Creatine as a Conditionally Essential Nutrient

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From 1996 to 2011, the UK was the hardest hit country with 175 recorded cases of variant Creutzfeldt-Jakob Disease (vCJD). The cause of the outbreak was widely attributed to consumption of beef products contaminated with central nervous system tissue of animals aff cted with so called Mad Cow Disease. In response to fears over public health and public confidence in the meat supply, EU leaders enacted a ban on the use of animal protein sources in livestock feeds. Similar bans or partial bans were enacted in many other countries around the world. The total ban has since been modifi d to allow some exceptions, however, some consumers have indicated a preference for meat and eggs produced without animal proteins. This has given rise to a market for "Vegetarian" or "Animal Protein-free" (APF) livestock feeding programs.

While attractive markets exist for such APF products, many producers noticed a drop in animal performance when animal protein sources were removed from the diet. Historically, animal protein sources were known to have unidentifi d growth factors, and through the early part of the 20th century feed formulations called for a minimum amount of various animal proteins to support adequate performance. Many of these growth factors were later identifi d as vitamins or amino acids that were absent or deficient in the plant feedstuffs in use.

The role of creatine

Creatine and creatine phosphate, just as ADP (adenosine diphosphate) and ATP (adenosine triphosphate), are key substances for energy transmission in all living cells (Wyss and Kaddurah-Daouk 2000). The creatine/creatine phosphate system is restricted to vertebrates, and it functions as a backup to the ADP/ATP system to store and mobilize energy when required on short notice, particularly in muscle cells and brain cells. This is assumed to be the reason that we find creatine in animals only and not in plants.

Adenosine Triphosphate is the universal energy currency in all living cells. In addition to its energy transfer function, ATP and its hydrolysis products, ADP and AMP (adenosine monophosphate), interact with a vast number of enzymes and processes in the body including active transport of molecules across membranes, muscular contraction and activation of hormone receptor cascades. Decreases in cellular ATP concentration have recently been associated with increased protein degradation through up regulation of proteasome peptidase activity (Huang et al, 2010). The concentration of ATP in the cells is tightly regulated and cannot be elevated to extremes (Stryer 1990). In this context, creatine phosphate serves as a dynamic reservoir of high energy phosphate bonds that buff rs the cytosolic ATP / ADP ratio from rapid fluctuations that would be deleterious to protein deposition, the smooth functioning of myofibrils, cation pumps, and numerous other ATP-dependent functions (Walker 1979).

De novo synthesis of creatine occurs in the kidney. Arginine and Glycine combine to form Guanidino Acetic Acid (GAA) and Ornithine in a reaction catalyzed by arginine-glycine amidinotransferase (AGAT). Arginine is the rate-limiting factor for GAA and creatine biosynthesis. AGAT is the first step and is rate limiting for creatine synthesis. It is down regulated by creatine and is up regulated by growth hormone in rats (Guthmiller et al, 1994). GAA is transported by the bloodstream to the liver where the methylation of GAA to form creatine is catalyzed by guanido-

acetate N-methyltransferase with S-adenosylmethionine acting as the methyl donor (Daly 1985; Stead et al. 2001; Komoto et al. 2003). Creatine is then transported by the bloodstream to the target cells. The major portion (> 95 %) of the creatine pool (creatine phosphate / creatine) is found in the skeletal muscle, the rest in the heart and brain (Walker 1979).

GAA - precursor for creatine

Guanidino Acetic Acid (GAA) is the only immediate metabolic precursor for creatine in the body. It is a naturally occurring compound in all humans and vertebrates. It is also present in limited amounts in mixed diets and can be absorbed from the gut (Wyss and Kaddurah-Daouk1 2000).

Creatine can also be absorbed from the gut (Peral et al. 2002, Peral et al. 2005). Nutritional sources for creatine are protein-rich tissues of animal origin. As a natural constituent of mixed diets for humans creatine is most abundant in fish (3-10 g/kg) and meat (4-5 g/kg). An adult person of 70 kg body weight, depending on his/her nutritional state, contains about 120 grams of creatine, of which two thirds are in the form of "high-energy" creatine phosphate (Mertschenk et al. 2001). Creatine and its phosphorylated form, creatine phosphate, play an important role in cellular energy storage, buff ring and transport (Clark 2000).

The reversible reaction of the creatine/creatine phosphate system is catalyzed by creatine kinase. This enzyme must be regarded as a key enzyme in energy metabolism. The reaction catalyzed by this enzyme is the only enzymatic reaction known in vertebrate tissues for which creatine and its phosphorylated form, creatine phosphate are substrates.

Creatine is continuously converted to creatinine which is the degradation product of creatine. This is an irreversible non-enzymatic reaction in all cells containing creatine. Creatinine diffuses to the blood and is then excreted by the kidney at a rather constant rate of approximately 1.6 % of the creatine pool per day (Wyss and Wallimann 1994). The conversion of creatine and creatine phosphate to creatinine and its subsequent clearance via the kidney results in a loss of approximately 2 grams of creatine per day in humans.

The metabolism of GAA, creatine and creatinine represents a one-way street with no branches except the reversible creatine/creatine phosphate reaction. GAA cannot be converted back into the two amino acids it originates from, glycine and arginine. Creatine cannot be re-converted to GAA and, finally, creatinine cannot be re-converted to creatine. GAA has only one metabolic fate: creatine and thereafter creatinine.

When the diet is devoid of animal protein sources, creatine levels may be reduced. Lower creatine values in human blood serum were reported for vegetarians by Delanghe et al. (1989). Schek (2000) reported similar findings as well as lower creatinine excretion. Burke et al (2003) observed lower total creatine and creatine phosphate in human vegetarians compared to non-vegetarians. Supplementing both vegetarians and non-vegetarians with an oral creatine supplement resulted in greater total creatine, creatine phosphate, muscular strength and lean tissue mass. These increases were greater for vegetarians than for non-vegetarians.

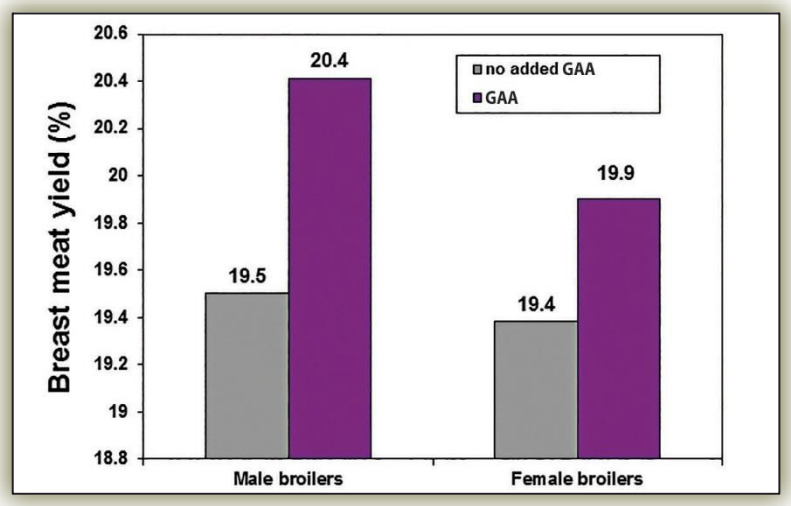
In a study conducted by CCL Research in The Netherlands, Cobb 500 male broilers were fed animal protein-free diets supplemented with GAA from a commercially available source at 0, 0.02, 0.04 or 0.06% of the diet. Muscle tissue analysis demonstrated a significant linear increase in creatine content up to the maximum supplementation rate.

Treatment		**I**	**II**	**III**	**IV**	
GAA suppl.		0%	0.02%	0.04%	0.06%	**p-value**
Analysis of muscle tissue						
GAA	(mg/kg)	23.7[a]	13.7[b]	6.2[c]	3.7[c]	< 0.001
Creatine	(mg/kg)	3986[c]	4006[c]	4357[b]	4560[a]	< 0.001
Creatinine	(mg/kg)	10.7[b]	13.0[a]	14.0[a]	14.5[a]	0.005

Requirements for GAA

The factorial approach distinguishes between requirements for maintenance and (growth) performance. For both fractions

	Feed intake (g)	Weight gain (g)	FCR (g/g)	FCR corr.* (g/g)	Mortality (%)
6% meat and bone meal diet	2700[a]	1671[a]	1.62[b]	1.61[b]	1.25
Vegetable diet	2612[b]	1610[b]	1.62[b]	1.60[b]	1.11
Vegetable diet with 0.06% GAA	2657[ab]	1693[a]	1.57[a]	1.56[a]	0.74
P value	0.066	0.006	0.008	0.001	0.798

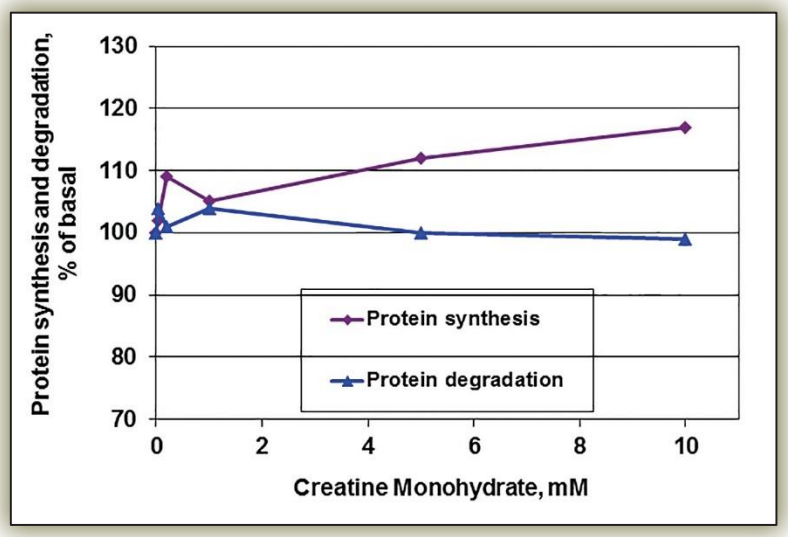


key figures enabling the calculation of requirements are needed. Some of them can be derived from the literature, however, most of the creatine and GAA data available were generated using human beings, laboratory animals or in-vitro assays.

A maintenance requirement is the amount of a nutrient or energy needed to avoid loss of weight in the absence of reproduction, product yield and work. GAA-requirements have to be seen in conjunction with creatine requirements. Balsom et al. (1994) and Wyss and Kaddurah-Daouk (2000) reported a daily degradation of 1.6-1.7 % of the creatine pool. Also Keshavarz and Fuller (1971) analyzed muscle creatine and creatine in excreta. Using these data a daily excretion of about 1.8 % of the creatine pool can be calculated. In his review from 2000, Harris pointed out that mammalian muscle contains 3-6 g creatine/kg. Balsom et al. (1994) reported values ranging between 4.5 and 5 g/kg in beef and pork. Chamruspollert et al. (2002) determined creatine in broiler muscle tissue with values ranging between 1.34 g/kg and 5.81 g/kg muscle. They found in one experiment that low dietary arginine resulted in low muscle creatine concentrations which might be explained by the fact that arginine is required for the GAA synthesis. The weighted mean of creatine concentrations from that experiment (excluding those values aff cted by dietary arginine) was 4.42 g creatine/kg muscle. An average of 4.5 g creatine/kg muscle can be assumed for broiler meat. Assuming that the vast majority of creatine is found in skeletal muscle (Balsom et al. 1994; Harris 2000; Wyss and Kaddurah-Daouk 2000) and that the proportion of muscle tissue in the living broiler represents about 50 % of total weight (Kallweit et al. 1988; Kirchgessner 1997), we can estimate an average creatine content of 2.25 g creatine/kg of live weight in broilers. Using the daily creatinine excretion estimate we can calculate a daily maintenance requirement of 38.3 mg/kg of live weight.

Performance of farm animals raised for meat production is defined as weight gain, muscle growth and protein retention, respectively. Apart from body growth, feather growth plays a small role, too, in poultry. However, as creatine is predominantly found in muscle tissue (Balsom et al. 1994; Harris 2000; Wyss and Kaddurah-Daouk 2000) feather growth can be neglected in the present approach.

Continuing the example of the 1 kg broiler an average daily weight gain of about 70 g can be expected (Aviagen 2014; Cobb-Vantress 2012). Since about 50 % of the gain is muscle (Kallweit et al. 1988; Kirchgessner 1997) containing on average 4.5 g creatine/kg (Balsom et al. 1994; Harris 2000, Chamruspollert et al. 2002), 157.5 mg creatine are required (net) per day for performance.



Adding up the net requirement for maintenance and performance gives the total net requirement. In the 1 kg broiler example this would be 195.8 mg creatine/day (38.3 mg for maintenance and 157.5 mg for weight gain performance). A considerable portion of the total net requirement is covered by de-novo synthesis of GAA and creatine. Based on observed performance of broiler on animal protein-free feeding programs, approximately two thirds of the daily requirement can be met without dietary sources of creatine activity. This would reduce the unmet needs of the 1 kg broiler from 195.8 mg creatine to 66.6 mg creatine daily corresponding to 60.3 mg GAA (assuming equimolar conversion of GAA to creatine). This shortfall would need to be met by dietary sources.

GAA supplementation has been seen to increase breast meat yield in broilers. One European broiler producer observed a statistically significant ($p<0.05$) increase of 1 percentage point in breast meat yield in male broilers supplemented with GAA. Females fed the GAA supplemented diets showed an increase of 0.5 percentage points, though the difference was non-significant.

In a trial conducted at the University of Sao Paulo, male Ross 308 broilers were fed diets containing 6% meat and bone meal (MB), an animal protein-free diet (APF), or an animal protein-free diet supplemented with GAA at 0.06% of the diet (GAA) from 15 to 35 days of age. Feed intake tended to be greater for the MB treatment versus the APF treatment while the GAA supplemented treatment was intermediate. Weight gain was similar between the MB and GAA and significantly greater than gain for APF. Feed to gain was significantly lower for GAA than for the other treatments.

Two studies give information about the involvement of creatine in protein deposition. Young et al (2007) found an increase in protein synthesis and decrease protein degradation in myotubes from pigs treated with creatine monohydrate in vitro. Huang et al (2010) reported an increase in proteasome peptidase activity and resulting in higher rates of protein degradation in cells with reduced ATP concentrations. Creatine may serve to reduce protein degradation and, therefore, protein accretion by buff r-ing normal ATP concentrations.

GAA for veggie and non-veggie diets

There is considerable evidence that creatine may be a conditionally dietary essential nutrient. Diets devoid of animal protein sources provide no creatine activity and force the animals to rely on de novo synthesis. **Even diets containing meat and fish products may not supply sufficient creatine to support optimal performance due to the maximum limits placed on the ingredient or due to the limitations of price of the ingredient in the formula.** Guanidino acetic acid is the immediate precursor of creatine, is efficiently absorbed from the gut, and has been demonstrated to increase tissue concentration of creatine. With direct supplementation of GAA, the optimal level of creatine can be reached, sparing ariginine. Supplementation of GAA to diets devoid of animal protein sources or lacking sufficient creatine levels to compliment de novo creatine synthesis has been observed to improve feed efficiency, growth rate and breast meat yield in broilers. ■

See www.evonik.com/animal-nutrition for references

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