Supplemental guanidino acetic acid affects energy metabolism of broilers

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The semi-essential nutrient creatine is an important molecule in the cellular energy metabolism for short term energy storage. Energy from surplus adenosine tri-phosphate (ATP) can quickly be overtaken by creatine and vice versa. Supplemental guanidino acetic acid (GAA) is a natural precursor of creatine and might be used as creatine source. Either no (negative control), 0.20, 0.40, or 0.60 g/kg GAA were added to pure vegetable basal starter, grower, and finisher diets and fed to 3120 male broilers (six pens with 130 birds per treatment) for 41 days. GAA supplementation significantly improved feed conversion ratio (0.40 g/kg GAA; p<0.05). At day 41 five birds per pen were slaughtered and breast meat samples were collected for biochemical evaluation. The muscle creatine content gradually increased from 3986 (negative control) to 4560 mg/kg breast meat (0.60 g/kg GAA; p<0.05). In contrast, the muscle GAA content stepwise decreased with increasing GAA supplementation (from 24 to 4 g/kg, p<0.05). One hour post mortem the muscle ATP content was increased up to 0.40 g/kg dietary GAA compared to the control whereas the ADP content was rather unaffected. The muscle AMP and IMP levels gradually declined with increasing dietary GAA up to 0.60 g/kg. Results indicate that supplemental GAA improves the cellular energy metabolism.

Keywords: guanidino acetic acid, creatine, ATP, broiler

Introduction

Creatine and its phosphorylated form phospho-creatine are key substances for the energy transmission in all living cells of vertebrates (Wyss and Kaddurah-Daouk, 2000). The creatine/phospho-creatine system functions as a backup to the adenosine tri-phosphate (ATP) / adenosine di-phosphate (ADP) system in order to store and mobilise energy when required on short notice, particularly in muscle cells.

Guanidino acetic acid (GAA) is the only immediate precursor for creatine in the body and is thus a naturally occurring compound in vertebrate animals and thus also in chicken. GAA is formed from the amino acids glycine and arginine mainly in the kidney and is then transported to the liver where most of it is transformed to creatine. Although, these syntheses mainly occur in kidney and liver, respectively, all cells and thus also muscle cells are equipped with the respective enzymes (Wyss and Kaddurah-Daouk, 2000). The major portion (>95 %) of the creatine pool is found in the skeletal muscle. Part of this creatine pool (1.5-2.0 % per day) is irreversibly transformed into creatinine which is then excreted with urine. This indicates the need of a steady refilling of the creatine pool either by de-novo synthesis or by nutrition.

Creatine and GAA are not found in plants and consequently under pure vegetable feeding conditions all creatine must be derived by *de-novo* synthesis. Based on data from human research on vegetarians (Schek, 2000) but also on experience after the ban of animal by-products in broiler nutrition in 2001 when poultry performance (especially feed conversion) was impaired (Richter, 2004), it is assumed that the potential of the *de-novo* synthesis for optimum creatine supply is limited. Creatine can thus be seen as a semi-essential substance.

An experiment was conducted in which graded levels of GAA (in form of CreAmino™, Degussa Feed Additives) added to a vegetable basal diet were fed to growing broilers in order to study the effects on performance and several bio-chemical parameters.

Materials and methods

A total of 3120 male, day-old Cobb 500 broiler chicks were equally placed into 24 floor pens with 130 birds each. The floor was covered with wood shavings. A computerized climate control allowed for a linear decrease of the temperature from 34 °C at placement to 19 °C at day 41. From day one until day 11 the lighting schedule was 23 h light and 1 h dark. At days 12 and 13, 14 and 15, and 16 to 41 the dark time was increased to 5, 8, and 12 hours, respectively. Dark periods were split into 2 parts.

The experimental arrangement comprised 4 dietary treatments with 6 replicates each. Basal starter (day 1-10), grower (day 11-28) and finisher diets (day 29-41) consisting of ingredients only of plant origin (Treatment I) were supplemented with no (Treatment I), 0.02 % (II), 0.04 % (III), or 0.06 % (IV) GAA. The GAA source was CreAmino™. The main ingredients of the diets were corn, wheat, and soybean meal (Table 1). Pelleted diets and water were offered *ad libitum*.

Table 1 Ingredients and nutrient composition of the basal starter, grower, and finisher diet.

Ingredients, %	Starter 1-10 d	Grower 11-28 d	Finisher 29-41 d	Energy and nutrients, %	Starter 1-10 d	Grower 11-28 d	Finisher 29-41 d
Corn	32.00	27.50	23.50	Energy, kcal ME/kg	3004	3110	3207
Wheat	34.94	39.40	43.16	Ether extract	4.3	6.0	7.2
Corn gluten 60%	2.48	1.00	1.73				
Soybean meal	20.09	17.24	14.34	Crude Protein	20.0	18.8	18.5
Rapeseed meal	3.00	6.00	7.50	Dig. Lys**	1.06	1.01	1.00
Soybean oil	1.11	0.90	1.85	Dig. Met + Cys	0.86	0.84	0.81
Animal fat	0.56	2.63	3.00	Dig. Thr	0.67	0.65	0.65
Monocalcium Phosphate	0.89	0.38	0.27	Dig. Trp	0.19	0.18	0.18
Limestone	0.63	0.48	0.46	Dig. Arg	1.05	1.00	0.95
Salt	0.14	0.11	0.11				
Premix with minerals, vitamins, free amino				Calcium	0.69	0.57	0.54
acids, and enzymes.	4.96	5.27	4.68	Available phosphorus	0.39	0.29	0.27

** Digestible Lysine; according to Dutch CVB table

Chick weights were recorded per pen at start of the experiment, and after each period. Feed consumption was also recorded per pen per phase.

At day 41 two birds per pen with body weights close to the pen average were selected for further investigation. Carcasses were stored at 4 °C. Breast meat samples were taken from the cooled carcasses and frozen. These samples were analysed for their GAA, creatine, and creatinine content by HPLC procedures in the Degussa laboratory. Further breast meat samples were taken 1 hour and 4 hours after slaughter and immediately frozen in liquid nitrogen by researchers from the University of Aarhus. In these samples several phosphorylated compounds including phospho-creatine, ATP, ADP, AMP, and inosine mono-phosphate (IMP) were analysed by HPLC in the laboratory of the University of Aarhus.

Data was subjected to analysis of variance using SAS 8.2 using the following model:

$Y_{ij} = \mu + T_i + e_{ij}$
with T_i = Treatment (i = I, II, III, IV), and e_{ij} = residual error.

The level of significance was chosen at $p<0.05$.

Results and discussion

Broilers performed well and final weights ranged between 2636 g and 2691 g (Table 2). Increasing supplementation of GAA particularly improved feed conversion ratio by 0.03 to 0.05 kg/kg indicating an improvement of the efficiency of the nutrient and energy utilisation.

Table 2 Effects of increasing levels of supplemental GAA on daily gain, feed intake, and feed conversion ratio in 1 to 41 days old male Cobb 500 broilers.

Treatment	I	II	III	IV	p-value
GAA addition	0 %	0.02 %	0.04 %	0.06 %	
Weight 41 days (g)	2651 [ab]	2636 [b]	2691 [a]	2653 [ab]	0.021
Feed intake (g/day)	103	102	102	102	0.844
FCR (2100)*	1.45 [a]	1.44 [a]	1.40 [b]	1.42 [ab]	0.039

[(a.b)]Means within a row with different superscripts differ significantly (P<0.05).
* standardised to 2100g body weight

In Table 3 breast meat contents of GAA, creatine, and creatinine are shown. Muscle creatine content increased by about 14 % when supplemental GAA was increased up to 0.06 % in the diet suggesting dietary GAA to be a potent creatine source. In contrast, the muscle GAA content declined with increasing dietary supply. The *de-novo* synthesis of GAA is regulated by a feed back mechanism. Increased serum concentration of creatine suppresses the enzymatic activity of the L-arginine:glycine amidinotransferase (AGAT) which catalyses the formation of GAA from glycine and arginine (Wyss and Kaddurah-Daouk, 2000). This feedback repression of AGAT by creatine is the most important enzyme regulating mechanism in the biosynthesis of creatine. However, in the current case GAA derived from the feed and creatine was formed mainly in the liver. As mentioned earlier also the muscle tissues are equipped with all enzymes needed for creatine synthesis. It is thus hypothesised that the AGAT activity in the muscles has been gradually down regulated by the increasing amounts of incoming creatine with increasing dietary GAA supply. Consequently, the muscle GAA content gradually decreased with increasing dietary GAA concentration.

Increasing concentrations of creatine in the muscle was also accompanied by increasing levels of creatinine from treatment I to IV. However, the concentrations were all below 15 mg/kg suggesting that creatinine is efficiently transported out of the cells.

Table 3 Effects of graded levels of supplemental dietary GAA on the GAA, creatine and creatinine content of breast meat which has been obtained from the carcasses of 41 days old male Cobb 500 broilers.

Treatment	I	II	III	IV	p-value
GAA addition	0 %	0.02 %	0.04 %	0.06 %	
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

[(a.b,c)]Means within a row with different superscripts differ significantly (P<0.05).

Table 4 shows concentrations of some phoshorylated substances in breast meat both one and four hours post mortem. The substances are in an order from high energy value (phospho-creatine) to low energy value (inosine-mono-phosphate, IMP). Measured at one hour post mortem, the level of high energy molecules increased with increasing GAA supplementation (in case of ATP even significantly) whereas the level of substances with lower bound energy declined at least up to 0.04 % GAA supplementation. These effects suggest a more efficient storage of energy due to GAA supplementation which is available on short notice for metabolic purposes such as protein synthesis. For example, Young et al. (2007) supplementing increasing doses of creatine monohydrate to swine cell cultures observed an increased protein synthesis whilst protein degradation was unchanged.

After four hours post mortem these treatment effects almost disappeared (Table 4). The metabolic processes under anaerobic conditions caused a general shift towards an accumulation of low energy substances especially IMP.

Table 4 Effects of graded levels of supplemental dietary GAA on the GAA, creatine and creatinine content of breast meat of 41 days old male Cobb 500 broilers.

Treatment	I	II	III	IV	p-value
GAA addition	0 %	0.02 %	0.04 %	0.06 %	
1 hour post mortem					
Phospho-creatine (µmol/g)	2.35	2.52	2.72	2.79	0.766
ATP (µmol/g)	3.10^{b}	3.31^{b}	4.83^{a}	4.30^{ab}	0.050
ADP (µmol/g)	1.61	1.82	1.67	1.60	0.636
AMP (µmol/g)	0.81^{a}	0.81^{a}	0.57^{b}	0.46^{b}	0.007
IMP (µmol/g)	4.06^{a}	3.65^{ab}	2.65^{b}	2.67^{b}	0.052
4 hours post mortem					
Phospho-creatine (µmol/g)	0.93	1.21	0.97	1.40	0.223
ATP (µmol/g)	0.13	0.32	0.33	0.43	0.599
ADP (µmol/g)	0.51	0.70	0.70	0.80	0.453
AMP (µmol/g)	0.93	1.08	0.85	0.86	0.349
IMP (µmol/g)	7.10	6.96	6.93	6.74	0.890

$^{(a,b)}$Means within a row with different superscripts differ significantly ($P<0.05$).

In the experiment introduced above it was observed that supplemental GAA

1. improved particularly feed conversion ratio in 1-41 days old male broilers.
2. increased the muscle creatine content.
3. increased the content of energy rich phosphorylated creatine and ATP whereas energy poor substances such as IMP were reduced.

It is therefore concluded that supplemental GAA serves as an efficient creatine source improving the muscle energy metabolism. Effects on total muscle creatine content but also on phosphorylated creatine and ATP suggest an improved energy availability which in turn might affect the metabolic utilisation of nutrients for growth. Decreases in feed conversion might reflect these effects.

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