NON RUMINANT NUTRITION

The effect of supplementing glycine and serine to a low crude protein diet on growth and skin collagen abundance of nursery pigs¹

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Abstract

A total of 96 newly weaned barrows (initial body weight [BW]: 6.3 ± 0.5 kg) were used to determine the effect of a low crude protein (CP) diet supplemented with Gly and Ser on growth and skin collagen abundance. Barrows were assigned to one of three experimental diets in a three-phase feeding program fed for 35 days ($n = 8$; pen was the experimental unit): 1) corn-soybean meal diet (CON; 20.3% to 23.1% CP; as-fed, analyzed contents); 2) low CP diet (14.8% to 21.4% CP) supplemented with Gly and Ser (G + S) to the same concentrations as CON; 3) low CP diet supplemented with Glu to maintain the same CP concentration as the G + S diet (GLU; 15.0% to 22.1% CP). On days 21 and 35, eight pigs per treatment were euthanized for the determination of physical and chemical body composition and skin collagen abundance. Pigs fed the CON diet had greater overall ADG and final BW compared to pigs fed GLU and G + S ($P < 0.01$). Over the entire 35-day experimental period, ADFI was not influenced by dietary treatment but G:F tended to be greater for pigs fed CON than G + S ($P = 0.084$), while intermediate values were observed for GLU. Carcass weights on days 21 and 35 were greater for pigs fed CON than G + S or GLU ($P < 0.01$). Viscera weights on day 21 were greater for CON than G + S and GLU ($P < 0.05$) and on day 35 were greater for CON than G + S ($P < 0.05$) with intermediate values observed for GLU. The N intake (g/d) between days 0 and 35 was greater for CON than G + S or GLU ($P < 0.05$) and N retention in combined carcass and viscera was greater for CON than G + S ($P < 0.01$) with intermediate values observed for GLU. No treatment effects were observed for efficiency of N utilization. Between days 0 and 21 however, the efficiency of using dietary N for N retention in carcass and viscera tended to be less for pigs fed CON vs. GLU (73.8% vs. 91.6%), while intermediate values were observed for G + S (84.3%; $P = 0.095$). Pigs fed CON and G + S diets had greater skin collagen abundance than pigs fed GLU on days 21 and 35 ($P < 0.01$). Supplementing low CP diets with Glu or with Gly and Ser at the levels used in the current study did not maintain ADG or combined carcass and viscera N retention and only the G + S diet supported skin collagen abundance not different from pigs fed CON. The importance of meeting essential AA requirements for growth are well accepted, but supplementing specific NEAA may be needed when feeding reduced CP diets to newly weaned pigs to support secondary indicators of AA status, such as skin collagen abundance.

Key words: collagen, glycine, low crude protein diets, nursery pigs, serine

Introduction

Commercially available crystalline amino acids (CAA) are becoming an economically viable option for reducing the protein concentration of swine diets, especially for the diets of newly weaned pigs, where high quality but expensive protein sources are often used. Closely matching estimated AA requirements and reducing dietary CP concentration will improve AA and N utilization efficiencies for net protein gain and thereby, reduce the amount of N excreted into the environment (NRC, 2012; Millet et al., 2018).

It is well established that CAA supplementation (e.g., Lys, Met, Thr, Trp) to a diet with CP reduced by 4 percentage units can be sufficient to support ADG not different from pigs fed a conventional corn- and soybean meal-based diet (Kerr et al., 1995). Reducing the dietary CP concentration, however, also reduces the supply of dietary N and nonessential AA (NEAA) from whole-protein sources. Therefore, it is plausible that inadequate endogenous synthesis of NEAA may perturb optimal growth when pigs are fed reduced CP diets supplemented with essential CAA. This suggestion is supported by the recent observation of increased body protein gain or body weight gain in response to including dietary Gly in low protein diets fed to young pigs (Powell et al., 2011; Wang et al., 2014a). Gly is generated endogenously from Ser and Thr but is in high demand for synthesis of collagen, purines, glutathione, creatine, and choline, among other compounds, especially in growing animals (Wu et al., 2014). Collagen represents more than 25% of total protein in the body (Waterlow, 1984) and of that, Gly accounts for approximately 33% of the AA residues (Gregg and Rogers, 1986). In the physiological concentration range, Gly has been shown to stimulate collagen synthesis in vitro (de Paz-Lugo et al., 2018).

It was hypothesized that supplementation of Gly may be obligatory when feeding low CP diets, and that skin collagen abundance will be a sensitive indicator of Gly adequacy. The objective of this study was to determine the effect of Gly and Ser supplementation in a low CP diet on growth, N retention in carcass and viscera, and skin collagen abundance of pigs during the nursery period.

Materials and Methods

Animals and dietary treatments

The study was conducted at the University of Guelph's Arkell Swine Research Station in Guelph, ON, Canada. The experimental protocol was approved by the University of Guelph Animal Care Committee and followed the Canadian Council of Animal Care guidelines for the care and use of farm animals (CCAC, 2009). Ninety-six, newly weaned Yorkshire × Landrace × Duroc barrows (initial body weight [BW] 6.3 ± 0.5 kg [mean $\pm$ SD]; ~21 d of age) were used for the study. Four pigs were housed per pen, initial BW was balanced among pens, and littermates were delegated to separate pens where possible. Pigs were weighed individually each week of the study for the determination of ADG and feed refusals were weighed each week per pen to determine ADFI.

At weaning (study day 0), pens were randomly allocated to one of three dietary treatments: 1) conventional corn- and soybean meal-based diet (CON); 2) low CP diet supplemented with Gly and Ser (G + S) to the same concentrations as CON; 3) low CP diet supplemented with Glu to maintain the same CP concentration and essential-to-total nitrogen ratio as the G + S diet (GLU; $n = 8$ pens per treatment). All diets were formulated to meet or exceed estimated essential AA requirements (NRC,

2012); dietary treatments were fed over a three-phase feeding program (phase 1 through phase 3; fed for 7, 14, and 14 d, respectively). The CON diet was formulated as a conventional corn- and soybean meal-based diet with the inclusion of easily digestible protein sources (e.g., whey, blood plasma, and blood meal) in phases 1 and 2. The low CP diets were formulated to reduce dietary CP concentration by approximately 4, 6, and 6 percentage units in phases 1, 2, and 3, respectively using cornstarch. Pigs were fed ad libitum and had free access to water throughout the study.

Serial slaughter

Before implementing dietary treatments, eight additional barrows were slaughtered to determine initial body composition. On days 21 and 35, one pig per pen with BW similar to the pen average (eight pigs per treatment) was slaughtered for the determination of physical body composition and carcass and viscera N concentrations; pigs were not fasted before slaughter. On days 21 and 35, pigs were weighed before electrical stunning, after which pigs were exsanguinated via severing major blood vessels in the neck; blood was collected and weighed. The heart, lungs, spleen, pancreas, kidneys, liver (drained gall bladder), and empty bladder were removed and weighed together; the kidneys and liver were also weighed individually. The gastrointestinal tract was weighed separately after the gut contents were removed. The visceral organs (combined with the empty gastrointestinal tract) and the carcass were placed in separate plastic bags per pig and stored at -20°C for 2 wk before grinding. Whole carcasses (including head, skin, hair, feet, and hooves) and viscera were ground separately, as described previously (Totafurno et al., 2019); subsamples of ground carcass and viscera were collected for freeze drying and subsequent N analysis.

Skin collagen abundance

From each slaughtered pig, one skin sample ($4\text{ cm} \times 4\text{ cm}$) was collected from the ham, superficial to the coxal joint. Samples were rinsed thoroughly with saline, blotted dry and placed immediately in a container of 10% buffered formalin solution for preservation. Before embedding, hair was removed with a razor and skin samples were separated with a scalpel between the dermis layer (i.e., connective tissue, hair follicles) and the hypodermis (i.e., subcutaneous fat, blood vessels; Kolarsick et al., 2011). Two subsamples ($2\text{ cm} \times 2\text{ cm} \times 0.2\text{ cm}$) were then collected from the dermis layer of each skin sample (i.e., where collagen resides; Sullivan et al., 2001) and enclosed in cassettes. The Animal Health Histology Lab (University of Guelph, Guelph, ON, Canada) embedded each sample in paraffin, sliced and mounted the sections on microscope slides, and stained the sections using Picrosirius red for the visualization of collagen I and III fibers (Junqueira et al., 1979).

Bright Field microscopy (Leica Microsystems Model: DMR, Concord, ON, Canada) was used to estimate skin collagen abundance; collagen fibers appear red on a pale-yellow background. Images were obtained with a $10\times$ objective lens and were subsequently projected onto a monitor using the Open Lab Imposition software (University of Warwick Science Park, Coventry, England, UK). Five pictures were taken for each sample. Processed collagen fiber images were then analyzed by ImageJ software developed for microscopy (Version 1.50 i; Schneider et al., 2012). To quantify collagen abundance, images were converted to a 32-bit grayscale and an automatic threshold was applied to each individual sample according to the instructions

provided by ImageJ; skin collagen abundance was calculated as a percentage area of each image (Rich and Whittaker, 2005).

Chemical analyses

Diet samples were collected from each 25-kg bag at manufacturing, pooled within diet and phase, and homogenized before analysis. Approximately 100 g of subsampled feed from each diet were shipped to Evonik Nutrition & Care GmbH Analytical Services (Frankfurt, Germany) and analyzed for total AA contents by ion-exchange chromatography coupled with post-column derivatization with ninhydrin (Llames and Fontanie, 1994; AOAC, 2006; Method 982.30). Diet samples were also sent to SGS Agrifood Laboratories (Guelph, ON, Canada) for DM (AOAC, 1997; Method 930.15), P and Ca (AOAC, 1997; Method 985.01), and N (via total combustion; LECO-FP 428; LECO Instruments Ltd., Mississauga, ON, Canada; AOAC, 1997; Method 990.03) analyses. Samples of freeze dried carcass and viscera were also sent to SGS Agrifood Laboratories for DM and N analyses, as described for the diets.

Calculations and statistical analysis

The daily rate of N retention in carcass and viscera was calculated as the difference between the end and beginning of the period in N masses in carcass and viscera, divided by the period length.

All statistical analyses for physical composition, whole-body N retention and utilization, skin collagen abundance and growth performance were conducted using the Proc GLIMMIX function of SAS (v.9.4, SAS Institute, Inc., Cary, NC), with pen as the experimental unit. Dietary treatment was the fixed effect, pig within pen was the random effect, and initial BW was used as a covariate. Differences among individual means were assessed using the Tukey–Kramer post hoc test. Differences with $P < 0.05$ were considered significant, whereas $0.05 \leq P \leq 0.10$ was considered a trend and $P > 0.10$ was considered not significant.

Results

The analyzed CP and AA of the experimental diets were generally comparable with calculated values (Tables 1 and 2). For the G + S and GLU diets, analyzed CP was approximately 12% greater than calculated values for phase 1, which resulted in greater analyzed AA (Lys) concentrations. This deviation may reflect an underestimation of the CP concentration in some of the ingredients used. The analyzed Gly concentration of the CON diet was less than expected, which resulted in ~28% lower Gly concentration for CON than G + S in phase 1. Conversely, the analyzed Gly and Ser concentrations of the GLU diet for all three phases were approximately 33% greater than expected; variability in AA from hydrolyzed feed samples is often large. All pigs used in this experiment remained healthy throughout the study except for one pig from CON that was removed due to lameness.

The initial BW of pigs was not different among treatment groups. The final BW for pigs fed the CON diet was greater than for pigs fed G + S or GLU diets ($P < 0.01$; Table 3). The ADG and ADFI in phases 1 and 3 were not influenced by dietary treatment, but in phase 2 (i.e., between days 8 and 21) and overall (i.e., between days 0 and 35) they were greater for pigs fed CON vs. pigs fed G + S or GLU diets ($P < 0.01$). The G:F was not influenced by dietary treatment in phases 1 or 3 or overall, but in phase 2 tended to be greater for pigs fed the CON diet than for pigs fed

G + S ($P = 0.058$), while intermediate values were observed for pigs fed GLU.

Carcass weights on days 21 (i.e., at the end of phase 2) and 35 (i.e., at the end of phase 3) for pigs fed the CON diet were greater than for pigs fed the G + S or GLU diets ($P < 0.01$; Table 3). Viscera weights on day 21 were greater for pigs fed the CON diet vs. those fed the G + S and GLU diets ($P < 0.05$). On day 35, viscera weights were greater for pigs fed the CON diet vs. those fed G + S ($P < 0.05$), while intermediate values were observed for GLU. On day 21, the weights of blood and empty gastrointestinal tract were greater for pigs fed the CON diet vs. those fed G + S ($P < 0.05$), while intermediate values were observed for GLU. On day 35, the weight of blood was greater for the CON diet vs. those fed G + S ($P < 0.01$), while intermediate values were observed for GLU; there were no differences in gastrointestinal weights on day 35. Liver weights on days 21 and 35 were greater for pigs fed the CON diet than pigs fed GLU ($P < 0.05$), while intermediate values were observed for G + S. Kidney weights were not affected by dietary treatment on day 21, but on day 35 were greater for pigs fed the CON diet vs. pigs fed G + S ($P < 0.05$), while intermediate values were observed for GLU.

Daily N intakes in phases 1 and 2 combined (i.e., between days 0 and 21) and over the entire experimental period (i.e., between days 0 and 35) were greater for pigs fed the CON diet vs. pigs fed G + S or GLU diets ($P < 0.01$; Table 4). In phases 1 and 2 combined, daily carcass N retention was greater for pigs fed the CON diet vs. pigs fed G + S or GLU diets ($P < 0.01$). Over the entire experimental period, carcass N retention rate was greater for pigs fed the CON diet vs. pigs fed G + S ($P < 0.01$), while intermediate values were observed for pigs fed GLU. Viscera N retention rate was not influenced by dietary treatment in phases 1 and 2 combined; over the entire experimental period, viscera N retention tended to be greater for pigs fed the CON diet than pigs fed the G + S diet, while intermediate values were observed for GLU ($P = 0.081$). Combined carcass and viscera N retention in phases 1 and 2 were greater for pigs fed the CON diet compared with pigs fed G + S or GLU diets ($P < 0.01$). Over the entire experimental period, combined carcass and viscera N retention was greater for pigs fed the CON diet vs. those fed the G + S diet, while intermediate values were observed for GLU ($P < 0.01$). The N utilization efficiency for N retention (%) in combined carcass and viscera in phases 1 and 2 tended to be less for pigs fed CON vs. those fed GLU, while intermediate values were observed for pigs fed G + S ($P = 0.095$), but was not influenced by dietary treatment over the entire experimental period. On days 21 and 35, pigs fed CON and G + S diets had greater skin collagen abundance vs. pigs fed GLU ($P < 0.01$; Figure 1).

Discussion

The objectives of the current study were to explore the effect of a low CP diet supplemented with Gly and Ser (G + S) on growth, N retention in carcass and viscera, and skin collagen abundance of nursery pigs. In the current study, it was shown for the first time that pigs fed a low CP diet supplemented with Gly and Ser maintained skin collagen abundance not different to that of pigs fed a high CP (CON) diet, while pigs fed a low CP diet without additional Gly and Ser had reduced skin collagen abundance. In addition to protein retention, NEAA are important for a myriad of other metabolic pathways (Yu et al., 1985). Others have demonstrated that NEAA, specifically Gly, play many important roles in animal metabolism including, but not limited to, synthesis of purines, glutathione, porphyrins, choline,

Table 1. Ingredient composition and nutrient concentrations of a conventional corn- and soybean meal-based diet (CON) or low protein diets with supplemental Gly and Ser (G + S) or Glu (GLU) fed to nursery pigs (as-fed)

Item	Phase 1*			Phase 2			Phase 3		
	Dietary treatment								
	CON	G + S	GLU	CON	G + S	GLU	CON	G + S	GLU
Ingredient composition, %									
Soybean meal	22.00	19.00	19.00	25.00	15.00	15.00	31.57	11.00	11.00
Corn	11.00	21.00	21.00	32.00	60.00	60.00	60.00	74.00	74.00
Cornstarch	3.40	21.45	20.73	9.26	13.21	12.53	2.24	4.91	4.45
Barley	24.00	—	—	10.00	—	—	—	—	—
Whey, dried	25.00	25.00	25.00	10.20	—	—	—	—	—
Blood plasma, AP920	4.80	2.00	2.00	5.00	—	—	—	—	—
Blood meal, spray dried	3.00	2.00	2.00	2.00	—	—	—	—	—
Monocalcium phosphate	1.69	1.70	1.70	1.33	1.40	1.40	0.90	0.90	0.90
Sodium bicarbonate	0.40	0.40	0.40	0.10	0.30	0.30	—	0.40	0.40
Limestone	0.72	0.80	0.80	0.98	1.23	1.23	1.18	1.18	1.18
Salt	0.60	0.60	0.60	0.70	0.60	0.60	0.38	0.42	0.42
Swine premix†	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Animal-vegetable fat	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
L-Lys-HCl	0.14	0.63	0.63	0.17	1.17	1.17	0.38	1.04	1.04
DL-Met	0.15	0.22	0.22	0.13	0.24	0.24	0.14	0.18	0.18
L-Cys-HCl	—	0.18	0.18	—	0.28	0.28	—	0.22	0.22
L-Thr	0.01	0.24	0.24	0.03	0.48	0.48	0.11	0.41	0.41
L-Trp	—	0.03	0.03	—	0.11	0.11	—	0.10	0.10
L-Ile	—	0.16	0.16	—	0.31	0.31	—	0.26	0.26
L-Val	—	0.16	0.16	—	0.43	0.43	—	0.36	0.36
L-Leu	—	0.14	0.14	—	0.46	0.46	—	0.28	0.28
L-His	—	0.07	0.07	—	0.21	0.21	—	0.16	0.16
L-Arg	—	—	-	—	—	—	—	—	—
L-Phe	—	0.16	0.16	—	0.33	0.33	—	0.26	0.26
L-Tyr	—	0.04	0.04	—	0.14	0.14	—	0.11	0.11
L-Glu	—	—	1.65	—	—	1.68	—	—	1.16
L-Gly	—	0.65	—	—	0.52	—	—	0.31	—
L-Ser	—	0.27	—	—	0.48	—	—	0.39	—
Calculated nutrient contents‡									
CP, %	23.65	19.67	19.67	22.84	16.47	16.47	20.54	14.77	14.77
NE, kcal/kg	2,458	2,580	2,581	2,494	2,605	2,607	2,514	2,646	2,637
Standardized ileal digestible amino acid, %									
Lys	1.53	1.53	1.53	1.43	1.43	1.43	1.24	1.24	1.24
Arg	1.21	0.90	0.90	1.25	0.68	0.68	1.22	0.60	0.60
His	0.64	0.53	0.53	0.61	0.50	0.50	0.48	0.43	0.43
Ile	0.81	0.78	0.78	0.78	0.73	0.73	0.74	0.64	0.64
Leu	1.83	1.53	1.53	1.77	1.43	1.43	1.51	1.25	1.25
Met	0.44	0.44	0.44	0.41	0.41	0.41	0.41	0.36	0.36
Met + Cys	0.84	0.84	0.84	0.79	0.79	0.79	0.69	0.69	0.69
Cys	0.40	0.40	0.40	0.37	0.37	0.37	0.28	0.33	0.33
Phe	1.02	0.89	0.89	1.00	0.84	0.84	0.87	0.74	0.74
Phe + Tyr	1.68	1.41	1.41	1.67	1.32	1.32	1.43	1.16	1.16
Thr	0.89	0.89	0.89	0.84	0.84	0.84	0.74	0.74	0.74
Trp	0.31	0.25	0.25	0.29	0.23	0.23	0.22	0.20	0.20
Val	1.13	0.97	0.97	1.04	0.91	0.91	0.80	0.80	0.80
Glu	1.81	1.71	3.34	2.30	1.89	3.55	3.15	1.76	2.90
Gly	1.18	1.18	0.53	0.92	0.92	0.41	0.68	0.68	0.38
Ser	1.00	1.00	0.73	0.97	0.97	0.50	0.85	0.85	0.46
Gly + Ser	2.17	2.17	1.27	1.89	1.89	0.91	1.53	1.53	0.84
E:TN¶	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48

*Phases 1, 2, and 3 were fed between days 0 and 7, 8 and 21, and 22 and 35 after weaning, respectively.

[†]Supplied per kg of complete diet: vitamin A, 10,000 IU as retinyl acetate (2.5 mg) and retinylpalmitate (1.7 mg); vitamin D3, 1,000 IU as cholecalciferol; vitamin E, 56 IU as dl- α -tocopherol acetate (44 mg); vitamin K, 2.5 mg as menadione; choline, 500 mg; pantothenic acid, 15 mg; riboflavin, 5 mg; folic acid, 2 mg; niacin, 25 mg; thiamine, 1.5 mg; vitamin B6, 1.5 mg; biotin, 0.2 mg; vitamin B12, 0.025 mg; Se, 0.3 mg from Na₂SeO₃; Cu, 15 mg from CuSO₄·5H₂O; Zn, 104 mg from ZnO; Fe, 100 mg from FeSO₄; Mn, 19 mg from MnO₂; and I, 0.3 mg from KI (DSM Nutritional Products Canada, Inc., Ayr, ON, Canada).

[‡]Calculated using ingredient values according to the [NRC \(2012\)](#).

^{||}Essential AA N:Total N ratio.

Table 2. Analyzed crude protein, minerals, and total amino acid contents of the experimental diets (% as-fed)

Item	Phase 1*			Phase 2			Phase 3		
	Dietary treatment								
	CON	G + S	GLU	CON	G + S	GLU	CON	G + S	GLU
CP	23.05 (23.65) [†]	21.43 (19.67)	22.14 (19.67)	22.72 (22.84)	17.23 (16.47)	17.78 (16.47)	20.32 (20.54)	14.77 (14.77)	15.01 (14.77)
Ca	0.84	0.86	0.83	0.65	0.77	0.73	0.70	0.61	0.61
P	0.75	0.74	0.77	0.64	0.58	0.61	0.51	0.49	0.50
Lys	1.50 (1.53)	1.67 (1.53)	1.63 (1.53)	1.47 (1.43)	1.50 (1.43)	1.44 (1.43)	1.30 (1.24)	1.25 (1.24)	1.27 (1.24)
Arg	1.30 (1.21)	1.08 (0.90)	1.13 (0.90)	1.33 (1.25)	0.83 (0.68)	0.85 (0.68)	1.28 (1.22)	0.73 (0.60)	0.78 (0.60)
His	0.62 (0.64)	0.58 (0.53)	0.58 (0.53)	0.60 (0.61)	0.49 (0.50)	0.49 (0.50)	0.50 (0.48)	0.43 (0.43)	0.43 (0.43)
Ile	0.91 (0.81)	0.93 (0.78)	0.93 (0.78)	0.88 (0.78)	0.76 (0.73)	0.79 (0.73)	0.82 (0.74)	0.67 (0.64)	0.70 (0.64)
Leu	1.91 (1.83)	1.75 (1.53)	1.77 (1.53)	1.90 (1.77)	1.50 (1.43)	1.57 (1.43)	1.63 (1.51)	1.32 (1.25)	1.38 (1.25)
Met	0.46 (0.44)	0.44 (0.44)	0.44 (0.44)	0.40 (0.41)	0.38 (0.41)	0.36 (0.41)	0.38 (0.41)	0.34 (0.36)	0.32 (0.36)
Phe	1.14 (1.02)	1.06 (0.89)	1.07 (0.89)	1.11 (1.00)	0.89 (0.84)	0.94 (0.84)	0.97 (0.87)	0.78 (0.74)	0.83 (0.74)
Thr	1.04 (0.89)	1.08 (0.89)	1.08 (0.89)	0.99 (0.84)	0.91 (0.84)	0.89 (0.84)	0.82 (0.74)	0.75 (0.74)	0.77 (0.74)
Trp	0.32 (0.31)	0.29 (0.25)	0.30 (0.25)	0.30 (0.29)	0.24 (0.23)	0.25 (0.23)	0.24 (0.22)	0.22 (0.20)	0.21 (0.20)
Val	1.17 (1.17)	1.11 (0.97)	1.12 (0.97)	1.14 (1.04)	0.94 (0.91)	0.96 (0.91)	0.91 (0.80)	0.81 (0.80)	0.84 (0.80)
Glu [‡]	3.70 (1.81)	3.38 (1.71)	4.19 (3.34)	3.61 (2.30)	2.33 (1.89)	3.58 (3.55)	3.39 (3.15)	2.16 (1.76)	3.05 (2.90)
Gly	0.85 (1.18)	1.09 (1.18)	0.72 (0.53)	0.83 (0.92)	0.97 (0.92)	0.54 (0.41)	0.79 (0.68)	0.69 (0.68)	0.51 (0.38)
Ser	1.10 (1.00)	1.07 (1.00)	0.97 (0.73)	1.10 (0.97)	0.93 (0.97)	0.68 (0.50)	0.95 (0.85)	0.82 (0.85)	0.61 (0.46)

*Phases 1, 2, and 3 were fed between days 0 and 7, 8 and 21, and 22 and 35 after weaning, respectively.

[†]Calculated nutrient contents are shown in parentheses.

[‡]During acid hydrolysis the amide N in Gln is removed, converting it to Glu. Therefore, analyzed values presented for Glu combine both Gln and Glu.

creatine, and collagen protein (Wang et al., 2013, Li and Wu, 2018). Collagen is recognized as the most prominent protein in the human body, with skin having the highest collagen content by weight (74%; Schultz, 2011). Of the AA that comprise collagen, 33% are Gly and 13% are Pro (Gregg and Rogers, 1986). Therefore, skin collagen abundance could be used as a sensitive measure of Gly adequacy and availability of Gly for metabolic fates beyond muscle protein synthesis. In addition, collagen enables the skin to resist mechanical stresses and aids in rapid wound healing (Meyer et al., 1982), which are important skin functions for all mammals, including pigs. Previous work demonstrated that Gly was the only NEAA with a positive endogenous synthesis rate when growing pigs were fed a low CP, NEAA-supplemented diet (10.8% CP; Mansilla, 2017) and that marginal intakes of dietary N elicited N conservation that appeared sufficient to maintain the endogenous synthesis of Gly in adult humans (Gibson et al., 2002). These studies indicate that de novo synthesis of Gly is highly conserved. In the current study, however, and for young pigs with high potential for protein synthesis, it is possible that de novo synthesis of Gly was not sufficient to maintain collagen production in pigs fed low CP diets without supplemental Gly and Ser. This observation lends support to the estimation of Meléndez-Hevia et al. (2009) indicating that endogenous Gly synthesis is inadequate to sustain collagen synthesis in adult

humans. The determination of alternative metabolic fates for Gly was beyond the scope of this study. However, specific NEAA are needed for a variety of functions beyond protein synthesis that should be considered when making nutrient recommendations, especially for young animals that have rapid growth rates and a relatively low capacity for feed intake.

The low CP diets (i.e., G + S and GLU) did not maintain ADG or final BW compared to pigs fed a conventional corn- and soybean meal-based diet (CON) in the current study, indicating that one or more AA or total N limited whole-body protein deposition. Combined carcass and viscera N retention were greater for pigs fed CON than those fed the G + S and GLU diets between days 0 and 21 and the efficiency of using dietary N for N retention was high for pigs fed the low CP diets. Therefore, N intake was limiting whole-body protein deposition between days 0 and 21 for pigs fed the low CP diets, which was exacerbated by reduced feed intake during phase 2 (i.e., between days 8 and 21). Conversely, over the entire experimental period (i.e., between days 0 and 35), the N utilization efficiency was below the biological maximum for all treatment groups, indicating that dietary N was no longer limiting protein deposition in the carcass and viscera after day 21. The pigs fed the GLU diet partially recovered the combined carcass and viscera N retention over the entire experimental period (i.e., intermediate retention to CON and G + S between

Table 3. Growth performance and physical body composition of nursery pigs fed a conventional corn- and soybean meal-based diet (CON) or low protein diets with supplemental Gly and Ser (G + S) or Glu (GLU)

Item	Dietary treatment*			SEM†	P value
	CON	G + S	GLU		
Initial BW, kg	6.4	6.5	6.3	0.2	0.610
Final BW, kg	23.1 ^a	21.1 ^b	21.1 ^b	0.3	0.001
ADG, g					
Phase 1; days 0 to 7	128	130	135	14	0.928
Phase 2; days 8 to 21	430 ^a	312 ^b	319 ^b	11	<0.001
Phase 3; days 22 to 35	725	680	681	17	0.130
Overall; days 0 to 35	480 ^a	421 ^b	421 ^b	10	<0.001
ADFI, g					
Phase 1; days 0 to 7	185	177	182	16	0.922
Phase 2; days 8 to 21	578 ^a	482 ^b	462 ^b	21	0.002
Phase 3; days 22 to 35	1,052	1,086	1,070	36	0.798
Overall; days 0 to 35	689	662	649	19	0.347
G:F					
Phase 1; days 0 to 7	0.68	0.72	0.75	0.04	0.493
Phase 2; days 8 to 21	0.75 ^a	0.66 ^b	0.70 ^{ab}	0.03	0.058
Phase 3; days 22 to 35	0.66	0.63	0.63	0.02	0.404
Overall; days 0 to 35	0.70	0.64	0.65	0.02	0.084
Physical body composition					
Day 21					
Carcass, kg	14.3 ^a	12.3 ^b	12.5 ^b	0.4	0.004
Viscera,‡ g	2,185 ^a	1,888 ^b	1,961 ^b	80	0.040
Blood, g	710 ^a	569 ^b	597 ^{ab}	39	0.042
Gastrointestinal tract, g	1,272 ^a	1,058 ^b	1,161 ^{ab}	49	0.019
Liver, g	409 ^a	351 ^{ab}	342 ^b	18	0.037
Kidney, g	86	77	87	4	0.247
Day 35					
Carcass, kg	22.4 ^a	19.0 ^b	20.4 ^b	0.6	0.001
Viscera, g	3,373 ^a	2,912 ^b	3,186 ^{ab}	120	0.015
Blood, g	1,159 ^a	918 ^b	1,079 ^{ab}	44	0.003
Gastrointestinal tract, g	1,886	1,718	1,852	84	0.348
Liver, g	593 ^a	505 ^b	528 ^{ab}	22	0.029
Kidney, g	130 ^a	110 ^b	124 ^{ab}	5	0.039

*Phases 1, 2, and 3 were fed between days 0 and 7, 8 and 21, and 22 and 35 after weaning, respectively.

†Maximum value of the standard error of the means.

‡Viscera included heart, lungs, spleen, pancreas, kidneys, liver (drained gall bladder), and empty bladder and gastrointestinal tract.

^{ab}Means followed by different superscripts in the same row are different according to a Tukey's multiple range test ($P < 0.05$).

days 0 and 35). The partitioning of N was altered however, as pigs fed the GLU diet had intermediate weights of visceral organs but were unable to maintain skin collagen abundance. This is in contrast to pigs fed the G + S diet, as skin collagen abundance was not different from CON at the end of phases 2 (day 21) or 3 (day 35).

In the current study, analyzed dietary CP concentrations were reduced by approximately 5 percentage units in phases 2 and 3 with the Lys:CP ratio reaching 8.5%. Powell et al. (2011) found that supplementation of Gly as a source of N to achieve 1.66% total Gly and Ser to a diet reduced by 5 percentage units of CP improved ADG and G:F for growing pigs (i.e., between 21 and 40 kg BW) but supplementation of Gly to achieve 1.35% total Gly and Ser did not. Therefore, it is possible that dietary supplementation of Gly and Ser greater than the levels used in the current study are required for improved nursery pig (i.e., between 6 and 22 kg BW) growth performance and whole-body protein deposition when CP is reduced by more than 4 percentage units. In addition, Yue and Qiao (2008) reported that growth performance of nursery pigs (7 to 10 kg BW) was not affected when dietary CP concentration was reduced by 4 percentage units (19% vs. 23% CP) and when diets were balanced for all essential AA, but performance was negatively

affected when dietary CP concentration was reduced further to 17% and the Lys:CP ratio reached 8.1%. Similarly, Gloaguen et al. (2014) showed no reduction in growth performance for pigs between 12 and 22 kg when the dietary CP was reduced from 17% to 13% and the Lys:CP ratio was 7.4%. However, increasing the Lys:CP ratio to 8.2% with a further reduction of dietary CP to 12% reduced growth performance. The Lys:CP ratios in G + S and GLU diets used in the current study were 7.7%, 8.7%, and 8.4% in phases 1, 2, and 3, respectively, which were higher than the optimum 7.4% indicated by Gloaguen et al. (2014). These elevated Lys:CP ratios suggest that supplementation of Gly + Ser or Glu to the low CP diets was not sufficient to meet the NEAA needs for growth and N retention for these pigs.

Currently, the NRC (2012) does not provide specific recommendations for NEAA in any stage of production for swine, despite evidence supporting the notion that dietary Gly, along with other NEAA, are necessary to maximize protein accretion, particularly in young pigs (Wang et al., 2014a, 2014b). With the continuous genetic improvement of swine for leaner and faster growing phenotypes, and the potential use of low CP diets to alleviate competition between humans and livestock for dietary protein sources and to reduce environmental N

Table 4. Whole-body N retention and utilization of nursery pigs fed a conventional corn- and soybean meal-based diet (CON) or low protein diets with supplemental Gly and Ser (G + S) or Glu (GLU)

Item	Dietary treatment*			SEM†	P value
	CON	G + S	GLU		
Digestible N intake,‡ g/d					
Phase 1 and 2; days 0 to 21	14.3 ^a	9.2 ^b	9.1 ^b	0.5	<0.001
Overall; days 0 to 35	22.0 ^a	16.1 ^b	16.1 ^b	0.6	<0.001
Carcass N retention, g/d					
Phase 1 and 2; days 0 to 21	9.2 ^a	7.1 ^b	7.0 ^b	0.5	0.006
Overall; days 0 to 35	11.8 ^a	8.6 ^b	9.8 ^{ab}	0.7	0.009
Viscera N retention, g/d					
Phase 1 and 2; days 0 to 21	1.3	1.0	1.1	0.2	0.134
Overall; days 0 to 35	1.5	1.2	1.3	0.1	0.081
Carcass + viscera N retention, g/d					
Phase 1 and 2; days 0 to 21	10.5 ^a	8.1 ^b	8.1 ^b	0.6	0.007
Overall; days 0 to 35	13.3 ^a	9.9 ^b	11.1 ^{ab}	0.7	0.007
Apparent N utilization efficiency, %					
Phase 1 and 2; days 0 to 21	73.8	84.3	91.6	5.5	0.095
Overall; days 0 to 35	61.2	61.0	69.2	4.5	0.362

*Phases 1, 2, and 3 were fed between days 0 and 7, 8 and 21, and 22 and 35 after weaning, respectively.

†Maximum value of the standard error of the means.

‡Estimated using predicted N digestibility; [NRC, 2012](#).

^{ab}Means followed by different superscripts in the same row are different according to a Tukey's multiple range test ($P < 0.05$).

Figure 1. Skin collagen abundance of nursery pigs on days 21 (end of phase 2) and 35 (end of phase 3) after weaning fed a conventional corn- and soybean meal-based diet (CON) or low protein diets with supplemental Gly and Ser (G + S) or Glu (GLU).

excretion, the term “nonessential” amino acids should be reevaluated to encompass nutritional, metabolic, and functional needs and considering specific physiological states (i.e., [Reeds, 2000](#)). The reduction in dietary protein concentration requires careful assessment of the NEAA and N needs, since the dietary and endogenous supplies of these compounds are reduced when replacing whole-protein sources with individual CAA. The reduction in dietary NEAA may have consequences for multiple biosynthetic pathways (e.g., collagen production) and the availability of NEAA for protein retention in skeletal muscle, which have unknown long-term consequences for the pig.

In summary, supplementing low CP diets with either Gly and Ser or Glu at the levels used in the current study was insufficient to support ADG and N retention in combined carcass and viscera of nursery pigs. Only Gly and Ser supplementation to nursery diets reduced by 5 percentage units of CP supported skin collagen abundance not different from pigs fed conventional

corn- and soybean meal-based nursery diets. The importance of meeting essential AA requirements for growth are well accepted, but supplementing specific NEAA may be needed when feeding reduced CP diets to newly weaned pigs to support secondary indicators of AA status, such as skin collagen abundance, and growth performance.

Conflict of interest statement

J.K.H. is an employee of Evonik Nutrition & Care GmbH. Remaining authors declare that they have no conflicts of interest.

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