Effects of graded levels of creatine and guanidino acetic acid in vegetable-based diets on performance and biochemical parameters in muscle tissue

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Fish meal is still allowed as feed ingredient in broiler diets, however, recently its availability decreased while prices increased. When switching to pure vegetable diets, poultry producers often observe a reduction in performance which may partly be explained by a lack of creatine supply. Playing an important role in energy metabolism of animals, creatine is not found in plants. Supplemented creatine (as Creapure™) or guanidino acetic acid (GAA, as CreAmino™) which is the only natural precursor of creatine, may balance the animal's demand for this semi-essential nutrient. A trial with male broilers was conducted in order to investigate the potential of both supplements to resolve a performance reduction under vegetarian nutrition conditions. The arrangement comprised a negative control (vegetable-based), treatments with 0.04, 0.08 and 0.12% creatine, treatments with 0.031, 0.063, 0.094, and 0.126% supplemental GAA, and a positive control (50 (1-21 days) and 30 g/kg fish meal (22-42 days)). Overall weight gain and feed conversion in the positive control were superior to the negative control ($p<0.05$). Increasing levels of supplemented creatine and GAA improved performance until almost reaching the levels of the positive control. Muscle creatine content in breast meat was lower in the negative control compared to the positive control ($p<0.05$), but graded creatine and GAA supplementation increased the content to even higher levels than in the positive control (highest inclusion $p<0.05$). Results suggest that both supplemental creatine and GAA have the potential to counteract performance declines due to feeding pure vegetable diets. Data further suggest that GAA can be used as an efficient creatine source.

Keywords: guanidino acetic acid; creatine; broiler; vegetable diets

Introduction

Creatine is a naturally occurring component in the animal's body tissue and plays a major role in energy metabolism. Creatine, which is produced naturally in the body from guanidino acetic acid (GAA), which in turn is synthesised from arginine and glycine, is mainly used in muscle tissues (Wyss and Kaddurah-Daouk, 2000). It is assumed that about 2/3 to 3/4 of the daily requirement is synthesised by de-novo while the remainder must be supplied by feed. As long as animal by-products, which are rich in creatine, formed a substantial part of the ration, no signs for a creatine deficiency could be detected. With the absence of animal proteins in pure vegetable diets the risk for a creatine deficiency increased. The objective of an experiment was to investigate the effects of graded dietary levels of supplemental creatine (creatine monohydrate supplemented as Creapure™) and GAA (supplemented as CreAmino™) on growth, feed intake, feed conversion and breast meat yield of male and female broilers in comparison to a negative (pure vegetable diet) and positive control (meat and bone meal containing diet).

Materials and methods

A total of 2240 one-day old, male Ross 308 broilers were allocated to 56 pens with 40 broilers per pen. Feed and water were supplied ad libitum. Environmental conditions during the trial were appropriate to the age of the birds and followed the breeder's recommendation.

The experiment consisted of nine dietary treatments including a negative and a positive control, three graded levels of creatine added to the negative control diet (0.04, 0.08, and 0.12% supplemented as Creapure™), and three levels of GAA equimolar to the creatine levels (0.031, 0.063, 0.094% supplemented as CreAmino™) plus an extra GAA treatment with 0.126% GAA. Birds were fed pelleted starter and grower diets from 0-21 days and from 22-42 days of age, respectively. The negative control was completely vegetarian whereas the starter and grower diet of the positive control contained 50 and 30 kg/tonne fish meal, respectively (Table 1). Amino acid, creatine and GAA analyses of the diets confirmed a successful feed production. Both positive and negative control were replicated seven times while the other treatments were replicated six times.

Body weights of the birds were recorded per pen at placement, day 21 and day 42. Feed consumption was recorded at days 21 and 42. Three birds of median weight from each pen (a total of 114) were killed at day 42 of age for carcass evaluation. The whole breast fillets were removed, weighed and frozen at -20°C. Muscle creatine and GAA content was determined by HPLC procedure.

Data from the nine treatments were analysed by standard ANOVA procedures (GenStat Release 6.1) and LSD test was used for a multiple range test. $p<0.05$ was considered significant.

Table 1 Composition and calculated nutrient concentrations (g/kg) of the basal starter (0-21 days) and grower (22-42 days) diets. Aliquots from the negative control diet were supplemented with graded levels of creatine (0.04, 0.08, 0.12%) and guanidino acetic acid (0.031, 0.063, 0.094, 0.126%).

Ingredients	Starter nC	Starter pC	Grower nC	Grower pC	Calculated nutrients	Starter nC	Starter pC	Grower nC	Grower pC
Wheat	303.5	411.9	252.5	362.2	ME (MJ/kg)	12.7	12.7	13.1	13.1
Corn	250.0	140.0	300.0	200.0	Crude fat	55	60	68	75
Corn gluten meal	50.0	-	50.0	-	Crude fiber	27	27	27	27
Soybean meal	271.8	261.2	231.7	262.4	Crude protein	225	225	210	210
Peas	40.0	60.0	70.0	50.0					
Fish meal	-	50.0	-	30.0	Lys	12.6	12.8	11.5	11.7
Soybean oil	35.0	40.4	46.7	55.2	Met+Cys	9.3	9.4	8.7	8.7
Limestone	6.6	4.4	6.6	5.1	Thr	8.3	8.4	7.7	7.7
Dicalcium-P	26.3	19.6	26.7	22.6					
Sodium chloride	3.0	2.2	3.0	2.5	Dig. Lys	11.5	11.5	10.5	10.5
Na-bicarbonate	1.0	0.8	1.0	0.9	Dig. Met+Cys	8.5	8.5	7.9	7.9
DL-Methionine	2.1	2.3	1.9	2.1	Dig. Thr	7.4	7.4	6.8	6.8
Biolys	4.4	1.1	3.7	1.0					
L-Threonine	0.3	0.1	0.2	0.0	Calcium	10.0	10.0	10.0	10.0
Vitamins and minerals	6.0	6.0	6.0	6.0	Phosphorus	8.6	8.6	8.6	8.6

nC: negative control; pC: positive control

Results and Discussion

Compared to breeder's recommendation (Aviagen, 2002; 2634g gain / 1.676 FCR for male Ross 308 at day 42) performance of the broilers in the trial was on a high level (Table 2). Mortality was at a reasonable level (5.5%) and was not correlated to treatments.

During the entire period of the trial total feed intake of the broilers was on average 4442 g/bird and there were no significant differences between treatments (Table 2). In contrast, there were significant treatment effects on both body weight gain and feed conversion ratio (FCR). Breast meat yield also showed no treatment effects which is in contrast to Lemme et al. (2007b) reporting increasing breast meat yield with increasing dietary GAA levels. However, this was particularly observed in female broilers.

Compared to the positive control, weight gain and FCR were significantly lower in the negative control although diets of both treatments were formulated to contain the same energy, mineral, and digestible amino acid levels. Similar findings were reported by Lemme et al. (2007b) where the differences between positive and negative control occurred particularly in female broilers. Although, fish meal is allowed as only animal by-product in broiler rations, its high price and sometimes low availability forces feed producers to produce pure vegetable diets.

Table 2 Mean feed intake (g), weight gain (g/bird), feed conversion ratios (g feed/g gain), and breast meat yield (g/bird) of the broilers fed diets supplemented with creatine monohydrate (CMH), GAA, or fish meal (positive control) for 42 days.

Treatment	Feed intake	Weight gain	Feed conversion ratio	Breast meat yield
1 (negative control)	4493	2557 [bc]	1.757 [b]	487.6
2 (0.400 g CMH/tonne)	4484	2519 [c]	1.781 [b]	472.5
3 (0.800 g CMH/tonne)	4507	2703 [abc]	1.668 [ab]	511.5
4 (1.200 g CMH/tonne)	4519	2810 [a]	1.608 [b]	567.6
5 (0.314 g GAA/tonne)	4423	2685 [abc]	1.650 [a]	505.1
6 (0.628 g GAA/tonne)	4446	2664 [abc]	1.676 [ab]	501.7
7 (0.942 g GAA/tonne)	4518	2735 [ab]	1.651 [a]	514.2
8 (1.256 g GAA/tonne)	4372	2660 [abc]	1.645 [a]	511.6
9 (positive control)	4397	2744 [a]	1.606 [a]	478.1
SEM[1]	128.9	75.12	0.100	18.3

Values are the means of 6 (7 for treatments 1 and 9) replicate pens, 3 birds per pen for carcass analyses
Values within a column with no common superscripts differ significantly ($p<0.05$)
[1] Pooled standard error of means

When arranging the trial setup, it was speculated that these performance differences between negative and positive control were due to the missing creatine source in all vegetable diets because creatine is exceptionally found in products of animal origin. The lower creatine level found in broiler meat from the negative control compared to that found in meat of the positive control also indicated that the de-novo synthesis was not sufficient (Table 3). Schek (2000) also measured much more creatine in serum of non-vegetarian than in vegetarian human beings. Adding increasing levels of creatine to the diet gradually resolved weight gain and feed conversion back to the level of the positive control (Table 2). Moreover, the performance data are of linear nature and suggest even a further performance improvement with higher inclusion levels. This provides evidence that creatine obviously plays an important role for optimum growth and nutrient utilisation in growing chicken and that de-novo synthesis of creatine in the kidney and liver is not sufficient for optimum supply of the animals. Analyses of the muscle creatine content support this conclusion (Table 3). Increasing supply with supplemental creatine almost linearly increased the muscle creatine level from 4665 mg/kg (neg. control) to 5893 mg/kg (at 0.12% creatine). The incorporation into the muscle tissue appeared to be quite efficient as in the positive control muscle creatine content was at 5215 mg/kg.

However, creatine as a feed supplement is not stable both during storage and at lower pH due to its chemical properties. An alternative to supplemental creatine is supplemental GAA (CreAmino™) which meets the technical requirements for a feed supplement. In the metabolism GAA is the only precursor of creatine which is transformed to creatine by methylation in the liver. From there creatine is transported to the target tissues. About 95% of the creatine pool is located in muscle tissue (Wyss and Kaddurah-Daouk, 2000). Muscle creatine concentrations suggest that supplemented GAA was efficiently transformed to creatine in the liver which subsequently was transported to the muscles. From the data in Table 3 it can be concluded that supplemental GAA is as efficient as supplemental creatine in terms of creatine incorporation into the muscle. Moreover, this finding is in line with linear increases of muscle creatine levels with increasing dietary GAA provision as observed by Lemme et al. (2007a). In this respect, supplemental GAA can be seen as an efficient creatine source particularly when compared to the positive control and the creatine supplemented treatments.

In this trial, the effects of supplemental GAA on weight gain and feed conversion were not as pronounced compared to those of supplemental creatine. However, supplementation of 0.094% GAA resulted in body weight gain close to that of the positive control. Feed conversion was generally significantly improved by 10 points with GAA supplementation when compared to the negative control. Lemme et al. (2007a,b) reported significant improvements in FCR of up to five and seven points, respectively, in 42 days old broilers. While Lemme et al. (2007b) also reported significant increase in body weight gain due to GAA supplementation effects were rather small in the trial by Lemme et al. (2007a).

Table 3 Creatine (mg/kg) and guanidino acetic acid (GAA) content in breast meat of male broilers fed diets supplemented with creatine monohydrate (CMH), GAA, or fish meal (positive control) for 42 days.

Treatment	Creatine content in muscle	GAA content in muscle
1 (negative control)	4665 [e]	7.59 [a]
2 (0.400 g CMH/tonne)	4713 [e]	6.96 [a]
3 (0.800 g CMH/tonne)	5472 [c]	1.15 [b]
4 (1.200 g CMH/tonne)	5893 [a]	0.00 [b]
5 (0.314 g GAA/tonne)	5337 [cd]	1.30 [b]
6 (0.628 g GAA/tonne)	5370 [cd]	1.78 [b]
7 (0.942 g GAA/tonne)	5322 [cd]	1.21 [b]
8 (1.256 g GAA/tonne)	5689 [b]	0.91 [b]
9 (positive control)	5215 [d]	1.41 [b]
SEM[1]	93.2	1.72

Values are the means of 6 (7 for treatments 1 and 9) replicate pens, 3 birds per pen for muscle analyses
Values within a column with no common superscripts differ significantly ($p<0.05$)
[1] Pooled standard error of means

Muscle GAA content was high in the negative control and diminished with supplementation of either creatine source (Table 3). This is in line with results by Lemme et al. (2007a) where graded levels of dietary GAA also gradually decreased muscle GAA levels. It is hypothesised that this effect is due to a feedback suppression of GAA synthesis in the tissues due to high creatine levels because increased plasma creatine suppressed the activity of the L-arginine:glycine amidinotransferase catalysing the formation of GAA from glycine and arginine.

From the current trial it is concluded that

- pure vegetable diets lower performance compared to diets containing animal by-products.
- this performance difference can largely be resolved by both supplemental creatine and GAA.
- diets supplemented with creatine and GAA as well as with fish meal increase muscle creatine and decrease muscle GAA content compared to all vegetable diets.
- GAA is an effective creatine source.

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