Performance of pigs kept under different sanitary conditions affected by protein intake and amino acid supplementation[1,2]

Y. van der Meer,*†[3] A. Lammers,‡ A. J. M. Jansman,§ M. M. J. A. Rijnen,† W. H. Hendriks,* and W. J. J. Gerrits*

*Animal Nutrition Group, Wageningen University, Wageningen, 6708 WD, the Netherlands; †De Heus Animal Nutrition, Ede, 6717 VE, the Netherlands; ‡Adaptation Physiology Group, Wageningen University, Wageningen, 6708 WD, the Netherlands; and §Wageningen UR, Livestock Research, Wageningen, 6708 WD, the Netherlands

ABSTRACT: There is growing evidence that requirements for particular AA increase when pigs are kept under low sanitary conditions. The extent to which reduction in growth performance is related to these increased requirements is unclear. To evaluate this relationship, an experiment (2 × 2 × 2 factorial arrangement) was performed with 612 male pigs (9 per pen) kept under low sanitary conditions (LSC) or high sanitary conditions (HSC) and offered ad libitum access to either a normal CP concentration diet (NP; 17, 15, and 15% CP for the starter, grower, and finisher phase, respectively) or a low CP concentration diet (LP; 20% CP reduced relative to NP for each phase), each of which containing a basal AA profile (AA-B) or a supplemented AA profile (AA-S). The supplemented diet type contained 20% more Met, Thr, and Trp relative to Lys on an apparent ileal digestible basis compared with the basal diet type. Pigs were followed for a complete fattening period and slaughtered at a targeted pen weight of 110 kg. Haptoglobin concentrations in serum (0.92 g/L for LSC and 0.78 g/L for HSC) and IgG antibody titers against keyhole limpet hemocyanin (3.53 for LSC and 3.08 for HSC) collected in the starter, grower, and finisher phases and pleuritis scores at slaughter (0.51 for LSC and 0.20 for HSC) were greater for LSC pigs compared with HSC pigs ($P \leq 0.01$), illustrating that sanitary conditions affected health conditions. The ADG and G:F were greater for HSC pigs compared with LSC pigs ($P \leq 0.01$). The number of white blood cells (WBC) was higher in (AA-S)–fed pigs compared with (AA-B)–fed pigs when kept at LSC but not at HSC [SS (sanitary conditions) × AA interaction, $P = 0.04$]. Pigs fed NP had a lower number of WBC compared with pigs fed LP ($P = 0.02$). The number of platelets in pigs fed AA-S diets was higher compared with pigs fed AA-B diets ($P \leq 0.01$). A 20% reduction in dietary supplementation of Met, Thr, and Trp relative to Lys decreased G:F more in LSC pigs than in HSC pigs (interaction, $P = 0.03$), illustrating that dietary requirements for these AA differ depending on sanitary conditions. This study, performed under practical conditions, shows that AA requirements are dependent on sanitary conditions. Furthermore, supplementation of diets with particular AA may improve performance, especially under poor hygienic conditions. Dietary protein concentration as well as Met, Thr, and Trp supplementation can modify immune status, which may influence resistance to subclinical and clinical diseases.

Key words: amino acid, immune system, performance, pig, protein, sanitary conditions

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INTRODUCTION

A potential growth reduction with decreasing dietary protein intake can be ameliorated through the supplementation of limiting AA in the diet, thereby restoring growth at a lower CP intake (Kerr and Easter, 1995; Gloaguen et al., 2014). The optimal AA profile, however, differs depending on animal and environmental conditions (Le Floc'h et al., 2004). Activating the immune system can increase the requirements for nu-

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[3]Corresponding author: yvonne.vandermeer@wur.nl

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trients, such as AA. If the enhanced requirements are not compensated for by greater intake, repartitioning of dietary protein or AA away from development and growth tissues occurs (Le Floc'h et al., 2009) toward use by the immune processes. This shift of nutrients, together with an often-observed reduction in feed intake in case of immune stimulation, reduces pig performance (Klasing and Johnstone, 1991; Pastorelli et al., 2012a).

Despite the growing evidence that particular AA requirements are dependent on immune system activation, it remains unclear to what extent reduced performance during (subclinical) infections is related to changes in AA requirements and whether dietary supplementation of these AA can reverse the performance loss. Moreover, most evidence for increased AA requirements is obtained in studies in which pigs were repeatedly challenged with lipopolysaccharide (**LPS**; e.g., Kim et al., 2012; de Ridder et al., 2012; Rakhshandeh et al., 2010) or Complete Freund's Adjuvant (Kampman-van de Hoek et al., 2015; Le Floc'h et al., 2008), and it can be questioned to what extent these results can be extrapolated to pigs in commercial conditions (Pastorelli et al., 2012b). Therefore, we studied, under practical conditions, if the performance and immune status of pigs kept under different sanitary conditions is influenced by protein intake and AA supplementation. We hypothesized that increased provision of Met, Thr, and Trp would increase performance of pigs, particularly when kept under low sanitary conditions and low dietary protein intake.

MATERIAL AND METHODS

The experimental protocol was approved by the Animal Care and Use Committee of Wageningen University, the Netherlands.

Experimental Design

In a 2 × 2 × 2 factorial arrangement, groups of pigs were allocated to either high sanitary conditions (**HSC**) or low sanitary conditions (**LSC**) and were offered ad libitum access to 2 different diets, a normal CP concentration diet (**NP**) or a low CP concentration diet (**LP**), each having either a basal dietary AA profile (**AA-B**) or supplemented dietary AA profile containing 20% more Met, Thr, and Trp compared with the basal profile (**AA-S**).

Animals and Treatments

In total, 612 (Topigs 20 × Tempo; Topigs, Helvoirt, The Netherlands) newborn boar piglets were selected on a commercial nursery farm in the Netherlands and allocated to either the LSC or HSC treatment.

Per nursery room, half of the boar piglets were selected for LSC and the other half for HSC treatment. Only HSC piglets received vaccinations in the first 9 wk of age. The HSC piglets were vaccinated, at 1 to 2 wk of age, against *Mycoplasma hyopneumoniae* (Porcilis M Hyo; MSD Animal Health, Boxmeer, the Netherlands); at 4 to 5 wk of age against *M. hyopneumoniae*, porcine circovirus type 2 (**PCV2**), and porcine reproductive and respiratory syndrome (**PRRS**; Porcilis M Hyo, Porcilis Circo, and Porcilis PRRS, respectively; MSD Animal Health) and *Lawsonia intracellularis* (Enterisol Ileitis Boehringer Ingelheim B.V., Alkmaar, the Netherlands); at 6 to 7 wk of age against *Actinobacillus pleuropneumoniae* (**APP**; Porcilis APP; MSD Animal Health) and influenza A virus (Gripovac3; Merial B.V., Velserbroek, the Netherlands); and at 8 to 9 wk of age against APP and influenza A virus (Porcilis APP and Gripovac3, respectively) by subcutaneous injection in the neck or, in the case of Enterisol, by oral drench. Piglets of both LSC and HSC treatments were housed in the same rooms until weaning (±24 d of age). After weaning, LSC and HSC pigs were group housed in different rooms to prevent cross-vaccination by the 2 living vaccines used in the HSC piglets (Enterisol Ileitis and Porcilis PRRS).

The HSC and LSC pigs were separately transported to the experimental farm (Vlierbos V.O.F., Neerloon, the Netherlands). As it was not possible to obtain all measurements during the study on 612 pigs on a single day, the LSC and HSC groups were split into 2 batches of 324 (180 from the LSC treatment and 144 from the HSC treatment) and 288 pigs (144 from the LSC treatment and 144 from the HSC treatment) arriving 1 wk apart. Therefore, pigs of batch 1 and 2 arrived at the experimental farm at an age of 10 and 11 wk, respectively.

Upon arrival, all pigs were individually weighed and, within sanitary condition treatment and batch, allocated to their pen based on BW to minimize variation between pens and within pens (17.3 ± 0.06 kg for LSC batch 1, 18.1 ± 0.07 kg for LSC batch 2, 15.9 ± 0.07 kg for HSC batch 1, and 17.4 ± 0.07 kg for HSC batch 2). The LSC pigs of each batch were housed in 5 LSC rooms and the HSC pigs were housed in 4 HSC rooms located in the same building. Each room had separate manure pits and separate ventilation regulation and contained 8 pens with 9 pigs per pen (0.8 m^2 space/pig), except for 1 LSC room, where 4 out of 8 pens were left empty. In addition, the HSC and LSC rooms were separated by a wall in the central corridor.

High sanitary condition rooms were intensively cleaned in 4 steps before arrival of the pigs: twice with foam (MS Topfoam LC Alk; MS Schippers, Bladel, the Netherlands) and high pressure washing and then treated twice with a disinfectant (MS Megades and MS Oxydes; MS Schippers). In addition, a strict hygiene protocol was adhered to when entering the HSC rooms, which included showering, change of clothes, and use of a hairnet and face mask. People were not allowed to have access to a pig farm 48 h before entering HSC rooms. High sanitary condition animals received a preventative antibiotic injection (Fenflor; AUV Veterinary Services B.V., Cuijk, the Netherlands; 1 mL/pig, submuscular at Day 1 and 3 of the experiment) and were dewormed every 5 wk during the experiment starting at arrival (Flutelmium 0.6% premix; AUV Veterinary Services B.V.; topdressing, 1.5 mg Flubendazol/kg BW for 5 subsequent days).

Rooms for the LSC pigs were not cleaned after a previous batch of commercial finisher pigs left the facility 2 d before, and no hygiene protocol was applied. Starting at 5 wk after arrival, fresh manure of another commercial pig farm was spread in the LSC pens every 2 wk until end of the experiment to enhance antigenic pressure. Low sanitary condition pigs did not receive any medication or preventive treatment.

The experimental period lasted from December 11, 2013, until April 16, 2014. Animals were monitored for the complete fattening period, divided in 3 phases, that is, starter (0–34 d), grower (35–49 d), and finisher phases (from Day 50 until a target average pen weight of 110 kg BW). At the end of each phase, pigs were individually weighed.

Diets and Feeding

Pigs were allocated to 2 diets, NP (17, 15, and 15% CP for the starter, grower, and finisher phases, respectively) or LP (20% CP reduced relative to NP for each phase), each of which contained a basal or a supplemented AA profile. This resulted in 4 dietary treatments: Low protein - basal amino acid diet, low protein- supplemented amino acid diet, normal protein - basal amino acid die, and normal protein - supplemented amino acid diet. Each diet was fed to the pigs in both sanitary regimes resulting in 8 treatment groups.

The apparent ileal digestible (**AID**) Lys to NE ratio of the diets was reduced in each subsequent phase of the experiment to follow a 3-phase feeding system. For the NP, the ratio was based on the Lys to NE requirements for boars according to the NRC (2012). The values for Lys to NE requirements were multiplied by 0.95 to make sure that the dietary energy concentration was not limiting the growth performance of the pigs. This resulted in diets for the starter, grower, and finisher phases containing 0.90, 0.81, and 0.75 g AID Lys/MJ of NE. For the LP, the inclusion level of all protein-containing ingredients was decreased by 20% relative to the NP and replaced by maize starch and Opticell (Agromed Austria GmbH, Kremsmünster, Austria), resulting in 0.72, 0.65, and 0.60 g AID Lys/MJ of NE.

The basal AA profile (AA-B) was designed based on a factorial approach to cover the requirements for body protein deposition based on results from Bikker et al. (1994), Le Bellego and Noblet (2002), and the NRC (2012) and to cover losses associated with basal endogenous AA in ileal digesta based on results from Jansman et al. (2002) and the NRC (2012), related to losses of AA in skin and hair based on resluts from the NRC (2012), and AA losses related to cell turnover based on resluts from Moughan (1998). All values were expressed in the same units for a pig of 50 kg BW with an assumed protein deposition of 138 g/d. The Met + Cys (45% of AID Lys) and Trp (15% of AID Lys) concentrations in AA-B diets, obtained in this manner, were adjusted to 51% Met + Cys and 18% Trp based on results from Knowles et al. (1998) and Jansman et al. (2010), as we considered these to be far below the requirement values (CVB, 2011; NRC, 2012). The supplemented AA profile (AA-S) was derived from the AA-B profile by increasing the Met, Thr, and Trp ratio relative to Lys by 20%. These AA were increased in particular as they are believed to be important as building blocks for proteins, for example, acute-phase proteins, synthesized in case of immune system activation (Melchior et al., 2004; Le Floc'h et al., 2008, 2012; Rakhshandeh et al., 2010), because of their function as precursors for important immune related components and antioxidants, and also because of their effects on several immune processes. Methionine is known to be an important methyl donor (Burke et al., 1951) and antioxidant (Wu, 2009), Thr plays an important role in mucus synthesis for gut integrity and immune function (Wu, 2009), and Trp is known as a precursor of melatonin and serotonin, both known to inhibit inflammatory cytokines (Wu, 2009).

The ingredient and nutrient composition of the diets is shown in Tables 1, 2, and 3. All diets were isocaloric on a NE basis and contained TiO_2 as an indigestible marker. Diets were analyzed for AA composition by acid hydrolysis at 110°C for 23 h and ion-exchange chromatography with postcolumn derivatization with ninhydrin (ISO13903; ISO, 2005a) and Trp by alkaline hydrolysis at 110°C for 20 h ion-exchange chromatography with fluorescence detection (MOD.0094 version G; ISO 13904; ISO, 2005c).

Per pen (9 pigs), 1 feeder was used and feed and water were offered ad libitum. The feed was provided as pellets via a computerized automatic system (Fancom Multiphase; Fancom B.V., Panningen, the Netherlands), which registered the mass of feed delivered per pen per day. At the end of each phase (starter, grower, and finisher), remainders of the diet

Table 1. Ingredients and nutrient composition of the starter diets

	LP[1]		NP[1]	
Item	AA-B[2]	AA-S[2]	AA-B	AA-S
Ingredient, g/kg of feed				
Maize	320.00	320.00	400.00	400.00
Soybean meal	182.02	182.00	227.54	227.54
Barley	160.00	160.00	200.00	200.00
Wheat	45.53	45.53	56.91	56.91
Maize starch	206.79	204.64	40.65	37.90
Sugarcane molasses	20.00	20.00	20.00	20.00
Limestone	13.94	13.94	14.11	14.11
Monocalcium phosphate	9.99	9.99	8.93	8.93
Soybean oil	10.65	10.98	15.98	16.38
Vitamin + mineral mix[3]	5.00	5.00	5.00	5.00
Salt	3.19	3.19	3.83	3.83
l-Lysine HCl	1.95	1.94	2.35	2.35
Titanium dioxide	2.50	2.50	2.50	2.50
Sodium bicarbonate	2.58	2.58	1.34	1.34
l-Threonine	0.60	1.46	0.63	1.71
l-Tryptophan	0.03	0.31	0.00	0.37
dl-Methionine	0.23	0.94	0.23	1.13
Cellulose[4]	15.00	15.00	0.00	0.00
Nutrients calculated, g/kg				
NE, MJ/kg[5]	9.80	9.80	9.80	9.80
DM	889.60	889.80	893.20	884.90
CP	138.00	136.00	168.00	167.00
Starch[4]	474.10	472.30	410.00	407.70
Lys[6]	8.60	8.60	10.50	10.50
Thr[6]	5.40	6.20	6.60	7.40
Trp[6]	1.70	1.90	2.00	2.30
Met + Cys[6]	4.30	4.80	5.20	5.90
Ile[6]	5.60	5.60	6.90	6.80
Arg[6]	8.30	8.40	10.50	10.30
Phe[6]	6.60	6.60	8.20	8.10
His[6]	3.40	3.40	4.20	4.20
Leu[6]	10.9	11.00	13.60	13.40
Tyr[6]	4.40	4.50	5.70	5.70
Val[6]	6.40	6.40	7.90	7.70

[1]LP = low CP concentration diet; NP = normal CP concentration diet.

[2]AA-B = basal dietary AA profile; AA-S = supplemented dietary AA profile containing 20% more Met, Thr, and Trp compared with the basal profile.

[3]Supplied the following per kilogram of diet: 3.0 mg riboflavin, 20 mg niacin, 10 mg d-pantothenic acid, 150 mg choline chloride, 0.015 mg cyanocobalamin, 40 mg dl-α-tocopheryl acetate, 1.5 mg menadione, 6,000 IU retinyl acetate, 1,200 IU cholecalciferol, 0.2 folic acid, 1.0 thiamin, 1.0 pyridoxine HCl, 50 m manganese oxide, 267 mg iron SO4·H2O, 60 mg copper SO4·5H2O, 140 mg zinc SO4·H2O, 0.44 mg disodium selenium trioxide, and 1.0 potassium iodate.

[4]Opticell (Agromed Austria GmbH, Kremsmünster, Austria).

[5]Based on chemical composition, digestibility, and energy values for pigs from the Centraal Veevoeder Bureau livestock feed table (CVB, 2011).

[6]Analyzed values.

per pen were collected and weighed to determine the feed intake per pen per phase. The computerized feeding system was calibrated before the trial started and after each phase.

Table 2. Ingredients and nutrient composition of the grower diets

	LP[1]		NP[1]	
Item	AA-B[2]	AA-S[2]	AA-B	AA-S
Ingredient, g/kg of feed				
Maize	400.00	400.00	500.00	500.00
Soybean meal	138.15	138.15	172.69	172.69
Barley	171.36	171.36	214.19	214.19
Maize starch	199.19	197.24	43.34	40.86
Wheat	20.00	20.00	20.00	20.00
Sugarcane molasses	13.11	13.11	13.33	13.33
Limestone	8.62	8.62	7.67	7.67
Monocalcium phosphate	9.19	9.48	10.20	10.57
Soybean oil	5.00	5.00	5.00	5.00
Vitamin + mineral mix[3]	3.95	3.95	3.61	3.61
Salt	2.45	2.45	2.92	2.92
l-Lysine HCl	2.50	2.50	2.50	2.50
Titanium dioxide	1.56	1.56	2.00	2.00
Sodium bicarbonate	3.87	3.87	1.52	1.52
l-Threonine	0.72	1.49	0.74	1.72
l-Tryptophan	0.21	0.46	0.22	0.55
dl-Methionine	0.12	0.76	0.07	0.87
Cellulose[4]	20.00	20.00	0.00	0.00
Nutrients calculated, g/kg				
NE, MJ/kg[4]	9.84	9.84	9.84	9.84
DM	883.70	885.90	882.80	887.70
CP	124.00	124.00	152.00	152.00
Starch[5]	497.10	495.50	448.90	446.90
Lys[6]	8.00	7.90	9.70	10.00
Thr[6]	5.10	5.90	5.90	7.00
Trp[6]	1.56	1.75	1.91	2.16
Met + Cys[6]	3.98	4.52	4.76	5.58
Ile[6]	4.80	4.70	5.90	5.80
Arg[6]	7.20	7.00	9.00	9.00
Phe[6]	5.80	5.70	7.10	7.10
His[6]	3.00	3.00	3.70	3.60
Leu[6]	10.0	9.90	12.3	12.3
Tyr[6]	3.80	3.90	5.00	5.00
Val[6]	5.70	5.80	6.90	6.90

[1]LP = low CP concentration diet; NP = normal CP concentration diet.

[2]AA-B = basal dietary AA profile; AA-S = supplemented dietary AA profile containing 20% more Met, Thr, and Trp compared with the basal profile.

[3]Supplied the following per kilogram of diet: 3.0 mg riboflavin, 20 mg niacin, 10 mg d-pantothenic acid, 150 mg choline chloride, 0.015 mg cyanocobalamin, 40 mg dl-α-tocopheryl acetate, 1.5 mg menadione, 6,000 IU retinyl acetate, 1,200 IU cholecalciferol, 0.2 folic acid, 1.0 thiamin, 1.0 pyridoxine HCl, 50 m manganese oxide, 267 mg iron SO4·H2O, 60 mg copper SO4·5H2O, 140 mg zinc SO4·H2O, 0.44 mg disodium selenium trioxide, and 1.0 potassium iodate.

[4]Opticell (Agromed Austria GmbH, Kremsmünster, Austria).

[5]Based on chemical composition, digestibility, and energy values for pigs from the Centraal Veevoeder Bureau livestock feed table (CVB, 2011).

[6]Analyzed values.

Digestion of DM and N

At wk 13, 18, and 24 of age, pigs in 4 pens per room and 4 rooms per sanitary treatment were sampled for feces by rectal stimulation. In 3 subsequent

Table 3. Ingredients and nutrient composition of the finisher diets

Item	LP[1]		NP[1]	
	AA-B[2]	AA-S[2]	AA-B	AA-S
Ingredient, g/kg of feed				
Maize	360.10	360.10	450.10	450.10
Soybean meal	115.70	115.70	144.60	144.60
Barley	240.00	240.00	300.00	300.00
Maize starch	187.20	185.30	36.40	34.10
Wheat	20.00	20.00	20.00	20.00
Sugarcane molasses	12.20	12.20	12.50	12.50
Limestone	7.40	7.40	6.50	6.50
Monocalcium phosphate	14.00	14.30	13.20	13.60
Soybean oil	5.00	5.00	5.00	5.00
Vitamin + mineral mix[3]	2.30	2.30	2.60	2.60
Salt	2.40	2.40	2.80	2.80
l-Lysine HCl	2.50	2.50	2.50	2.50
Titanium dioxide	5.30	5.30	3.00	3.00
Sodium bicarbonate	0.00	0.60	0.00	0.70
l-Threonine	0.70	1.40	0.70	1.60
l-Tryptophan	0.10	0.40	0.10	0.40
dl-Methionine	0.10	0.10	0.00	0.00
Cellulose[4]	25.00	25.00	0.00	0.00
Nutrients calculated, g/kg				
NE, MJ/kg[5]	9.84	9.84	9.84	9.84
DM	885.90	886.50	881.60	887.70
CP	132.00	126.00	151.00	148.00
Starch[5]	541.70	509.40	541.70	540.20
Lys[6]	8.00	7.60	8.90	8.90
Thr[6]	5.30	5.60	5.90	6.50
Trp[6]	1.51	1.62	1.68	1.91
Met + Cys[6]	4.03	4.22	4.68	5.04
Ile[6]	4.90	4.60	5.70	5.60
Arg[6]	7.30	6.80	8.20	8.20
Phe[6]	6.00	5.60	6.80	6.80
His[6]	3.10	2.90	3.50	3.50
Leu[6]	10.10	9.60	11.90	11.30
Tyr[6]	4.00	3.70	4.70	4.70
Val[6]	6.00	5.70	6.60	6.60

[1]LP = low CP concentration diet; NP = normal CP concentration diet.

[2]AA-B = basal dietary AA profile; AA-S = supplemented dietary AA profile containing 20% more Met, Thr, and Trp compared with the basal profile.

[3]Supplied the following per kilogram of diet: 3.0 mg riboflavin, 20 mg niacin, 10 mg d-pantothenic acid, 150 mg choline chloride, 0.015 mg cyanocobalamin, 40 mg dl-α-tocopheryl acetate, 1.5 mg menadione, 6,000 IU retinyl acetate, 1,200 IU cholecalciferol, 0.2 folic acid, 1.0 thiamin, 1.0 pyridoxine HCl, 50 m manganese oxide, 267 mg iron SO4·H2O, 60 mg copper SO4·5H2O, 140 mg zinc SO4·H2O, 0.44 mg disodium selenium trioxide, and 1.0 potassium iodate.

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days, 1 sample per pig was collected and samples were pooled per pen and stored at −20°C. Sampling pens were equally distributed over dietary treatments.

Frozen feces samples were dried at 103°C in an oven for 24 h to determine DM content (method 930.15; AOAC; ISO, 1999) and were analyzed for N by the Kjeldahl method (ISO 5983; ISO, 2005b). Before Ti analysis (Short et al., 1996; Myers et al., 2004), samples were freeze-dried and ground to pass a 1-mm screen using a Retsch ZM 100 mill (Retsch GmbH, Haan, Germany). Apparent total tract digestibility (**ATTD**) for DM and N was calculated using TiO_2 as an indigestible marker (Kotb and Luckey, 1972).

Blood Sampling

At the start of the experiment, 2 pigs with an average weight per pen were selected for blood sampling at 13, 18, and 24 wk of age from the vena cava. Selected pigs were sampled during each of the 3 phases. Per sampling moment, two 9-mL tubes per animal were filled: 1 EDTA tube for blood cell counts (Vacuette; Greiner Bio-One, Kremsmünster, Austria) and 1 serum tube for acute-phase protein and natural antibody (**Nab**) analysis (Vacuette). Blood samples collected in EDTA tubes were immediately stored on ice and transported to the lab where blood cell counts were performed using a Microcell counter (Sysmex pocH- iV Diff; Toa Medical Electronics Co., Ltd., Kobe, Japan). Blood samples in serum tubes were allowed to clot for 1 h at room temperature, after which serum was collected after centrifugation for 10 min at 5,251 × *g* at room temperature and stored at −20°C pending analysis of haptoglobin (Tridelta Phase Haptoglobin Assay, catalog number TP-801; Tridelta Development, Ltd., Maynooth, Ireland), pig major acute-phase protein (Cusabio Pig-MAP, ELISA, catalog number CSB-E13425p; Cusabio Biotech Co, Ltd., Wuhan, Hubei Province, China), and Nab titers against keyhole limpet hemocyanin (**KLH**) types IgG and IgM using ELISA.

Oral Fluid Sampling for Presence of Respiratory Pathogens

In each room with 8 pens of pigs, 2 pens with the normal protein - basal amino acid diet were selected for oral fluid sampling by using a swine oral fluid test kit (Tego oral fluids kit; ITL Corporation, Melbourne, Australia). At wk 14, 20, and 24 of age (1 time point per phase), a clean rope was hanged in the pen at pigs' shoulder height and securely tied. Pigs were allowed to chew on the rope for 30 min. Subsequently, the rope was removed from the pen (by wearing gloves) and placed in a clean pouch bag. The bag was closed and the fluid was extracted from the rope by squeezing the rope through the bag. Oral fluid was collected from the

Table 4. Performance parameters of growing pigs kept under different sanitary conditions and fed diets differing in protein content and AA supplementation, (bold *P*-values are significant and underlined *P*-values are considered as tendency)

	LSC[1]				HSC[1]											
	LP[2]		NP[2]		LP		NP			*P*-value[5]						
Item	AA-B[3]	AA-S[3]	AA-B	AA-S	AA-B	AA-S	AA-B	AA-S	SEM[4]	SC	CP	AA	SC × CP	SC × AA	CP × AA	SC × CP × AA
No. of pens[6]	9	9	9	9	8	8	8	8								
Start, kg BW	17.6	18.0	17.6	17.6	16.6	16.6	16.7	16.6	0.1	**<0.0001**	0.23	0.43	0.15	0.21	0.10	0.41
ADG, g/d																
0–34 d	602	591	655	652	618	660	697	724	16	**0.01**	**<0.0001**	0.22	0.53	0.06	0.86	0.60
35–49 d	962	992	1,023	1,088	979	1,024	1,113	1,132	33	0.25	**<0.0001**	**0.02**	0.22	0.65	0.89	0.39
17–110 kg BW	825	846	864	884	860	886	925	969	18	**0.03**	**<0.0001**	**0.01**	0.10	0.49	0.66	0.65
ADFI, g/d																
0–34 d	1,246	1,225	1,218	1,198	1,201	1,280	1,230	1,259	20	0.17	0.39	0.21	0.27	**0.01**	0.42	0.40
35–49 d	2,122	2,123	2,120	2,070	2,100	2,222	2,220	2,244	51	0.11	0.45	0.49	0.17	0.17	0.27	0.69
17–110 kg BW	1,950	1,924	1,901	1,890	1,910	1,989	1,940	1,998	37	0.25	0.66	0.24	0.13	**0.04**	0.90	0.63
G:F, g/g																
0–34 d	0.48	0.48	0.54	0.54	0.52	0.52	0.57	0.57	0.009	**0.003**	**<0.0001**	0.62	0.75	0.94	0.59	0.96
35–49 d	0.46	0.47	0.48	0.52	0.47	0.46	0.5	0.51	0.013	0.93	**<0.0001**	0.11	0.91	0.09	0.60	0.81
17–110 kg BW	0.42	0.44	0.46	0.48	0.45	0.44	0.48	0.48	0.004	**0.002**	**<0.0001**	**0.01**	0.70	**0.03**	0.32	0.15

[1]LSC = low sanitary conditions; HSC = high sanitary conditions.

[2]LP = low CP concentration diet; NP = normal CP concentration diet.

[3]AA-B = basal dietary AA profile; AA-S = supplemented dietary AA profile containing 20% more Met, Thr, and Trp compared with the basal profile.

[4]SEM = pooled SEM. Means are presented as least squares means.

[5]SC = sanitary conditions. Considered significant when $P \leq 0.05$ and considered a tendency when $0.05 < P \leq 0.10$.

[6]A pen contained 9 pigs.

bag in a clean sample tube after the corner of the bag was torn off. Oral fluid was refrigerated until further analysis. Oral samples were analyzed with multiplex PCR for presence of *Mycoplasma hyorhinis*, porcine respiratory corona virus, PRRS virus, *M. hyopneumoniae*, influenza A virus, porcine cytomegalo virus, and PCV2 (IVD GmbH, Hannover, Germany).

Anti-Keyhole Limpet Hemocyanin IgM and IgG Assessment

Antibody titers were determined as described by de Koning et al. (2015) with the minor modification that a 4-step dilution (40, 160, 640, 2,560 times diluted) of the sera was made instead of a 3-step dilution.

Observations at Slaughter

All pigs per pen were slaughtered in the week in which the average BW of the pigs in that pen was close to the target weight of 110 kg. In the slaughterhouse, lungs were collected, examined, and scored by a pathologist for pleuritis (0 to 2 scale, in which 0 = absence of pleuritis, 1 = adhesion of lung tissue with film-like tissue, and 2 = lung tissue completely grown together) and pneumonia lesions (0 to 3 scale, in which 0 = absence of pneumonia, 1 = one spot of pneumonia, 2 = a few spots of pneumonia, and 3 = diffuse deviation of pneumonia spots). Carcass weight, backfat thickness, and muscle thickness were measured in the slaughterhouse. Both fat and muscle thickness were measured at 6 cm from the back midline between the third and fourth last rib. Lean meat and dressing percentages were calculated per pig from the parameters obtained at slaughter using the following formulas: lean meat (%) = 66.86 − 0.6549 × (fat, mm) + 0.0207 × (muscle, mm) (Engel et al., 2012) and dressing (%) = (carcass weight/live weight) × 100% (Watkins et al., 1990).

Statistical Analysis

Data were analyzed as a 2 × 2 × 2 factorial arrangement using the GLM procedure for parameters measured at slaughter and the Mixed Model procedure for other performance parameters and serum and blood data (SAS 9.3; SAS Inst. Inc., Cary, NC) with pen as experimental unit for all parameters. For all data, the normality of the distribution of Studentized residuals was assessed by the Shapiro–Wilk statistic. If required, transformation of data was performed to obtain normal distribution of residuals. Values are presented as least squares means ± SEM, and effects were

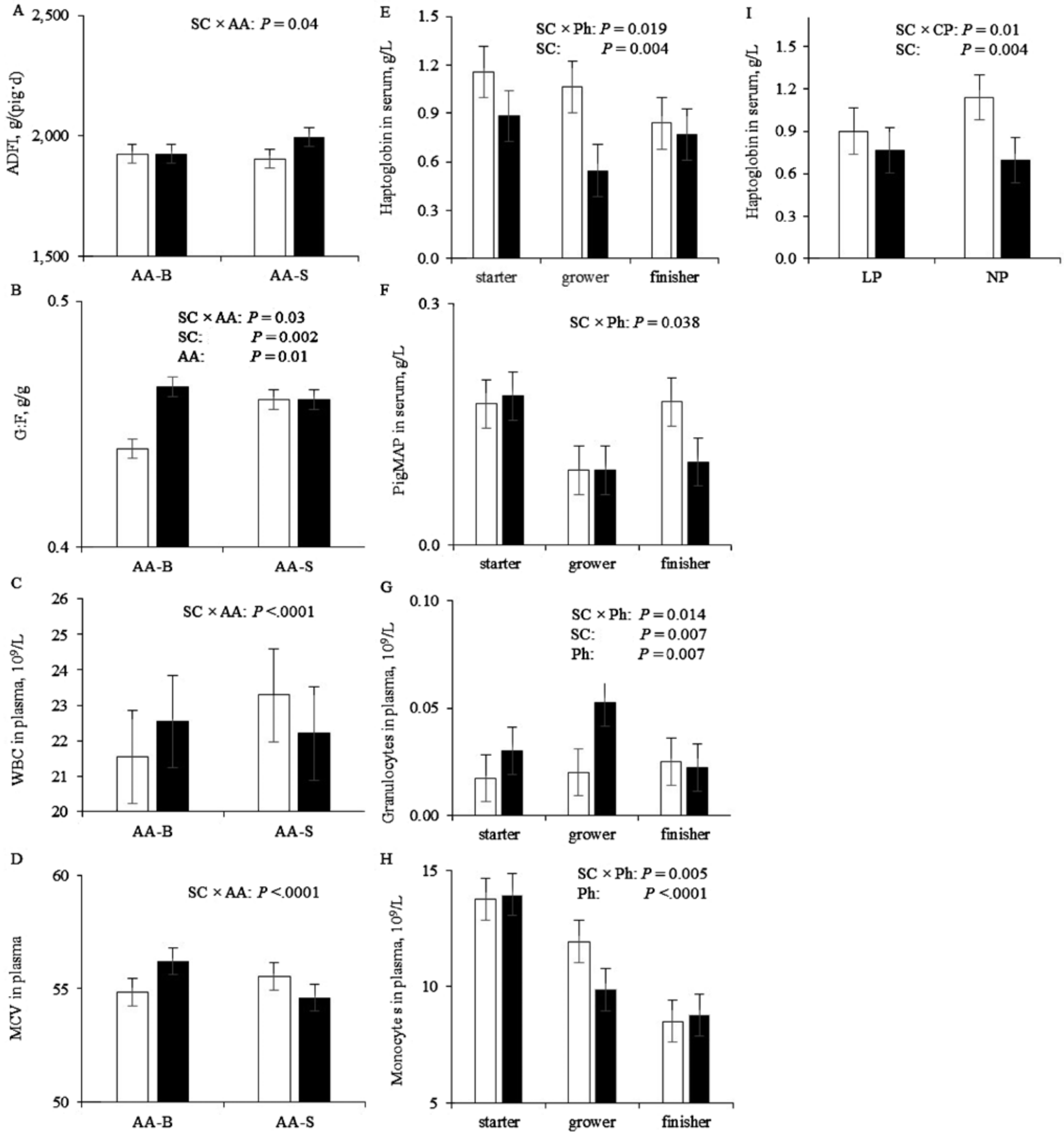

Figure 1. Interactions between low sanitary condition (LSC) and high sanitary condition (HSC) pigs and a basal dietary AA profile (AA-B) or a supplemented dietary AA profile containing 20% more Met, Thr, and Trp compared with the basal profile (AA-S), for ADFI (A), G:F (B), white blood cell (WBC) number (C), and mean cell volume (MCV; D). SC = sanitary conditions. Interactions between LSC and HSC pigs and phase (Ph) for serum haptoglobin concentration (E), serum PigMAP concentration (F), plasma granulocyte number (G), and plasma monocyte number (H). Interaction between LSC and HSC pigs and a low CP concentration diet (LP) or a normal CP concentration diet (NP) for serum haptoglobin concentration (I). The open bars represent the LSC treatment and the filled bars represent the HSC treatment. Bars represent least squares means ± SEM. *P*-values were considered significant when $P \leq 0.05$.

considered significant at $P \leq 0.05$ and a trend was defined as $0.05 < P \leq 0.10$. Sanitary condition, dietary CP level, and AA profile, batch, and their interactions were used as fixed effects. Phase was added in the model as a fixed effect and phase × sanitary condition as an interaction for all blood parameters and ATTD of N and DM. The effect of room within sanitary status was used as a random effect to correct for differences between rooms. The Kenward–Roger statement was used to correct for the degrees of freedom for batch. The difference between individual start BW and average BW of the treatment group (sanitary conditions and batch) was used as covariate in the model in the first statistical evaluations but finally omitted because of absence of statistical significance.

RESULTS

Two pigs selected for blood sampling died during the grower phase and. as such. the data of these pigs are missing for the finisher phase. Data of another pig selected for blood sampling was omitted from the data set as this pig was treated with antibiotics in the starter phase due to lung problems. No other clinical signs of illness were observed during the experiment. All results are presented in Tables 4 through 8. For clarity, selected treatment interactions are represented in Fig. 1A through 1I.

Performance

Mean BW at start of the experiment was greater for LSC pigs (17.7 ± 0.1 kg) compared with HSC pigs (16.6 ± 0.1 kg; $P \leq 0.01$; Table 4). The ADG was (52 g/d) lower for LSC pigs compared with HSC pigs for the starter phase ($P \leq 0.05$) and (55 g/d) during the complete fattening period ($P \leq 0.01$) but not during the grower phase ($P > 0.10$). The LP pigs had (56 g/d) lower ADG in the complete fattening period compared with NP pigs (all, $P \leq 0.05$). The AA-B pigs tended to have (28 g/d) lower ADG compared with AA-S pigs in the starter phase when kept under HSC but not under LSC [sanitary conditions (**SC**) × AA, $P \leq 0.10$].

For ADFI, an interaction was present for SC × AA in the starter phase ($P \leq 0.01$) and over the complete fattening period ($P \leq 0.05$; Fig. 1A) but not in the grower phase ($P > 0.10$). The AA-B pigs had (54 g/d in the starter phase and 69 g/d over the complete fattening period) lower ADFI compared with AA-S pigs when kept under HSC but not under LSC.

The G:F was (0.035 g/g) lower for pigs kept under LSC compared with HSC for the starter phase ($P \leq 0.01$) and (0.013 g/g) for the entire grower–finisher period ($P \leq 0.01$) but not for the grower phase ($P > 0.10$). The G:F was 0.038 g/g lower for LP pigs compared with NP pigs in all phases (all, $P \leq 0.01$). The AA-B pigs had a (0.008 g/g) lower G:F compared with AA-S pigs in the entire grower–finisher period ($P \leq 0.01$). The greater G:F for AA-B pigs compared with AA-S pigs for the entire experimental period was (0.025 g/g) greater for LSC pigs compared with HSC pigs (SC × AA, $P \leq 0.05$; Fig. 1B). A tendency for a similar interaction was found for the G:F in the grower phase ($P \leq 0.10$).

Acute-Phase Proteins and Natural Antibodies against Keyhole Limpet Hemocyanin in Serum

In LSC pigs but not HSC pigs, reduction of dietary CP concentration reduced serum haptoglobin by 0.24 g/L (CP × SC, $P \leq 0.01$; Table 5; Fig. 1I). The LSC pigs showed lower haptoglobin concentrations over time whereas HSC pigs had lower concentrations during the grower phase compared with the starter phase and showed greater concentrations again in finisher phase (SC × phase, $P \leq 0.05$; Fig. 1E). The LSC pigs had (0.29 g/L) greater serum haptoglobin concentrations compared with the HSC pigs ($P \leq 0.01$).

The LSC pigs had (0.03 g/L) lower PigMAP concentrations in the grower phase compared with the starter phase and (0.09 g/L) greater concentrations again in the finisher phase compared with the grower phase, whereas the HSC pigs had (0.09 g/L) lower concentrations in the grower phase compared with the starter phase (SC × phase, $P \leq 0.05$; Fig. 1F).

Keyhole limpet hemocyanin–specific IgM antibody titers in serum tended to be (0.04) lower for AA-S–fed pigs compared with AA-B–fed pigs ($P \leq 0.10$). Keyhole limpet hemocyanin–specific IgG antibody titers were (0.45) greater for LSC pigs compared with HSC pigs ($P \leq 0.05$).

Blood Cell Counts

The number of white blood cells (**WBC**) was (1.8 × 10^9/L) greater (Table 6) in AA-S–fed pigs compared with AA-B–fed pigs when kept under LSC but not under HSC (SC × AA, $P \leq 0.05$; Fig. 1C). Over time, the concentration of WBC in pigs decreased (by 4.1 × 10^9/L) but the number of red blood cells consistently increased (with 0.5 × 10^{12}/L) in all treatment groups ($P \leq 0.01$). Pigs fed the NP had a (3.1%) lower number of WBC compared with pigs fed the LP ($P \leq 0.05$). Hemoglobin concentration was (0.1 mmol/L) lower in AA-S–fed pigs compared with AA-B–fed pigs, particularly under HSC (SC × AA, $P \leq 0.05$, and $P \leq 0.05$ for AA). Hemoglobin concentrations increased with age in all treatment groups (0.74 mmol/L); however, this increase was greater in HSC pigs compared with LSC pigs (SC × phase, $P \leq 0.05$). Pigs fed NP had a (0.1 mmol/L) greater hemoglobin concentration than pigs fed LP ($P \leq 0.05$). Mean cell volume was (0.7, 10^{-15}L) greater for AA-S–fed pigs compared with AA-B–fed pigs in LSC but this was reversed in HSC (SC × AA, $P \leq 0.01$; Fig. 1D). The mean cell volume was (0.4, 10^{-15}L) greater in pigs fed NP compared with pigs fed LP ($P \leq 0.05$). The number of platelets decreased (by 258 × 10^9/L) in pigs over time for all treatments, but the decrease was (60%) greater in HSC pigs compared with LSC pigs (interaction, $P \leq 0.05$).

Table 5. Concentrations of acute-phase protein and natural antibody titers against keyhole limpet hemocyanin (KLH) in serum of pigs kept under different sanitary conditions and fed 1 of 4 diets, with a low or normal protein level and a basal or supplemented AA profile (bold *P*-values are significant and underlined *P*-values are considered as tendency)

		LSC[2]				HSC[2]												
		LP[3]		NP[3]		LP		NP			*P*-value[6]							
Item	Phase[1]	AA-B[4]	AA-S[4]	AA-B	AA-S	AA-B	AA-S	AA-B	AA-S	SEM[5]	SC	CP	AA	SC × phase	SC × CP	SC × AA	CP × AA	SC × CP × AA
No. of pens[7]		9	9	9	9	8	8	8	8									
Haptoglobin, g/L	Starter	1.15	0.75	1.36	1.37	0.91	0.79	0.86	0.98	0.16	**0.004**	0.25	0.91	**0.02**	**0.01**	0.42	0.12	0.74
	Grower	0.97	0.97	1.21	1.11	0.63	0.47	0.46	0.62									
	Finisher	0.83	0.73	0.85	0.95	0.85	0.95	0.58	0.69									
PigMAP, g/L	Starter	0.17	0.14	0.14	0.25	0.18	0.16	0.19	0.21	0.03	0.25	0.19	0.55	**0.04**	0.99	0.35	0.22	0.39
	Grower	0.07	0.08	0.10	0.12	0.08	0.09	0.11	0.09									
	Finisher	0.19	0.19	0.16	0.17	0.11	0.10	0.09	0.11									
KLH[8]–IgM	Starter	6.59	6.29	6.68	6.60	6.40	6.78	6.59	6.42	0.20	0.68	0.47	0.07	0.32	0.92	0.32	0.47	0.20
	Grower	7.50	7.27	7.47	7.09	7.06	7.03	7.31	6.99									
	Finisher	7.85	7.46	7.80	7.69	7.78	7.65	7.90	7.84									
KLH–IgG	Starter	3.12	3.30	3.08	2.97	2.78	2.46	2.47	3.02	0.28	**0.04**	0.75	0.62	0.09	0.42	0.87	0.81	0.08
	Grower	3.64	4.13	3.67	3.75	3.10	2.98	3.15	2.99									
	Finisher	3.62	3.70	3.82	3.52	3.46	3.59	3.36	3.59									

[1]The experiment consisted of 3 different phases: starter, grower, and finisher.

[2]LSC = low sanitary conditions; HSC = high sanitary conditions.

[3]LP = low CP concentration diet; NP = normal CP concentration diet.

[4]AA-B = basal dietary AA profile; AA-S = supplemented dietary AA profile containing 20% more Met, Thr, and Trp compared with the basal profile.

[5]SEM = pooled SEM. Means are presented as least squares means.

[6]SC = sanitary conditions. Considered significant when $P \leq 0.05$ and considered a tendency when $0.05 < P \leq 0.10$.

[7]All animals were group housed in pens with 9 animals per pen. Two animals per pen were selected for blood sampling.

[8]Keyhole limpet hemocyanin is a protein produced by a sea snail. Pigs have natural antibodies against this protein.

The concentration of platelets in pigs fed AA-S diets was (121×10^9/L) greater compared with pigs fed AA-B diets ($P \leq 0.01$).

White Blood Cell Distribution

The AA-S–fed pigs had (0.7×10^9/L) a greater number of blood lymphocytes compared with AA-B–fed pigs when provided a LP, but this was reversed (-0.5×10^9/L) when pigs were provided a NP (CP × AA, $P \leq 0.05$). The number of lymphocytes increased (0.9×10^9/L) with age for all treatment groups ($P \leq 0.01$). The number of monocytes (2.9×10^9/L) decreased over time for all treatment groups, particularly for LSC pigs (SC × phase, $P \leq 0.01$; Fig. 1H). The AA-S–fed pigs had a (0.6×10^9/L) greater monocyte number compared with AA-B–fed pigs ($P \leq 0.05$). The NP-fed pigs had a (0.8×10^9/L) lower monocytes number compared with LP-fed pigs ($P \leq 0.05$). The concentration of granulocytes increased (by 0.01×10^9/L) over time for LSC pigs and, in the HSC pigs, increased (0.02×10^9/L) from the starter to the grower phases but decreased (0.03×10^9/L) again in the finisher phase (SC × phase, $P \leq 0.05$; Fig. 1G). The concentration of granulocytes was (0.01×10^9/L) greater for HSC pigs than for LSC pigs ($P \leq 0.01$).

Carcass Observations at Slaughter and Lung Scores

Results obtained at slaughter are presented in Table 7. Carcass weight was (3.8 kg) lower for LP pigs compared with NP pigs but not dressing percentage ($P \leq 0.01$ and $P > 0.05$, respectively). The LP pigs had (1.2 mm) greater backfat thickness ($P \leq 0.01$) and (0.8%) lower lean meat percentage ($P \leq 0.01$) compared with NP pigs. The AA-B pigs had (1.5 mm) lower muscle thickness ($P \leq 0.01$; 0.5 mm), greater backfat thickness ($P \leq 0.01$), and a (0.4%) lower lean meat percentage ($P \leq 0.01$) compared with AA-S pigs.

Pleuritis scores were (0.3) greater for LSC pigs compared with HSC pigs ($P \leq 0.01$). Percentage of lung surface with pleuritis was also (1.2%) greater for LSC pigs compared with HSC pigs ($P \leq 0.01$). The AA-B pigs had a (1%) greater percentage of lung surface with pleuritis compared with AA-S pigs when kept in LSC, but for HSC pigs, this was reversed (SC × AA, $P \leq 0.05$). The LP pigs had (0.2) greater pneumonia scores compared with NP pigs when kept under HSC; however, when kept under LSC, this was reversed (SC × CP, $P \leq 0.05$). The AA-B pigs tended to have greater pneumonia scores compared with the AA-S pigs in all cases except in LSC pigs fed a LP (CP × AA, $P \leq 0.01$).

Table 6. Cell count in fresh blood of pigs kept under different sanitary conditions and fed 1 of 4 diets, with a low or normal protein level and a basal or supplemented AA profile (bold *P*-values are significant and underlined *P*-values are considered as tendency)

		LSC[2]				HSC[2]													
		LP[3]		NP[3]		LP		NP			*P*-value[6]								
Item[1]	Phase	AA-B[4]	AA-S[4]	AA-B	AA-S	AA-B	AA-S	AA-B	AA-S	SEM[5]	SC	CP	AA	Phase	SC × phase	SC × CP	SC × AA	CP × AA	SC × CP × AA
No. of pens[7]		**9**	**9**	**9**	**9**	**8**	**8**	**8**	**8**										
WBC, 10^9/L	Starter	24.3	26.6	20.8	25.0	26.0	25.0	25.1	23.4	1.31	0.89	0.02	0.15	<0.0001	0.08	0.60	0.04	0.18	0.46
	Grower	22.7	24.9	22.5	22.0	21.9	22.7	21.3	19.9										
	Finisher	19.4	21.2	19.5	20.0	19.6	21.4	21.4	20.8										
Lymphocytes, 10^9/L	Starter	10.7	11.3	9.4	10.3	11.0	11.3	11.2	10.4	0.67	0.43	0.10	0.69	0.005	0.88	0.13	0.05	0.02	0.30
	Grower	11.0	11.9	10.9	10.8	11.4	11.8	11.7	10.9										
	Finisher	11.2	12.5	11.2	11.1	11.1	11.6	13.2	10.9										
Monocytes, 10^9/L	Starter	13.6	15.3	11.3	14.8	15.0	13.9	13.9	13.0	0.900	0.34	0.01	0.049	<0.0001	0.005	0.55	0.13	0.69	0.56
	Grower	11.7	13.1	11.6	11.3	10.4	10.8	9.5	8.8										
	Finisher	8.1	8.7	8.4	8.9	8.2	9.7	8.1	9.1										
Granulocytes, 10^9/L	Starter	0.02	0.01	0.01	0.03	0.02	0.03	0.04	0.03	0.011	0.007	0.27	0.88	0.007	0.01	0.39	0.61	0.74	0.10
	Grower	0.02	0.02	0.01	0.03	0.05	0.07	0.05	0.04										
	Finisher	0.04	0.02	0.02	0.02	0.04	0.03	0.01	0.01										
RBC, 10^{12}/L	Starter	5.7	5.6	5.6	5.5	5.4	6.0	5.5	5.4	0.13	0.83	0.83	0.95	<0.0001	0.30	0.95	0.08	0.44	0.36
	Grower	5.9	5.8	5.9	5.6	5.7	6.0	5.9	5.8										
	Finisher	6.0	5.9	6.0	6.1	6.1	6.1	6.0	6.3										
Hb, mmol/L	Starter	6.4	6.3	6.5	6.4	6.3	6.3	6.5	6.0	0.14	0.13	0.01	0.03	<0.0001	0.045	0.62	0.046	0.18	0.07
	Grower	6.6	6.6	6.8	6.6	6.8	6.7	7.2	6.7										
	Finisher	6.8	6.9	6.9	7.3	7.2	7.0	7.3	7.2										
Ht, %	Starter	47.8	30.9	31.4	30.9	30.3	31.0	31.0	29.3	3.7	0.49	0.45	0.28	0.64	0.23	0.41	0.40	0.48	0.29
	Grower	31.6	31.6	32.3	31.2	31.9	32.3	33.0	31.6										
	Finisher	32.9	32.9	33.4	34.3	34.0	33.5	34.3	34.5										
MCV, 10^{-15}L	Starter	54.8	55.1	55.8	56.1	56.0	54.4	56.0	54.1	0.59	0.48	0.04	0.06	0.13	0.24	0.31	<0.0001	0.52	0.95
	Grower	54.0	54.7	54.8	55.3	56.0	54.3	56.4	54.9										
	Finisher	54.5	56.0	55.2	56.0	56.2	55.0	56.7	55.0										
PTL, 10^9/L	Starter	614	712	562	721	571	703	744	1,219	94.8	0.92	0.23	**0.002**	**<0.0001**	**0.02**	0.14	0.46	0.60	0.78
	Grower	522	606	520	652	438	551	439	559										
	Finisher	489	555	480	494	405	508	447	401										

[1]WBC = white blood cells; RBC = red blood cells; Hb = haemoglobin; Ht = haematocrit; MCV = mean cell volume; PTL = platelets.

[2]LSC = low sanitary conditions; HSC = high sanitary conditions.

[3]LP = low CP concentration diet; NP = normal CP concentration diet.

[4]AA-B = basal dietary AA profile; AA-S = supplemented dietary AA profile containing 20% more Met, Thr, and Trp compared with the basal profile.

[5]SEM = pooled SEM. Means are presented as least squares means.

[6]SC = sanitary conditions. Considered significant when $P \leq 0.05$ and considered a tendency when $0.05 < P \leq 0.10$.

[7]All animals were group housed in pens with 9 animals in total. Two animals per pen we selected for blood sampling.

Table 7. Slaughter results of fattening boars kept under different sanitary conditions and fed diets with 2 CP levels and 2 AA profiles (bold *P*-values are significant and underlined *P*-values are considered as tendency)

	LSC[1]				HSC[1]											
	LP[2]		NP[2]		LP		NP			*P*-value[5]						
Item	AA-B[3]	AA-S[3]	AA-B	AA-S	AA-B	AA-S	AA-B	AA-S	SEM[4]	SC	CP	AA	SC × CP	SC × AA	CP × AA	SC × CP × AA
No. of pens[6]	9	9	9	9	8	8	8	8								
BW,[7] kg	109.7	108.0	112.8	114.3	108.1	111.9	113.1	116.3	1.85	0.40	**0.0007**	0.20	0.98	0.17	0.62	0.48
Carcass weight, kg	84.7	82.3	86.4	88.2	82.0	85.7	86.3	88.8	1.55	0.78	**0.001**	0.20	0.95	0.12	0.49	0.23
Muscle,[7] mm	55.4	55.6	55.3	59.1	55.3	56.6	55.8	56.6	0.90	0.78	0.12	**0.008**	0.19	0.42	0.18	0.07
Backfat,[7] mm	15.3	13.9	13.3	12.9	14.7	14.3	13.4	13.7	0.33	0.45	**<0.0001**	**0.047**	0.25	0.07	0.08	0.88
Lean meat,[7] %	58.1	58.9	59.3	59.7	58.4	58.7	59.2	59.1	0.23	0.53	**<0.0001**	**0.03**	0.26	0.11	0.13	0.90
Dressing, %	77.1	76.2	76.6	77.2	75.9	76.6	76.3	76.4	0.35	0.18	0.51	0.63	0.73	0.25	0.32	**0.02**
Pleuritis score[8]	0.44	0.50	0.59	0.43	0.15	0.21	0.12	0.33	0.08	**<0.0001**	0.43	0.51	0.96	0.10	0.87	0.09
Pleuritis lung, %[9]	1.82	1.26	2.55	1.04	0.39	0.96	0.06	0.36	0.60	**0.004**	0.39	0.77	0.83	0.49	0.66	0.58
Pneumonia score[8]	0.53	0.65	0.79	0.74	0.94	0.80	0.86	0.57	0.15	0.32	0.65	0.33	**0.02**	0.07	0.16	0.83
Pneumonia lung, %[9]	1.42	1.26	3.17	2.14	1.81	1.93	2.48	0.70	1.40	0.78	0.22	0.15	0.12	0.27	0.16	0.46

[1]LSC = low sanitary conditions; HSC = high sanitary conditions.

[2]LP = low CP concentration diet; NP = normal CP concentration diet.

[3]AA-B = basal dietary AA profile; AA-S = supplemented dietary AA profile containing 20% more Met, Thr, and Trp compared with the basal profile.

[4]SEM = pooled SEM. Means are presented as least squares means.

[5]SC = sanitary conditions. Considered significant when $P \le 0.05$ and considered a tendency when $0.05 < P \le 0.10$.

[6]A pen contained 9 pigs.

[7]Body weight expressed 1 d before slaughter day; muscle, backfat, and lean meat is expressed as corrected for carcass weight, by including carcass weight as a covariate in the statistical model.

[8]Pleurits was scored on a scale of 0 to 2 and pneumonia was scored on a scale of 0 to 3.

[9]Percentage of lung surface affected by pleuritis or pneumonia.

Dry Matter and N Digestion

The effect of experimental treatments on the ATTD of DM and N were consistent over the duration of the experiment and are presented averaged over all phases (Table 8). In general, treatment differences, albeit significant, were small. Apparent total tract digestibility for DM was (0.3%) lower for LSC pigs compared with HSC pigs during all phases (all, $P \le 0.05$). The AA-B pigs had (0.4%) lower ATTD of DM compared with AA-S pigs ($P \le 0.01$). Apparent total tract digestibility for N was (0.98%) greater for HSC pigs compared with LSC pigs during all phases (all, $P \le 0.01$), regardless the dietary CP content or AA profile. Apparent total tract digestibility for N increased

Table 8. Apparent fecal digestibility (%) of DM and N in fattening boars kept under different sanitary status and fed 1 of 4 experimental diets with either a low or normal protein level and a basal or supplemented AA profile (bold *P*-values are significant)

	LSC[1]				HSC[1]												
	LP[2]		NP[2]		LP		NP			*P*-value[5]							
Item	AA-B[3]	AA-S[3]	AA-B	AA-S	AA-B	AA-S	AA-B	AA-S	SEM[4]	SC	CP	AA	Phase[6]	SC × CP	SC × AA	CP × AA	SC × CP × AA
No. of pens[7]	4	4	4	4	4	4	4	4									
DM	88.1	88.5	87.9	88.6	88.8	88.9	88.3	88.5	0.16	**0.047**	0.10	**0.03**	**<0.0001**	0.21	0.19	0.51	0.70
N	82.3	82.1	82.9	83.1	83.6	83.4	84.2	83.1	0.43	**0.02**	0.14	0.28	**<0.0001**	0.31	0.30	0.62	0.31

[1]LSC = low sanitary conditions; HSC = high sanitary conditions.

[2]LP = low CP concentration diet; NP = normal CP concentration diet.

[3]AA-B = basal dietary AA profile; AA-S = supplemented dietary AA profile containing 20% more Met, Thr, and Trp compared with the basal profile.

[4]SEM = pooled SEM. Means are presented as least squares means.

[5]SC = sanitary conditions. Considered significant when $P \le 0.05$.

[6]Results are presented as average values of measurement of digestibility at 3 time points, once in each of the 3 experimental phases.

[7]A pen contained 9 pigs.

from 80.6 (starter phase) to 83.9 (grower phase) and 84.7% finisher phase ($P \leq 0.01$). There were no interactions between phase and other independent variables.

Oral Fluid Samples

Oral fluid samples of pigs in all sampled pens (18 pens in total) were positive for *M. hyorhinis* and negative for PRRS, *M. hyopneumoniae*, influenza A virus, and porcine cytomegalo virus in all phases. All pens were negative for porcine respiratory corona virus in the starter and grower phases, but in the finisher phase, 4 out of 9 LSC pens and 3 out of 9 HSC pens became positive. All pens were negative for PCV2 in the starter phase, but LSC pigs became positive in grower phase (7 out of 9 pens) and finisher phase (all, 9 pens). Only 1 out of 9 HSC pens became positive for PCV2 in the grower phase and was negative again in the finisher phase. The rest of the sampled HSC pigs were negative for PCV2 in all phases.

DISCUSSION

The main objective of the present experiment was to evaluate, under practical conditions, if diets with low or normal CP level and basal or supplemented Met, Thr, and Trp have differential effects on pig performance and immune status at different sanitary conditions.

Effect of Sanitary Conditions on Performance and Immune Status

A contrast in sanitary conditions was generated by imposing a combination of differences in hygiene, antibiotic treatment, deworming, and a vaccination protocol, all applied to piglets originating from the same farm. Over the duration of the experiment, HSC pigs showed greater ADG (50 g/d) and G:F (0.013 kg/kg) than LSC pigs. Low sanitary condition pigs had greater serum haptoglobin levels and greater KLH–IgG titers than HSC pigs. This indicates that LSC pigs had a more active immune system compared with HSC pigs. In addition, at slaughter, higher pleuritis scores were observed in LSC pigs compared with HSC pigs. Taken together, the absence of clinical signs of illness of pigs in either treatment group and the lower ADG and G:F, elevated haptoglobin concentrations, increased KLH-specific IgG titers, greater pleuritis occurrence, and oral fluid samples positive for PCV2 in the grower and finisher phases in the LSC groups illustrate a difference in subclinical health status between the LSC and HSC pigs (Le Floc'h et al., 2006; van den Berg et al., 2007; Piñeiro et al., 2009).

We expected that the sanitary regimes used in our study would affect PigMAP and haptoglobin concentrations in a similar way, as these are both considered to be positive acute-phase proteins and are expected to increase in response to immune stimulation (Murata et al., 2004). Haptoglobin appeared more responsive to the difference in sanitary conditions than PigMAP, which is in line with Heegaard et al. (1998) and Kampman-van de Hoek et al. (2016). In addition, PigMAP levels found here (0.14 g/L serum) were low compared with the values found by Piñeiro et al. (2009) and Kampman-van de Hoek et al. (2016; 0.9 and 1.4 g/L serum, respectively), whereas the haptoglobin concentrations reported by these authors were similar to values reported in the present study.

The KLH antigen is a relevant antigen to measure Nab levels as pigs had no previous exposure to KLH. Natural antibodies are defined as antigen-specific antibodies that are present in the absence of intentional immunization with specific antigens (KLH, in this case; Star et al., 2007). The IgG titer against KLH was greater for LSC pigs than for HSC pigs. Because Nab play a role in the first line of defense against pathogens, the increase in Nab levels might be an adaptive response of the pigs to the higher infection pressure at LSC. Keyhole limpet hemocyanin–specific antibodies were shown to be cross-reactive with antigens of pathogens (Hamilton et al., 1999). Therefore, it can not be excluded that antigen-specific Nab are products of adaptive immune responses and cross-reactive with structurally related antigens. Specific memory B cells were shown to be activated in a polyclonal but antigen-independent way (Lanzavecchia et al., 2006). This way of activation is triggered by non-specific microbial-associated molecular patterns, such as lipopolysaccharide or bacterial or viral nucleotide motifs. This B cell activation mechanism is likely responsible for the lifelong presence of circulating specific antibodies, which forms an important part of the first line of defense. It can easily be envisaged that memory B cells of pigs under LSC become more activated by microbial associated molecular patterns than under HSC, resulting in higher levels of KLH-specific IgG levels. For IgM titers against KLH, there were no differences between LSC and HSC pigs. In the study of Ploegaert et al. (2010), a similar result in IgM and IgG Nab was found. Ploegaert et al. (2010) studied genetic and phenotypic correlation of Nab titers in dairy cattle and found greater estimates for environmental variation in the IgG than in the IgM isotype of Nab. Most probably, the LSC in the present study, as an environmental factor, stimulated KLH-specific IgG responses. Immunoglobulin M titers are believed to be influenced by genetic factors (Ploegaert et al., 2010), which explains the absence of differences in IgM against KLH between sanitary regimes.

As the HSC pigs were vaccinated against several pathogens in their first 10 wk of life, vaccination and/or antibiotic treatment might have modulated immune functions of these animals. Our results show that despite the vaccination of the HSC pigs, LSC pigs had significantly higher values for haptoglobin and IgG antibodies against KLH, showed reduced ADG and G:F, and had significant higher pleuritis scores, indicating that the effects of the LSC conditions outweighed the potential effects of the vaccinations or antibiotic treatment of the HSC pigs. However, the vaccinations of the HSC pigs may have reduced the immunological contrasts between the pigs kept under the different sanitary conditions. If this is the case, the observed interactions between diet and sanitary conditions could even be larger when vaccinations are omitted. In addition, vaccinations or antibiotic treatment may have affected other (undetermined) immune parameters as well.

Sanitary Conditions and Growth Performance

At start of the experiment, the BW of LSC pigs was 1.1 kg greater compared with that of HSC pigs, likely related to the vaccination program of the HSC pigs prior to arrival at the experimental farm. During the starter phase, HSC pigs compensated for this lower starting weight, or the ADG of the LSC pigs was negatively influenced by the LSC.

A lower ADG of LSC pigs was also found in other studies evaluating a contrast in sanitary conditions (Williams et al., 1997; Le Floc'h et al., 2009; Pastorelli et al., 2012a). This decreased ADG can be explained by the competition for use of nutrients between the immune system and for use in deposition in organs and body tissues. In general, it is perceived that use by the immune system has a high priority (Humphrey and Klasing, 2004; Le Floc'h et al., 2004). From a meta-analysis, Pastorelli et al. (2012b) concluded that the major portion of the reduction in ADG (12.2% lower ADG of the total 16.3% lower ADG in pigs in poor housing conditions compared with unchallenged animals) was related to a decreased feed efficiency (in Pastorelli et al. [2012b], indicated as "maintenance") rather than to a decrease in feed intake. This was also observed in the present experiment, where G:F was affected but ADFI was not influenced by sanitary conditions. The former indicates a greater maintenance requirement or a lower growth efficiency for LSC pigs as suggested by Pastorelli et al. (2012b).

Signs of respiratory problems, such as pleuritis or pneumonia, are related to reduced performance (Saco et al., 2011). The LSC pigs had greater scores for pleuritis and greater percentage of lung surface with pleuritis at slaughter compared with HSC pigs, which might have negatively affected growth and G:F of LSC pigs. From the meta-analysis of Pastorelli et al. (2012b), it appeared that a reduction in ADFI is the major contributor to reduced ADG in case of respiratory problems. In contrast, in our study, ADFI is not the major contributor to reduced ADG, indicating that respiratory problems are likely not the cause of the decrease in G:F in LSC pigs or that conclusions about the respiratory problems drawn by Pastorelli et al. (2012b) are not representative for our study. Pastorelli et al. (2012b) used many different challenge studies for the meta-analysis, differing from studies conducted under more practical conditions such as the present study.

The greater G:F of the HSC pigs might partly be due to a greater apparent total N digestion in these animals compared with LSC pigs. When assuming similar postabsorptive efficiencies for absorbed AA, the observed increase in N digestibility would typically explain approximately 20% of the observed increase in ADG, hence leaving 80% unaccounted for. Differences in ATTD for N correspond with results by Kampman-van de Hoek et al. (2016), who found a reduction in ATTD for N of 3.7% in LSC growing pigs compared with HSC pigs ($P \leq 0.01$). The reduced ATTD for N might be due to intestinal infections, intestinal damage, or an increased digesta passage rate (Sandberg et al., 2006; Pastorelli et al., 2012b). The small difference found in ATTD of DM might suggest that the ATTD of GE also differed to a similar extent.

Greater ADG and G:F were expected for NP-fed pigs compared with the LP-fed pigs. It is well known that additional AA under conditions where AA are limiting but sufficient energy is available lead to greater ADG and improved G:F (Noblet et al., 1987). Supplementation of extra AA by increase in dietary CP (LP vs. NP) or by specifically supplementing Met, Thr, and Trp (AA-B vs. AA-S diet type) resulted in increased ADG, suggesting that one of these AA was limiting.

The increased G:F in AA-S pigs vs. AA-B pigs, particularly in LSC pigs (SC × AA interaction; Fig. 1B), confirms our hypothesis that the dietary AA-S profile better matches the AA requirements of pigs under LSC. It should be noted, however, that this effect was not yet present in the starter phase. Our results are in accordance with other studies that show that immune stimulation by different challenges lead to increased requirements for specific AA compared with unchallenged pigs (Grimble and Grimble, 1998; Le Floc'h et al., 2004; Klasing, 2007; Rakhshandeh et al., 2014). As a consequence, these animals require more AA for their immune system. The increased demand for these AA (Met, Thr, and Trp) for LSC pigs was expected to be greater for pigs fed the LP. This interaction, however, was absent. Therefore, the results of our study do not confirm our hypothesis that at low levels of protein intake, the requirements for Thr, Trp, and Met relative to Lys are increased. As illustrated by the meta-analysis by Pastorelli et al. (2012b), the type of challenge has a major impact on the response of pigs. The contrast in sanitary conditions, as applied in our study, illustrates that also in absence of clinical disease, requirements for Met, Thr, and Trp are persistently affected over the entire weight range. This is in agreement with observations by Kim et al. (2012) for Met, using a repeated LPS model, but not in a study by de Ridder et al. (2012) for Trp, also using a repeated LPS model, in which the response to incremental intake of Trp was demonstrated to be transient.

Dietary protein concentration did not affect ADFI in our study. Apparently, the exchange of protein for starch did not affect the satiating potential of the diets. This is in agreement with Le Bellego and Noblet (2002) and Kerr et al. (2003); however, research in humans has indicated satiating effects of dietary proteins to exceed that of carbohydrates and fats (Andersen and Moore, 2004).

A remarkable result is the interaction between SC and AA profile in ADFI, particularly observed during the starter phase. High sanitary condition AA-S pigs had greater ADFI compared with HSC AA-B pigs, whereas pigs in LSC ate the same amount, regardless of AA supplementation. The greater ADFI for HSC AA-S pigs resulted in a tendency for ADG in the same direction as the change in ADFI. This result illustrates that pigs in HSC were probably more limited in their growth by the AA-B profile than LSC pigs receiving the same profile, which was expected to be the other way around due to the effect of immune stimulation of pigs housed in LSC. We speculate that 1 of the 3 supplemented AA, probably Met, has restricted growth, particularly inhibiting HSC pigs to exploit their full ADG potential following a period of restricted growth before the start of the trial. Methionine is thought to be the limiting AA because the Met:Lys ratio deviates most from recommended values (e.g., CVB, 2011) compared with Thr and Trp. The growth restriction in HSC pigs before the start of the trial was possibly caused by the vaccination strategy and illustrated by a lower BW of HSC pigs at the onset of the trial. The AA profile of the basal diets may not have been sufficient to support compensatory growth following a period of growth restriction.

Dietary Protein Effect on Immune Status

Increasing the dietary protein concentration increased serum haptoglobin concentrations in LSC pigs but not in HSC pigs. The effect of protein scarcity on total serum protein concentrations has been demonstrated in mice (Cooper et al., 1974). Houdijk et al. (2007) found a reduced C-reactive protein and haptoglobin response for infected pigs fed a low protein diet. This may reflect a sensitive response of acute-phase proteins to protein scarcity (Houdijk et al., 2007) or a high priority for the use of AA for protein gain in young pigs, occurring at the expense of the synthesis of haptoglobin. It is unexpected that this interaction was not observed for WBC counts, which were consistently higher for LP pigs compared with NP pigs ($P = 0.02$), being unaffected by SC. Possible differences in migration of WBC into the lymphatic system or tissues complicates clear conclusions on this point (Ganusov and Auerbach., 2014; Marelli-Berg et al., 2010).

Dietary AA Effect on Immune Status

Supplementation of AA in diets has been shown to influence the immune system by increased variation of lymphocytes, increased production of antibodies and cytokines, and activation of lymphocytes, natural killer cells, and macrophages (Daly et al., 1990; Li et al., 2007; Negro et al., 2008). Although increasing the dietary protein concentration reduced monocyte counts, supplementation of Met, Thr, and Trp increased monocyte counts, regardless of SC. Elevated levels of Trp in the AA-S–fed pigs might have stimulated production of monocytes, as Trp is known to play a role in functionality of monocytes and lymphocytes (Melchior et al., 2004). The administration of 300 mg Trp to rats increased monocyte phagocytosis and the innate immune response (Esteban et al., 2004). Overall, there was no clear interaction for sanitary conditions and AA profile present on the measured blood parameters.

In summary, poor sanitary conditions imposed in our study, in a practical setting, reduced ADG over the entire BW range of 17 to 110 kg. Low sanitary conditions increased pleuritis scores at slaughter and increased indicators for the innate immune response and serum haptoglobin concentrations. The vaccination strategy for the HSC pigs in early life may have triggered a compensatory performance response (particularly ADFI) during the starter phase. Dietary supplementation of Met, Thr, and Trp improved the G:F, particularly under LSC, illustrating that dietary requirements for these AA are affected by sanitary conditions. Furthermore, this study provides indications that dietary protein concentration and Met, Thr, and Trp supplementation modify immune status.

Our study suggests that dietary supplementation of Met, Thr, and/or Trp, in addition to provision of AA for covering basal requirements for protein deposition, is beneficial for animal performance, particularly under poor sanitary conditions.

Further research should focus on defining determinants of sanitary status and identification of the requirement of individual AA to be supplemented.

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