PAPER

Anticatabolic activity of alpha-ketoglutaric acid in growing rats

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Abstract

This study evaluated the anti-catabolic effect of α-ketoglutaric acid (AKG) in rats. Thirty Sprague Dowley male rats were divided into 3 groups of 10 animals each and fed *ad libitum* with protein-free diet (PFD) supplemented with 0, 3, and 6 g/kg feed of AKG for 14 consecutive days. The AKG administration had no effect on the growth performance of rats in the global trial period (0-14 d). After 7 d of excreta collection, the losses of endogenous nitrogen (N), both urinary and total, linearly reduced (P<0.05) following the administration of AKG, ranging from 35.00 mg/d to 28.00 mg/d, and from 45.28 mg/d to 36.36 mg/d, respectively. The villi and microvilli heights were lower when animals received 3 g/kg of AKG respecting to other tested AKG levels. Indeed, enterocytes length linearly increased (P<0.05) as the level of AKG increased in the diet by 25.0% (3 g/kg) and 49.0% (6 g/kg). The AKG levels quadratically influenced (P<0.05) the essential amino acids (EAA) concentrations in the blood, being EAA lower for animals treated with 6 g/kg of AKG (-21.6%) compared to the animals fed the control PFD and the PFD with 3 g/kg of AKG. In conclusion, AKG was effective in reducing catabolism and EAA depletion.

Introduction

Available data strongly support the notion that glutamine (Gln) should be an essential part of parenteral nutrition in various diseased states (Griffiths, 2001). Following severe traumas, a reduction in muscle protein synthesis is observed in conjunction with a lowering of the Gln free fraction (Nordgren *et al.,* 2002); the latter is very important in the gastrointestinal tract as it supplies a large amount of the energy required by the intestinal mucosa and it is essential for maintaining the metabolism, the structure and the functions of the mucosa (Reeds and Douglas, 2001).

Alpha-ketoglutarate (AKG) can be rapidly transaminated, by glutamate dehydrogenase, to glutamic acid which can be further aminated, by glutamine synthetase, to form Gln. Unlike free Gln, AKG has a good water solubility, is non-toxic and relatively stable in water solutions, and thus represents an effective alternative Gln source for the composition of parenteral solutions (Filip and Pierzynowski, 2007). In addition, AKG can prevent uncontrolled nitrogen (N) loss from muscle protein in human patients suffering from post-surgical trauma, burns or chronic malnutrition, controlling the amino acid (AA) blood profile and reducing the blood urea in patients undergoing hemodialysis (Blomqvist *et al.,* 1995; Riedel *et al.,* 1996).

Ornithine (Orn) and AKG are central to intermediary metabolism, and as such ornithine α-ketoglutarate (OKG, a salt formed of two molecules of Orn and one of AKG) is a potential precursor of several potential modulating agents. OKG action is complex, involving its components, Orn and AKG, and its metabolites generated *in vivo*, namely Gln, arginine, proline and polyamines (Cynober, 2004). OKG also acts as a potent stimulator of the secretion of anabolic hormones such as insulin, growth hormone and IGF-1/Sm-C (Moukarzel *et al.,* 1994). As shown by Jeevanandam *et al.* (1991) on rats with OKG, the diets supplemented with AKG could have a significant value in reducing the N losses with relevant effects on the environmental impact of most important animal species. In order to provide a contribute to understand anti-catabolic effect of AKG, a study was carried out on growing rats fed protein-free diets supplemented with two levels of the test product.

Materials and methods

Animals and diets

Thirty male Sprague Dowley rats of 42 days old, purchased from a small animal production farm (Morini Stefano, Reggio Emilia, Italy), were individually housed in an environmental-controlled room (22±1°C) with a 12-h light/dark cycle. The animals were acclimated to the digestibility cages and fed *ad libitum* for 3 days with a meal commercial complete diet (Nossan, Milano, Italy) with the following analytical composition (% as-fed basis): dry matter 89.0%, crude protein 23.0%, ether extract 4.1%, crude fibre 7.3%, ash 11.2%, N-free extract 43.4%. Two days before the beginning of the N balance, the rats were fed a mixture of 30% complete diet and 70% protein-free diet (PFD). The day of the start of the balance trial (0 d) the rats were weighed (136.4±5.76 g body weight, BW), assigned randomly to three treatments (10 animals each) and fed *ad libitum* PFD where 0 (control diet), 3 and 6 g/kg feed of AKG were added. The animals had free access to water.

The composition and the analytical characteristics of the PFD are shown in Table 1. The analyses of PFD were performed according to the AOAC (1997) procedures, while the gross energy was determined by adiabatic bomb (Martillotti *et al.,* 1987).

Growth performance

Individual rats and feeders were weighed at 0 d, 7 d, and 14 d to calculate the daily weight losses and feed intake during the three experimental periods (0-7 d, 7-14 d, and 0-14 d). Rats were not fasted before weight measurements. None of the rats had diarrhoea or were subjected to veterinary drug treatment.

Nitrogen balance

The animals were housed individually in digestibility cages (type 3700MO, Tecniplast, Varese, Italy) with plastic funnels for the separate collection of urine and faeces. The nitrogen (N) balance was carried out during the

two weeks of the trial in two subsequent periods of 5 consecutive days (one period for each week) with total excreta collection. Daily urine was collected in a plastic tube containing 1 mL of 3% sulfuric acid, pooled separately among single animals for each period and immediately frozen (-20°C) to minimize bacterial activity and ammonia loss until N determination. Similarly pooled faecal samples were dried daily at 60°C and subsequently homogenized in a 1-mm screen using a Retsch type ZM100 centrifugal grinding mill (Retsch, Haan, Germany) before N analysis. The N content of the faeces and urine were determined using the Kjeldahl method (AOAC, 1997).

Blood collection

On day 14, six rats per treatment were fasted for 12 h after weighing and then anaesthetized by intraperitoneal injection of 0.1 mg/g of ketamine chlorohydrate 5% (Ketavet 50, batch No. 13, Gellini, Latina, Italy) for blood collection. Blood was drawn by cardiac puncture using 3 mL heparinized (lithium heparin) disposable syringes by Becton Dickinson Acute Care (USA).

Blood was centrifuged and the surnatant was frozen at -80°C until urea and free amino acid analyses according to Stein and Moore's technique (1954) using a model 3A30 Carlo Erba amino acid analyzer (Carlo Erba, Corsico, Italy). The analytical method requires a starting deproteinization procedure of samples by precipitation with picric acid; 5 mL of the sample was placed in a tube with 25 mL of picric acid (1%) and 1 mL of norleucine (2500 nM/mL) as internal standard. After 15 min of centrifugation at 5000-6000 rpm, 25 mL of surnatant was subsequently passed through a Dowex 2×10 resin column with 100-200 meshes in the Cl$^-$ form. The column was washed three times with 5 mL of 0.02 N HCl then the resulting mixture collected in a flask and dried in a Rotavapor at room temperature adding 5 mL of lithium buffer (pH 2.2). The amount of products and samples used are correlated to the type of analyzer. The CV of the analysis was less than 5%.

Intestinal tract collection

On day 14 the same animals kept for blood collection were killed and the entire small intestine of each animal was removed for morphological examination through a mid-line abdominal incision, rinsed and extended on the bench with minimal stretching. The 2-3 cm upstream of the ileo-cecal valve was sectioned and gut tissue measuring about 4×5 mm was sampled. The tissues were rinsed with a PBS solution (0.15 M; made by $Na_2HPO_4 \times 12\ H_2O$, 2.89 g/L; KH_2PO_4, 0.2 g/L; KCl, 0.2 g/L; NaCl, 8 g/L), fixed in glutaraldehyde (2.5% v/v) in cacodylate buffer (0.15 M) for 3 h at 4°C, rinsed with 0.15 M cacodylate buffer and post-fixed in osmium tetraoxide at 1% (w/v) for 1 h at 4°C. The samples underwent progressive dehydration using 75, 85, 95, and 100% ethanol for 12 h each at room temperature and were subjected to drying under vacuum. The tissues were examined at 25 kV with a scanning electron microscope (HITACHI-2300; Nanovision, Milano, Italy) to measure enterocyte, villus and microvilli length, and gut tissue thickness. These measures were taken according to the Pluske *et al.* (1996). Images were captured using a Kevex 4416-4561 processor (Kevex Instruments, San Carlos, CA, USA) and were processed with the Digital Image Processing System (Point electronic GmbH, Hallee, Germany) (Piva *et al.*, 2002).

Statistical analysis

Data were analyzed by ANOVA using the GLM procedure of SAS (2003). The linear and quadratic effects of the nature of the response to the feeding of AKG were tested. Differences were considered statistically significant at P<0.05, while 0.05<P<0.10 were considered a near-significant trend. The experimental unit was the cage.

Results and discussion

Growth performance

The fast physiological response of the growing animals to PFD is shown by a BW reduction (Table 2). The AKG acid administration had no effect on the growth performance of rats. Also Filip *et al.* (2008), in their study on dietary sup-

Table 1. Composition of the protein-free diet and analytical characteristics.

Composition	%	Analytical characteristics	
Starch	42.0	Dry matter, %	91.82
Sucrose	42.0	Total nitrogen, % DM	0.04
Hydrogenated oil	10.0	Ether extract, % DM	10.00
Cod liver oil	2.0	Crude fibre, % DM	2.03
Mineral premix°	3.0	Ash, % DM	2.67
Vitamin premix#	1.0	Gross energy, kJ/g DM	20.03

°Composition per 1 kg premix: $CaHPO_4$, 500 mg; NaCl, 74 mg; $K_3C_6H_5O_7.H_2O$, 220 mg; $K2SO_4$, 52 mg; MgO, 24 mg; $MnCO_3$, 3.5 mg; $Fe_6C_6H_5O_7.5H_2O$, 6 mg; $ZnCO_3$, 1.6 mg; $CuCO_3$, 0.3 mg; KJO_3, 0.01 mg; $Na_2SeO_3.5H_2O$, 0.1 mg; $CrK(SO_4)_2.12H_2O$, 0.55 mg. #Composition per 1 kg premix: tiamine HCl, 600 mg; riboflavin, 600 mg; piridossine HCl, 700 mg; nicotinic acid, 3000 mg; calcium pantotenate, 1600 mg; folic acid, 200 mg; biotine, 20 mg; vit. B_{12}, 1 mg; vit. A, 400,000 U; vit. D_3, 100,000 U; vit. E, 5000 U; vit. K, 5 mg.

Table 2. Main effect means of alpha-ketoglutarate acid on growth performance of rats.°

	Dietary concentration of AKG acid, g/kg feed			√MSE#	P	Contrast	
	0	3	6			Linear	Quadratic
Live weight, g							
0 d	136.8	136.2	136.3	5.06	ns	ns	ns
7 d	113.1	113.3	114.4	4.57	ns	ns	ns
14 d	106.3	107.3	108.6	4.24	ns	ns	ns
Daily weight losses in the period, g							
0-7 d	3.39	3.27	3.13	0.545	ns	ns	ns
7-14 d	0.96	0.86	0.83	0.306	ns	ns	ns
0-14 d	2.18	2.06	1.96	0.274	ns	ns	ns
Daily feed intake in the period, g							
0-7 d	6.20	6.18	5.88	0.951	ns	ns	ns
7-14 d	7.49	7.50	7.76	0.890	ns	ns	ns
0-14 d	6.80	6.78	6.75	0.909	ns	ns	ns

AKG, alpha-ketoglutarate. °The values represent least square means of 10 cages with one animal each; #root of mean-square error (MSE); ns, not significant.

plementation with AKG in rats, found no influence of AKG on the daily gain and feed intake while Jeevanandam *et al.* (1996) reported lower weight gain per day and feed intake in rats fed a diet supplemented with AKG compared with rats fed the control diet.

In Beza's equation (1986) $Y = A \times t^{(-0.062\pm0.002)}$, obtained in rats aged from 23 to 275 days that had been fed with PFD for 16 days, t is the time of PFD administration and A is the age of the animals (in our case 59 d). The value of the angular coefficient b=-0.062 shows that the weight loss (Y) at the end of PFD administration (assessed in fasting conditions) is independent of the age of the animal and is not constant over time. It rather relates to the initial weight loss (assessed in fasting conditions), which, on the contrary, depends on the age of the animal.

In our study, 14 days after PFD administration, the loss of body mass (Y), calculated according to the Beza's equation, was 50 g while it was supposed to be 34 g by Beza's (1986) with animals of the same weight. Nevertheless, the real losses of body mass were of 30.5, 28.9 and 27.7 g for the PFDs supplemented with 0, 3 and 6 g/kg feed of AKG acid, respectively. Therefore, the real values obtained by the study were better than those expected. The differences between the estimated values and the real values are probably due to the different experimental conditions of our trial compared with those of Beza's trial: the duration of the assessment period and the number of animals used for calculating the means, the weight measurements were not performed on fasting animals since they were also subjected to balance testing, and the protein content of the diet administered in the pre-experimental period was higher (23% in our study and 4.8% in Beza's experiment) with probably a consequently lower depletion of labile protein reserve.

The highest and fastest weight losses were found after the first 7 d of PFD administration in rats, without a significant treatment effect. On the other hand, lower daily feed intakes were also observed during the first trial period (0-7 d) for all three diets, without a significant treatment effect. Similarly, Sant'Ana *et al.* (2001), Kern *et al.* (2002) and Dönmez and Karsli (2004) in their studies on protein restricted rats found a depression of the weight gain associated to protein deficient diets.

Nitrogen balance

The endogenous urinary N results from irreversible reactions involved in the breakdown and replacement of various protein compounds and derivates in the body. The endogenous faecal N is mainly N in digestive secreta and in desquamated epithelial cells. Both the urinary and faecal endogenous fractions represent N, which has been already adsorbed and utilized by the animal. The amount of endogenous N varies depending on animal species, age, live weight, protein content of the diet before PFD and the so-called labile protein reserves (Chawalibog *et al.*, 2008).

The amounts of endogenous fecal and urinary N excreted during balance testing are shown in Table 3. At the end of the first period of excreta collection, the amount of endogenous N excreted through the urine linearly reduced (P<0.05) following the administration of AKG acid, ranging from 35.00 mg/d to 28.00 mg/d, respectively. The reduced amount of urinary N excreted owing to the AKG acid administration in the first balance period might be due to reduced glutamine degradation in the intestinal mucosa, since this is the main precursor of urinary NH_3 (Schmidt and Marindale, 2003). The inclusion of AKG acid in the diet linearly reduced (P<0.05) the total N excretion, with values ranging from 45.28 mg/d to 36.36 mg/d. Nevertheless, no AKG acid effect was observed in the second week of excreta collection.

The reduced N losses observed in the first week of excreta collection owing to the AKG acid administration could be due to the beneficial effects of AKG on the intestinal growth and integrity (Hou *et al.*, 2011). The gastrointestinal tract actively participates in N recycling in the gut and plays an important role in nutrition and health (Bergen and Wu, 2009). The beneficial effects of AKG may be explained by its key role in the metabolism of amino acids, particularly Gln (Chen *et al.*, 2009; Xi *et al.*, 2011). There is now evidence that AKG can inhibit glutamine degradation and enhance protein synthesis in intestinal epithelial cells (Yao *et al.*, 2011) with consequently lower labile protein reserves depletion.

Intestinal tract

Even if rats suffer from protein deficiency, they can still retain a high metabolic activity. This is most likely due to the fact that they continue to take in a sufficient amount of energy from carbohydrates. Protein deficiency, however, changes the intestinal architecture without altering its absorption capacity. The absorption capacity is preserved with physiological adaptation and change in the turnover rate of the

Table 3. Faecal and urinary nitrogen excretion.°

	Dietary concentration of AKG acid, g/kg feed			√MSE[#]	P	Contrast	
	0	3	6			Linear	Quadratic
Daily N excretion during the 1st balance period, mg							
Faecal excretion	10.28	8.54	7.36	3.500	ns	ns	ns
Urinary excretion	35.00	28.36	28.00	6.571	<0.05	<0.05	ns
Total N excretion	45.28	36.90	36.36	8.224	<0.05	<0.05	ns
Daily N excretion during the 2nd balance period, mg							
Faecal excretion	9.30	9.22	9.37	2.613	ns	ns	ns
Urinary excretion	21.37	19.37	19.24	8.854	ns	ns	ns
Total N excretion	30.67	28.60	28.61	11.082	ns	ns	ns
Daily mean N excretion during the two balance periods, mg							
Faecal excretion	9.79	8.88	8.36	2.387	ns	ns	ns
Urinary excretion	28.18	23.87	23.97	6.705	ns	ns	ns
Total N excretion	37.97	32.75	32.33	8.147	ns	ns	ns
Daily water intake in the two periods, g							
1st period	10.25	10.55	8.94	3.045	ns	ns	ns
2nd period	9.15	9.11	9.00	3.622	ns	ns	ns
Mean of the two periods	9.66	9.59	8.74	3.143	ns	ns	ns

AKG, alpha-ketoglutarate; N, nitrogen. °The values represent least square means of 10 cages with one animal each; [#]root of mean-square error (MSE); ns, not significant.

enterocytes (Drozdowski and Thomson, 2006). The data obtained from the intestinal measurements carried out in our study show that the administration of AKG acid affects the intestinal adaptation, but not the absorption capacity, in rats fed a PFD. In fact, the administration of AKG acid did not change the thickness of the intestinal tissue but the length of villi, microvilli and enterocytes changed significantly (Table 4). Both villi and microvilli heights were influenced quadratically ($P < 0.05$) by AKG acid administration. In particular, the lower values were observed when animals received 3 g/kg feed of AKG acid respecting to other tested AKG acid administration levels. Indeed, enterocytes length linearly increased ($P < 0.05$) as the level of AKG acid increased in the diet by 25.0% (3 g/kg feed) and 49.0% (6 g/kg feed), respectively.

Enterocytes are largely dependent on Gln for their energy metabolism, rapid division, and proliferation. Therefore, the improvement of the enterocytes height was probably due to the higher availability of Gln resulting from the AKG acid administration (Pierzynowski and Sjodin, 1998; Drozdowski and Thomson, 2006).

Our data show that AKG acid provided at 3 g/kg feed has a negative effect on villi and microvilli length as reported by Montoya *et al.* (2006). On the contrary, the data obtained with AKG acid at 6 g/kg feed are similar to those of the control group. On the base of the available studies in literature, we are not able to justify these results. Nevertheless, studies indicate that OKG administration improves gut morphology and function, and enhances intestinal adaptation under stress conditions (Dumas *et al.,* 1998; Cynober, 1999). The positive action of the OKG on the gut would seem be due to the synergic effect exerted by its constituents (Cynober *et al.,* 1990). This suggests that the simultaneous administration of Orn and AKG is necessary to obtain the same effects found with OKG.

Free amino acids

The absence of protein in the diets and the inclusion of 6 g/kg feed of AKG acid had a quadratic effect ($P < 0.05$) on the blood urea concentration (15.56 mg/100 mL) that reduced in comparison with the control diet (19.40 mg/100 mL) (Table 5). This implies energy saving since urea contains 2.52 kcal/g (5.45 kcal/g of N).

The blood urea level provides useful indications on the metabolic utilization efficiency of feed proteins. The presence of high blood urea levels is generally due to excess proteins and/or unbalanced amino acid ratios in feed proteins. In our case, the AKG acid (at a con-

centration of 6 g/kg feed), which reached the liver via the portal blood, may have reduced the available NH_3 for the synthesis of urea, incorporating it to form glutamate or glutamine. Nevertheless, no variation in blood concentration of these products was observed. Glutamine was reported as the most probable

donor of amino groups required for AA synthesis (Lobley *et al.,* 2001). This might explain the constant values of glutamate and glutamine, and the variations in concentration of some AA found in the blood of the rats fed PFD supplemented with 6 g/kg feed of AKG acid in comparison with the other two PFDs.

Table 4. Intestinal measurements.

	Dietary concentration of AKG acid, g/kg feed			√MSE°	P	Contrast	
	0	3	6			Linear	Quadratic
Tissue thickness, mm	0.425	0.462	0.506	0.1547	ns	ns	ns
Villi height, μm	410.9	315.8	371.5	74.18	<0.05	<0.05	<0.05
Microvilli height, μm	0.969	0.882	1.007	0.0786	<0.05	<0.05	<0.05
Enterocytes height, μm	18.20	22.75	27.11	3.961	<0.05	<0.05	ns

AKG, alpha-ketoglutarate. °Root of mean-square error (MSE); ns, not significant.

Table 5. Plasma free amino acids (mg/100 mL).°

	Dietary concentration of AKG acid, g/kg feed			√MSE#	P	Contrast	
	0	3	6			Linear	Quadratic
Ammonia	0.74	0.90	0.67	0.222	ns	ns	ns
Urea	19.40	18.31	15.56	2.505	<0.05	ns	<0.05
EAA							
Threonine	1.16	1.14	0.87	0.245	0.05<P<0.10	ns	<0.05
Valine	1.54	1.70	1.21	0.258	<0.05	ns	<0.05
Methionine	0.48	0.40	0.33	0.076	<0.05	ns	<0.05
Isoleucine	0.74	0.71	0.51	0.166	<0.05	ns	<0.05
Leucine	1.46	1.23	1.06	0.224	<0.05	ns	<0.05
Phenylalanine	0.89	0.97	0.59	0.211	<0.05	ns	<0.05
Lysine	5.86	5.82	4.89	0.906	ns	ns	ns
NEAA							
Serine	9.28	8.62	7.97	1.187	ns	ns	ns
Glicine	3.76	4.12	3.97	0.610	ns	ns	ns
Alanine	7.15	5.78	5.72	1.473	ns	ns	ns
Tyrosine	0.54	0.62	0.44	0.145	0.05<P<0.10	ns	<0.05
Histidine	2.65	2.56	2.61	0.375	ns	ns	ns
Arginine	2.51	2.22	2.07	0.651	ns	ns	ns
Aspartic acid	0.50	0.66	0.30	0.276	0.05<P<0.10	ns	<0.05
Asparagine	0.92	0.96	1.02	0.134	ns	ns	ns
Glutamine	20.06	19.63	19.02	2.723	ns	ns	ns
Glutamic acid	3.59	3.83	3.90	0.906	ns	ns	ns
Taurine	2.14	2.43	1.80	0.474	0.05<P<0.10	ns	<0.05
Hydroxyproline	0.38	0.33	1.00	0.170	<0.05	ns	<0.05
Proline	2.03	1.91	1.41	0.300	<0.05	ns	<0.05
Citrulline	1.51	1.51	1.27	0.463	ns	ns	ns
Ornithine	1.32	1.54	1.24	0.420	ns	ns	ns
Ethanolamine	0.56	0.78	0.44	0.192	<0.05	<0.05	<0.05
ΣEAA	12.14	11.96	9.45	1.679	<0.05	ns	<0.05
Σ NEAA	58.89	57.47	53.17	5.347	ns	ns	ns
Σ AA	71.03	69.43	62.61	6.774	0.05<P<0.10	ns	<0.05
Σ EAA/Σ AA	0.17	0.17	0.15	0.013	<0.05	ns	<0.05

°The concentration of tryptophan and cystine were under the method sensibility; AKG, alpha-ketoglutarate; #root of mean-square error (MSE); EAA, essential amino-acids; NEAA, non-essential amino-acids; ns, not significant.

The variations in AA blood profile found in our trial with the administration of the PFD added with 6 g/kg feed of AKG acid could be summarized as follows (Table 5): Valine, Methionine, Isoleucine, Leucine, Phenylalanine, Hydroxyproline and Proline decreased with a quadratic trend ($P<0.05$). On average, the values measured in animals receiving 6 g/kg feed of AKG acid were 25.3, 25.0, 29.7, 21.1, 36.6, 64.0 and 28.4% lower than values observed in the control PFD and in the PFD with 3 g/kg feed of AKG acid.

Other differences found between the PFD with 6 g/kg feed of AKG acid and the other two PFDs were only tending to significance ($0.05<P<0.10$) for blood concentrations of threonine, tyrosine, aspartic acid and taurine.

The ethanolamine blood concentration was quadratically ($P<0.05$) influenced by AKG acid levels, being higher for the PFD with 3 g/kg feed of AKG than for the control PFD and the PFD with 6 g/kg feed of AKG acid (0.78 mg/100 mL *vs.* 0.56 and 0.44 mg/100 ml; respectively). Generally, the AKG acid levels quadratically influenced ($P<0.05$) the essential AA concentrations in the blood, being EAA lower for animals treated with 6 g/kg of AKG acid (-21.6%) compared to the animals fed the control PFD and the PFD with 3 g/kg feed of AKG acid. Also, the total AA concentrations tendentially ($0.05<P<0.10$) decreased with a quadratic pattern ($P<0.05$).

Free amino acids play a key role in N metabolism since the metabolically active N is absorbed and transported from one organ to the other in this form. A change in the blood amino acid (AA) profile may be the initial sensory signal for recognition of dietary AA deficiency which has long been known to suppress feed intake in rats (Koehnle *et al.,* 2004). Nevertheless, a PFD decreases N excretion by increasing the rate of reutilization of amino acids from endogenous proteins for protein synthesis (Nielsen *et al.,* 1994).

Our results show that AKG acid at a concentration of 6 g/kg feed could reduce the mobilization and/or catabolism of the essential AA. In particular, a high conservation of methionine and phenylalanine was observed to the detriment of hydroxyproline. On the other hand, if glutamine was reported as the most probable donor of amino groups required for AA synthesis, of the oxo-acid acceptors those of methionine and phenylalanine were reported as preferred acceptors (Blarzino *et al.,* 1994; Lobley *et al.,* 2001). Methionine plays important roles in the body and may be the first limiting AA (Storm and Orskov, 1984). Similarly, much of the adsorbed phenylalanine is often removed through the liver and has been suggested as a limiting AA for acute phase protein synthesis (Le Floc'h *et al.,* 1999). Thus, mechanisms that protect these AA against hepatic oxidation are likely to be beneficial.

Conclusions

Our study, carried out on growing rats fed protein-free diets supplemented with three levels of AKG acid (0, 3 e 6 g/kg feed) for a period of 14 days, reported the following results: after 7 d of trial, AKG acid both at 3 and 6 g/kg feed can reduce endogenous N losses; AKG acid at 3 g/kg feed can reduce the heights of the villi and microvilli; AKG acid, both at 3 and 6 g/kg feed, can increase the height of the enterocytes; AKG acid at 6 g/kg feed can reduce the mobilization and/or catabolism of the essential AA and the blood NH_3 level with consequent energy saving.

It can be deduce from these results that AKG acid can positively interfere with the N balance, affect the intestinal adaptation, but not the adsorption capacity, and have anti-catabolic effect. The 6 g/kg feed dose seems to be the most effective. In fact, AKG at 6 g/kg feed, compared with AKG at 3 g/kg feed, showed an anticatabolic effect without to affect adversely the intestinal architecture.

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