-0.047), calculated yield grade (r=-0.161), TBARS (r=-0.060), tenderness (r=-0.070 for initial tenderness and r=-0.098

for overall tenderness), and connective tissue amount (r=-0.107) but positively correlated with KPH fat percentage and flavor (r=0.102 for beef flavor and r=0.069 for livery/metallic flavor). As for anserine, the positive correlations with KPH fat percentage (r=0.162), ribeye area (r=0.043), TBARS (r=0.107), overall tenderness (r=0.060), connective tissue amount (r=0.064), and beef flavor (r=0.062) and the negative correlations with WBSF (r=-0.122) and painty/fishy flavor (r=-0.172) were found. The large sample size of this study (n=2,342) allowed us to detect weak correlations. Although all these correlations were reported at statistically significant level ($P<0.05$), we should notice that the absolute values of correlation coefficients were very small (less than 0.25) in most instances.

Partial correlation coefficients also were calculated to examine the relationship between nutraceuticals and meat quality traits after accounting for group variation caused by gender, feeding location, and birth seasons. Table 8 and Table 9 report partial correlation coefficients among traits. After removing group differences, creatine was only significantly correlated with ribeye area (r=0.061), marbling (r=-0.129) and calculated yield grade (r=-0.068). Creatinine showed no significant correlation with TBARS, fat thickness, and calculated yield grade ($P>0.05$), which indicated that the previously reported moderate simple correlations might over-estimate the relationship among them. Weak partial correlations, however, were found among creatinine and ribeye area (r=0.078), tenderness (r=-0.053 for initial tenderness and r=-0.052 for overall tenderness), and flavor (r=0.074 for beef flavor and r=-0.063 for painty/fishy flavor). Similarly, carnosine was correlated weakly

with hot carcass weight (r=-0.047), KPH fat percentage (r=0.049), marbling scores (r=-0.072), WBSF (r=-0.131), tenderness (r=-0.119 for initial tenderness and r=-0.100 for overall tenderness), connective tissue amount (r=-0.065), juiciness (r=-0.077 for initial juiciness and r=-0.067 for sustained juiciness), and painty/fishy flavor (r=-0.102). As for anserine, the significant partial correlation was observed only with hot carcass weight (r=-0.076). Compared with simple correlations, even smaller absolute values were observed for partial correlation coefficients.

Because creatine, creatinine, carnosine, and anserine were distributed only in muscle tissue (Hipkiss, 2009a; Wyss and Kaddurah-Daouk, 2000), the negative associations with traits related to intramuscular fat content, such as marbling and tenderness, were expected. Our data, however, showed the correlations among them to be very weak, especially after removing the group differences (partial correlation coefficients). At physiological concentrations in muscle, carnosine can scavenge reactive oxygen species and have anti-oxidant effects (Hipkiss, 2009a). Therefore, it is believed that carnosine can reduce products of lipid peroxidation (TBARS) (Derave et al., 2010). Our study did not show strong negative correlation between carnosine content and TBARS (r=-0.060 for simple correlation coefficient ($P<0.05$) and r=-0.031 for partial correlation coefficient ($P>0.05$)). It should be noticed that samples in this study showed small TBARS values (with the average at 0.19 ± 0.01 mg/kg) compared with the threshold value of acceptability at 2 mg/kg, which indicated little lipid oxidation opportunity was allowed during sample collection and

processing (Garmyn et al., 2011). The low TBARS values might explain the weak correlations between carnosine and TBARS in this study.

Acceptance on meat quality by consumers should be considered fully during the selection of meat with better health value. For instance, concerns are raised about leanness in the pork industry because of the positive association between intramuscular fat content and tenderness (Wood J.D., 1986; Wood J.D., 2008). The weak correlations among creatine and carnosine with carcass and palatability traits observed in our study indicate that beef quality, especially tenderness, juiciness, and flavor will not be strongly affected by selecting for higher creatine and carnosine contents. Because few other studies directly calculated the correlation coefficients among creatine, carnosine, and meat quality traits, it is difficult to compare our results with other studies. The relationship between these nutraceuticals and meat quality, however, can be inferred from the experiments in which creatine or carnosine were used as dietary supplements. Dietary administration of creatine monohydrate was reported to have influences on postmortem pH, water-holding capacity, and meat color but to have little or no impacts on other pork quality parameters, especially on tenderness and juiciness (Berg and Allee, 2001; O'Quinn et al., 2000; Stahl et al., 2001). Similar results were observed on chicken with creatine supplement (Nissen and Young, 2006). In the study of Derave et al. (2010), who investigated the influence of dietary supplement with carnosine on meat quality of finishing pigs, carcass traits including carcass weight, yield grade, longissimus muscle area, back fat thickness, shear force, and marbling score were not

affected by the dose up to 100 mg carnosine per kg diet for 8 weeks. Despite the potential difference among species, these results supported for the weak correlations observed in our study.

Implication

Beef is good source of dietary creatine and carnosine when compared with vegetarian diets or other red meat product. Beef creatine and carnosine contents are affected by gender and feeding locations. The correlations, although weak, among creatine and carnosine contents with carcass quality and beef palatability provide the possibility of optimal selection of healthful beef without compromising production efficiency or consumer satisfaction.

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Tables

Table 1. Simple statistics for creatine, creatinine, carnosine, and anserine concentrations in longissimus muscle of Angus cattle[1]

Item	**Mean**	**Minimal**	**Maximal**
Creatine (mg/g)	5.26	2.58	6.86
Creatinine (mg/g)	0.207	0.034	0.553
Carnosine (mg/g)	3.72	2.28	5.72
Anserine (mg/g)	0.668	0.140	1.218

[1] Traits were expressed as mg per g wet tissue

Table 2. Effect of gender on creatine, creatinine, carnosine, and anserine concentrations in longissimus muscle of Angus cattle[1] from Iowa.

Item	Bull	Steer	Heifer	P
Creatine (mg/g)	5.30±0.03 [a]	5.12±0.02 [b]	5.22±0.06 [a,b]	<0.0001
Creatinine (mg/g)	0.167±0.006 [a]	0.178±0.005 [a]	0.235±0.015 [b]	0.0018
Carnosine (mg/g)	3.76±0.05 [a]	3.77±0.05 [a]	4.12±0.10 [b]	0.0003
Anserine (mg/g)	0.68±0.01 [a]	0.71±0.01 [b]	0.65±0.03 [a]	<0.0001

[a,b] Means within a row lacking a common superscript differ ($P<0.05$).

[1] Traits, per gram wet tissue, are expressed as least squares means±SE.

Table 3. Effect of gender on carcass traits of Angus cattle[1] from Iowa.

Item	Bull	Steer	Heifer	P
Hot carcass weight, kg	352.46±2.50[a]	320.10±4.83[b]	268.94±5.98[c]	<0.0001
KPH fat[2], %	2.25±0.03 [a]	2.30±0.02 [a]	1.96±0.07 [b]	<0.0001
Ribeye area[3], cm^2	86.26±0.58[a]	78.65±0.52[b]	72.39±1.29[c]	<0.0001
12th rib fat thickness[4], cm	0.99±0.03[a]	1.09±0.04[a]	0.88±0.07[b]	<0.0001
Marbling score[5]	5.41±0.07[a]	6.44±0.06[c]	5.91±0.16[b]	<0.0001
Calculated yield grade[6]	2.61±0.04 [b]	2.84±0.03 [a]	2.36±0.09 [c]	<0.0001

[a,b,c] Means within a row lacking a common superscript differ ($P<0.05$).

[1] Traits are expressed as least squares means±SE.

[2] Estimated of percentage of kidney, pelvic, and heart fat, % of carcass weight.

[3] *Longissimus dorsi* cross sectional area at carcass split between the 12th and 13th ribs.

[4]Measured at 3/4 the lateral distance across the *Longissimus dorsi* from the spine at carcass split between the 12th and 13th ribs.

[5]3.0=traces; 4.0=slight; 5.0=small; 6.0=modest; 7.0=moderate; 8.0=slightly abundant; 9.0=moderately abundant (based on USDA system).

[6]Yield grade= 2.5 + (6.35 × fat thickness, cm) + (0.2 × KPH %) + (.0017 ×hot carcass weight, kg) - (2.06 × ribeye area, cm^2)

Table 4. Effect of gender on Warner-Bratzler Shear Force (WBSF), thiobarbituric acid reactive substances (TBARS), and palatability traits of beef from Angus cattle[1] fed in Iowa.

Item	Bull	Steer	Heifer	P
WBSF, kg	3.61±0.06 [a]	3.49±0.05 [a]	2.80±0.15 [b]	<0.0001
TBARS[2], mg/kg	0.126±0.005 [a]	0.143±0.004 [b]	0.187±0.008 [c]	<0.0001
Initial tenderness[3]	5.38±0.10[a]	5.69±0.07[b]	5.73±0.15[a,b]	0.0004
Overall tenderness[3]	5.25±0.10[a]	5.69±0.07[b]	5.73±0.15 [a,b]	<0.0001
Connective tissue[4]	5.32±0.10[a]	5.66±0.08[b]	5.76±0.15[a,b]	<0.0001
Initial juiciness[5]	5.38±0.07	5.41±0.05	5.27±0.11	0.4002
Sustained juiciness[5]	5.06±0.07	5.10±0.05	4.83±0.10	0.0603
Beef flavor[6]	2.43±0.04 [a]	2.56±0.03 [b]	2.39±0.06 [a]	<0.0001
Painty/fishy flavor[6]	1.19±0.03 [a]	1.11±0.02 [b]	1.25±0.05 [a]	<0.0001
Livery/metallic flavor[6]	1.07±0.02	1.08±0.01	1.12±0.02	0.5129

[a,b] Means within a row lacking a common superscript differ ($P<0.05$).

[1] Traits are expressed as least squares means±SE.

[2] Expressed as mg of malonaldehyde per kg of sample.

[3] 1=Extremely tough; 2=Very tough; 3=Moderately tough; 4=Slightly tough; 5=Slightly tender; 6=Moderately tender; 7=Very tender; 8=Extremely tender.

[4] 1=Abundant; 2=Moderately abundant; 3=Slightly abundant; 4=Moderate; 5=Slight; 6=Traces; 7=Practically none; 8=None.

[5] 1=Extremely dry; 2=Very dry; 3=Moderately dry; 4=Slightly dry; 5=Slightly juicy; 6=Moderately juicy; 7=Very juicy; 8=Extremely juicy.

[6] 1=Not detectable; 2=Slightly detectable; 3=Strong.

Table 5. Effect of feeding location on creatine, creatinine, carnosine, and anserine concentrations in longissimus muscle of Angus steers[1].

Item	CA	TX	CO	IA	P
Creatine (mg/g)	5.56±0.04 [a]	5.03±0.04 [b]	----------	5.15±0.04 [b]	<0.0001
Creatinine (mg/g)	0.15±0.07	0.29±0.05	0.32±0.09	0.17±0.03	0.1972
Carnosine (mg/g)	3.53±0.03[b]	3.39±0.03 [a]	4.09±0.03 [d]	3.84±0.03 [c]	<0.0001
Anserine (mg/g)	0.68±0.01 [b,c]	0.64±0.01 [a,b]	0.63±0.01 [a]	0.70±0.01 [c]	0.0017

[a,b] Means within a row lacking a common superscript differ (P<0.05).

[1] Traits, per gram wet tissue, are expressed as least squares means±SE.

Table 6. The simple correlation coefficients(r) [1] between creatine, creatinine, carnosine, and anserine concentrations and carcass traits of Angus cattle

	HCW[2]	KPH fat[3]	Ribeye area[4]	Fat thickness[5]	Marbling[6]	Calc YG[7]
Creatine	0.028	0.035	0.085***	-0.133***	-0.235***	-0.127***
Creatinine	0.085***	-0.091***	-0.083***	0.284***	0.082***	0.271***
Carnosine	-0.014	0.143***	0.013	-0.239***	-0.047*	-0.161***
Anserine	-0.013	0.162***	0.043*	-0.042	0.013	-0.040

[1]Pearson correlation coefficients are calculated for HCW, KPH fat, ribeye area, fat thickness, and calculated yield grades. Spearman correlation coefficients are calculated for marbling scores. Level of significance: *P<0.05; **P<0.01; ***P<0.001.

[2] Hot carcass weight

[3] Estimated of percentage of kidney, pelvic, and heart fat, % of carcass weight

[4] *Longissimus dorsi* cross sectional area at carcass split between the 12th and 13th ribs.

[5]Measured at 3/4 the lateral distance across the *Longissimus dorsi* from the spine at carcass split between the 12th and 13th ribs.

[6]3.0=traces; 4.0=slight; 5.0=small; 6.0=modest; 7.0=moderate; 8.0=slightly abundant; 9.0=moderately abundant (based on USDA system).

[7] Yield grade= 2.5 + (6.35 × fat thickness, cm) + (0.2 × KPH %) + (.0017 ×hot carcass weight, kg) - (2.06 × ribeye area, cm^2)

Table 7. The simple correlation coefficients(r) [1] between creatine, creatinine, carnosine, and anserine concentrations, Warner-Bratzler shear force (WBSF), thiobarbituric acid reactive substances (TBARS), and palatability traits of beef from Angus cattle

	WBSF	TBARS[2]	Initial tenderness[3]	Overall tenderness[3]	Connective tissue[4]	Initial juiciness[5]	Sustained juiciness[5]	Beef flavor[6]	Painty/fishy flavor[6]	Livery/metallic flavor[6]
Creatine	-0.040	0.099***	0.016	0.041	0.096***	-0.135***	-0.102***	-0.066*	-0.134***	0.034
Creatinine	0.033	-0.419***	0.095***	0.056*	0.019	0.191***	0.133**	0.149***	0.124***	0.066**
Carnosine	0.006	-0.060*	-0.070**	-0.098***	-0.107***	0.005	-0.027	0.102***	0.021	0.069**
Anserine	-0.122***	0.107***	0.027	0.060*	0.064*	0.001	0.035	0.062*	-0.172***	0.016

[1] Pearson correlation coefficients are calculated for WBSF and TBARS. Spearman correlation coefficients are calculated for sensory panel scores. Level of significance: *P<0.05; **P<0.01; ***P<0.001.

[2] Expressed as mg of malonaldehyde per kg of sample.

[3] 1=Extremely tough; 2=Very tough; 3=Moderately tough; 4=Slightly tough; 5=Slightly tender; 6=Moderately tender; 7=Very tender; 8=Extremely tender.

[4] 1=Abundant; 2=Moderately abundant; 3=Slightly abundant; 4=Moderate; 5=Slight; 6=Traces; 7=Practically none; 8=None.

[5] 1=Extremely dry; 2=Very dry; 3=Moderately dry; 4=Slightly dry; 5=Slightly juicy; 6=Moderately juicy; 7=Very juicy; 8=Extremely juicy.

[6] 1=Not detectable; 2=Slightly detectable; 3=Strong.

Table 8. The partial correlation coefficients(r) [1] between creatine, creatinine, carnosine, anserine, and carcass traits of Angus cattle

	HCW[2]	KPH fat[3]	Ribeye area[4]	Fat thickness[5]	Marbling[6]	Calc YG[7]
Creatine	-0.017	0.030	0.061**	-0.040	-0.129***	-0.068**
Creatinine	0.002	0.036	0.078***	0.026	-0.030	-0.028
Carnosine	-0.047*	0.049*	-0.027	-0.025	-0.072*	-0.013
Anserine	-0.076***	0.028	-0.004	0.007	-0.004	-0.022

[1] The partial correlation coefficients are adjusted for gender, feeding location, and birth season. Pearson correlation coefficients are calculated for HCW, KPH fat, ribeye area, fat thickness, and calculated yield grades. Spearman correlation coefficients are calculated for marbling scores. Level of significance: *P<0.05; **P<0.01; ***P<0.001.

[2] Hot carcass weight

[3] Estimated of percentage of kidney, pelvic, and heart fat, % of carcass weight

[4] *Longissimus dorsi* cross sectional area at carcass split between the 12th and 13th ribs.

[5]Measured at 3/4 the lateral distance across the *Longissimus dorsi* from the spine at carcass split between the 12th and 13th ribs.

[6]3.0=traces; 4.0=slight; 5.0=small; 6.0=modest; 7.0=moderate; 8.0=slightly abundant; 9.0=moderately abundant (based on USDA system).

[7] Yield grade= 2.5 + (6.35 × fat thickness, cm) + (0.2 × KPH %) + (.0017 ×hot carcass weight, kg) - (2.06 × ribeye area, cm^2)

Table 9. The partial correlation coefficients(r) [1] between creatine, creatinine, carnosine, and anserine concentrations, Warner-Bratzler shear force (WBSF), thiobarbituric acid reactive substances (TBARS), and palatability traits of beef from Angus cattle

	WBSF	TBARS[2]	Initial tenderness[3]	Overall tenderness[3]	Connective tissue[4]	Initial juiciness[5]	Sustained juiciness[5]	Beef flavor[6]	Painty/fishy flavor[6]	Livery/metallic flavor[6]
Creatine	0.012	-0.019	-0.027	-0.035	0.022	-0.038	-0.015	-0.031	-0.005	0.006
Creatinine	0.037	-0.0001	-0.053*	-0.052*	-0.018	0.009	0.015	0.074**	-0.063*	0.025
Carnosine	-0.131***	-0.031	-0.119***	-0.100***	-0.065**	-0.077**	-0.067**	0.042	-0.102***	-0.011
Anserine	0.040	0.033	-0.042	-0.030	-0.022	0.017	0.039	0.036	-0.049	0.015

[1] The partial correlation coefficients are adjusted for gender, feeding location, and birth season. Level of significance: *P<0.05; **P<0.01; ***P<0.001. Pearson correlation coefficients are calculated for WBSF and TBARS. Spearman correlation coefficients are calculated for palatability traits.

[2] Expressed as mg of malonaldehyde per kg of sample.

[3] 1=Extremely tough; 2=Very tough; 3=Moderately tough; 4=Slightly tough; 5=Slightly tender; 6=Moderately tender; 7=Very tender; 8=Extremely tender.

[4] 1=Abundant; 2=Moderately abundant; 3=Slightly abundant; 4=Moderate; 5=Slight; 6=Traces; 7=Practically none; 8=None.

[5] 1=Extremely dry; 2=Very dry; 3=Moderately dry; 4=Slightly dry; 5=Slightly juicy; 6=Moderately juicy; 7=Very juicy; 8=Extremely juicy.

[6] 1=Not detectable; 2=Slightly detectable; 3=Strong.

GENERAL SUMMARY

Creatine and carnosine are both valuable nutraceuticals that naturally exist only in animal tissues. Creatine plays an important role in cellular energy metabolism. Mutation in genes involving creatine biosynthesis and transportation causes an inborn error of metabolism called creatine deficiency syndromes. Secondary creatine deficiency in brain cells can be developed by other CNS pathologies, including hyperammonemia, stroke, and gyrate atrophy of the choroid and retina. Creatine deficiency impairs neurological function. Recent studies show neuroprotective effects of creatine in neurodegenerative diseases. Carnosine performs multiple biological functions including pH buffering, anti-oxidation, anti-glycation, anti-aging, and chelation of divalent metal cations. In humans, creatine and carnosine can be obtained either endogenously by biosynthesis from primary precursors or exogenously from dietary sources. Creatinine is the spontaneously formed cyclic derivative of creatine. Anserine is an N-methylated analogue of carnosine found mainly in fish and birds. Beef is a good source of dietary creatine and carnosine compared with other meat products (Hipkiss, 2009; Pais et al., 1999).

In this study, I was interested in determining concentrations of creatine, creatinine, carnosine, and anserine in longissimus muscle of Angus cattle. An HPLC method was used to quantify their concentrations in 2,342 beef samples collected from Iowa, Texas, Colorado, and California. The creatine concentration averaged 5.26 ± 0.01 mg/g, and the carnosine concentration averaged 3.72 ± 0.01 mg/g. The amount of creatine present in a typical serving

(100 g) of beef is generally lower than that recommended for supplementation (e.g., a typical creatine supplement protocol asks for 5 g of creatine per day for four days and then a maintenance dose of 2 g per day). The carnosine in a typical serving (100 g) of beef is comparative with the amount in commercial capsule (200-500 mg). We observed significant gender effects on concentrations of creatine and carnosine. Bulls contained greater creatine content than did steers ($P < 0.05$) but produced lower amount of carnosine than did heifers ($P < 0.05$).

The correlations among beef creatine, creatinine, carnosine, and anserine with carcass and palatability traits also were investigated. The weak correlations observed in our study indicate that beef quality, especially tenderness, juiciness, and flavor would not be strongly affected by selecting for healthful beef with higher creatine and carnosine contents.

In our study, all beef samples were collected from Angus breed and the harvest ages were not well documented. In future research, the influences of breed and age could be further investigated. Besides, the inheritability of these nutraceuticals could be used to study the influences of genetic factors. The regulation of creatine and carnosine metabolism has not been well studied. In human, creatine deficiency syndromes are associated with mutations in AGAT, GAMT, and SLC6A8 genes (Braissant, 2010; Schulze, 2003). Carnosine synthase was identified as the ATP-grasp domain containing protein 1(ATP-GD 1) very recently (Drozak et al., 2010). Future studies about genetic factors (especially single polymorphism nucleotides (SNPs)) associated with the variation of beef creatine, creatinine, carnosine, and

anserine could contribute toward revealing the mechanism of creatine and carnosine homeostasis in both cattle and humans.

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