Increased dietary protein or free amino acids supply for heat stress pigs: effect on performance and carcass traits

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ABSTRACT: Heat stress (HS) pigs reduce their voluntary feed intake (VFI) and ingestion of indispensable amino acids (AA). Increasing the dietary crude protein (CP) content may help to correct the reduced AA intake by HS pigs, but it may further increase their body heat load. Increasing the AA intake by adding free AA to the diet does not affect the heat load of HS pigs. Two 21-d experiments were conducted. In Exp. 1, 30 pigs (31.1 ± 1.2 kg initial body weight) were used to determine the performance depression because of HS. Treatments were: thermo neutral pigs fed a 22% CP control diet (TN-C); HS pigs fed the control diet (HS-C); HS pigs fed a 14% CP, AA supplemented diet (HS-AA). HS pigs had lower ADG and Lys utilization efficiency, and consumed 20 and 25% less Lys and Thr, respectively, than the TN-C pigs ($P < 0.05$). In Exp. 2 (comparative slaughter), 25 pigs (33.6 ± 0.65 kg initial body weight) were used to evaluate the effect of extra dietary AA either as protein-bound or free AA on the performance and carcass traits of HS pigs. Treatments were: control wheat-SBM-free Lys, Thr and Met diet (CON); diet with 30% more CP than CON (HSxP); diet added with free AA to contain at least 25% more of each AA than the recommended level (HSxAA). Ambient

temperature (AT) ranged from 27.7 to 37.7°C, and body temperature (39.9 to 41.2°C) followed a similar daily pattern as the AT did. There was no dietary treatment effect on daily feed and NE intake ($P > 0.10$), but the Lys, Thr, and Met intake was higher in pigs fed the HSxP or HSxAA diets than in pigs fed the CON diet ($P < 0.05$). The daily weight gain (ADG) was not affected ($P > 0.10$) but G:F tended to be higher and the Lys utilization efficiency (ADG, g/g Lys intake) tended to be lower in HSxP pigs than in CON pigs ($P < 0.10$). The HSxAA pigs had higher ADG ($P < 0.05$), and tended to have higher weight of hot carcass and leg muscle, and the weight gain of hot carcass and leg muscle than the CON pigs ($P < 0.10$). The weight and daily weight gain of loin muscle was higher in the HSxAA than in the HSxP pigs ($P < 0.05$). Kidney weight and serum urea in HSxP pigs were higher than in CON and HSxAA pigs, but spleen weight was higher in HSxAA pigs than in CON and HSxP pigs ($P < 0.05$). These results confirm that HS reduces the VFI, and show that increased levels of AA either as free or protein-bound do not additionally reduce the VFI of HS pigs. These also show that extra free AA supply rather than protein-bound AA better ameliorate the reduced growth performance of HS pigs.

Key words: dietary amino acids, heat stress, pigs

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INTRODUCTION

Pigs are exposed to ambient temperature (AT) exceeding their thermo neutral (TN) zone in most areas of the world. Pigs exposed to 35°C or more show marked signs of heat stress (HS; Liu

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et al., 2009), affecting their voluntary feed intake (VFI) and growth rate (Renaudeau et al., 2011). In México, based on the models by St-Pierre et al. (2003), the estimated economic loss associated with the depressed performance of HS pigs exceeds US\$ 100 million/year.

HS pigs reduce their VFI to decrease the body heat production and maintain thermal homeostasis (Collin et al., 2001). The reduction in the VFI fluctuates between 20 and 40% compared with TN pigs (Quiniou et al., 2000; Pearce et al., 2013a), which translates into a lower nutrient intake, especially AA. A damaged intestinal mucosa (Pearce et al., 2013b) as well as a diminished abundance of intestinal AA transporters in HS pigs (Morales et al., 2014) suggest a reduced AA absorption. The combined low VFI and low AA absorption results in reduced AA availability for the growth of HS pigs (Morales et al., 2016a). Hence, increasing the dietary AA level could be a good strategy to overcome the low AA availability in HS pigs.

The AA level can be increased by elevating the content of protein or free AA in the diet. Increasing the dietary protein content may further increase the HS because the digestion of proteins may increase the body heat load (Pesta and Samuel, 2014), which could additionally affect the VFI. In contrast, dietary free AA may not generate additional heat. The effect of adding extra protein-bound or free AA on the performance of HS pig has not been reported. We hypothesized that increasing the dietary AA content as protein or as free AA may differently correct the low AA availability in HS pigs, and their depressed performance. Hence 2 studies were conducted to determine whether supplying extra protein-bound or free AA in the diet helps to correct the depressed performance of HS pigs.

MATERIALS AND METHODS

General Experimental Procedure

Two experiments were conducted with 55 cross-bred (Landrace × Hampshire × Duroc) pigs in the Northwestern part of México during the summer of the year 2016. The pigs were individually housed in pens (1.2 m wide, 1.2 m long, and 1.0 m high) with elevated iron-mesh floor, and equipped with a stainless-steel self-feeder and a nipple water drinker. The pigs were allowed 5 d to adjust to the pens and the AT before the beginning of each experiment; after that, they were weighed on a weekly basis, and ADFI was measured with the same frequency; ADG, G:F, Lys utilization efficiency (ADG, g/g Lys

intake), and MJ/g ADG were calculated accordingly. The formulation of the diets in both experiments (Table 1) was based mainly on wheat and soybean meal (SBM), which were analyzed for AA content (method 982.30; AOAC, 2006). The standardized ileal digestible (SID) contents of AA in the diets were calculated using the analyzed AA content and the SID coefficients for wheat and SBM reported by Stein et al. (2001). Feed and water were provided ad libitum all the time during the conduction of both experiments. The pigs used in the present experiments were cared for in accordance with the guidelines established in the Official Mexican Regulations on Animal Care (Ochoa, 2001).

Experiment 1

Thirty pigs (31.1 ± 1.2 kg initial BW) were used to determine the performance reduction because of their exposure to HS conditions, and to analyze the effect of the dietary CP content on their VFI. The treatments were: thermo neutral pigs fed a control 22% CP diet (TN-C); HS pigs fed the control diet (HS-C); HS pigs fed a 14% CP, AA supplemented diet (HS-AA). The control and the low protein diets were formulated to meet the SID requirement of the first (Lys) and the first 3 (Lys, Thr, and Met) limiting AA, respectively. Hence both diets met or exceeded the SID requirements of all indispensable AA for pigs within the body weight range of 25 to 50 kg with a predicted weight gain of 758 g/d (NRC, 2012). These diets were also added with vitamins and minerals to meet or exceed the NRC (2012) requirements. The TN-C pigs were maintained inside an air-conditioned room ($22 \pm 2^\circ\text{C}$) but the HS pigs were housed inside a room kept under natural AT conditions during the whole study. The AT and relative humidity inside the rooms was recorded with the aid of a Higrothermograph (Thermotracker HIGRO; iButtonLink LLC, Whitewater, WI, USA) set to record those values every 15 min during the whole study.

Experiment 2

The experiment was conducted with 25 pigs, 4 of which (31.8 ± 0.15 kg BW) were sacrificed as part of a comparative slaughter study. The remaining 21 pigs (33.6 ± 0.65 kg initial body weight) were maintained inside a room kept under natural AT conditions. The treatments were: control, 15.0% CP diet supplemented with free Lys, Thr, and Met, and added with vitamins and minerals, to meet or exceed the NRC (2012) requirements for pigs

Table 1. Composition of the experimental diets (% , as fed basis)¹

Ingredient	Exp. 1			Exp. 2	
	Control	LP-AA	CON	HSxP	HSxAA
Wheat	64.90	91.46	86.64	76.14	85.50
Soybean meal	30.30	4.00	10.00	20.50	10.00
L-Lys.HCl	-	0.80	0.56	0.56	0.88
L-Thr	-	0.27	0.14	0.14	0.29
DL-Met	-	0.11	0.06	0.06	0.18
L-Trp	-	0.04	-	-	0.03
L-Phe	-	0.09	-	-	0.08
L-Leu	-	0.25	-	-	0.18
L-Ile	-	0.13	-	-	0.07
L-Val	-	0.17	-	-	0.14
L-His	-	0.08	-	-	0.05
Calcium carbonate	1.25	1.40	1.40	1.40	1.40
Dicalcium phosphate	1.00	0.65	0.65	0.65	0.65
Iodized salt	0.35	0.35	0.35	0.35	0.35
Vitamin and Mineral Premix ²	0.20	0.20	0.20	0.20	0.20
Canola oil	2.00	-	-	-	-
Calculated content					
NE, MJ/kg ³	9.88	10.21	10.02	9.88	10.10
CP, %	21.72	13.91	15.10	18.98	16.05
SID ⁴ Arg	1.30	0.58	0.85	1.14	0.84
SID His	0.50	0.34	0.38	0.47	0.42
SID Ile	0.80	0.52	0.57	0.73	0.63
SID Leu	1.39	0.99	1.06	1.31	1.23
SID Lys	0.98	0.98	0.98	1.23	1.22
SID Met	0.28	0.28	0.28	0.36	0.40
SID Met + Cys	0.57	0.57	0.57	0.68	0.69
SID Phe	0.94	0.59	0.71	0.88	0.79
SID Thr	0.68	0.59	0.59	0.73	0.74
SID Trp	0.25	0.18	0.18	0.23	0.21
SID Val	0.88	0.64	0.66	0.82	0.80

¹Diets: Control, only protein-bound AA diet; LP-AA, low protein diet added with free AA; CON, Control diet; HSxP, CON + 30% protein as SBM; HSxAA, CON + 26% free AA.

²Supplied per kg of diet: Vitamin A, 4,800 IU; vitamin D₃, 800 IU; vitamin E, 4.8 IU; vitamin K₃, 1.6 mg; riboflavin, 4 mg; D-pantothenic acid, 7.2 mg; niacin, 16 mg; vitamin B₁₂, 12.8 mg; Zn, 64 mg; Fe, 64 mg; Cu, 4 mg; Mn, 4 mg; I, 0.36 mg; Se, 0.13 mg. The premix was supplied by Nutrionix, S.A., Hermosillo, México.

³Based on the ingredients NE values (MJ/kg): Wheat, 10.50; SBM, 8.12 (Sauvant et al. 2004).

⁴SID = Standardized ileal digestible.

within the BW range of 25 to 50 kg with a predicted weight gain of 758 g/d (CON); increased protein diet (HSxP); and increased free AA diet (HSxAA). The increase in the dietary content of protein or free AA was based on the fact that the VFI of HS pigs decreases between 20 and 40%. Also, results from Exp. 1 showed that HS pigs consumed 20 and 25% less Lys and Thr, respectively, compared to the TN pigs. In addition, data from a study we reported recently (Morales et al., 2014) indicate that pigs use some AA to ameliorate negative effects of HS. Thus we increased at least 26% the SID content of the indispensable AA to correct the reduced AA intake observed in Exp. 1 plus the amount of AA the pigs deviate from growth to fight HS. The HSxP

diet contained 30% more CP than the CON diet, by adjusting the wheat and SBM levels, without modifying the free AA levels. The HSxAA diet contained the same levels of wheat and SBM as in the CON diet, but it was added with free Lys, Thr, Met, Trp, Phe, Leu, Ile, His, and Val at levels that supplied at least 26% more of each AA, compared with the NRC (2012) requirements. The reason why 30% more CP was included in the HSxP diet and only 26% more free AA were included in the HSxAA diet was because of differences in the SID of AA between soybean proteins and free AA; the mean SID of AA in SBM proteins is only 88% while free AA are considered 100% SID. The NE content in the CON and HSxAA diets was 10.1 MJ of per kg,

whereas in the HSxP diet it was 9.88 MJ of per kg. All pigs had free access to purified water all the time during the 21 d study.

At the end of the study, 4 pigs from each treatment were also sacrificed to complete the comparative slaughter trial. The selection of pigs for slaughter was based on the initial and final BW; pigs from each treatment with BW closer to their respective treatment BW mean were selected. These pigs were sacrificed by exsanguination after they were knocked down by electrocution, and immediately eviscerated. The whole gastrointestinal tract of all pigs was emptied and the stomach, small and large intestines, as well as liver, heart, kidneys, and spleen were weighed. The right half carcass of all pigs was completely dissected after being stored at 2 to 4°C for 24 h, and the whole carcass, whole leg, leg muscles, whole loin, and loin muscles were weighed. The relative weight of each visceral organ and carcass component in the pigs sacrificed at the beginning, expressed as % of their respective BW, was computed and used to estimate their initial weight in pigs sacrificed at the end of the experiment. The average daily weight gain of the visceral organs, whole carcass and carcass components was calculated by subtracting the beginning from the final weights.

On day 20 of the experiment, blood samples (approximately 7 mL) were collected by venipuncture of the jugular vein from all pigs to analyze the serum concentration of urea (Chua and Tan, 1976). The sampling of blood was performed at 1700 h, when the AT reached its highest value in the day. Blood samples were kept on ice during the whole collection procedure. Immediately after collection, the blood samples were centrifuged at $1,500 \times g$, 4°C for 10 min to separate serum from blood cells. Serum samples were freeze-dried and stored at -20°C until analysis. Amino acid analyses of feed ingredients and diets of Exp. 2 (Table 2) were performed by HPLC with post-column ninhydrin derivatization and the use of a fluorescence detector, after acid hydrolysis with 6 N HCl (method 982.30E; AOAC, 2006); tryptophan was not analyzed.

Statistical Analyses

Analyses of the growth performance and carcass traits data were performed according to a randomized complete block design. The individual pig was considered as the experimental unit. In Exp. 1, 3 contrasts were constructed to evaluate the effect of AT and dietary CP level (C_1 , TN-C vs. HS-C; C_2 , TN-C vs. HS-AA; C_3 , HS-C vs. HS-AA). In Exp. 2,

3 contrasts were constructed to evaluate the effects of excess protein-bound AA, and excess free AA as follows: C_1 , CON vs. HSxP; C_2 , CON vs. HSxAA; C_3 , HSxP vs. HSxAA). In addition, differences in AT within the same day were analyzed by means of repeated measures and the multiple comparison test Tukey. Probability levels of $P \leq 0.05$, and $0.05 < P \leq 0.10$ were defined as significant differences and tendencies, respectively.

RESULTS

All pigs remained healthy during the experiment regardless the exposure to HS. The daily AT recorded inside the TN and HS rooms during the conduction of Exp. 1 and 2 is presented in Fig. 1. During Exp. 1, the AT inside the TN room was maintained relatively constant, from 19.5 to 23.5°C, but the AT inside the HS room varied substantially within the same day ($P < 0.05$), from 24.5°C (around 0500 h) to 42.6°C (around 1600 h). The highest AT (above 35°C), was recorded between 1000 and 1900 h. During Exp. 2, the AT inside the HS room also varied substantially within the same day ($P < 0.05$), from 27.7°C (around 0600 h) to 37.7°C (around 1600 h). The highest AT (above 35°C), was also recorded between 1130 and 1800 h. The body temperature measured in 6 ileal-cannulated pigs of

Table 2. Analyzed total content (%) of crude protein and amino acids in the experimental diets of Exp. 2 (as fed basis)^a

Item	Control	Increased protein	Increased free AA
CP	15.65	20.14	16.98
Indispensable amino acids			
Arg	0.80	0.98	0.83
His	0.36	0.44	0.42
Ile	0.61	0.78	0.69
Leu	1.04	1.28	1.25
Lys	1.09	1.38	1.41
Met	0.30	0.45	0.42
Phe	0.70	0.86	0.79
Thr	0.62	0.70	0.76
Val	0.70	0.85	0.85
Dispensable amino acids			
Ala	0.58	0.69	0.57
Asp	1.06	1.44	1.09
Glu	3.52	4.06	3.60
Pro	1.22	1.31	1.22
Gly	0.62	0.73	0.60
Ser	0.60	0.74	0.61
Tyr	0.40	0.43	0.41

^aCON, Control diet; HSxP, CON + 30% protein as SBM; HSxAA, CON + 26% free AA.

Figure 1. Average ambient temperature measured inside the thermo neutral and heat stress rooms with the aid of a hygro-thermograph, at 15-min intervals, during the conduction of Exp. 1 and 2.

similar BW and housed in the same HS room and at the same time, followed a daily pattern similar to that of the AT. The intestinal temperature registered between 1630 to 1930 h (41.2°C) was 1.3°C higher ($P < 0.01$) than that registered between 0600 and 0830 h (39.9°C).

The performance results of Exp. 1 are presented in Table 3. The ADG of the HS-C ($P = 0.006$) and the HS-AA ($P = 0.004$) pigs decreased in comparison with the TN-C pigs. The ADFI as well as the consumption of Lys, Thr, and Met tended to be lower in the HS-C pigs ($P < 0.10$), and were lower in the HS-AA pigs ($P \leq 0.01$) than in the TN-C pigs. The ADFI and the consumption of Lys and Met did not differ between the HS-C and the HS-AA pigs ($P > 0.10$), but the Thr intake was lower in the HS-AA pigs ($P = 0.004$). The G:F of the HS-C pigs tended to be lower ($P = 0.061$) and was lower ($P = 0.050$) compared with that of the TN-C and HS-AA pigs, respectively; there was no difference in G:F between the TN-C and the HS-AA pigs ($P > 0.10$). The ADG (g)/g of Lys intake or per MJ of NE intake was lower in the HS-C pigs than in the TN-C ($P = 0.050$), but it did not differ between the TN-C and the HS-AA pigs ($P > 0.10$).

The performance results of Exp. 2 are presented in Table 4. There were no differences in ADG, ADFI, and NE intake ($P > 0.10$) between pigs fed the CON or the HSxP diet, but the consumption of Lys, Thr, and Met was higher ($P < 0.01$); the G:F tended to be higher but the Lys utilization efficiency tended to be lower in pigs fed the HSxP diet ($P < 0.10$). Pigs fed the HSxAA diet had a higher ADG, increased consumption of Lys, Thr, and Met, G:F, and a lower NE efficiency (MJ/g of ADG) ($P < 0.05$) than those fed the CON diet, but the ADFI, the NE intake, and the Lys utilization efficiency did not differ between these pigs ($P > 0.10$). When comparing pigs fed the HSxP with those fed the HSxAA diets, there were no differences in performance ($P > 0.10$).

The absolute weight at the beginning as well as the absolute (kg) and relative weight (g/kg BW) at the end of the experiment of the hot carcass, whole leg, leg muscle, leg bone, whole loin, and loin muscle are presented in Table 5. The final absolute weight of the hot carcass and carcass components did not differ between pigs fed the HSxP or the CON diet ($P > 0.10$). However, the weight of the hot carcass and leg muscle tended to be higher ($P \leq 0.10$) in pigs fed the HSxAA diet compared to those fed the CON diet; no differences were observed in the other carcass components ($P > 0.10$). Pigs fed the HSxAA diet had heavier loin muscle than those fed the HSxP diet ($P < 0.05$), but no differences were found in the weight of these cuts or the whole carcass ($P > 0.10$) between these treatments. The relative weight of the loin muscle tended to be lower in pigs fed the HSxP diet than in pigs fed the CON or the HSxAA diet ($P = 0.100$). No other weight differences were observed in the carcass and remaining carcass components.

The daily weight gain of the hot carcass and the carcass components is presented in Table 6. There were no differences in those weights between pigs fed the HSxP and the CON diet ($P > 0.10$). But pigs fed the HSxAA diet gained more carcass weight ($P = 0.050$) and tended to gain more leg muscle weight ($P = 0.073$) than pigs fed the CON diet. Also, the loin muscle of pigs fed the HSxAA diet gained more weight than that of pigs fed the HSxP diet ($P < 0.05$).

The absolute (kg) and relative (g/kg body weight) of visceral organs at the end of the experiment is presented in Table 7. The absolute weight of visceral organs did not differ between pigs fed the HSxP or the CON diet ($P > 0.10$). Compared to the CON pigs, the HSxAA pigs tended to have a higher liver weight ($P = 0.100$), and had a heavier spleen ($P < 0.05$), but the weight of the stomach, small intestine, large intestine, heart, and kidney did not

Table 3. Performance of thermo neutral pigs fed ad libitum a high protein diet (TN-C) and heat stress pigs fed a high protein (HS-C) or a low protein diet supplemented with free amino acids (HS-AA; Exp. 1)

Item	Treatment			SEM	Contrasts, $P = a$		
	TN-C	HS-C	HS-AA		C ₁	C ₂	C ₃
Initial body weight, kg	31.1	31.7	30.7				
Daily weight gain, kg/d	0.938	0.777	0.770	0.037	0.006	0.004	0.884
Daily intake							
Feed, kg/d	1.683	1.525	1.462	0.065	0.100	0.008	0.226
SID Lys, g/d	16.65	14.94	13.92	0.59	0.100	0.010	0.269
SID Thr, g/d	11.44	10.37	9.21	0.42	0.090	<0.001	0.004
SID Met, g/d	4.71	4.27	3.98	0.19	0.100	0.010	0.269
NE, MJ/d	16.63	15.07	14.50	0.64	0.100	0.032	0.545
G:F	0.557	0.513	0.559	0.016	0.061	0.929	0.050
g of ADG/g Lys intake	56.86	52.34	55.52	1.54	0.050	0.546	0.161
g of ADG/MJ NE intake	56.40	51.92	53.29	1.51	0.050	0.163	0.529

^aContrasts: C₁, TN-C vs. HS-C; C₂, TN-C vs. HS-AA; C₃, HS-C vs. HS-AA.

differ among these 2 groups of pigs ($P > 0.10$). The kidney of pigs fed the HSxP diet was heavier but the spleen was lighter ($P \leq 0.05$) than in those fed HSxAA diet; no differences were observed in the weight of the other visceral organs ($P > 0.10$). The relative weight of the visceral organs did not differ between pigs fed the CON or the HSxP diet ($P > 0.10$). The relative weight of the kidneys tended to be lower ($P = 0.076$) and that of the spleen was higher ($P < 0.05$) in pigs fed the HSxAA diet than in those fed the CON diet. Also, the relative weight of the kidneys was lower and that of the spleen was higher in the HSxAA pigs than in the HSxP pigs ($P \leq 0.05$).

The daily weight gain of the visceral organs is presented in Table 8. The weight of these organs did not differ between pigs fed the HSxP or the CON diet ($P > 0.10$). Pigs fed the HSxAA diet

gained more spleen weight than pigs fed the CON diet ($P < 0.05$), but no difference was observed in the other organs weight gain ($P > 0.10$). Pigs fed the HSxP diet tended to have higher kidney weight gain than that of pigs fed the HSxAA diet ($P = 0.100$) but the gain weight of the other organs did not differ between these 2 groups of pigs ($P > 0.10$). The serum concentration of urea (Fig. 2) was higher ($P < 0.05$) in pigs fed the HSxP diet than in those fed the CON or the HSxAA diets.

DISCUSSION

The AT registered inside the HS room ranged from 24.5°C (between 0500 and 0700 h) to 42.5°C (between 1000 and 2000 h) during the conduction of Exp. 1, and from 27.7°C (around 0600 h) to 37.7°C (around 1600 h) in Exp. 2. Moreover, the body

Table 4. Performance of pigs exposed to heat stress conditions (21 d) and increased dietary amino acids supply as protein-bound (HSxP) or in free form (HSxAA; Exp. 2)

Item	Treatment ^a			SEM	Contrasts P -values ^b		
	CON	HSxP	HSxAA		C1	C2	C3
Initial BW, kg	33.55	33.34	33.94				
ADG, kg/d	0.644	0.714	0.761	0.035	0.219	0.050	0.369
Daily intake							
Feed, kg/d	1.639	1.669	1.656	0.059	0.689	0.805	0.869
Lys, g/d	18.00	22.53	22.73	0.80	0.002	0.001	0.871
Thr, g/d	10.2	12.76	12.24	0.44	0.001	0.006	0.421
Met, g/d	4.95	7.34	6.76	0.24	<0.001	<0.001	0.105
NE, MJ/d	16.53	16.14	16.29	0.59	0.650	0.781	0.858
G:F, kg/kg	0.343	0.437	0.477	0.036	0.087	0.022	0.453
ADG, g/g Lys intake	36.65	31.69	33.78	1.74	0.077	0.266	0.411
MJ/g ADG	39.87	44.26	47.16	2.30	0.203	0.045	0.391

^aTreatment: CON, heat stress pigs fed a standard diet; HSxP, heat stress pigs fed excess protein-bound amino acids; HSxAA, heat stress pigs fed excess free amino acids.

^bContrasts: C₁, CON vs. HSxP; C₂, CON vs. HSxAA; C₃, HSxP vs. HSxAA.

Table 5. Weight of carcass and some carcass main cuts of pigs sacrificed at both the beginning and after being exposed to heat stress conditions (21 d) and increased dietary amino acids supply as protein-bound (HSxP) or in free form (HSxAA; Exp. 2)^a

	Initial	Treatment ^b				Contrast <i>P</i> -values ^c		
Item	Sacrifice	CON	HSxP	HSxAA	SEM	C1	C2	C3
Absolute weight, kg								
Hot carcass	20.00	28.9	29.5	30.4	0.5	0.465	0.100	0.342
Whole Leg	2.98	5.02	5.13	5.29	0.12	0.558	0.193	0.429
Leg muscle	2.33	3.81	3.93	4.10	0.13	0.521	0.100	0.390
Leg bone	0.56	0.90	0.85	0.83	0.04	0.549	0.400	0.795
Whole Loin	2.28	4.52	4.49	4.67	0.11	0.851	0.367	0.286
Loin muscle	1.67	3.21	3.02	3.31	0.08	0.141	0.400	0.041
Relative, g/kg hot carcass								
Whole Leg	149.1	174.4	174.0	175.0	2.3	0.907	0.861	0.770
Leg muscle	116.3	132.1	133.2	134.9	1.8	0.682	0.321	0.539
Leg bone	28.0	31.4	29.0	27.6	1.5	0.308	0.100	0.547
Whole Loin	113.8	156.3	152.3	153.8	3.3	0.426	0.620	0.752
Loin muscle	83.6	110.1	102.1	109.1	3.6	0.100	0.758	0.100

^aThere were 4 observations per treatment. Treatment: CON, heat stress pigs fed a standard diet; HSxP, heat stress pigs fed excess protein-bound amino acids; HSxAA, heat stress pigs fed excess free amino acids.

^bContrasts: C₁, CON vs. HSxP; C₂, CON vs. HSxAA; C₃, HSxP vs. HSxAA.

temperature of pigs in Exp. 2 followed a pattern similar to that of the AT; the intestinal temperature recorded between 1500 and 1830 h (41.2°C) was 1.3°C higher than that between 0500 and 0700 h, which agrees with previous reports (Morales et al., 2016b; Cervantes et al., 2017). The BT data were collected from ileal-cannulated pigs fed 2 times a day as opposed to pigs in Exp. 2 that were fed ad libitum. Differences in the amount of feed consumed and time of feeding appear to affect the BT of TN pigs but we recently found that the BT of HS pigs is mostly affected by the AT (Cervantes et al., 2017). Hence, and in agreement with Liu et al. (2009), the pigs used in both experiments were exposed to HS conditions for at least 10 h a day.

Pigs exposed to HS usually reduce their VFI from 20% to 40% (e.g., Colin et al., 2001;

Morales et al., 2014) or even higher (47%; Pearce et al., 2013a) and thus, these pigs are expected to decrease their AA intake in a similar percentage. Indeed, pigs exposed to HS in Exp. 1, reduced Lys and Thr intake by 20 and 25%, respectively, in comparison with the TN pigs. These AA intake reductions were associated with a similar decrease in the ADG of HS pigs, which is explained by the fact that Lys and Thr are the first 2 limiting AA in wheat-SBM diets for growing pigs. In agreement with Morales et al. (2014), the lower efficiency variables (G:F, g of ADG/g Lys intake, and g of ADG/MJ NE intake) of the HS-C pigs compared with the HS-AA pigs suggest that HS not only affects the nutrient consumption but also the efficiency of nutrient utilization.

Table 6. Daily weight gain (g/d) of some carcass main cuts of pigs exposed to heat stress conditions (21 d) and increased dietary amino acids supply as protein-bound (HSxP) or in free form (HSxAA; Exp. 2)^a

Item	Treatment ^b			SEM	Contrast <i>P</i> -values ^c		
	CON	HSxP	HSxAA		C ₁	C ₂	C ₃
Hot carcass	410	453	507	32	0.360	0.050	0.262
Whole Leg	95.0	102.4	111.8	5.7	0.383	0.067	0.272
Leg muscle	69.1	76.5	86.1	5.9	0.401	0.073	0.281
Leg bone	15.6	13.9	13.5	2.2	0.602	0.511	0.888
Loin	105.4	105.5	115.7	8.6	0.994	0.418	0.423
Loin muscle	72.1	64.4	85.5	6.0	0.451	0.490	0.034

^aThere were 4 observations per treatment

^bTreatment: CON, heat stress pigs fed a standard diet; HSxP, heat stress pigs fed excess protein-bound amino acids; HSxAA, heat stress pigs fed excess free amino acids.

^cContrasts: C₁, CON vs. HSxP; C₂, CON vs. HSxAA; C₃, HSxP vs. HSxAA.

Table 7. Weight of visceral organs of pigs sacrificed at the beginning of the study and after being exposed to heat stress conditions (21 days) and increased dietary amino acids supply as protein-bound (HSxP) or in free form (HSxAA; Exp. 2)^a

Organ	Initial	Treatment ^b				Contrasts <i>P</i> -values ^c		
	Sacrifice	CON	HSxP	HSxAA	SEM	C ₁	C ₂	C ₃
Absolute weight, kg								
Stomach	0.268	0.346	0.347	0.354	0.01	0.963	0.700	0.744
Small intestine	1.150	1.371	1.361	1.386	0.11	0.951	0.929	0.881
Large intestine	0.843	1.104	1.160	1.042	0.11	0.725	0.693	0.464
Heart	0.180	0.216	0.212	0.221	0.02	0.891	0.884	0.778
Liver	1.056	1.116	1.186	1.251	0.05	0.412	0.100	0.445
Kidney	0.195	0.202	0.217	0.191	0.01	0.205	0.781	0.050
Spleen	0.065	0.068	0.080	0.109	0.003	0.379	0.014	0.047
Relative weight, g/kg BW								
Stomach	7.50	6.83	6.73	6.71	0.2	0.781	0.739	0.954
Small intestine	36.27	27.15	26.76	26.10	1.9	0.891	0.710	0.813
Large intestine	23.58	21.48	22.83	19.69	2.1	0.659	0.561	0.322
Heart	5.03	4.28	4.10	4.13	0.3	0.703	0.743	0.956
Liver	29.51	21.93	23.22	23.73	0.8	0.321	0.181	0.681
Kidney	5.46	4.00	4.24	3.63	0.1	0.209	0.076	0.012
Spleen	2.12	1.33	1.54	2.04	0.1	0.363	0.016	0.050

^aThere were 4 observations per treatment^bTreatment: CON, heat stress pigs fed a standard diet; HSxP, heat stress pigs fed excess protein-bound amino acids; HSxAA, heat stress pigs fed excess free amino acids.^cContrasts: C₁, CON vs. HSxP; C₂, CON vs. HSxAA; C₃, HSxP vs. HSxAA.

In theory, increasing the AA content in the diet beyond the NRC (2012) requirement would help to overcome the reduced AA consumption of pigs exposed to HS. However, from previous reports (Pesta and Samuel, 2014), it was speculated that an increase in the dietary CP level might result in higher BT that consequently could further reduce the VFI of HS pigs. In Exp. 2, the HSxP diet contained 30% more protein than the CON diet, which was formulated to meet 100% the NRC (2012) SID requirement of all AA. Increasing the dietary CP content from about 16% to 20% did not affect the

VFI. This might be partly due to a lower NE content (0.22 MJ/kg) in the HSxP diet compared with that of CON diet coupled with pigs being able to adjust their VFI to meet their energy requirement. Quiniou and Noblet (2012) reported significant changes in feed intake of pigs when the NE content in the diets increased by as low as 0.60 MJ/kg. These results indicate that the dietary CP content, at the levels included in the present experiment, has no effect on the VFI of HS pigs as opposed to what it might have been expected. Kerr et al. (2003) reported that low CP diets supplemented with

Table 8. Daily weight gain (g/d) of body components of pigs exposed to heat stress conditions (21 d) and increased dietary amino acids supply as protein-bound (HSxP) or in free form (HSxAA; Exp. 2)^a

Item	Treatment ^b				Contrasts <i>P</i> -values ^c		
	CON	HSxP	HSxAA	SEM	C ₁	C ₂	C ₃
Stomach	3.70	3.76	4.09	0.94	0.954	0.704	0.746
Small intestine	10.55	10.07	11.22	5.37	0.951	0.931	0.883
Large intestine	12.45	15.09	9.47	5.00	0.718	0.684	0.448
Heart	1.73	1.54	1.94	0.98	0.884	0.889	0.775
Liver	2.83	6.18	9.30	2.86	0.429	0.145	0.461
Kidney	0.34	1.05	0.19	0.48	0.324	0.453	0.100
Spleen	0.14	0.71	2.09	0.67	0.586	0.041	0.173

^aThere were 4 observations per treatment^bTreatment: CON, heat stress pigs fed a standard diet; HSxP, heat stress pigs fed excess protein-bound amino acids; HSxAA, heat stress pigs fed excess free amino acids.^cContrasts: C₁, CON vs. HSxP; C₂, CON vs. HSxAA; C₃, HSxP vs. HSxAA.

Figure 2. Concentration of urea in serum of heat stress pigs fed the control (CON) diet or a diet with increased protein (HSxP) or free amino acids (HSxAA) content.

free AA decrease heat production in growing pigs housed in a TN (23°C) or HS (33°C) environment. Conversely, we recently reported a small (0.07°C) but not significant difference in BT between TN pigs fed either a 22% or an 11% CP diet (Cervantes et al., 2017). This response may partially explain the lack of effect of dietary CP content on the VFI of pigs in the present experiment. Moreover, as expected, increasing the dietary content of AA by supplementing additional free AA did not further affect the VFI of HS pigs. The latter is explained by the fact that there was no need to release the added free AA for absorption and thus, no additional digestion-related heat was produced. Therefore, these results suggest that the reduced AA intake associated with the decreased VFI in HS pigs may be overcome by supplementing extra AA either as protein-bound or free AA without further affecting the VFI.

However, in Exp. 2, the growth response to the dietary extra AA was not the same for pigs fed the HSxP or the HSxAA diet, although these pigs consumed more AA than those fed the CON diet. The calculated consumption of SID Lys, Thr and Met was 25 and 26%, 25 and 20%, and 48 and 37% higher in pigs fed the HSxP or the HSxAA diets, respectively, than in pigs fed the CON diet. Nevertheless, compared to pigs fed the CON diet, the increased Lys, Thr and Met intake resulted in higher ADG (18%) only in pigs fed the HSxAA diet. This growth response of HS pigs to extra dietary free AA was differently observed in the whole carcass and some of its components. The daily weight gain of hot carcass tended to be 13% higher in pigs fed the HSxAA diet than those fed the CON diet. Interestingly, pigs fed the HSxAA diet tended to gain 17% more leg muscle weight than pigs fed the CON and gained 20% more loin muscle weight than pigs fed the HSxP diet. These results show that HS pigs respond to the supply of extra dietary free AA probably by increasing the synthesis of muscle proteins.

The growth response of pigs fed the HSxAA diet, associated with a theoretical increase in the synthesis of muscle protein may be explained by an increase in the AA availability. Morales et al. (2016a) reported that HS pigs increase the endogenous loss of some AA (Arg, His, and Thr) and decreased the SID of these AA (Morales et al., 2016b). These issues might partially explain the lack of effect of surplus protein-bound AA and the positive effect of surplus free AA on the ADG of HS pigs, although their impact is expected to be modest. Recently, we have reported 2 interesting findings. First, we observed a dramatic decrease in the expression of some AA transporters, including the one responsible for the absorption of Lys and Arg ($b^{0,+}$), in the jejunum of pigs because of their exposure to HS (Morales et al., 2014). Second, the expression of this AA transporter was about 2-fold higher in the duodenum of pigs fed a diet in which 65% of the total Lys content was supplied as free Lys, compared to pigs fed only protein-bound Lys (Morales et al., 2015). Accordingly the absorptive (2.5 h postprandial) serum concentration of Lys was about 3-fold higher in pigs fed the diet containing free Lys than in those fed the protein-bound Lys. These findings suggest that dietary free Lys is absorbed in the duodenum in contrast to protein-bound Lys that, according to Silk et al. (1985), is mostly absorbed in jejunum after its release during the digestion of protein that takes place in the jejunum. Hence, it is reasonable to believe that the free dietary Lys absorbed in duodenum does not compete for the absorption of protein-bound Lys released in the jejunum. Combining those findings with the performance results of Exp. 2, we hypothesize that the availability of Lys and perhaps that of Thr and Met is higher when these AA are supplied as free- and protein bound AA, compared when they are supplied exclusively as protein-bound AA. This hypothesis is supported by the fact that ADG (g/g Lys intake) tended to be lower in pigs fed the HSxP diet compared to the CON pigs, but no difference was observed between CON and the HSxAA diet. Moreover, the HSxAA diet was more AA balanced than the HSxP diet, as evidenced by the dietary analyzed AA profile and the lower urea serum concentration (Fig. 2) in pigs fed the HSxAA diet. This combination of factors may explain better the positive ADG response to surplus free AA and the lack of response to protein-bound AA.

The positive growth response to extra free AA was associated with the improvement in the G:F of pigs fed the HSxAA diet. Nevertheless, the growth response to extra free AA observed in Exp. 2 was

smaller than the performance reduction observed in Exp. 1, and the one recently reported for HS pigs compared with that of pigs housed under TN conditions (Collin et al., 2001; Cervantes et al., 2016). Apparently, the requirement of some AA such Arg, His, Thr, and Met increases because of the exposure of pigs to HS (Morales et al., 2016a, b), which might further reduce their availability for growth, beyond the reduction provoked by the decrease in their VFI. In Exp. 2, the HSxAA diet contained 24 and 25% more His and Thr, respectively, than their SID recommended levels (NRC, 2012), and the Thr intake by the HSxAA pigs was only 20% higher than the CON pigs. Moreover, the SID Lys content in the HSxAA diet was 25% higher than the recommended level. In addition, HS pigs have been shown to increase the synthesis of heat shock proteins (Hsp70 and Hsp90; Kamanga-Sollo et al., 2011) that may result in further decrease in AA availability for growth. Therefore, additional supply of indispensable AA, especially Lys, Thr, and His may be necessary to completely achieve the growth potential recovery of pigs exposed to HS.

The CON diet was formulated on the basis of the SID ideal protein concept (NRC, 2012) for the first 5 limiting AA (Lys, Thr, Met, Trp, and Val) but, inevitably, the levels of Ile, Leu, and Phe were slightly above, and Arg was highly above the NRC (2012) recommendations. These excesses made the CON diet somewhat unbalanced. Adding free Lys, Thr, Met, Trp, Val, Ile, Leu, Phe, and His in the HSxAA diet improved their balance with regards to the ideal AA ratio. In contrast, adding protein in the form of soybean meal to the HSxP diet, to increase 26% the SID content of the first limiting amino acid (Lys), also increased the SID content of the other indispensable amino acids by more than 26% (e.g., Arg, 64; Ile, 43; Leu, 32; Phe, 49; His, 38%). Thus, although the HSxP diet supplied more SID Lys, it also became more unbalanced than the CON diet. These AA profile differences may explain the higher relative weight of the kidney of pigs fed the CON (10%) or the HSxP (15%) diet compared with those fed the HSxAA diet. In agreement, Morales et al. (2015) reported that the kidney of growing pigs housed under TN conditions fed a 22% wheat-SBM diet weighed 43% more than those fed a 14% protein AA-supplemented diet. Excess ammonia in tissues resulting from the ingestion of excess and unbalanced protein needs to be eliminated through the kidneys (Wu, 1995) suggesting increased renal activity to eliminate that excess. However, we have no clear explanation for the 56% increment in the relative weight of spleen in pigs

fed the HSxAA diet. Mice fed an AA deficient diet (Bounous and Kongshavn, 1978) and chickens fed a Met-deficient diet (Wu et al., 2012) showed a reduction in the relative weight of the spleen, but the CON and the HSxP diet were not deficient in any of the indispensable AA.

In general, the results of these studies confirm the negative effect of HS on the consumption of AA and growth rate of pigs. These also show that the supplementation of extra AA either as protein-bound or in free form by increasing the SID Lys content for about 24% while maintaining other AA for the ideal AA ratio does not affect the VFI in HS pigs. However, these results also indicate that supplementing extra free AA rather than protein-bound AA may help to overcome the reduced consumption of AA and the growth performance of HS pigs observed in previous studies. Furthermore, because the potential growth of HS pigs fed the HSxAA diet was not fully recovered, additional research is needed to evaluate the effect of higher levels of AA in the diets for HS pigs.

Conflict of interest statement. None declared.

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