Effects of *Bacillus amyloliquefaciens* and *Bacillus subtilis* on ileal digestibility of AA and total tract digestibility of CP and gross energy in diets fed to growing pigs¹

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ABSTRACT: The objective of this experiment was to test the hypothesis that the apparent ileal digestibility (AID) of AA, CP, and GE, the apparent total tract digestibility (ATTD) of CP and GE, and the apparent hindgut digestibility of CP and GE by growing pigs may be improved by supplementing diets with two direct-fed microbials (DFM) containing different *Bacillus* strains. Twenty-four growing barrows (initial BW: 22.69 ± 1.48 kg) that had a T-cannula installed in the distal ileum were individually housed and randomly allotted to a three diet, three period design with 24 pigs and three 21-d periods. There were eight pigs per diet in each period for a total of 24 observations per diet. Three diets that were based on corn, soybean meal, and distillers dried grains with solubles were formulated. The control diet contained no DFM, but two additional diets contained two different *Bacillus* strains (*Bacillus amyloliquefaciens* or *Bacillus subtilis*). Feed was provided in mash form in two daily meals at 0800 and 1600 hours. The initial 12 d of each period was the adaptation period to the diet. Fecal and urine samples were collected from days 13 to 18, and ileal digesta were collected for 8 h on days

20 and 21. Results indicated that there were no differences among diets in ATTD of CP, but the AID of CP was reduced ($P < 0.05$) for the *B. subtilis* diet compared with control and *B. amyloliquefaciens* diets. Therefore, the apparent hindgut digestibility of CP was greater ($P < 0.005$) in pigs fed the *B. subtilis* diet compared with the other diets. The AID of total indispensable, total dispensable, and total AA was greater ($P < 0.05$) in the *B. amyloliquefaciens* diet compared with the control diet. There were no differences among diets in ATTD of GE, but the AID of GE was greater for the *B. amyloliquefaciens* diet than for the control and the *B. subtilis* diets ($P < 0.001$). Therefore, the apparent hindgut digestibility of GE was less ($P < 0.05$) in the *B. amyloliquefaciens* diet compared with the other diets. The DE (DM basis) for the *B. subtilis* diet was greater ($P < 0.05$) compared with the control and the *B. amyloliquefaciens* diets. In conclusion, supplementation of *Bacillus* spp. to diets fed to growing pigs may increase the AID of AA and GE, but there appears to be differences among strains of *Bacillus* spp. in their impact on AA and energy digestibility.

Key words: amino acids, digestibility, direct-fed microbials, energy, pig

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INTRODUCTION

Since the ban of antibiotics as growth promoters in the European Union in 2006, there has been increased interest in using direct-fed microbials (DFM) to support intestinal

health and growth performance of pigs. DFM are defined as live microorganisms that offer a health benefit for the host when administered in appropriate and regular quantities (Chaucheyras-Durand and Durand, 2010). Some commonly used DFM strains of bacteria are *Lactobacillus* spp., *Bifidobacterium* spp., *Propionibacterium* spp., *Enterococcus* spp., *Pediococcus* spp., and *Bacillus* spp. (Buntyn et al., 2016).

Addition of *Bacillus*-based DFM to diets may result in improved feed efficiency by weanling pigs (Cai et al., 2015; Jaworski et al., 2017) and growing pigs (Jørgensen et al., 2016). *Bacillus*-based DFM also enhances growth and feed intake in growing pigs (Wang et al., 2009) and reduces emission of methane (Prenafeta-Boldú et al., 2016) and ammonia (Wang et al., 2009) during decomposition of pig slurries. *Bacillus* spp. also produce extracellular enzymes that may enhance digestibility and absorption of nutrients (Santoso et al., 2001). Supplementing diets with *Bacillus*-based DFM enhanced apparent total tract digestibility (ATTD) of N in weanling pigs (Cai et al., 2015), but not in growing pigs (Wang et al., 2009), and improved the ATTD of fat and P in growing pigs (Jørgensen et al., 2016).

However, not all *Bacillus* strains are suitable for use as DFM. Larsen et al. (2014) characterized 245 bacterial isolates of *Bacillus* strains and concluded that isolates from *B. amyloliquefaciens*, *B. subtilis*, and *B. mojavensis* had superior characteristics in terms of heat resistance of spores, inhibitory activity against pathogenic bacteria, antibiotic resistance, production of glycosyl hydrolases, and biofilm formation. However, information about effects of *B. amyloliquefaciens* and *B. subtilis* on digestibility of AA and energy when fed to growing pigs is limited. Therefore, an experiment was conducted to test the hypothesis that DFM based on *Bacillus amyloliquefaciens* (DSM 25840) or *Bacillus subtilis* (DSM 25841) improve the apparent ileal digestibility (AID) of AA, CP, and GE, the ATTD of CP and GE, and apparent hindgut digestibility of CP and GE when fed to growing pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of L 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN).

Animals, Housing, and Experimental Design

Twenty-four growing barrows (initial BW: 22.69 ± 1.48 kg) were selected from 8 L with 3 barrows/L. Pigs were prepared with a T-cannula in the distal ileum (Stein et al., 1998). Pigs were allowed 7 d for recuperation after the surgery, and they were then allotted to a three diet, three-period design with 24 pigs, and three 21-d periods. There were eight pigs per diet in each period for a total of 24 observations per diet. No pig received the same diet more than once during the experiment and all pigs received all three diets. Pigs were housed in metabolism crates in an environmentally controlled room with a feeder and a nipple drinker. Crates had smooth sides and fully slatted floors. A screen floor and a urine funnel were installed below the slatted floor.

Diets and Feeding

Two sources of DFM provided by Chr Hansen A/S (Hørsholm, Denmark) were used and three diets were prepared (Table 1). The control diet was based on corn, soybean meal, and 20% distillers dried grains with solubles. Two additional diets were formulated by adding either *B. amyloliquefaciens* (DSM 25840) or *B. subtilis* (DSM 25841) to the control diet to supply 1.5×10^9 CFU per kg diet. Both DFM premixes were supplied in a corn base. 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 0.50% titanium dioxide as an indigestible marker. Manufactured feed was analyzed for DFM before the start of the experiment to confirm that each diet contained the expected levels of DFM (Table 2). All diets were fed to pigs in mash form. 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 $\text{kg}^{0.60}$; NRC, 2012), and two equal meals were provided every day at 0800 and 1600 hours. Water was available at all times.

Date Recording and Sample Collection

Pig weights were recorded at the beginning of each period and at the conclusion of the experiment and the amount of feed supplied each day was recorded. The initial 12 d of each period was considered the adaptation period to the diet. A color marker was included in the feed that was provided in the morning meal on day 13 and again in the diet

Table 1. Composition of the control diet and two diets containing direct-fed microbials (*B. amyloliquefaciens* and *B. subtilis*), as-fed basis

Ingredient, %	Control	<i>B. amyloliquefaciens</i>	<i>B. subtilis</i>
Ground corn	54.10	53.16	53.13
Soybean meal, 48% CP	23.00	23.00	23.00
Distillers dried grains with solubles	20.00	20.00	20.00
Ground limestone	1.20	1.14	1.17
Monocalcium phosphate, 21% P	0.60	0.60	0.60
Sodium chloride	0.40	0.40	0.40
DFM premix ¹	-	1.00	1.00
Vitamin-mineral premix ²	0.20	0.20	0.20
Titanium dioxide	0.50	0.50	0.50
Total	100.00	100.00	100.00

¹The DFM premix for the *B. amyloliquefaciens* diet was prepared by mixing 14.12 kg of ground corn and 0.88 kg of a DFM concentrate, that contained 2.56×10^9 CFU/kg; the DFM premix for the *B. subtilis* diet was prepared by mixing 14.56 kg of ground corn and 0.44 kg of a DFM concentrate that contained 5.12×10^9 CFU/g. Both DFM concentrates were produced by Chr. Hansen Holding A/S, Hørsholm, Denmark, and both diets were formulated to contain 1.5×10^9 CFU/kg complete diet.

²Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D₃ as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B₁₂, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 2. Analyzed composition of the control diet and in two diets containing direct-fed microbials (*B. amyloliquefaciens* and *B. subtilis*)

Item	Control	<i>B. amyloliquefaciens</i>	<i>B. subtilis</i>
DM, %	91.29	91.36	90.90
CP, %	19.99	19.96	19.90
GE, kcal	3,934	3,924	3,960
Titanium, ppm	2,960	2,810	2,770
Total spore count, CFU/g	$<4.0 \times 10^5$	1.72×10^9	1.89×10^9
<i>Indispensable AA, %</i>			
Arg	1.17	1.15	1.19
His	0.58	0.54	0.59
Ile	0.86	0.84	0.88
Leu	1.81	1.77	1.87
Lys	1.03	1.00	1.05
Met	0.31	0.33	0.31
Met + Cys	0.64	0.68	0.65
Phe	1.02	0.95	1.00
Thr	0.76	0.75	0.78
Trp	0.23	0.24	0.24
Val	1.00	0.98	1.03
<i>Dispensable AA, %</i>			
Ala	1.09	1.05	1.14
Asp	1.80	1.78	1.85
Cys	0.33	0.35	0.34
Glu	3.27	3.23	3.39
Gly	0.84	0.80	0.87
Pro	1.18	1.27	1.35
Ser	0.84	0.83	0.88
Tyr	0.65	0.63	0.65

that was provided in the morning meal of day 18. Fecal collections were initiated upon appearance of the first color marker in the feces and ceased upon

appearance of the second color marker according to the marker to marker approach (Adeola, 2001). Urine collections started 2 h after feeding the morning meal on day 13 and ceased 2 h after feeding the morning meal on day 18.

Ileal digesta were collected for 8 h (from 0800 to 1600 hours) on days 20 and 21 by attaching a plastic bag to the opened cannula barrel, and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta or at least once every 30 min, and immediately frozen at -20°C to prevent bacterial degradation of the AA in the digesta. On the completion of one experimental period, animals were deprived of feed overnight and the following morning, a new experimental diet was offered.

At the conclusion of the experiment, ileal digesta 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 (Kim et al., 2009), and fecal samples were dried in a forced air oven at 65°C and then ground through a 1-mm screen in a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ).

Chemical Analysis

Diets (Table 2), ileal digesta, and fecal samples were analyzed for DM (method 930.15; AOAC Int., 2007) and for CP by combustion (method 999.03; AOAC International, 2007) using a Leco FP 628 N

analyzer (LECO Corporation, Saint Joseph, MI). Aspartic acid was used as a calibration standard and CP was calculated as $N \times 6.25$. Urine samples were analyzed for CP using the Kjeldahl method (method 976.05; AOAC International, 2007) on a Kjeltec™ 8400 apparatus (FOSS Inc., Eden Prairie, MN). Diets and ileal digesta were also analyzed for AA 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. Samples were hydrolyzed with 6 N HCl for 24 h at 110 °C prior to analysis, but Met and Cys were analyzed as methionine sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis, and Trp 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 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 6400, Parr Instruments, Moline, IL) using benzoic acid as the internal standard.

Calculations and Statistical Analysis

AID values for AA in each diet were calculated using equation 1 (Stein et al., 2007):

$$\text{AID (\%)} = \left[1 - \frac{(\text{AA}_d / \text{AA}_f)}{\times (\text{Tif} / \text{Tid})} \right] \times 100 \quad (1)$$

where AID is the apparent ileal digestibility of an AA (%), AA_d is the concentration of that AA in the ileal digesta DM, AA_f is the AA concentration of that AA 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 AID for CP and GE was also calculated using this equation.

The ATTD of GE and CP was calculated (Adeola, 2001) and values for DE and ME were calculated by subtracting the GE analyzed in feces or feces and urine from the GE in the diets, respectively (NRC, 2012). The retention of N was calculated using equation 2 (Pedersen et al., 2007):

$$\text{Nr (\%)} = \left[\frac{[\text{Ni} - (\text{Nf} + \text{Nu})]}{\text{Ni}} \right] \times 100 \quad (2)$$

where Nr is the retention (%) of N, Ni is the intake (g) of N from days 13 to 18, Nf is the fecal output (g) of N from the feed provided from days 13 to 18, and Nu is the urinary output (g) of N from the feed provided from days 13 to 18. The biological value

of the protein in each diet was calculated as well using equation 3 (Rojas and Stein, 2013):

$$\text{BV (\%)} = \left[\text{Nr} / (\text{Ni} - \text{Nf}) \right] \times 100 \quad (3)$$

where BV is the biological value of the protein in the diet (%), Nr is the retention (%) of N, Ni is the intake (g) of N from days 13 to 18, and Nf is the fecal output (g) of N from the feed provided from days 13 to 18.

Based on the data for AID and ATTD of GE and CP, apparent hindgut digestibility was calculated by subtracting the ileal digestibility of GE and CP from the total tract digestibility of GE and CP (Cervantes-Pahm et al., 2014), and the disappearance of GE and CP was partitioned into disappearance in the small intestine and disappearance in the large intestine.

Normality of data was verified, and outliers were identified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Outliers were identified and eliminated as values that deviated from the treatment mean by more than three times the interquartile range. Two pigs from the *B. amyloliquefaciens* treatment were identified as outliers and removed. Data were analyzed using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC) in a randomized complete block design with the pig as the experimental unit. The statistical model included diet as the main effect and pig and period as random effects. Treatment means were separated by using the LSMEANS statement and the PDIF option with the Tukey's adjustment. Significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

There were no differences among diets in N intake, fecal, and urinary excretion of N, in N retention, or in biological value of the protein (Table 3). There were also no differences among diets in ATTD of CP, but the AID of CP was reduced ($P < 0.05$) for the *B. subtilis* diet compared with the control and the *B. amyloliquefaciens* diets. Therefore, the apparent hindgut digestibility of CP was greater ($P < 0.01$) in pigs fed the *B. subtilis* diet compared with pigs fed the other diets.

With the exception of His, Ile, Lys, and Phe, the AID of all indispensable AA was greater ($P < 0.05$) in the *B. amyloliquefaciens* diet compared with the control diet (Table 4). The AID of Arg, Met, and Trp was also greater ($P < 0.05$) in the *B. amyloliquefaciens* diet compared with the *B. subtilis* diet. The AID of Asp, Cys, Pro, and Ser was greater

Table 3. Nitrogen balance, apparent total tract digestibility (ATTD) of CP, apparent ileal digestibility (AID) of CP, and apparent hindgut digestibility (HGD) of CP in the control diet and in two diets containing direct-fed microbials (*B. amyloliquefaciens* and *B. subtilis*), as-fed basis¹

Item	Control	<i>B. amyloliquefaciens</i>	<i>B. subtilis</i>	SEM	P-value
N intake, g	265.3	268.0	270.9	30.4	0.677
N in feces, g	34.4	33.8	33.9	2.8	0.968
N in urine, g	65.0	62.7	63.5	10.5	0.910
N retention, ² %	62.9	65.4	64.2	1.5	0.419
Biological value, ³ %	28.9	28.9	28.3	3.8	0.806
ATTD CP, %	86.85	86.98	87.26	0.75	0.846
AID CP, %	74.94 ^a	74.94 ^a	71.86 ^b	0.74	0.003
HGD CP, %	11.78 ^b	11.97 ^b	15.79 ^a	1.41	0.004

^{a,b}Values within a row lacking a common superscript letter are different ($P \leq 0.05$).

¹Data are least squares means of 24 observations per treatment, except for *B. amyloliquefaciens* treatment that had 22 observations.

²Nitrogen retention was calculated as $\{[N \text{ intake} - (N \text{ in feces} + N \text{ in urine})]/N \text{ intake}\} \times 100$.

³Biological value was calculated as $[N \text{ retained}/(N \text{ intake} - N \text{ in feces})] \times 100$.

Table 4. Apparent ileal digestibility of AA in the control diet and in two diets containing direct-fed microbials (*B. amyloliquefaciens* and *B. subtilis*), as-fed basis¹

Item	Control	<i>B. amyloliquefaciens</i>	<i>B. subtilis</i>	SEM	P-value
<i>Indispensable AA, %</i>					
Arg	86.85 ^b	87.98 ^a	86.87 ^b	0.67	0.026
His	82.23	83.03	81.94	0.69	0.136
Ile	77.18	78.95	77.84	0.67	0.055
Leu	80.29 ^b	82.10 ^a	80.97 ^{ab}	0.62	0.037
Lys	75.81	76.46	75.31	1.90	0.250
Met	81.54 ^b	84.64 ^a	81.76 ^b	0.66	0.001
Phe	79.57	80.27	79.17	0.73	0.269
Thr	67.72 ^b	71.15 ^a	69.48 ^{ab}	1.06	0.015
Trp	78.36 ^b	81.78 ^a	79.07 ^b	1.04	0.006
Val	72.00 ^b	74.63 ^a	73.32 ^{ab}	0.91	0.022
<i>Dispensable AA, %</i>					
Ala	73.54	75.50	74.64	1.05	0.076
Asp	72.42 ^b	74.79 ^a	73.27 ^{ab}	0.83	0.047
Cys	64.70 ^b	70.14 ^a	63.78 ^b	1.52	0.001
Glu	79.44	81.61	79.66	0.99	0.076
Gly	62.21	63.61	62.46	1.42	0.580
Pro	74.32 ^c	79.01 ^a	76.73 ^b	1.10	0.001
Ser	75.19 ^b	78.08 ^a	77.27 ^a	0.93	0.013
Tyr	80.41	82.21	80.91	0.68	0.076
All indispensable AA, %	78.28 ^b	80.04 ^a	78.74 ^{ab}	0.69	0.045
All dispensable AA, %	74.51 ^b	77.29 ^a	75.52 ^{ab}	0.92	0.032
All AA, %	76.27 ^b	78.56 ^a	77.01 ^{ab}	0.77	0.036

^{a-c}Values within a row lacking a common superscript letter are different ($P \leq 0.05$).

¹Data are least squares means of 24 observations per treatment, except for *B. amyloliquefaciens* treatment that had 22 observations.

($P < 0.05$) in the *B. amyloliquefaciens* diet compared with the control diet, and the AID of Cys and Pro was greater ($P < 0.05$) in the *B. amyloliquefaciens* diet than in the *B. subtilis* diet. The AID of Pro was less ($P < 0.01$) in the control diet compared with the *B. amyloliquefaciens* and *B. subtilis* diets. There were no differences among the three diets in the AID for the other dispensable AA. The AID of total indispensable, dispensable, and total AA was

greater ($P < 0.05$) in the *B. amyloliquefaciens* diet compared with the control diet.

There were no differences in GE intake or GE excretion in feces among pigs fed control, *B. amyloliquefaciens*, or *B. subtilis* diets (Table 5), but pigs fed the *B. subtilis* diet tended ($P = 0.084$) to have greater urine excretion of GE than pigs fed control and *B. amyloliquefaciens* diets. The DE (DM basis) in the *B. subtilis* diet was greater ($P < 0.05$)

Table 5. Concentrations of DE, ME, apparent total tract digestibility (ATTD) of GE, apparent ileal digestibility (AID) of GE, and apparent hindgut digestibility (HGD) of GE in the control diet and in two diets containing direct-fed microbials (*B. amyloliquefaciens* and *B. subtilis*), as-fed basis¹

Item	Control	<i>B. amyloliquefaciens</i>	<i>B. subtilis</i>	SEM	P-value
GE intake, kcal	6,528	6,555	6,739	105	0.344
GE in feces, kcal	1,046	1,026	1,011	26	0.812
GE in urine, kcal	201	202	235	7	0.084
DE, diet, kcal/kg	3,298 ^b	3,304 ^b	3,359 ^a	26	0.042
DE, diet, kcal/kg DM	3,609 ^b	3,615 ^b	3,696 ^a	32	0.011
ME, diet, kcal/kg	3,177	3,196	3,222	28	0.214
ME, diet, kcal/kg DM	3,480	3,498	3,545	29	0.068
ATTD GE, %	83.82	84.20	84.83	0.65	0.313
AID GE, %	64.97 ^b	68.63 ^a	66.38 ^b	0.68	0.001
HGD GE, %	18.83 ^a	14.56 ^b	18.46 ^a	0.91	0.002

^{a-b}Values within a row lacking a common superscript letter are different ($P \leq 0.05$).

¹Data are least squares means of 24 observations per treatment, except for *B. amyloliquefaciens* treatment that had 22 observations.

than the DE in the control and *B. amyloliquefaciens* diets. The ME (DM basis) in the *B. subtilis* diet also tended to be greater ($P = 0.068$) than the ME in the control and the *B. amyloliquefaciens* diets.

There were no differences among diets in ATTD of GE, but the AID of GE was greater ($P < 0.001$) in the *B. amyloliquefaciens* diet than in the control and the *B. subtilis* diets. Therefore, the apparent hindgut digestibility of GE was less ($P < 0.05$) in the *B. amyloliquefaciens* diet compared with the other diets.

DISCUSSION

The genus *Bacillus* is spore forming bacteria and the spores are resistant to physical and environmental factors such as heat, desiccation, and UV radiation (Cutting, 2011). *Bacillus* spp. have some advantages over other non-spore formers such as *Lactobacillus* spp. because they can be stored at room temperature in a desiccated form without affecting viability (Cutting, 2011), and the spores can survive the low pH of the stomach (Barbosa et al., 2005). These characteristics make *Bacillus* spp. potentially useful as DFM although it is recognized that simply surviving the stomach does not guarantee beneficial effects to the host animal. Larsen et al. (2014) demonstrated that among 245 bacterial isolates, strains of *B. amyloliquefaciens*, *B. subtilis*, and *B. mojavensis* have the strongest probiotic potential. It was also reported that weaned pigs fed diets containing *B. subtilis* (DSM 25841) had improved growth rate and reduced gut permeability compared with pigs fed a control diet without *B. subtilis* (Kim et al., 2018), and weaned pigs infected with an enterotoxigenic F4+ *Escherichia coli* strain had reduced incidence of

diarrhea when fed diets containing *B. subtilis* (DSM 25481) or *B. amyloliquefaciens* (DSM 25840; Röhe et al., 2017). As a consequence, *B. amyloliquefaciens* (DSM 25840) and *B. subtilis* (DSM 25841) were used in this experiment.

The observation that supplementation of *Bacillus amyloliquefaciens* results in improved AID of most indispensable AA, total indispensable AA, total dispensable AA, and of total AA has not been previously reported. The reason the AID of CP did not increase although the AID of most indispensable AA increased may be that absorption of non-protein N from the diets did not change or that more endogenous protein was synthesized. The increase in the AID of AA may be a result of the fact that *B. amyloliquefaciens* synthesizes a protease, which may have aided in the hydrolysis of proteins in the small intestine (unpublished data, Chr. Hansen A/S). *Bacillus amyloliquefaciens* also synthesizes α -amylase, cellulase, and metalloproteases (Gould et al., 1975; Gangadharan et al., 2008; Lee et al., 2008). However, the increased AID of GE that was observed for diets supplemented with *B. amyloliquefaciens* may be a result of increased AID of AA. This result is in agreement with data indicating that increasing AA digestibility also results in an increase in AID of GE (Stein and Bohlke, 2007; Rojas et al., 2016).

Bacillus subtilis also secretes α -amylase (Duran-Paramo et al., 2000) and addition of *B. subtilis* and *Clostridium butyricum* to diets fed to growing pigs may increase the ATTD of energy and N (Meng et al., 2010). Addition of *Bacillus subtilis* to diets fed to broiler chickens resulted in increased apparent retention of GE and CP (Sen et al., 2012). The observations that the DE in the diet containing *B. subtilis* was greater than in the control diet and

that the ME tended to be greater support the results by Sen et al. (2012) and indicates that *B. subtilis* also increases energy utilization in pigs. This may be a result of the synthesis of α -amylase by *B. subtilis*, because α -amylase hydrolyses the α -D-(1,4) glycosidic linkages in starch (McDonald et al., 2011). However, it is also possible that *B. subtilis* synthesizes fiber hydrolyzing enzymes, such as pectinase and xylanase (Priest, 1977), which may have contributed to increased fermentation of dietary fiber and subsequent synthesis and absorption of VFA. Pigs fed *Bacillus*-based DFM containing diets had an increased expression of glucagon-like peptide-2 receptors in the liver (Jaworski et al., 2017), which may be a result of increased expression of maltase-glucoamylase and sucrose-isomaltase enzymes (Petersen et al., 2002). This may improve the energy utilization of diets containing *Bacillus* spp.

The increased apparent hindgut digestibility of CP from pigs fed the *B. subtilis* diet may be a result of absorption of ammonium from the hindgut as the absorption of AA from the cecum or colon is negligible. The hindgut data also rely on the assumption that the indigestible marker travels through the hindgut at the same rate as the digesta, which may not always be the case (Olukosi et al., 2012; De Vries et al., 2014).

In conclusion, addition of *B. amyloliquefaciens* to diets fed to growing pigs resulted in increased AID of some indispensable and dispensable AA, of total AA, and GE compared with the control diet, whereas addition of *B. subtilis* resulted in increased DE and a tendency for an increased ME of the diet. Therefore, supplementation of diets fed to growing pigs with *B. amyloliquefaciens* or *B. subtilis* may result in improved utilization of AA and energy.

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