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Use of protected methionine in diets for finishing pigs

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ABSTRACT

Objective: To evaluate the substitution of regular DL-methionine (RM) by protected DL-methionine (PM) in the productive performance of finishing pigs.

Design/Methodology/Approach: 50 pigs (56.85±2.02 kg of live weight) distributed in a completely randomized experimental design were used. The treatments consisted of the substitution of MR by MP (0, 25, 50, 75, and 100%) in diets for finishing I and II pigs.

Results: As the MP in the diet increased, the gain of final live weight (LWF) and backfat thickness (BFT) in finishing I increased linearly ($p \leq 0.05$), while feed:gain ratio (FGR) decreased linearly ($p = 0.07$). PM at finishing II phase linearly ($p \leq 0.10$) improved feed intake, FGR, and *longissimus* muscle area.

Study Limitations/Implications: Feeding pigs by including protected amino acids improves their productive response.

Findings/Conclusions: The use of protected methionine in diets for finishing I pigs improves some productive response variables.

Keywords: Synthetic amino acids, absorption, bioavailability, pigs.

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INTRODUCTION

Adding synthetic amino acids (AA) to pig diets prevents deficiencies and the excess of crude protein, decreasing N excretion and environmental pollution (Gloaguen *et al.*, 2014). In pig production, the second or third limiting AA related to the production of feed is methionine (Met); including a synthetic version of this AA is essential when the main ingredients do not meet the requirement (NRC, 2012).

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Synthetic AAs are more rapidly absorbed than protein AAs and this may result in a transient imbalance between AAs in the systemic circulation of pigs, which may influence AA bioavailability (Yang and Liao, 2019). Likewise, the metabolic inefficiency of AA can be largely attributed to catabolizing enzymes that are mainly present in pancreatic juice and some of the other digestive organs (Wu, 2009).

The use of protected AA which can resist stomach conditions and allow a slow release in the intestine can help to overcome the above-mentioned bioavailability limitations (Piva *et al.*, 2007). Protected AAs are used in ruminants in order to overcome ruminal conditions and be used in the small intestine. Commercially available protected AAs include lysine and methionine, which have successfully improved productive parameters in ruminants (Lee *et al.*, 2012; Zanton *et al.*, 2014). Feeding protected methionine (PM) to pigs instead of regular methionine (RM) increased weight gain and feed intake (Figueroa-Velasco *et al.*, 2020). However, it was not determined whether using a single source or a combination of both (regular and protected) achieved a better result. Meanwhile in finishing pigs, the use of protected lysine (PL) instead of regular lysine (RL) did not affect growth and carcass characteristics (Prandini *et al.*, 2013).

Sun *et al.* (2020a; 2020b) observed that the use of PL and PM improves the balance of absorbed AA and contributes to the reduction of AA supplemental levels in the diet of broiler chickens and laying hens, improving the efficiency and bioavailability of AA, without negative effects on productive performance. For their part, Prandini *et al.* (2013) mention that PL —compared to RL and lysine bound to the proteins of the macro ingredients— improved AA bioavailability in pigs, as a result of its slower release and absorption rate. This could reduce the crude protein (CP) content and improve the efficiency of the use of synthetic AA in the diet.

Owing to the favorable results obtained by the use of protected AA in ruminants and non-ruminants, the substitution or combination of RM by PM in diets for pigs was assumed to improve the productive response, as a consequence of the greater bioavailability and the synchronization of absorption of the AA obtained from the intact protein of the ingredients. Therefore, the objective of this study was to evaluate the effect of substituting regular synthetic DL-Met (RM) by protected DL-Met (PM) on the productive variables, carcass characteristics, and urea concentration of finishing pigs.

MATERIALS AND METHODS

The study was carried out at the Experimental Farm of *Colegio de Postgraduados*, in Texcoco, State of Mexico, Mexico, at an altitude of 2,241 m. The climate is temperate sub-humid with summer rains, with a mean annual temperature of 15.2 °C and an average annual rainfall of 644.8 mm.

Pigs were managed according to the technical specifications for the production, care, and use of lab animals, complying with the Official Mexican Standard NOM-062-ZOO-1999 (SAGARPA, 2001).

Fifty hybrid pigs (Landrace × Yorkshire × Duroc) were used in this study. The group consisted of 35 barrows and 15 sows, with 56.85±2.02 kg of initial live weight (LWI). Treatments consisted of substituting regular synthetic Met (RM, 0% PM control diet) by

25, 50, 75, and 100% protected methionine (PM). PM concentrations for finishing stage I were: 0, 0.025, 0.050, 0.075, and 0.100% MS of the diet. For finishing stage II, they were: 0, 0.046, 0.093, 0.139, and 0.185% MS of the diet. The evaluation period in both stages was 28 d. PM concentrations were established taking into consideration the requirements suggested by the NRC (2012). The PM contained 85% encapsulated DL-methionine (Mepron® Evonik, Germany).

The diets (Table 1) were formulated with the Excel Solver command (Microsoft Excel, 2007), based on sorghum-soybean meal, added with synthetic AA [L-lysine-HCl, DL-

Table 1. Basal diets used to evaluate the substitution of regular methionine by protected methionine in finishing I and finishing II pigs.

Ingredient (%)	Finishing I	Finishing II
Sorghum grain	82.51	82.96
Soybean meal	14.48	13.48
Soybean oil	0.66	0.77
L-lysine (50%)	0.64	0.71
DL-methionine	0.10	0.16
Protected methionine (PM)	0.00	0.00
L-threonine	0.07	0.10
L-tryptophan	0.00	0.14
Vitamins premix[†]	0.17	0.17
Minerals premix[¶]	0.17	0.17
NaCl	0.30	0.30
$CaCO_3$	0.86	1.00
Calculated content (%)		
Metabolizable energy (Mcal kg^{-1})	3.30	3.300
Crude protein	15.20	15.00
Calcium	0.59	0.64
Phosphorus	0.34	0.33
Lysine	0.85	0.93
Threonine	0.52	0.57
Tryptophan	0.15	0.16
Methionine	0.31	0.37
Methionine+Cysteine	0.48	0.53
Determined content (%)		
Crude protein	14.95	14.75
Calcium	0.56	0.58
Phosphorus	0.30	0.30

[†] Contribution per kg of feed: Vitamin A, 15,000 IU; vitamin D3, 2,500 IU; vitamin E, 37.5 IU; vitamin K, 2.5 mg; thiamin, 2.25 mg; riboflavin, 6.25 mg; niacin, 50 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.0375 mg; biotin, 0.13 mg; choline chloride, 563 mg; pantothenic acid, 20 mg; folic acid, 1.25 mg.
[¶] Contribution per kg of feed: Fe, 150 mg; Zn, 150 mg; Mn, 150 mg; Cu, 10 mg; Se, 0.15 mg; I, 0.9 mg; Cr, 0.2 mg.

methionine (Evonik Industries AG., Parsippany, NJ, USA), L-threonine (Jefo Nutrition Inc., Saint-Hyacinthe, Quebec, Canada), L-tryptophan (CPB Aurum, Mexico)]; these AAs covered the requirements suggested by the NRC (2012).

The feed of the finishing stage II included 5 mg kg^{-1} of ractopamine (Paylean™, Elanco, Mexico) and the formulation requirements of the diets were adjusted based on the recommendation of the NRC (2012) when this β-adrenergic is added.

The pigs were housed in individual pens equipped with a hopper feeder and nipple drinker. Food and water were provided *ad libitum*. The pens were cleaned and the health status of the pigs was inspected on a daily basis.

Backfat thickness (BFT) and *longissimus* muscle area (LMA) were measured at the tenth rib with real-time ultrasound (SonoVet 600, Medison, Inc., Cypress, CA, USA) at the beginning and at the end of each phase. These data —along with the initial live weight (LWI) and final live weight (LWF) were used to estimate the fat free lean gain (FFLG), as well as the lean meat percentage (LMP), the initial lean meat percentage (LMPI), and the final lean meat percentage (LMPF), using the equation number five proposed by Burson and Berg (2001).

At the end of each stage, blood samples were taken from the anterior vena cava, using 10-mL Vacutainer® tubes with heparin (BD Vacutainer, Franklin Lakes, NJ, 07417, USA). The samples were placed on ice. Subsequently, they were centrifuged at 1286 g for 20 min using a Sigma 2-16K centrifuge (Osterade am Harz, 37520, Germany) to separate the plasma from cells. Plasma from each sample was transferred to a polypropylene tube and stored in a freezer (EUR251P7W Tappan, Electrolux Home Products North American, Augusta, GA, 30907, USA) at −20 °C, until the urea was determined.

Laboratory analysis. The plasma urea nitrogen concentration (PUN) of the blood samples was determined with a spectrophotometer (Varian Cary UV vis Spectrophotometer, Victoria, Australia) using the methodology proposed by Chaney and Marbach (1962). The crude protein (PC) of the feed was determined by the macro-Kjeldahl method (AOAC, 2005). The calcium (Ca) and phosphorous (P) concentration of the feed was determined with an atomic absorption spectrophotometer (Perkin Elmer 4000, Lambda 2 series, Perkin Elmer Inc., Norwalk, CT, USA), following Karl *et al*. (1979).

Response variables. The following variables were analyzed: growth performance [average daily feed intake (ADFI), average daily gain (ADG), feed:gain ratio (FGR), LWF, and FFLG)], carcass characteristics (BFT, LMP, and LMA), and PUN.

Statistical analysis. The experimental design was a completely randomized with five treatments and 10 replicates. Each pig housed in an individual pen was considered an experimental unit. The normality and homogeneity of the data were evaluated with the Shapiro-Wilk and Levene's tests, respectively. An analysis of variance was performed with the data using the GLM procedure of SAS (2010) and the effect of PM concentration was determined with orthogonal polynomials to detect linear or quadratic trends ($p \leq 0.10$). Initial live weight and sex were used as covariates ($p \leq 0.10$) for LWF, ADG, ADFI, FGR, FFLG, and LMP. Meanwhile, the initial LMA and BFT were used as covariates ($p \leq 0.10$) of their respective final measurements.

RESULTS AND DISCUSSION

In finishing stage I, ADG (p=0.009), LWF (p=0.01), and BFT (p=0.01) had a linear increase and FGR had a linear decrease (p=0.07) as the level of PM inclusion in the diet increased (Table 2). The percentage of lean meat in the carcass showed a quadratic trend (p=0.06), as a result of the substitution of RM by PM, increasing when 25 or 50% of RM was replaced by PM. The use of RM or PM did not affect (p>0.10) the other carcass characteristics.

Table 3 shows the results of finishing stage II. Adding PM did not seem to have an effect (p>0.10) on ADG, LWF, FFLG, BFT, and LMP. However, ADFI (p=0.08) and LMA (p=0.09) increased and FGR had a linear decrease (p=0.10) in response to the substitution for RM in the diet by an increasing amount of PM. The PUN behaved in a quadratic manner (p=0.06): the highest concentrations were recorded with the control treatment and the treatments with the highest amount of PM; meanwhile, replacing 25 and 50% of the RM with PM resulted in the lowest concentration.

In the present experiment, proportional improvements in growth performance were observed during finishing stage I in response to the substitution of RM by PM. However, only improvements in FGR and LMA were observed during finishing stage II. These differences between both stages match the findings of Figueroa-Velasco *et al.* (2020) who observed that the positive effect of using PM in fattening pig feed is differential at every stage. Pigs fed with PM at starting and finishing phases increased their feed intake and weight gain, while PM improved protein synthesis (lean meat percentage and longissimus muscle area) in finishing pigs (Figueroa-Velasco *et al.*, 2020).

During finishing II stage, the higher ADFI was reflected in lower FGR in pigs fed with a higher PM concentration, although there was no statistical improvement in ADG. However, the treatments with the highest PM content had the highest LMA values. The

Table 2. Response of finishing I pigs (50 to 75 kg of weight) fed with five levels of protected methionine.

	Treatment (Protected methionine, %)					SEM	p Value	
	0	25	50	75	100		Lineal	Quadratic
Growth performance								
ADG (kg)	0.68	0.70	0.75	0.76	0.83	0.03	0.009	0.57
ADFI (kg d^{-1})	2.61	2.63	2.65	2.56	2.77	0.15	0.60	0.61
FGR	3.74	3.79	3.53	3.35	3.31	0.21	0.07	0.94
LWI (kg)	53.56	58.53	56.32	56.58	58.19	0.97	-	-
LWF (kg)	72.53	78.05	77.43	77.80	81.47	2.49	0.01	0.57
FFLG (kg d^{-1})	0.23	0.23	0.25	0.23	0.27	0.01	0.23	0.66
Carcass characteristics and plasma urea nitrogen								
BFT (mm)	9.40	10.59	10.46	11.51	10.21	0.40	0.07	0.02
LMA (cm^2)	26.81	28.71	28.55	28.09	27.57	1.10	0.79	0.20
LMP	52.69	53.56	53.57	53.05	52.29	0.55	0.48	0.06
PUN (mg dL^{-1})	13.44	14.17	13.11	11.71	13.49	1.12	0.51	0.67

SEM: standard error of the mean; ADG: average daily gain; ADFI: average daily feed intake; FGR: feed:gain ratio; LWI: initial live weight; LWF: final live weight; FFLG: fat free lean gain; BFT: backfat thickness; LMA: longissimus muscle area; LMP: lean meat percentage.

Table 3. Response of finishing I pigs (75 to 100 kg of weight) fed with five levels of protected methionine.

	Treatment (Protected methionine, %)					SEM	p value	
	0	25	50	75	100		Lineal	Quadratic
Growth performance								
ADG (kg)	0.77	0.73	0.78	0.78	0.79	0.03	0.47	0.80
ADFI (kg d^{-1})	2.82	2.80	2.88	2.84	3.10	0.12	0.08	0.12
FGR	3.67	3.69	3.67	3.65	3.98	0.10	0.10	0.13
LWF (kg)	96.88	97.50	99.36	97.83	99.56	3.18	0.50	0.78
FFLG (kg d^{-1})	0.24	0.25	0.27	0.25	0.28	0.01	0.16	0.81
Carcass characteristics and plasma urea nitrogen								
BFT (mm)	14.48	13.57	14.05	14.03	14.47	0.61	0.82	0.34
LMA (cm^2)	32.36	33.16	33.76	32.39	34.67	0.74	0.09	0.72
LMP	50.88	51.68	52.34	50.95	51.42	0.46	0.81	0.12
PUN (mg dL^{-1})	25.27	19.89	19.52	23.71	22.28	1.80	0.71	0.06

EEM: standard error of the mean; ADG: average daily gain; ADFI: average daily feed intake; FGR: feed:gain ratio; LWF: live weight final; FFLG: fat free lean gain; BFT: backfat thickness; LMA: longissimus muscle area; LMP: lean meat percentage.

higher ADFI could have been caused by a higher bioavailability of methionine and the resulting imbalance with other AAs led the pig to consume more feed to compensate their diminished bioavailability. The imbalance of AAs is confirmed by the highest PUN in the treatments that contained the highest amount of PM. The PUN is a biological indicator of the efficient use of the AA of the diet, because this blood metabolite is very sensitive to changes in the contribution of CP and AA in the feed (Lents *et al.*, 2013). This imbalance caused a greater intake of food —in order to be able to ingest a greater quantity of other AAs—, since the growth potential allowed pigs to continue synthesizing muscle protein, resulting in a greater LMA accumulation.

The use of PM can increase its availability and absorption in the animal's organism (Piva *et al.*, 2007). This can be potentially reflected in a better productive response, since a greater availability of methionine in standard diets for pigs improves ADG, LW, FGR, BFT, and LMP (Chen *et al.*, 2014; Shen *et al.*, 2014). Piva *et al.* (2007) showed that protected AA can be fully and slowly released in the intestine, which could allow a greater constancy in plasma concentration between time lapses in feed intake (Prandini *et al.*, 2013).

The better metabolic efficiency of microencapsulated (protected) AAs compared to synthetic AAs traditionally used in pig feed may be due to their slower rate of release and absorption in the gastrointestinal tract (Prandini *et al.*, 2013). This allows for greater bioavailability by reducing the transient imbalance between AAs in the systemic circulation (Wu, 2009). Furthermore, synthetic AAs are rapidly absorbed through the gastrointestinal tract and are available for metabolic purposes (Wu, 2009).

Meanwhile, protein-bound AAs are released into the gastrointestinal lumen after the digestive processes take place, at a rate that varies among feed ingredients (Wu, 2009; NRC, 2012). The differential absorption rate of protein-bound and synthetic AAs may decrease the use of AAs for protein synthesis throughout the body. This imbalance could

lead to the oxidation of rapidly absorbed crystalline AAs, as well as of AAs absorbed at a later stage from intact proteins (Yen *et al.*, 2004).

CONCLUSION

The substitution of regular synthetic methionine by protected methionine in the diet of finishing I pigs (50-75 kg of weight) improves weight gain and feed:gain ratio. However, the dietary use of protected methionine in pigs from 75 to 100 kg does not have a clear effect on the productive variables and carcass characteristics.

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