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Bioavailability of di-peptide DL-methionyl-DL-methionine in comparison to DL-methionine in weaned and growing pigs

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ABSTRACT

The relative bioavailability (RBV) of a dipeptide DL-methionyl-DL-methionine (DL-Met-Met) was compared with DL-methionine (DL-Met) in growing pigs (experiment 1; N-balance study) and in weaned pigs (experiment 2; performance study). In experiment 1, 42 barrows with an initial body weight (BW) of 21.0 ± 1.37 kg were assigned to 7 dietary treatments with 6 replicate/pigs per treatment in a nitrogen (N) balance study to evaluate the RBV of DL-Met-Met to DL-Met. A basal diet (BD) was formulated to be adequate for all amino acids with the exception of Met + Cys which was 68% of the requirement [4.7 g/kg standardized ileal digestible (SID) Met + Cys; 11.5 g/kg SID Lys] for 20–25 kg pigs. Three graded levels of DL-Met (0.3, 0.6 and 0.9 g/kg) and DL-Met-Met (0.306, 0.612 and 0.919 g/kg) were supplemented to the BD to create diets 2–7. In experiment 2, a total of 189 weaned pigs (initial BW of 10.2 ± 0.98 kg) were assigned to 7 dietary treatments with 9 replicates/pens of 3 pigs per treatment. The dietary treatments consisted of a Met-deficient BD (5.3 g/kg SID Met + Cys; 13.0 g/kg SID Lys) and the same 3 graded levels of DL-Met and DL-Met-Met as in Exp. 1. In experiment 1, supplementation with DL-Met or DL-Met-Met linearly decreased ($P \le 0.01$; linear) urinary N excretion and increased ($P \le 0.02$; linear) N retained (g/day), N retention (% of intake and % of absorbed). However, there was no effect of Met sources on all N balance parameters. Based on the slope-ratio regression the RBV for DL-Met-Met compared to DL-Met was estimated 111% [95% confidence interval (CI): 63-158%] for N retained (g/d), 109% (95% CI: 57-160%) for N retention (% of intake) and 98% (95% CI: 43–154%) for N retention (% of absorbed) on an equi-molar basis. In experiment 2, the overall average daily gain (ADG) and gain:feed ratio (G:F) increased linearly ($P < 0.01$) by supplementation with DL-Met or DL-Met-Met. The average daily feed intake increased by supplementation with DL-Met ($P = 0.02$) and DL-Met-Met ($P = 0.09$). For ADG, the RBV for DL-Met-Met was estimated 104% (95% CI: 66-141%) on an equi-molar basis by the slope-ratio. Based on G:F, the RBV for DL-Met-Met was estimated 117% (95% CI: 61–174%) on an equi-molar basis. The results of both experiments indicate that the bioavailability DL-Met-Met is not different and at least equally bioavailable as DL-Met as a Met source for pigs.

Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; BD, basal diet; BW, body weight; CI, confidence interval; DL-Met, DL-methionine; DL-Met-Met, DL-methionyl-DL-methionine; Exp, experiment; G:F, gain:feed ratio; Met, methionine; RBV, relative bioavailability; SID, standardized ileal digestible

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1. Introduction

Methionine (Met) is the second or third limiting amino acids (AA) in typical swine diets, and supplemental Met sources are commonly used to supply adequate dietary level of Met + Cys. Different supplemental Met products are available which include DL-methionine (DL-Met; 99% purity), L-methionine (99% purity), liquid or calcium salt of methionine hydroxy analogue free acid (MHA-FA, 88% active substance, Kim et al., 2006; MHA-Ca, 84% active substance, Opapeju et al., 2012). A new Met source as a dipeptide DL-methionyl-DL-methionine (DL-Met-Met; 97% purity; AQUAVI® Met-Met produced by Evonik Nutrition & care GmbH) is currently available mainly for shrimp.

It is well accepted that supplemental (crystalline) AA are well utilized by animals including pigs (Nørgaard et al., 2016). Di- and tri-peptides are transported into the enterocyte intact by peptide transporter, PepT1 and then hydrolysed to free amino acids by peptidases (Leibach and Ganapathy, 1996; Gilbert et al., 2008). It has been reported that di- and tri-peptides are absorbed rapidly and efficiently by the intestine without initial pancreatic digestion in sea bass (Infante et al., 1997). Feeding a peptide product (dried hydrolysate of pig intestines) improved growth performance of weaned pigs (Cho et al., 2010). As reported by Sleisinger et al. (1976) the intestinal transport systems of small peptides are different from those of free AA and this might minimize the competition for transport sites. Compared with other Met sources, dipeptide of DL-Met-Met due to its low water solubility (Niu et al., 2018), may presumably be less unstable and better synchronize with protein bound AA in terms of absorption along the small intestine.

Bioavailability is the extent to which an ingested nutrient in a particular source is digested and absorbed in a form that can be utilized in metabolism by the animal and it is commonly determined by a slope-ratio model (Littell et al., 1997). Recently, a greater relative bioavailability (RBV) for DL-Met-Met (AQUAVI® Met-Met) relative to DL-Met has been reported in white shrimp (Niu et al., 2018). However, there is a lack of information about the RBV of DL-Met-Met relative to DL-Met in pigs. Therefore, the RBV of DL-Met-Met in comparison to DL-Met was evaluated in 20–28 kg pigs using a nitrogen (N)-balance assay (Exp. 1) applying N deposition as the basis for RBV comparison. Additionally, a classical dose-response growth trial (Exp. 2) was conducted with 10–22 kg pigs for comparing RBV of DL-Met-Met with DL-Met using average daily gain (ADG) and gain:feed ratio (G:F) to confirm the results obtained in Exp. 1.

2. Materials and methods

The protocol for this experiment was approved by the Institutional Animal Care and Use Committee of Sao Paulo State University. Two experiments were conducted using crossbred pigs (Agroceres Pig Improvement Company, Rio Claro, SP, Brazil: AGPIC 337™ x Camborough™) in a 12-h of natural light program at the facility of the São Paulo State University.

2.1. Experiment 1 (N-balance assay)

2.1.1. Animals, housing and experimental design

Forty-two barrows with an average initial body weight (BW) of 21.0 ± 1.37 kg were used in two batches of 21 pigs each. Each batch of pigs served as a block. Three pigs were randomly allotted to each of 7 experimental diets within each block resulting in a total of 6 replicate pigs per dietary treatment. Pigs were housed individually in adjustable metabolism crates (0.60 × 1.60 m) in a temperature controlled room (22 °C). Each crate was equipped with a single low-pressure drinking nipple and a stainless steel self-feeder.

2.1.2. Dietary treatments

A basal diet was formulated based on corn and soybean meal to be adequate for all AA with the exception of Met + Cys which was 68% of the requirement [4.7 g/kg standardized ileal digestible (SID) Met + Cys; 11.5 g/kg SID Lys] for 20–25 kg pigs (NRC, 2012; Table 1). Three graded levels of DL-Met (0.3, 0.6 and 0.9 g/kg) or DL-Met-Met (0.306, 0.612 and 0.919 g/kg) based on the purity of the products, were added to the basal diet for achieving the target Met dietary levels to create diets 2–7. The Met + Cys inclusion levels ranged from 68 to 80% of the requirement, while the (Met + Cys)/Lys ratio increased from 0.41 to 0.48%, from the basal to the most supplemented diet, respectively. Supplemental DL-Met (MetAMINO®) and DL-Met-Met (AQUAVI® Met-Met) were obtained from a commercial company (Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany). The diets were formulated on the basis of analyzed AA contents of ingredients and the SID coefficients of AA (AMINODat 4.0 Platinum version, 2010).

2.1.3. Feeding and collection

Nitrogen balance trial lasted for 12 days consisting of a 7-day adaptation period to the metabolism crates, meal regimen and experimental diets and a 5-day period of quantitative collection of feces and urine. Each metabolism crate had a collection tray for urine collection and a fine-mesh net just above the tray for fecal collection thus, allowing for separate but total collection of feces and urine. Pigs were weighed at the beginning and end of the adaptation period and at the end of collection. Daily feed allowance during the adaptation and collection periods was set at 4% (as-fed basis) of the average BW of all pigs at the beginning and end of adaptation period, respectively, corresponding to approximately 3 times their daily energy requirement for maintenance (i.e., 0.44 MJ ME/kg of $BW^{0.75}$/day) (NRC, 1998) to ensure similar feed intake.

The daily feed allotment was divided into two equal portions and offered at 0800 and 1600 h. Diets were fed as a mash and pigs had unlimited access to water at all times. On the morning of day 8, each pig received 100 g of feed mixed with 3 g of ferric oxide as an indigestible marker to identify the start of fecal collection. Upon complete consumption of the marked diet, the remainder of the