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Short communication

Apparent ileal digestibility of amino acids by pigs is not affected by increasing dietary calcium from deficient to excess concentrations, but phosphorus digestibility is reduced

S.A Lee [1], H.H. Stein [*,2]

Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA

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ABSTRACT

The objective was to test the hypothesis that the concentration of calcium (Ca) in diets affects apparent ileal digestibility (AID) of amino acids (AA) by pigs due to interactions between Ca, phosphorus (P), and AA. Six corn-soybean meal-based diets with Ca included at 50–175 % of the requirement were formulated. Dietary Ca was increased by increasing inclusion levels of calcium carbonate, whereas the concentration of P was constant among diets. Six growing barrows (initial body weight: 81.43 ± 1.14 kg) were equipped with a T-cannula in the distal ileum and allotted to a 6 × 6 Latin square design with 6 diets. Ileal digesta were collected and analyzed for Ca, P, crude protein, and AA. The statistical model included diet as fixed variable and period and animal as random effects. Contrast coefficients were used to test linear and quadratic effects of increasing dietary Ca. The AID of dry matter was not affected by the level of Ca in the diets. The AID of Ca linearly ($P < 0.001$) increased, but the AID of P decreased in a quadratic manner ($P = 0.022$) with increasing Ca in diets. The AID of crude protein and all indispensable and dispensable AA was not affected by dietary Ca levels.

1. Introduction

Dietary interactions between calcium (Ca) and phosphorus (P) include precipitation of Ca and P in the intestinal tract of pigs and the chelation of Ca ions to phytate, which result in a reduction of P digestibility (Stein et al., 2011; Lee et al., 2020). Previous data indicate that Ca is usually over-formulated in diets for pigs and poultry (Walk, 2016). It is also possible that digestible amino acids (AA) in diets are affected by dietary Ca and if that is the case, the interaction between dietary Ca and digestible AA needs to be considered in diet formulation. Phytate may also reduce AA digestibility (Hill and Tyler, 1954; Cowieson et al., 2017; Misiura et al., 2018) because of Ca-phytate complexes that may reduce AA digestibility. However, adding Ca to diets may increase activation of proteases as co-factors (Konietzny and Greiner, 2003), which potentially results in increased AA digestibility. In contrast, pH in the digesta may be increased by adding Ca to diets, which likely will have a negative effect on activation of protein-digesting enzymes (Pontoppidan et al., 2007). Thus, there are several factors related to dietary Ca concentration that may influence digestibility of AA. However, to our knowledge,

Abbreviations: AA, amino acids; AID, apparent ileal digestibility; Ca, calcium; DM, dry matter; P, phosphorus.

* Corresponding author.
E-mail address: hstein@illinois.edu (H.H. Stein).
[1] https://orcid.org/0000-0001-9351-7196.
[2] https://orcid.org/0000-0002-4855-661X.

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no data demonstrating the effects of increasing dietary Ca on digestibility of AA in pigs have been reported. Therefore, the objective of this experiment was to test the hypothesis that the concentration of Ca in diets affects apparent ileal digestibility (AID) of AA by pigs.

2. Materials and methods

The protocol was submitted to the Institutional Animal Care and Use Committee at the University of Illinois (Urbana, IL, USA) and approved prior to the initiation of the experiment (protocol #: 18190). Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used and pigs remained healthy during the experiment.

2.1. Diets and feeding

Six corn-soybean meal-based diets containing Ca from 50 % to 175 % of the requirement were formulated (Tables 1 and 2). Dietary Ca was increased by increasing inclusion levels of calcium carbonate. All diets were formulated to contain P at the requirement for standardized total tract digestible P by 75–100 kg pigs (i.e., 2.4 g/kg; NRC, 2012). Likewise, AA were included at or above the requirement for 75–100 kg pigs (NRC, 2012). Vitamins and minerals except Ca were included in all diets to meet or exceed current requirement estimates. All diets also contained 4.0 g/kg chromic oxide as an indigestible marker. Pigs were fed their respective diets at 3.2 times the maintenance requirement for metabolizable energy (i.e., 197 kcal metabolizable energy per kg body weight$^{0.60}$). Water was available at all times.

2.2. Animal, housing, and sample collection

Six growing barrows (initial body weight: 81.43 ± 1.14 kg) were equipped with a T-cannula in the distal ileum (Stein et al., 1998) and allotted to a 6 × 6 Latin square design with 6 diets and six 7-day periods in each square. Therefore, there were 6 replicate pigs per diet. Pigs were cannulated when they had a body weight of approximately 20 kg and had been used in a different experiment prior to being allotted to diets in this experiment. A non-experimental corn-soybean meal diet was fed to all pigs for 12 days between the 2 experiments. Pigs were individually housed in a research facility that was constructed to house cannulated pigs. Pens (1.2 × 1.5 m) had a feeder, a drinking nipple, and fully slatted floors. Pig weights were recorded at the beginning of each period and the end of experiment. The average final body weight of pigs was 124.27 kg.

Each experimental period lasted 7 days. The initial 5 days of each period were considered an adaptation period. Ileal digesta were collected on days 6 and 7 for 9 h using standard procedures (Stein et al., 1998). Cannulas were opened at the beginning of collection and a 225-mL plastic bag was attached to the cannula barrel using a cable tie. Digesta flowing into the bag were collected and bags were replaced whenever they were full or at least once every 30 min. All samples were stored at − 20 °C after collection. At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was collected for analysis. Ileal digesta samples were lyophilized and finely ground using a coffee grinder.

Table 1
Composition of experimental diets, as-fed basis.

	Ca concentration, % of the requirement					
Item	50	75	100	125	150	175
Ingredient, g/kg						
Ground corn	713.7	713.7	713.7	713.7	713.7	713.7
Soybean meal, 480 g/kg crude protein	230.0	230.0	230.0	230.0	230.0	230.0
Soybean oil	20.0	20.0	20.0	20.0	20.0	20.0
Cornstarch	16.6	13.3	10.0	6.6	3.3	–
Calcium carbonate	0.2	3.5	6.8	10.2	13.5	16.8
Dicalcium phosphate	6.5	6.5	6.5	6.5	6.5	6.5
Sodium chloride	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral premix[a]	5.0	5.0	5.0	5.0	5.0	5.0
Chromic oxide	4.0	4.0	4.0	4.0	4.0	4.0
Calculated composition						
Metabolizable energy[b], MJ/kg	14.30	14.25	14.19	14.13	14.08	14.02

[a] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1660 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_D$-pantothenic acid as $_D$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

[b] Calculated from NRC (2012).

Table 2
Analyzed nutrient composition of experimental diets, as-fed basis.

	Ca level, % of the requirement					
Item, g/kg	50	75	100	125	150	175
Dry matter	877.1	878.0	882.7	880.9	876.1	880.7
Calcium	2.2	3.3	4.9	6.1	6.8	8.7
Phosphorus	4.7	4.5	4.5	4.7	4.4	4.5
Crude protein	160.7	153.6	159.8	154.0	160.4	156.0
Indispensable amino acids						
Arg	10.1	9.9	9.6	9.9	10.8	10.3
His	4.3	4.2	4.0	4.2	4.5	4.3
Ile	7.3	7.1	6.8	6.9	7.5	7.2
Leu	14.7	14.5	13.7	13.8	14.6	14.2
Lys	8.6	8.4	8.1	8.5	9.2	8.8
Met	2.7	2.5	2.3	2.4	2.6	2.5
Phe	8.3	8.1	7.7	7.9	8.5	8.2
Thr	6.1	6.0	5.8	5.9	6.4	6.1
Trp	2.3	2.6	2.5	2.4	2.4	2.5
Val	8.0	7.9	7.7	7.8	8.4	8.1
Total	72.4	71.2	68.2	69.7	74.9	72.2
Dispensable amino acids						
Ala	8.7	8.6	8.2	8.2	8.7	8.5
Asp	16.2	15.8	15.2	15.6	17.0	16.2
Cys	2.9	2.6	2.4	2.6	2.6	2.7
Glu	29.8	29.3	27.6	28.3	30.6	29.4
Gly	6.8	6.7	6.5	6.7	7.2	6.9
Pro	9.9	10.0	9.5	9.4	10.2	9.7
Ser	6.8	6.9	6.4	6.6	7.1	6.9
Tyr	5.6	5.5	5.3	5.3	5.7	5.6
Total	86.7	85.4	81.1	82.7	89.1	85.9
Total amino acids	161.1	158.7	151.5	154.6	166.1	160.3

Table 3
Coefficients for apparent ileal digestibility of dry matter, calcium, phosphorus, and amino acids.[a]

	Ca level, % of the requirement							P-value	
Item	50	75	100	125	150	175	SEM	Linear	Quadratic
Dry matter	0.744	0.728	0.748	0.753	0.732	0.746	0.011	0.772	0.890
Calcium	0.420	0.415	0.549	0.575	0.555	0.645	0.034	<0.001	0.602
Phosphorus	0.450	0.378	0.365	0.362	0.337	0.376	0.022	0.014	0.022
Crude protein	0.802	0.797	0.807	0.805	0.794	0.804	0.010	0.972	0.908
Indispensable amino acids									
Arg	0.904	0.900	0.901	0.899	0.894	0.901	0.005	0.160	0.319
His	0.865	0.854	0.854	0.859	0.845	0.856	0.008	0.156	0.317
Ile	0.828	0.829	0.831	0.834	0.819	0.832	0.009	0.860	0.861
Leu	0.847	0.845	0.847	0.850	0.835	0.846	0.008	0.473	0.972
Lys	0.809	0.814	0.823	0.824	0.810	0.821	0.010	0.511	0.403
Met	0.854	0.848	0.858	0.853	0.841	0.853	0.010	0.618	0.951
Phe	0.838	0.836	0.837	0.840	0.823	0.837	0.008	0.420	0.804
Thr	0.738	0.742	0.751	0.753	0.731	0.743	0.013	0.938	0.405
Trp	0.846	0.832	0.844	0.862	0.836	0.852	0.012	0.447	0.996
Val	0.796	0.795	0.798	0.803	0.783	0.795	0.011	0.620	0.729
Total	0.834	0.832	0.836	0.839	0.824	0.835	0.008	0.697	0.865
Dispensable amino acids									
Ala	0.776	0.780	0.788	0.792	0.772	0.784	0.012	0.799	0.427
Asp	0.811	0.810	0.814	0.816	0.801	0.811	0.008	0.596	0.735
Cys	0.719	0.723	0.739	0.739	0.721	0.735	0.016	0.511	0.482
Glu	0.858	0.855	0.862	0.856	0.850	0.860	0.009	0.840	0.853
Gly	0.657	0.659	0.669	0.665	0.647	0.654	0.023	0.723	0.622
Pro	0.807	0.794	0.806	0.802	0.785	0.771	0.021	0.056	0.295
Ser	0.819	0.817	0.821	0.821	0.802	0.816	0.008	0.247	0.812
Tyr	0.837	0.837	0.841	0.841	0.824	0.837	0.008	0.460	0.809
Total	0.811	0.808	0.815	0.814	0.800	0.807	0.009	0.474	0.626
Total amino acids	0.818	0.815	0.821	0.822	0.807	0.817	0.009	0.568	0.700

SEM = standard error of the means.

[a] Least mean squares of each diet represent 6 observations, respectively, with the exception of the 2 diets containing Ca at 50 % and 125 % of requirement for pigs ($n = 5$).

2.3. Chemical analysis

Samples of diet and ileal digesta samples were analyzed for dry matter (DM; method 930.15; AOAC Int, 2019). Nitrogen in diet and ileal digesta samples was determined using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI, USA; AOAC Int, 2019; method 990.03), and crude protein was calculated as analyzed N × 6.25. All diets and ileal digesta samples were analyzed for AA on a Hitachi Amino Acid Analyzer, Model No. L8800 [Hitachi High Technologies America, Inc; Pleasanton, CA, USA; methods 982.30 E(a,b, c); AOAC Int, 2019]. Concentrations of Ca and P in diet and ileal digesta samples were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; AOAC Int, 2019) and wet digestion with nitric acids (method 3050 B; U.S. Environmental Protection Agency, 1996). Chromium in diet and ileal digesta samples was analyzed using Inductive Coupled Plasma Atomic Emission Spectrometric method (method 990.08; AOAC Int, 2019).

2.4. Calculations and statistical analysis

Apparent ileal digestibility of DM, Ca, P, crude protein, and AA was calculated for all diets (Stein et al., 2007). Data were analyzed using the PROC MIXED procedure (SAS, 2018). The model included diet as fixed effect and period and animal as random effects. Mean values were calculated using the LSMeans statement. Polynomial contrasts were used to test for linear and quadratic effects of increasing dietary Ca. Pig was the experimental unit and results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.10$.

3. Results

Pigs remained healthy during the experiment and very limited feed refusals were observed. The AID of DM was not affected by the concentration of Ca in diets (Table 3). The AID of Ca increased (linear, $P < 0.001$), but the AID of P decreased (quadratic, $P = 0.022$) with increasing Ca in diets. The AID of crude protein and all indispensable and dispensable AA was not affected by dietary Ca concentration.

4. Discussion

Analyzed crude protein, AA, Ca, and P in all diets agreed with calculated values. Likewise, the AID of AA, Ca, and P agreed with values for a corn-soybean meal diet (NRC, 2012; Zhang et al., 2016).

Commercial diets for pigs and poultry contain on average 220 g per kg more Ca than formulated (Walk, 2016). The reason for this excess likely is that the concentration of Ca in some raw materials is underestimated and that Ca sometimes is used as a carrier in feed additives, which may not be appreciated in the formulation. An excess of Ca in diets results in significant reductions in average daily gain of weanling pigs (Gonzalez-Vega et al., 2016a; Lagos et al., 2019a) as well as in growing-finishing pigs (Gonzalez-Vega et al., 2016b; Merriman et al., 2017; Lagos et al., 2019b). The reason for this reduction in growth performance of pigs has not been determined and we, therefore, hypothesized that Ca may reduce AA digestibility and thereby reduce the gain of pigs.

Phytate may reduce AA digestibility because phytate sometimes interacts with endogenous losses of AA, dietary proteins, and digestive enzymes (Hill and Tyler, 1954; Cowieson et al., 2017). This may be a consequence of the fact that Ca ions are needed as co-factors for activation of proteases (Konietzny and Greiner, 2003) and the binding of Ca to phytate prevents this activation. Therefore, it is possible that the addition of Ca to diets increases activation of proteases. However, it is also possible that high Ca in diets increases pH of the digesta, which may negatively affect activation of proteases (Pontoppidan et al., 2007). Regardless, if activation of proteases is influenced by dietary Ca concentrations it is likely that digestibility of AA also is impacted by dietary Ca. However, the observation from this experiment that there was no impact of dietary Ca on AA digestibility demonstrates that the impact of Ca on AID of AA is not measurable under the conditions of this experiment where dietary P was constant among diets.

The observation that the AID of P decreased and the AID of Ca increased by increasing dietary Ca was in agreement with previous data (Stein et al., 2011; Lee et al., 2020). The reduction in the AID of P is a consequence of the interactions among Ca, P, and phytate that take place in the small intestine and result in precipitation of Ca-P complexes in the intestinal tract of pigs. As a consequence, excess dietary Ca may indirectly result in a deficiency of absorbed P because digestibility of P is reduced. It is, therefore, necessary that diets that contain excess Ca also contain P above the requirement to prevent P from being deficient.

Calcium may also be chelated to phytate to form non-digestible Ca-phytate complexes (Nelson and Kirby, 1987; Selle and Ravindran, 2008). Nevertheless, the reason for the increase in the AID of Ca by increasing dietary Ca is that the endogenous loss of Ca contributes to a reducing proportion of Ca in the ileal digesta as dietary Ca increases.

5. Conclusion

Apparent ileal digestibility of amino acids was not influenced by increasing dietary calcium from deficient levels to levels that were well in excess of the requirement. Results, therefore, implied that the negative effects of excess calcium on growth performance of pigs that have been reported from numerous experiments are not a result of reduced digestibility of amino acids. However, the negative effect of excess calcium on the digestibility of phosphorus was confirmed in this experiment. It is, therefore, most likely that the reduced growth performance of pigs fed diets containing excess calcium is a result of a phosphorus deficiency caused by reduced absorption of phosphorus.

Author contributions

SAL and **HHS** conceptualized the experiments. **SAL** conducted the experiment and summarized data. **HHS** contributed with data interpretation. **SAL** wrote the first draft of the manuscript. **HHS** edited the final version of the manuscript. **HHS** supervised the project.

Conflict of interest

The authors have no conflicts of interest.

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