Effectiveness and optimum level of protected methionine in fattening pig diets

Efectividad y nivel óptimo de metionina protegida en dietas para cerdos en engorde

Eficácia e nível ideal de metionina protegida em dietas para suínos de engorda

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Abstract

Background: Methionine (Met) requirements have not been clearly established for fattening pigs due to their metabolic interrelationships and its bioavailability for protein synthesis. **Objective:** To determine the optimum level of regular crystalline or protected Met in pig diets from nursery to finishing. **Methods:** A total of 48 crossbred pigs (11.74±1.72 kg of initial body weight) were used. The treatments consisted of adding four levels (0.00, 0.05, 0.10, 0.15% in addition to dietary content) and two types of Met (regular and protected) to pig diets. **Results:** Nursery, Finishing I, and II pigs fed protected Met increased daily feed intake (DFI; p≤0.10). Protected Met raised daily weight gain (DWG) in nursery pigs and increased backfat thickness (BT) in nursery and grower pigs (p≤0.10). In Finishing I pigs, protected Met increased DWG and improved carcass characteristics (p≤0.10). In nursery and grower pigs, an extra 0.15% Met decreased feed:gain ratio (FGR; p≤0.10). In grower and Finishing II pigs fed extra 0.05% Met improved DWG and extra 0.10% Met reduced plasma urea concentration (p≤0.10). **Conclusions:** Feeding protected Met in pig diets increases DWG, DFI and BT. Increasing 0.05-0.15% Met level improves FGR, DWG, potentially reducing nitrogen excretion to the environment.

Keywords: *bioavailability; carcass traits; growth performance; nutrient requirements; pig; protected methionine; protein; swine; synthetic amino acids; urea.*

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Resumen

Antecedentes: los requerimientos de metionina (Met) para cerdos en crecimiento no han sido claramente establecidos, lo que se debe a sus relaciones metabólicas y su biodisponibilidad para la síntesis proteica. **Objetivo:** determinar el nivel óptimo de Met regular o protegida en dietas para cerdos en crecimiento. **Métodos:** Se utilizaron un total de 48 cerdos híbridos (11,74±1,72 kg peso vivo inicial). Los tratamientos consistieron en niveles incrementales (0,00, 0,05, 0,10, 0,15% adicionales al contenido de la dieta) y dos tipos de Met (regular y protegida) en la dieta. **Resultados:** los cerdos en iniciación, Finalización I, y II, alimentados con Met protegida tuvieron un mayor consumo diario de alimento (DFI; p≤0,10). La Met protegida aumentó la ganancia diaria de peso (DWG) durante la etapa de iniciación, e incrementó el grosor de la grasa dorsal (BT) en iniciación y levante (p≤0,10). Durante Finalización I, la Met protegida aumentó la DWG y mejoró las características de la canal (p≤0,10). Durante iniciación y levante, 0,15% extra de Met disminuyó la conversión alimenticia (FGR; p≤0,10). Los cerdos en levante y Finalización II alimentados con 0,05% extra de Met mejoraron la DWG y con 0.10% extra de Met redujeron la concentración de urea en plasma (p≤0,10). **Conclusiones:** el uso de Met protegida incrementa DWG, DFI y BT. El aumento del nivel de Met de 0,05-0,15% mejora FGR y DWG, y podria disminuir la excreción de nitrógeno al ambiente.

Palabras clave: *aminoácidos sintéticos; biodisponibilidad; características de la canal; cerdos; comportamiento productivo; metionina protegida; proteína; requerimientos nutricionales; suínos; urea.*

Resumo

Antecedentes: os requisitos de metionina (Met) para suínos de engorda não foram claramente estabelecidos devido às suas relações metabólicas e sua biodisponibilidade para a síntese de proteínas. **Objetivo:** determinar o nível ideal de Met regular ou protegida em dietas para suínos de engorda. **Métodos:** foram utilizados 48 suínos híbridos (11,74±1,72 kg de peso vivo inicial). Os tratamentos consistiram em quatro níveis (0,00, 0,05, 0,10, 0,15% mais) e dois tipos (regular e protegida) de Met em dietas para suínos de engorda. **Resultados:** suínos no início, finalização I e II alimentados com Met protegida aumentaram o consumo diário de ração (DFI; p≤0,10). Met protegido aumentou o ganho de peso diário (DWG) em suínos na iniciação e, aumentou espessura da gordura dorsal (BT) em suínos em iniciação e crescimento (p≤0,10). Nos suínos finalização I, Met protegido aumentou DWG e melhorou as características do canal (p≤0,10). Para suínos de iniciação e crescimento, 0,15% extra de Met diminuiu conversão alimentar (FGR; p≤0,10). No crescimento e finalização II, a adição de 0,05% de Met melhoraram o DWG e com 0,10% reduziram a concentração de uréia (p≤0,10). **Conclusões:** o uso de Met protegida melhora DWG, DFI e BT. O nível de Met aumentado de 0,05-0,15% melhora FGR e DWG; além disso, a excreção de nitrogênio ambiental pode ser diminuída.

Palavras-chave: *aminoácidos sintéticos; biodisponibilidade; características de carcaça; comportamento produtivo; metionina protegida; necessidades nutricionais; porcos; proteína; suínos; uréia.*

Introduction

Methionine (Met) is the second or third-limiting aminoacid (AA) in conventional diets for growing pigs, which can lead to its deficiency, reduced availability for protein synthesis and limited growth (Bauchart-Thevret *et al.*, 2009; NRC, 2012; Chen *et al.*, 2014). The Met levels present in most dietary ingredients for pigs are insufficient; thus, it must be provided from a synthetic source (Santos *et al.*, 2011). Additionally, bioavailability and bioefficacy of synthetic DL-Met isomers show inconsistent results in growth performance of pigs (Shen *et al.*, 2014; Kong *et al.*, 2016). These could be attributed to the fact that free synthetic AA are very sensitive to the acidic conditions of the stomach and are rapidly absorbed in the digestive tract (Sato *et al.*, 1984, Stein *et al.*, 2007), decreasing their metabolic efficiency.

Dietary inclusion of protected AA provides resistance to stomach conditions and allows for slow intestinal release, which could help to overcome the above-mentioned limitations (Piva *et al.*, 2007). Protected AA utilization has been reported in ruminants, improving productive performance (Zanton *et al.*, 2014). Protected lysine, in substitution for HCl·lysine, enhances the bioavailability of this AA in pig diets without negatively affecting growth performance and carcass characteristics (Prandini *et al.*, 2013).

Supplementing Met to pig diets has been associated with positive effects on the immune system. (Opapeju *et al.*, 2012; de Oliveira *et al.*, 2015). Although pigs can tolerate some shortage or surplus of Met (Pena *et al.*, 2008; Santos *et al.*, 2011; Ying *et al.*, 2015) its excess can affect growth performance and carcass characteristics, while low Met levels can increase fat deposition, reducing protein synthesis (Conde-Aguilera *et al.*, 2014). Appropriate dietary concentration of Met depends on each analyzed variable (Zhang *et al.*, 2013).

The objectives of this study were to determine the optimum level of Met in pig diets and to evaluate the effects of regular synthetic or protected DL-Met on growth performance, carcass characteristics and plasma urea nitrogen (PUN) concentration.

Materials and Methods

Ethical Considerations

The experimental procedures followed the standards for ethics, biosafety and animal well-being of the Official Mexican Standard (NOM-062-ZOO, 1999) for the use of animals in experimentation.

Location

The experiment was conducted at the Swine Unit of the Experimental Farm of *Colegio de Postgraduados*, located in Montecillo, State of Mexico (98º 48' 27'' W and 19º 48' 23'' N). The climate is temperate, semi-arid, with 15.8 °C average annual temperature, infrequent frosts, 663.7 mm average annual rainfall, and 2,250 m altitude (Estación Agro-Meteorológica, 2016).

Animals and experimental design

Forty-eight crossbred pigs (Yorkshire x Landrace x Duroc) terminal line fattening barrows and gilts (11.74±1.72 kg initial BW) were used in a completely randomized design. The treatments consisted in the dietary supplementation with four levels of Met from two types (protected and regular Met) during four growing stages: Nursery (0.48, 0.53, 0.58, and 0.63%), Grower (0.38, 0.43, 0.48, and 0.53%), Finishing I (0.31, 0.36, 0.41, and 0.46%) and Finishing II (0.23, 0.28, 0.33, and 0.38%). These levels were obtained by adding synthetic or protected Met to the control diet. The Met concentrations of synthetic Met fulfilled the dietary requirements of Met+Cys suggested by the NRC (2012). The protected Met used contained 85% encapsulated DL-methionine (Mepron® Evonik Industries, Germany).

Diets and animal handling

Basal diets contained sorghum-soybean meal and were supplemented with crystalline AA [L-lysine·HCl, DL-methionine (Evonik

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Industries, Parsippany, NJ, USA), L-threonine (Jefo Nutrition Inc, Saint-Hyacinthe, Québec, Canada) and L-tryptophan (CPB Aurum, México, DF)] to meet or exceed the nutritional requirement for each stage of growth (NRC, 2012; Table 1). Pigs were individually housed in 1.2×1.5 m pens, on concrete floor partially covered with plastic slats, and equipped with a single feeder and a nipple drinker. The animals were injected with a deworming and vitamin product (Endovet polivitaminado® 15 µl kg^{-1} of body weight) before the start of the study. Pens were cleaned and the health of pigs verified throughout the experimental period on a daily basis. Ventilation was provided with curtains. Feed and water were provided *ad libitum.*

Table 1. Ingredients and nutrient composition of experimental diets.

	Stage			
Ingredient, %	**Nursery**	**Grower**	**Finishing I**	**Finishing II**
Sorghum grain	68.70	77.21	81.90	90.40
Soybean meal	26.49	18.66	14.14	6.50
Soybean oil	1.39	1.11	1.02	0.61
Biolys*	0.88	0.73	0.64	0.61
DL-Methionine	0.23	0.16	0.11	0.06
L-Threonine	0.16	0.10	0.08	0.06
L-Tryptophan	0.01	0.00	0.01	0.00
Vitamins**	0.20	0.20	0.20	0.20
Minerals***	0.15	0.15	0.15	0.15
Common salt	0.30	0.30	0.30	0.30
Calcium carbonate	0.83	0.56	0.51	0.87
Orthophosphate	0.65	0.82	0.91	0.23
Phytase	0.01	0.01	0.01	0.01
Nutrient composition calculated (%)				
Metabolizable energy, Mcal kg^{-1}	3.30	3.30	3.30	3.30
Crude protein	20.00	16.83	15.00	12.06
Lysine	1.28	1.00	0.85	0.63
Methionine	0.48	0.38	0.31	0.23
Met + Methionine+Cysteine	0.71	0.57	0.48	0.36
Threonine	0.76	0.60	0.52	0.40
Tryptophan	0.21	0.17	0.15	0.11
Calcium	0.74	0.67	0.67	0.64
Phosphorus	0.36	0.31	0.31	0.35
Nutrient composition determined (%)				
Crude protein	18.95	16.75	14.75	12.35
Calcium	0.68	0.62	0.62	0.64
Phosphorus	0.30	0.30	0.30	0.33

*50.7% lysine (Biolys® Evonik). **Supplied by kg of feed: 5.0×106 IU vitamin A; 1.0×106 IU vitamin D3; 2.0×104 IU vitamin E; 2 g vitamin K3; 1 g tiamine; 5 g de rivoflavin; 2 g pyridoxine; 25 g niacin; 15 g D-calcium panthotenate; 3 g folic acid; 225 g choline chloride; 0.3 g antioxidant; 15 mg B12; 180 mg biotin (REKA® Lapisa Animal Nutrition). *** Supplied by kg of feed: 0.2 g Se; 0.1 g Co; 0.3 g I; 10 g Cu; 100 g Zn; 100 g Fe; 100 g Mn (REKA® Lapisa Animal Nutrition).

Variables measured

The variables analyzed were: daily weight gain (DWG), daily feed intake (DFI) and feed:gain ratio (FGR). In addition, on the first and last day of the experiment, the backfat thickness (BT) and *longissimus* muscle area (LMA) were measured using real time ultrasound (Sonovet 600) with a 3.5 MHz transducer (Medison, Inc., Cypress, California, USA). These data, together with the initial (BWi) and final (BWf) body weight, were used to determine fat free lean gain (FFLG) and lean meat percentage (LMP) using the NPPC equations (Burson and Berg, 2001). At the last day of each stage, blood samples were taken from the vena cava in heparinized tubes (Vacutainer®), then set in ice until centrifuged at 2,500 *g* during 20 min (IEC Centra 8R, International Equipment Company, USA), to separate plasma from cell package. Plasma was transferred to polypropylene tubes and stored in a freezer (EUR251P7W Tappan, Electrolux Home Products North America, USA) at -20 °C until determination of PUN concentration by UV spectrophotometry (Spectrophotometer Cary 1E UV-vis, Varian, Australia; Chaney and Marbach, 1962).

Statistical analysis

The experimental design was completely randomized with a 4×2 factorial arrangement of treatments. Factors were: four levels and two types of Met (regular and protected). Six replicates per treatment were used. Shapiro-Wilk and Levene's tests were used to check normal distribution and homogeneity of variance for all variables. Data were analyzed with the GLM procedure, and Tukey's test ($p \leq 0.10$) was used to compare treatment means (SAS, 2011). Initial body weight was used as a covariate ($p \leq 0.10$)

Results

Table 2 presents the growth performance results of nursery pigs. No interaction was found between level and type of Met on growth performance ($p > 0.10$). The FGR was reduced ($p = 0.10$) with the highest level of Met (0.63%). The use of protected Met increased DFI, DWG, BWf and BT ($p = 0.07$).

Grower pigs consuming the highest levels of Met showed reduced DFI ($p = 0.08$; Table 3). A possible interaction was observed between level and type of Met for DFI ($p = 0.10$). The FGR improved ($p = 0.05$) when using 0.48-0.53% Met, although the best response by the interaction between Met level and type was observed with 0.53% of protected Met ($p = 0.05$). The highest DWG ($p = 0.10$) was observed with 0.43% Met, regardless of Met type ($p > 0.10$). The PUN was reduced with 0.48% Met ($p = 0.10$). Protected Met reduced LMP ($p = 0.09$) and increased BT ($p = 0.02$).

Table 4 shows the results of Finishing I pigs. The lowest BT was observed with 0.41-0.46% regular Met ($p = 0.09$). Protected Met increased DFI ($p \leq 0.01$), DWG ($p = 0.04$), FFLG ($p \leq 0.01$), LMP ($p = 0.07$), and LMA ($p = 0.02$).

The results of Finishing II pig variables are shown in Table 5. Protected Met increased DFI ($p = 0.02$). The DWG, BWf, and FFLG improved with 0.28% protected Met ($p = 0.02$). PUN was reduced ($p = 0.02$) using 0.33% Met, regardless of Met type.

Discussion

Protected Met increased DWG in nursery and finishing pigs, raised DFI in nursery and finishing pigs, improved FGR in grower pigs, increased BT in nursery and grower pigs, and improved carcass characteristics in Finishing I pigs. Prandini *et al.* (2013) reported better metabolic efficiency of protected AAs compared with traditional synthetic AAs in pig diets due to slower release and absorption rates, which allows for higher metabolic utilization. Protected Met could increase availability and absorption of this amino acid in pigs (Piva *et al.*, 2007; Schwab and Orway, 2016) leading to better productive performance, including DWG, final BW, and FGR (Opapeju *et al.*, 2012; Shen *et al.*, 2014; Chen *et al.*, 2014). In the present study, the increased digestibility of protected Met possibly led to increased BT evidencing a possible excess and imbalance among AAs, which results in removal and excretion of nitrogen excess and retention of carbon skeletons as fat (García et al., 2010).

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Table 2. Treatment means for growth performance of nursery pigs fed four levels and two types of methionine.

% Met	Met type	DFI (kg d^{-1})	DWG (kg d^{-1})	FGR	BWi (kg)	BWf (kg)	FFLG (kg d^{-1})	LMP (%)	BT (mm)	LMA (cm^2)	PUN (mg dL^{-1})
0.48	Regular	0.87	0.43	2.03	11.81	23.79	0.18	45.94	4.23	11.27	13.98
0.48	Protected	0.95	0.45	2.10	11.49	24.41	0.17	45.30	4.63	11.37	15.49
0.53	Regular	0.87	0.42	2.09	12.22	23.47	0.17	46.20	4.21	11.22	16.70
0.53	Protected	0.98	0.48	2.03	11.63	25.31	0.18	44.31	4.94	11.32	14.89
0.58	Regular	0.82	0.40	2.05	11.71	23.01	0.17	46.53	3.63	10.74	13.46
0.58	Protected	0.88	0.46	1.94	11.55	24.49	0.18	45.86	4.67	11.94	13.23
0.63	Regular	0.89	0.47	1.84	12.21	25.02	0.19	45.26	4.76	11.81	12.38
0.63	Protected	0.93	0.49	1.88	11.34	25.52	0.20	46.82	4.33	12.77	15.66
SEM		0.05	0.03	0.07	0.87	0.88	0.01	0.64	0.32	0.62	2.13
					Main effects						
0.48		0.91	0.44	2.07^a	11.65	24.11	0.17	45.63	4.43	11.33	14.77
0.53		0.93	0.45	2.06^a	11.92	24.39	0.17	45.26	4.57	11.27	15.80
0.58		0.85	0.42	2.00^{ab}	11.63	23.75	0.17	46.20	4.15	11.34	13.35
0.63		0.91	0.48	1.88^b	11.78	25.27	0.19	46.04	4.55	12.29	14.02
SEM		0.04	0.02	0.04	0.60	0.59	0.01	0.43	0.22	0.41	1.25
	Regular	0.86^b	0.43^b	2.01	11.99	23.82^b	0.17	45.99	4.21^b	11.26	14.13
	Protected	0.94^a	0.47^a	1.99	11.50	24.94^a	0.18	45.58	4.64^a	11.85	14.82
SEM		0.02	0.01	0.03	0.42	0.41	0.006	0.31	0.16	0.30	0.91

Met, Methionine; DFI, daily feed intake; DWG, daily weight gain; FGR, feed:gain ratio; BWi, initial body weight; BWf, final body weight; FFLG, fat free lean gain; LMP, lean meat percentage; BT, backfat thickness; LMA, *Longissimus* muscle area; PUN, plasma urea nitrogen concentration; SEM, standard error of the mean. Means with different superscript letters ($^{a, b, c, d}$) within columns differ statistically ($p \leq 0.10$).

In our experiment, an extra 0.10-0.15% Met was the best level for FGR in nursery and grower pigs. Supplementation with extra 0.02-0.12% Met has been reported to improve FGR in postweaning and growing pigs (Opapeju *et al.*, 2012; Chen *et al.*, 2014; de Oliveira *et al.*, 2015). This could be explained by met being a functional AA for intestinal growth and function, beyond its role as a precursor for protein synthesis (Wu *et al.*, 2013). In grower pigs, although DFI was reduced with 0.48 and 0.53% Met, FGR was improved with these levels.

Grower and Finishing II Pigs fed extra 0.05% Met resulted in higher DWG. In growing pigs, 0.01-0.15% additional Met reduced DFI, thus improving FGR. In Finishing I pigs, 0.10-0.15% extra Met reduced BT. In Finishing II pigs, the addition of 0.05% Met improved FFLG. The Met requirement found in this study is higher than that recommended by the NRC (2012). A higher Met level could represent extra benefits in terms of productive performance. Methionine supplementation (0.02, 0.04, and 0.06%) in growing pig diets has resulted in improved daily gain, feed intake (Opapeju *et al.*, 2012), and feed: gain ratio (Opapeju *et al.*, 2012; de Oliveira *et al.*, 2015). On the contrary, a Met deficiency in pigs can lead to lower DWG (Conde-Aguilera *et al.*, 2014), growth suppression of the intestinal mucosa, reduction of intestinal epithelium, and increase of oxidative stress (Bauchart-Thevret *et al.*, 2009; Chen *et al.*, 2014). Several studies have showed that pigs can tolerate some Met deficiency (Santos *et al.*, 2011; Ying *et al.*, 2015) or excess levels in the diet (Pena *et al.*, 2008).

Table 3. Treatment means for growth performance of grower pigs fed four levels and two types of methionine.

% Met	Met type	DFI (kg d^{-1})	DWG (kg d^{-1})	FGR	BWf (kg)	FFLG (kg d^{-1})	LMP (%)	BT (mm)	LMA (cm^{2})	PUN (mg dL^{-1})
0.38	Regular	1.58^{abc}	0.76	2.06^{abc}	48.44	0.25	41.59	7.56	20.33	17.99
0.38	Protected	1.72^{ab}	0.73	2.34^{a}	52.63	0.28	39.73	8.35	19.92	17.05
0.43	Regular	1.78^{a}	0.82	2.15^{ab}	51.15	0.29	41.51	7.70	21.95	19.47
0.43	Protected	1.71a^{b}	0.81	2.10^{abc}	51.81	0.28	40.17	8.37	20.39	15.07
0.48	Regular	1.37^{c}	0.72	1.89^{bc}	51.68	0.28	40.55	7.20	20.03	14.02
0.48	Protected	1.53^{abc}	0.75	2.04^{abc}	50.41	0.27	40.85	7.93	20.72	16.13
0.53	Regular	1.50^{abc}	0.76	1.96^{bc}	49.92	0.26	40.82	7.32	19.86	18.10
0.53	Protected	1.41^{bc}	0.76	1.86^{c}	51.02	0.26	39.97	7.42	19.05	20.69
SEM		0.08	0.03	0.07	2.13	0.02	0.82	0.37	0.92	1.91
					Main effects					
0.38		1.65^{a}	0.75^{b}	2.20^{a}	50.54	0.27	40.66	7.95	20.13	17.52^{ab}
0.43		1.75^{a}	0.82^{a}	2.13^{ab}	51.48	0.28	40.84	8.03	21.17	17.27^{ab}
0.48		1.45^{b}	0.74^{b}	1.97^{bc}	51.05	0.27	40.70	7.57	20.38	15.07^{b}
0.53		1.46^{b}	0.76^{ab}	1.91^{c}	50.47	0.26	40.40	7.38	19.45	19.39^{a}
SEM		0.05	0.02	0.05	1.52	0.01	0.57	0.26	0.62	1.33
	Regular	1.56	0.77	2.01	50.30	0.27	41.11^{a}	7.44^{b}	20.55	17.39
	Protected	1.60	0.76	2.09	51.46	0.27	40.18^{b}	8.02^{a}	20.02	17.24
SEM		0.03	0.01	0.03	1.04	0.01	0.38	0.17	0.44	0.89

Met, Methionine; DFI, daily feed intake; DWG, daily weight gain; FGR, feed:gain ratio; BWi, initial body weight; BWf, final body weight; FFLG, fat free lean gain; LMP, lean meat percentage; BT, backfat thickness; LMA, *Longissimus* muscle area; PUN, plasma urea nitrogen concentration; SEM, standard error of the mean. Means with different superscript letters ($^{a, b, c, d}$) within columns differ statistically ($p \leq 0.10$).

It has been also suggested that Met deficiencies could not affect growth performance, carcass characteristics and protein synthesis due to an adaptation of tissue metabolism when facing insufficient dietary met supply (Castellano *et al.*, 2015). The results of the present study confirm that increasing Met levels improve growth performance variables in pigs. Protein deposition is affected when Met is provided at insufficient levels (de Oliveira *et al.*, 2015); therefore, supplementation of extra Met (+0.12%) is required for optimal protein synthesis (Chen *et al.*, 2014). Additionally, proper Met levels might reduce carcass fat content in pigs with Met deficiency (Conde-Aguilera *et al.*, 2014; Castellano *et al.*, 2015). In our study, adding extra 0.10% Met in growing and Finishing II pigs reduced PUN. This lower PUN concentration is associated with a lower synthesis and excretion of urea originated by AA excess, indicating better utilization of metabolic nitrogen (Qin *et al.*, 2015).

In conclusion, substituting synthetic Met with protected Met increases DWG and DFI without negatively affecting FGR. Additionally, protected Met could alter lipid metabolism, considering it increases BT. These results indicate that increasing 0.05-0.15% Met improves FGR, DWG, FFLG, and reduces BT and PUN; potentially reducing the excretion of nitrogen in feces and urine to the environment.

Table 4. Treatment means for growth performance of Finishing I pigs fed four levels and two types of methionine.

% Met	Met type	DFI (kg d^{-1})	DWG (kg d^{-1})	FGR	BWf (kg)	FFLG (kg d^{-1})	LMP (%)	BT (mm)	LMA (cm^2)	PUN (mg dL^{-1})
0.31	Regular	4.44	0.83	2.99	73.62	0.27	38.63	11.06[a]	27.73	8.31
0.31	Protected	2.70	0.94	2.92	73.98	0.31	39.31	11.50[a]	29.14	11.60
0.36	Regular	2.55	0.81	3.15	74.19	0.27	38.21	11.22[a]	26.74	11.97
0.36	Protected	2.54	0.86	2.95	73.70	0.29	39.21	10.13[ab]	27.02	9.65
0.41	Regular	2.30	0.77	3.03	72.45	0.28	38.57	9.33[b]	25.67	10.22
0.41	Protected	2.56	0.74	3.01	74.94	0.30	39.09	10.50[ab]	28.39	10.63
0.46	Regular	2.41	0.80	2.98	73.97	0.29	38.93	9.39[b]	26.50	7.87
0.46	Protected	2.50	0.83	3.06	74.29	0.30	39.30	10.50[a]	28.33	10.21
SEM		0.07	0.04	0.13	1.02	0.01	0.37	0.35	0.92	1.77
					Main effects					
0.31		2.56	0.88	2.95	73.80	0.29	38.97	11.28[a]	28.43	9.95
0.36		2.54	0.83	3.05	73.94	0.28	38.71	10.67[ab]	26.88	10.81
0.41		2.43	0.80	3.02	73.69	0.27	38.83	9.92[b]	27.03	10.43
0.46		4.45	0.82	3.02	74.11	0.30	39.12	10.10[b]	27.42	9.04
SEM		0.05	0.03	0.09	0.70	0.01	0.27	0.25	0.63	1.21
	Regular	2.42[b]	0.80[b]	3.04	73.56	0.27[b]	38.58[b]	10.25[b]	26.66[b]	9.59
	Protected	2.58[a]	0.86[a]	2.99	74.22	0.30[a]	39.22[a]	10.73[a]	28.22[a]	10.52
SEM		0.04	0.02	0.06	0.50	0.006	0.18	0.16	0.44	0.86

Met, Methionine; DFI, daily feed intake; DWG, daily weight gain; FGR, feed:gain ratio; BWi, initial body weight; BWf, final body weight; FFLG, fat free lean gain; LMP, lean meat percentage; BT, backfat thickness; LMA, *Longissimus* muscle area; PUN, plasma urea nitrogen concentration; SEM, standard error of the mean. Means with different superscript letters ([a, b, c, d]) within columns differ statistically ($p \leq 0.10$).

Table 5. Treatment means for growth performance of Finishing II pigs fed four levels and two types of methionine.

% Met	Met type	DFI (kg d^{-1})	DWG (kg d^{-1})	FGR	BWf (kg)	FFLG (kg d^{-1})	LMP (%)	BT (mm)	LMA (cm^2)	PUN (mg dL^{-1})
0.23	Regular	2.60	0.75^b	3.47	95.17^b	0.24^b	37.28	15.56^{ab}	33.72	14.64^{ab}
0.23	Protected	3.04	0.78^{ab}	3.98	96.08^{ab}	0.25^b	38.08	14.67^{ab}	35.24	10.04^{ab}
0.28	Regular	2.80	0.78^{ab}	3.59	96.33^b	0.28^b	37.38	14.58^{ab}	33.46	13.60^{ab}
0.28	Protected	2.99	0.97^a	3.10	102.14^a	0.39^a	37.55	13.74^{ab}	34.63	9.06^b
0.33	Regular	2.75	0.83^{ab}	3.38	97.51^{ab}	0.28^{ab}	37.58	13.20^{ab}	33.07	9.84^b
0.33	Protected	2.84	0.84^{ab}	3.41	98.02^{ab}	0.30^{ab}	36.97	16.52^a	34.28	9.60^b
0.38	Regular	2.59	0.74^b	3.57	94.81^b	0.26^b	38.15	12.94^b	33.77	12.04^{ab}
0.38	Protected	2.68	0.75^b	3.59	95.35^b	0.23^b	27.16	14.40^{ab}	32.24	16.44^a
SEM		0.13	0.03	0.23	1.48	0.03	0.66	1.02	0.17	1.82
					Main effects					
0.23		2.82	0.76b	3.72	95.62^b	0.24^b	37.68	15.11	34.48	12.34^{ab}
0.28		2.90	0.88^a	3.34	99.24^a	0.33^a	37.46	14.16	34.05	11.33^{ab}
0.33		2.80	0.83^{ab}	3.40	97.77^{ab}	0.29^{ab}	37.28	14.86	33.68	9.72^b
0.38		2.63	0.75^b	3.58	95.08^a	0.25^b	37.65	13.68	33.01	14.23^a
SEM		0.08	0.03	0.15	0.97	0.02	0.44	0.67	0.12	1.22
	Regular	2.69^b	0.77^b	3.50	95.95^b	0.26^b	37.60	14.07	33.50	12.53
	Protected	2.89^a	0.84^a	3.52	97.90^a	0.29^a	37.44	14.83	34.10	11.29
SEM		0.06	0.02	0.10	0.65	0.01	0.28	0.47	0.78	0.78

Met, Methionine; DFI, daily feed intake; DWG, daily weight gain; FGR, feed:gain ratio; BWi, initial body weight; BWf, final body weight; FFLG, fat free lean gain; LMP, lean meat percentage; BT, backfat thickness; LMA, *Longissimus* muscle area; PUN, plasma urea nitrogen concentration; SEM, standard error of the mean. Means with different superscript letters ($^{a, b, c, d}$) within columns differ statistically ($p \leq 0.10$).

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

José. L Figueroa-Velasco participated in the experimental design, supervision of the experimental protocol and writing of the manuscript. David Trujano-San Luis conducted the experimental work, laboratory analysis, and wrote the manuscript. José A. Martínez-Aispuro participated in the experimental design, data collection, statistical analysis and writing of the manuscript. María T. Sánchez-Torres designed and supervised the study, and revised the final manuscript. María M. Crosby-Galván collaborated with laboratory analysis, revised the research proposal and the final manuscript. Agustín Ruíz-Flores revised the research proposal, statistical analysis and the final manuscript. Jose L. Cordero-Mora obtained samples and data, supervised the health status of the pigs and revised the final version of the manuscript.

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