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Research Report

Effects of a direct fed microbial and enzyme premix on ileal digestibility of amino acids, fat, and starch, and total tract digestibility of GE and fiber in diets fed to growing pigs

Maryane S. F. Oliveira and Hans H. Stein

University of Illinois

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OBJECTIVE

The objective of this experiment was to determine the apparent ileal digestibility (**AID**) of amino acids, fat, and starch, and apparent total tract digestibility (**ATTD**) of GE and fiber in diets supplemented with direct fed microbials or enzyme premix fed to growing pigs.

MATERIALS AND METHODS

The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign.

One source of direct fed microbials (**DFM**) and one source of an enzyme premix were provided by Carval animal nutrition. The basal diet was formulated based on corn and soybean meal and 2 additional diets were formulated by adding either 0.01% DFM or 0.01% enzyme premix to the basal diet. Vitamins and minerals were included in all diets to meet or exceed the estimated nutrient requirements for growing pigs (NRC, 2012). All diets also contained 0.40% titanium dioxide as an indigestible marker.

Twenty-four growing barrows that were the offspring of Line 359 boars mated to Camborough sows (Pig Improvement Company, Hendersonville, TN) with an average initial BW of 58.7 ± 9.7 kg that had a T-cannula installed in the distal ileum were allotted to a randomized complete block design with 24 pigs and 3 diets for a total of 8 pigs per diet. Pigs were housed in metabolism crates in an environmentally controlled room. Crates had smooth sides and fully slatted floors. A screen floor and a urine funnel were installed below the slatted floor and a feeder and a nipple drinker were installed in each crate. All pigs were fed their assigned diets in a daily amount of 3.4 times the estimated energy requirement for maintenance (i.e., 197 kcal ME per kg$^{0.60}$; NRC, 2012). Two equal meals were provided every day at 0800 and 1600 h. Water was available at all times.

The initial 12 days were considered an adaptation period to the diet. A color marker was included in the feed that was provided in the morning on d 13 and again in the diet that was provided in the morning of d 18. Fecal collections were initiated upon appearance of the first color marker in the feces and ceased upon appearance of the second color marker using the marker to marker approach (Adeola, 2001). Urine collections started 2 hours after feeding the morning meal on d 13 and ceased 2 hours after feeding the morning meal on d 18.

Ileal digesta was collected for 9 hours (from 0800 to 1700 h) on days 20 and 21 using standard operating procedures. In short, a plastic bag was attached to the cannula barrel and digesta flowing into the bag was collected. Ileal digesta was frozen at – 20°C to prevent bacterial degradation of the AA in the digesta.

At the conclusion of the experiment, ileal, fecal and urine samples were thawed, mixed within animal and diet, and a sub-sample was collected for chemical analysis. Ileal digesta samples were lyophilized and finely ground prior to chemical analysis. Urine samples were also lyophilized and fecal samples dried in a forced air oven at 65°C and then ground using a Wiley Mill with a 1 mm screen.

Diets, ileal digesta, and fecal samples were analyzed for DM (method 930.15; AOAC Int., 2007). Diets and ileal digesta were also analyzed for CP by combustion (method 999.03; AOAC Int., 2007) using a Rapid N cube (Elementar Americas Inc, Mt. Laurel, NJ) with aspartic acid as the internal standard. Diets and ileal digesta samples were analyzed for AA as well on a Hitachi Amino Acid Analyzer (Model L8800, Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for post-column derivatization and nor-leucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C, but methionine and cysteine were analyzed as methionine sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis and tryptophan was determined after NaOH hydrolysis for 22 h at 110°C [method 982.30 E (a, b, c); AOAC Int., 2007]. Diets and ileal digesta were also analyzed for acid-hydrolyzed ether extract (**AEE**) using 3*N* HCl (Ankom^{HCl}, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (Ankom^{XT15}, Ankom Technology, Macedon, NY), and diets and ileal digesta were analyzed for starch as well using the glucoamylase procedure (method 979.10; AOAC Int., 2007). Diets and ileal digesta samples were analyzed for titanium as well (Myers, et al., 2004) and diets, ileal digesta samples, fecal samples, and urine samples were analyzed for GE on an isoperibol bomb calorimeter (Model 6300, Parr Instruments, Moline, IL) using benzoic acid as the internal standard. Diets and fecal samples were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY).

The AID values for AA, AEE and starch in each diet was calculated using equation [1] (Stein et al., 2007):

$$\text{AID (\%)} = [1 - [(\text{Nd}/\text{Nf}) \times (\text{Tif}/\text{Tid})] \times 100 \qquad [1]$$

where AID is the apparent ileal digestibility value of an nutrient (%), Nd is the concentration of that nutrient in the ileal digesta DM, Nf is the N concentration of that N in the feed DM, Tif is the titanium concentration in the feed DM, and Tid is the titanium concentration in the ileal digesta DM.

The ATTD of GE and fiber was also calculated (NRC, 2012). The energy lost in feces and urine was calculated and the quantities of DE and ME in each diet was calculated as well (Adeola, 2001).

Data were analyzed using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC) with the pig as the experimental unit. The statistical model included diet as the main effect and pig as random effect. Treatment means were separated by using the LSMEANS statement and the PDIFF option of PROC MIXED. Statistical significance and tendency was onsidered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

There was no influence by the supplementation of DFM or enzyme premix in the diets on AID of DM, AEE, starch, and amino acids (Table 3). However the AID of CP had a tendency to be lower ($P = 0.06$) in pigs fed the basal diet compared with pigs fed diets supplemented with DFM or enzyme premix. Likewise, the addition of DFM or enzyme premix to the diets did not affect the daily feed intake, daily DM intake, daily GE intake, fecal output, and excretion of GE in feces (Table 4), but the excretion of GE in urine was greater ($P < 0.05$) in pigs fed diets containing DFM compared with other two diets. The ATTD of GE, DM, ADF, and NDF, and DE in diets were not influenced by the supplementation of DFM or enzyme premix in the diets. However, the ME in the diet supplemented with DFM had a tendency to be lower ($P = 0.06$) than in the other two diets.

In conclusion, the apparent ileal digestibility of dry matter, fat, starch, and amino acids, and the apparent total tract digestibility of energy and fiber were not affect by the supplementation of DFM or enzyme premix in the diets.

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Table 1. Ingredient composition of the control diet, as-fed basis[1]

Ingredient, %	Control
Ground corn	67.00
Soybean meal, 48% CP	28.15
Soybean oil	2.00
Ground limestone	0.90
Dicalcium phosphate	1.00
Sodium chloride	0.40
Vitamin-Mineral premix[2]	0.15
Titanium dioxide	0.40
Total	100.00

[1]Two additional diets were formulated by adding 0.01% of either DFM or enzyme premix to the basal diet.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganoussulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

Table 2. Analyzed composition of the experimental diets, as-fed basis[1]

Item	Basal Diet	Basal + DFM	Basal + enzyme premix
DM, %	91.16	91.20	91.13
GE, kcal/kg	3,875	3,877	3,899
CP, %	16.14	16.67	17.03
AEE, %	5.94	6.45	6.47
Starch, %	40.01	39.58	45.98
ADF, %	2.47	2.64	3.15
NDF, %	6.94	7.04	8.32
Indispensable AA, %			
Arg	1.18	1.12	1.11
His	0.48	0.45	0.45
Ile	0.82	0.77	0.78
Leu	1.54	1.49	1.47
Lys	1.01	0.95	0.96
Met	0.27	0.24	0.25
Phe	0.93	0.89	0.89

Thr	0.67	0.64	0.64
Trp	0.20	0.19	0.21
Val	0.90	0.85	0.85
Dispensable AA			
Ala	0.90	0.87	0.86
Asp	1.81	1.71	1.72
Cys	0.30	0.26	0.26
Glu	3.21	3.05	3.03
Gly	0.76	0.71	0.72
Pro	1.06	1.03	1.01
Ser	0.77	0.74	0.74
Tyr	0.62	0.62	0.60

Table 3. Apparent ileal digestibility (AID) of dry matter, crude protein, acid hydrolyzed ether extract, starch, and amino acids in diets supplement with Direct Fed Microbials (DFM) or enzyme premix[1]

Item	Basal Diet	Basal + DFM	Basal + enzyme premix	SEM	*P*-value
Dry matter	72.3	73.5	73.4	0.71	0.480
Crude protein	72.2	76.0	76.6	1.33	0.065
AEE[2]	68.7	63.2	68.6	3.28	0.197
Starch	94.7	94.5	96.0	0.67	0.227
Indispensable AA					
Arg	89.9	90.9	90.4	0.47	0.338
His	85.2	86.4	86.5	0.59	0.146
Ile	79.9	81.0	81.4	1.01	0.615
Leu	81.3	82.7	82.6	0.87	0.472
Lys	80.4	80.9	81.8	0.93	0.590
Met	83.5	82.7	84.0	1.12	0.692
Phe	81.2	82.5	82.7	0.91	0.483
Thr	70.3	72.7	71.8	1.26	0.403
Trp	80.3	80.0	82.1	1.00	0.294

Val	75.6	76.4	76.6	1.04	0.815
Mean	81.1	82.2	82.3	0.84	0.586
Dispensable AA					
Ala	73.8	75.0	74.6	1.54	0.854
Asp	76.9	79.0	78.5	1.11	0.422
Cys	71.4	70.6	71.4	1.43	0.914
Glu	80.7	83.0	82.6	1.61	0.409
Gly	61.4	66.8	65.2	1.83	0.132
Pro	78.3	81.5	80.5	0.94	0.084
Ser	80.0	80.9	80.4	0.92	0.777
Tyr	81.6	83.6	83.0	0.71	0.147
Mean	77.6	79.9	79.4	1.12	0.354
All AA	79.2	80.9	80.7	0.98	0.437

[1]Each least squares mean represents 8 observations, except for Basal diet (n=7).

[2]AEE = acid hydrolyzed ether extract.

Table 4. Apparent total tract digestibility (ATTD) of dry matter, gross energy, acid detergent fiber, neutral detergent acid, and DE and ME in diets supplement with Direct Fed Microbials (DFM) or enzyme premix[1]

Item	Basal Diet	Basal + DFM	Basal + enzyme premix	SEM	*P*-value
Daily feed intake, kg	2.26	2.22	2.21	0.08	0.863
Daily DM intake, kg	2.06	2.02	2.01	0.07	0.861
Daily GE intake, kcal	8,791	8,610	8,606	319.37	0.900
Daily fecal output, g	199	206	209	15.11	0.905
GE in feces, kcal	888	899	939	72.2	0.871
Daily urine output, kg	4.44	5.36	3.16	0.74	0.134
GE in urine, kcal	197[b]	162[b]	161[b]	66.70	0.011
ATTD of GE, %	89.60	89.27	88.80	0.75	0.679
ATTD of DM, %	90.55	90.20	89.90	0.61	0.709
ATTD of ADF, %	61.59	62.04	65.03	3.19	0.705
ATTD of NDF, %	59.52	61.58	63.35	3.05	0.685
DE, diet, kcal/kg	3,472	3,462	3,462	29.35	0.949
ME, diet, kcal/kg	3,360	3,273	3,401	38.21	0.063

[a-b]Within a row, means without a common superscript are different ($P < 0.05$).

[1]Each least squares mean represents 8 observations, except for Basal diet (n = 7).