Influence of differently processed yeast (*Kluyveromyces fragilis*) on feed intake and gut physiology in weaned pigs

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ABSTRACT: Two feeding trials were conducted to investigate the effects of hydrolyzed (HY) or non-hydrolyzed (NHY) yeast (*Kluyveromyces fragilis*) in isoenergetic and isonitrogenous diets in the postweaning period. In experiment 1, a total of 550 unsexed pigs (6.5 ± 0.5 kg BW), weaned at 24 ± 2 d of age, were allocated to five treatment groups, receiving either a control diet (CON) or diets with 1%, 3%, and 5% HY (groups HY1, HY3, and HY5, respectively), or a diet with 3% NHY (group NHY3). In experiment 2, a total of 48 male and female pigs (6.2 ± 0.3 kg BW, weaned at d 25) were allocated to three dietary groups ($n = 8$ replicates with two pigs) receiving a control diet (CON) or diets with 1% NHY or 1% HY. Eight animals were sacrificed 2 wk after weaning for histological investigations in the jejunum and colon, determination of apparent ileal digestibility (AID) of CP and ether extract (EE), and electrophysiological measurements in the jejunal tissue after addition of carbachol or L-glutamine using Ussing chambers. In experiment 1, different treatments had no significant effect on pig performance, but

diet HY1 tended to increase ADG and G:F in wk 2 after weaning ($P < 0.1$). In experiment 2, diet HY1 increased feed intake in wk 2 ($P < 0.05$), whereas NHY yeast had no effect on feed intake. Villus height, villus/crypt ratio in jejunum ($P < 0.05$), and crypt depth in colon ($P < 0.01$) were increased in group HY1. Crypt depth in jejunum and small intestinal length were not affected by different treatments. The AID of CP and EE tended to increase in group HY1 ($P < 0.1$) compared with groups CON and NHY. In the Ussing chamber experiments, no changes in basal electrophysiological parameters were observed, and the reactions of the treatment groups to carbachol and L-glutamine were comparable. ADFI was positively correlated with different parameters of intestinal morphology (villus height, villus/crypt ratio, crypt depth in colon, length of small intestine), AID of CP, EE, and performance. The results suggest that a supplementation of 1% HY based on *K. fragilis* to pig diets may positively influence ADFI and intestinal morphology in pig in the early postweaning period (d 1 to 14).

Key words: feed intake, gut physiology, hydrolyzed yeast, *Kluyveromyces fragilis*, performance, weaned pigs

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INTRODUCTION

Weaning is a stressful and critical period for pigs caused by changes of nutritional, social, and

environmental factors. As a result, low and variable feed intake is often observed within the first days or weeks after weaning, which may have a negative impact on intestinal physiology and performance (Pluske et al., 1997). Among other factors, the composition and palatability of weaning diets play an important role in controlling

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weaning-related disorders. Both, sweet and umami flavors trigger taste stimuli and promote feed intake in weaned pigs (Roura et al., 2008). Yeast extracts, which represent the soluble yeast constituents, are known to improve palatability of food due to their high amounts of umami-taste amino acids, peptides, and nucleotides such as 5'-guanosine monophosphate and 5'-inosine monophosphate (Sommer, 1998; Chae et al., 2001; Foster, 2011). It was demonstrated that in weaned pigs yeast extracts derived from *Saccharomyces cerevisiae* have improved performance and were comparable with positive effects obtained from blood plasma (Carlson et al., 2005; Pereira et al., 2012; Rigueira et al., 2013). However, due to economic reasons, yeast extracts are primarily used in human nutrition. Therefore, for the use in animal nutrition, other yeast products based on whole yeast cells containing both yeast extract and insoluble yeast fragments (e.g., yeast cell wall) may provide an alternative to yeast extracts. The use of *S. cerevisiae* cells or parts thereof (e.g., yeast cell wall) is well described for swine nutrition (Becker and Nehring, 1967; Kogan and Kocher, 2007), but only little is known about yeast products derived from *Kluyveromyces fragilis* and the impact of a hydrolysis process. *Kluyveromyces* spp.-based yeast products have gained increasing attention on the commercial production field because of its physiological properties such as high growth rate (Lane and Morrissey, 2010). Compared with *S. cerevisiae*, yeasts based on *K. fragilis* such as the tested yeast products seem to be high in CP and amino acids, for example, glutamic acid (Table 1). It could be hypothesized that the enzymatic hydrolysis may increase the availability of intracellular compounds such as umami-taste amino acids and enhance feed consumption in the postweaning period. Thus, the

study aimed to investigate the effects of *K. fragilis*, either intact or enzymatically hydrolyzed on productive parameters and gut physiology in weaned pigs.

MATERIALS AND METHODS

The effects of different yeast prototypes of *K. fragilis* (hydrolyzed [HY] or non-hydrolyzed [NHY]) were investigated in two feeding trials in weaned pigs. Experiment 1 was used as a pretest for experiment 2 and carried out to test the effect of different dosages of HY and one commonly used dosage of NHY yeast on pig performance. NHY yeasts such as brewers or fodder yeast are commonly dosed at approximately 3% (2% to 5%) in pig diets (Becker and Nehring, 1967). In main experiment 2, the effects of HY and NHY on intestinal physiology after weaning were investigated. All procedures were approved by the local state office of occupational health and technical safety "Landesamt für Gesundheit und Soziales Berlin" (LaGeSo Reg. Nr. A100/13 and 0194/15).

Animals, Housing, Diets, and Sampling

In experiment 1, a total of 550 postweaning pigs weighing 6.5 ± 0.5 kg (DanZucht $\times$ Piètrain, 24 ± 2 d of age) were randomly allocated to five different treatment groups balanced for BW, litter, and sex. Exact numbers of replicates per treatment ($n = 4$ or 5 , respectively) with 25 pigs per replicate are shown in Table 4. The relative low numbers of replicates per treatment had practical reasons and were accepted as experiment 1 had a pretest character to find the best dosage for HY in experiment 2. During the suckling period, the pigs had access to creep feed from d 12 of age until weaning. The

Table 1. Chemical composition of *S. cerevisiae* and the tested *K. fragilis* yeast

		Yeast based on <i>S. cerevisiae</i> ¹	Tested yeasts (<i>K. fragilis</i>)		
			Specification ²	HY ³	NHY ³
Organic matter	(g/ kg DM)	900–930	>920	948	923
Ash	(g/ kg DM)	70–100	33	32	69
Crude protein	(g/ kg DM)	450–600	532	559	496
Fiber	(g/ kg DM)	0.0–30	<10	58	33
EE	(g/ kg DM)	1.0–30	65	5.3	17
Lysine	(g/100 g CP)	6.3–9.7	8.6	7.9	7.1
Methionine	(g/100 g CP)	1.2–3.5	1.6	2.1	1.5
Cystine	(g/100 g CP)	0.3–1.7	0.8	1.3	1.4
Threonine	(g/100 g CP)	4.6–7.0	5.4	5.1	4.5
Glutamic acid	(g/100 g CP)	4.9–11.0	12.8	9.2	8.5

¹References (Spark, 2004; Freeland and Gale, 1947; Martini et al., 1979).

²Calculated from the manufacturer's specification based on as-fed basis (92% DM).

³Analyzed values.

lighting regime (natural/artificial) consisted of a 16-h light (about 50 lx) and 8-h dark cycle. The average housing temperature (pig level) was maintained at 28°C during the first 2 wk postweaning. In the following 4 wk, the temperature was gradually reduced up to 24°C by approximately 1°C per week. Relative humidity ranged between 55% and 65%.

Pigs had ad libitum access to feed (mash form) and drinking water. Pigs were fed isoenergetic and isonitrogenous diets either without yeast (negative control, group CON) or with graded levels of the HY at 1%, 3%, or 5% (groups HY1, HY3, and HY5, respectively). As a positive control, the basal diet was supplemented with 3% NHY (group NHY3). Both yeast products derived from the yeast

strain *K. fragilis* and were supplied by Prosol Spa, Madone, Italy.

The 42-d feeding phase was divided into two periods, d 1 to 14 and d 15 to 42 of experiment, where two different diets were offered. The diets were calculated to meet the nutritional requirements of postweaning pigs as recommended by GfE (2006). The composition and nutritional characteristics of the basal diet are given in Tables 2 and 3. The basal mixture contained no further zootechnical feed additives. BW, ADG, ADFI, and G:F per pen were recorded weekly. Feed consumption and ADG were calculated by dividing feed intake and BW per pen, respectively, by the number of pigs per pen. Throughout the experiment, all pigs were monitored daily for clinical signs of

Table 2. Ingredients (% as-fed basis) and calculated nutrient composition (% as-fed basis) of the experimental diets for experiments 1 and 2

Item	Experiment 1										Experiment 2		
	Period 1 (25–38 d of age)					Period 2 (39–66 d of age)					Period 1 (25–38 d of age)		
	CON	HY1	HY3	HY5	NHY3	CON	HY1	HY3	HY5	NHY3	CON	HY1	NHY1
Wheat	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Soybean meal, 44% CP	24.50	23.34	20.87	18.45	20.87	21.67	20.42	18.00	15.58	18.00	24.50	23.34	23.34
Corn	17.43	17.62	18.17	18.68	18.17	17.78	17.99	18.44	18.95	18.44	17.40	17.59	17.59
Barley	10.00	10.00	10.00	10.00	10.00	13.00	13.00	13.00	13.00	13.00	10.00	10.00	10.00
Soybean oil	3.01	2.99	2.90	2.80	2.90	2.85	2.85	2.77	2.68	2.77	3.01	2.99	2.99
Limestone	1.61	1.63	1.68	1.72	1.68	1.56	1.56	1.57	1.62	1.57	1.61	1.63	1.63
Monocalcium phosphate	1.40	1.36	1.26	1.18	1.26	1.20	1.18	1.17	1.08	1.17	1.40	1.36	1.36
Premix ¹	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
L-Lysine-HCl	0.55	0.56	0.58	0.61	0.58	0.52	0.54	0.56	0.58	0.56	0.55	0.56	0.56
DL-Methionine	0.15	0.15	0.16	0.16	0.16	0.11	0.11	0.12	0.12	0.12	0.15	0.15	0.15
L-Threonine	0.10	0.10	0.11	0.12	0.11	0.10	0.10	0.11	0.12	0.11	0.10	0.10	0.10
L-Tryptophan	0.05	0.05	0.07	0.08	0.07	0.04	0.05	0.06	0.07	0.06	0.05	0.05	0.05
Titanium dioxide	–	–	–	–	–	–	–	–	–	–	0.03	0.03	0.03
Hydrolyzed yeast ²	–	1.00	3.00	5.00	–	–	1.00	3.00	5.00	–	–	1.00	–
Non-hydrolyzed yeast ²	–	–	–	–	3.00	–	–	–	–	3.00	–	–	1.00
Energy (MJ ME/kg) ³	13.8					13.6					13.8		
Dry matter	87.7					87.6					87.7		
Crude protein	19.1					18.0					19.1		
SID Lysine	1.23					1.14					1.23		
SID methionine + cystine	0.68					0.61					0.68		
SID threonine	0.66					0.63					0.66		
SID tryptophan	0.25					0.22					0.25		

CON, control; HY1, 1% HY; HY3, 3% HY; HY5, 5% HY; NHY3, 3% NHY; NHY1, 1% NHY.

¹Premix provided per kg diet: 4,800 IU vitamin A (acetate), 1,440 IU vitamin D₃, 96 mg vitamin E (α -tocopheryl acetate), 2.4 mg vitamin K₃ (MSB), 3.0 mg vitamin B₁ (mononitrate), 5.0 mg vitamin B₂ (crystalline riboflavin), 30 mg niacin (niacinamide), 4.8 mg vitamin B₆ (HCl), 24 μ g vitamin B₁₂, 300 μ g biotin (commercial, feed grade), 12 mg pantothenic acid (Ca d-pantothenate), 1.2 mg folic acid (crystalline commercial feed grade), 960 mg choline (chloride), 60 mg Zn (sulfate), 60 mg Fe (carbonate), 72 mg Mn (sulfate), 12 mg Cu (sulfate-pentahydrate), 0.24 mg Se (Na-selenite), 0.54 mg I (Ca-iodate), 1.56 g Na (NaCl), and 0.66 g Mg (sulfate).

²Yeast products (HY, NHY) derived from the yeast strain *K. fragilis* and were supplied by Prosol Spa (Madone, Italy).

³Calculated by using the equation given by DLG (2013).

Table 3. Analyzed nutrient composition of the experimental diets (% as-fed basis) for experiments 1 and 2

Item	Experiment 1					Experiment 2							
	Period 1 (25–38 d of age)					Period 2 (39–66 d of age)					Period 3 (25–38 d of age)		
	CON	HY1	HY3	HY5	NHY3	CON	HY1	HY3	HY5	NHY3	CON	HY1	NHY1
Dry matter	92.01	91.93	91.80	91.67	91.97	91.68	91.75	91.67	91.68	91.51	90.21	89.58	89.70
Crude protein	20.10	20.24	20.50	21.08	20.64	18.68	18.92	19.36	19.85	19.42	20.49	20.33	20.32
Crude fiber	3.97	4.09	4.15	4.48	4.22	4.18	4.33	4.42	4.50	4.36	3.58	3.44	3.84
Crude ash	5.90	5.87	5.80	5.81	5.83	5.69	5.71	5.68	5.64	5.66	5.72	5.52	5.62
EE	5.97	5.90	5.95	5.93	5.89	4.71	4.82	4.88	4.79	7.82	4.89	4.96	4.62
Calcium	0.97	0.95	0.94	0.95	0.96	0.88	0.89	0.90	0.88	0.87	1.02	0.97	0.98
Phosphorus	0.66	0.70	0.68	0.69	0.70	0.66	0.71	0.68	0.70	0.68	0.60	0.59	0.61

CON, control; HY1, 1% HY; HY3, 3% HY; HY5, 5% HY; NHY3, 3% NHY; NHY1, 1% NHY.

disease and necessary medical treatments were documented. Additionally, the fecal consistency was scored daily on a pen basis using following scores: 1, liquid diarrhea; 2, pasty feces falling out of shape upon contact with surfaces; 3, formed feces, soft to cut; 4, well-formed feces, firm to cut; and 5, hard and dry feces.

In experiment 2, 48 postweaning pigs (DanZucht × Piétrain, 25 d of age, 6.17 ± 0.32 kg BW) were randomly allocated to three treatment groups ($n = 8$ cages with one male and one female pig each; a cage was considered as experimental unit). Animals had ad libitum access to feed (mash form) and drinking water. Based on the outcome of experiment 1, 1% HY (group HY1) and 1% NHY (group NHY1) were used in comparison with a control diet (group CON). Feed composition and nutritional characteristics are given in Table 2 and 3. Light regime, temperature, and humidity were identical to experiment 1. The trial lasted in total 2 wk, which corresponded to an age of pigs of 25 to 38 d. All pigs were individually identified by ear tags and weighed at d 1, 7, and 14 of experiment. Feed intake per pen and individual weight gain were documented weekly. Throughout the experiment, all pigs were monitored daily for any abnormalities, abnormal behaviors, and clinical signs of disease. Individual body temperatures were checked daily at d 1 to 4 and at d 7 of experiment. No antibiotics were administered before and during the experiment.

At $d 14 \pm 1$ after weaning, one pig per pen was euthanized for tissue and digesta sampling 4 h after the last meal. Pigs were anesthetized with 20 mg/kg BW of ketamine hydrochloride (Ursotamin, Serumwerk Bernburg AG, Germany) and 2 mg/kg BW of azaperone (Stresnil, Janssen-Cilag, Neuss, Germany) and euthanized by intracardial injection of 10 mg/kg BW of T61 (Intervet, Unterschleißheim, Germany). The gastrointestinal

tract was removed, the small intestine was taken, and length of small intestine (SIL) was recorded. To have enough material for the nutrient analysis to estimate their apparent ileal digestibility (AID) the total ileal digesta were collected from the still very small pigs and stored at -80°C . A total section of 25 cm from the mid-jejunum (starting 2 m from the pylorus) was taken and stored in ice-cold gassed (95% O_2 ; 5% CO_2) modified Krebs–Ringer buffer solution (pH adjusted to 7.4, containing in mmol per liter: NaCl, 115; KCl, 5; CaCl_2 , 1.5; MgCl_2 , 1.2; NaH_2PO_4 , 0.6; Na_2HPO_4 , 2.4; NaHCO_3 , 25; and mannitol, 20). Two 10 cm segments were used for functional analysis in Ussing chambers. Two pieces of the jejunum (2 cm each) and one piece of colon tissue were used for histological examinations (0.5 cm length each).

Histological Investigations

For the morphometric analysis, jejunal and colonic sections were cut open longitudinally, placed on cork boards, and subsequently fixed in 4% formaldehyde (11% formaldehyde [37%], 89% phosphate buffered saline) for 48 h. Samples were dehydrated by passing through an ascending concentration of ethanol (70% for 48 h, 80% for 48 h, and 96% for 2 h, isopropanol for 2 h). After passing the ascending concentrations of ethanol, samples were transferred into the clearing agent xylene for 2 h to remove the ethanol from the tissue. After infiltration with solidified paraffin wax (24 h), the tissue was embedded, cut at $5 \mu\text{m}$ with a sledge microtome (Typ 1400, Leitz, Wetzlar, Germany) and subsequently stained with hematoxylin–eosin. Villus height, crypt depth, villus/crypt ratio in jejunum, and crypt depth in colon were determined by light microscopy (BX43F, Olympus, Germany) in well-oriented villus and crypt units, respectively. Two slides per pig were used and the average values

taken for a minimum of 10 villi and crypts. The villus/crypt ratio was calculated as the villus height divided by the crypt depth.

Ussing Chamber Experiments

Ussing chamber experiments were performed as described previously (Kröger et al., 2013) with some modifications to test the reactivity of the gut tissue to carbachol as secretagogue and L-glutamine. The stripped jejunal epithelium (without serosal and muscle layers) was immediately mounted in Ussing chambers with an exposed area of 1.31 cm² and bathed in modified Krebs–Ringer buffer solution. The Krebs–Ringer buffer solution was prepared at 38°C to have a temperature of 37°C in the Ussing chambers. A microcomputer-controlled voltage/current clamp (K. Mussler Scientific Instruments, Aachen, Germany) was used to obtain electrical measurements. L-Glutamine (final concentration: 20 mmol/L) was added to the mucosal side of two chambers per animal. Mannitol was included in the solution of both sides. After the I_{sc} reached again a constant baseline, carbachol (final concentration 10 mmol/L) was applied to the serosal compartment of the same chambers to determine the response toward secretagogues. Basal values were obtained as average mean values of the last 3 min after a 15-min equilibration period under short-circuit condition and before the addition of L-glutamine. The change of short-circuit current (ΔI_{sc}) and tissue conductance (ΔG_t) after addition of the substrates was determined as indirect measure of electrolyte transport and integrity of the sample. For carbachol, ΔI_{sc} and ΔG_t were measured by subtracting values obtained 3 min after addition of carbachol from basal I_{sc} and G_t , respectively. For L-glutamine, the determination of ΔI_{sc} and ΔG_t was calculated by the difference between values obtained 6 s before and 30 s after addition.

Chemical Analyses

Proximate analysis (DM, ash, CP, ether extract [EE]) in diets and ileal digesta was determined as described by Naumann and Bassler (2004). Calcium and potassium in diets were analyzed by atomic absorption spectrometry in an AAS vario 6 spectrometer (Analytik Jena, Jena, Germany) as described by Pieper et al. (2015).

Titanium dioxide (TiO₂) contents in diets and digesta were measured according to the method described by Short et al. (1996). Briefly, 0.1 g of freeze-dried digesta samples was incinerated for

48 h at 500°C in beakers. The ash was dissolved in 10 mL boiling 7.4 M H₂SO₄ and the solution was transferred in a new beaker containing 25 mL of distilled H₂O. The solution was filtered into 100 mL volumetric flasks, and 20 mL H₂O₂ (30%) was added to each flask followed by dilution to 100 mL with distilled H₂O. Absorbance of the resulting solutions was measured at 410 nm on a spectrometer and compared with a standard curve for determination of the initial TiO₂ content.

To calculate the AID of CP and EE, the following quotation was used:

$$\text{AID (\%)} = 100 - \frac{\text{TiO}_2 \text{ in feed (\%)}}{\text{TiO}_2 \text{ in digesta (\%)}} \cdot \frac{\text{Nutrient in digesta (\%)}}{\text{Nutrient in feed (\%)}} \cdot 100$$

Statistical Methods

Normally distributed data were analyzed either by one-way ANOVA (Performance, Ussing chamber) followed by Tukey-HSD test or by one-way ANCOVA (Gut architecture, AID). For ANCOVA, BW (d 14) was considered as covariate. Results are presented as means $\pm$ SD unless otherwise stated. For not normally distributed data (e.g., fecal scores), Pearson chi-square test was applied to test for group differences. In addition, Pearson correlation analysis was performed to evaluate functional correlation among feed intake at different time periods and data representing results of performance, gut morphometry, nutrient digestibility, and parameters measured in Ussing chamber. The statistical analyses were performed with the software package SPSS (IBM SPSS Version 21). Differences at $P < 0.05$ were considered as significant; P -values ranging from 0.06 to 0.10 were accepted as trends.

RESULTS

Experiment 1

Body weights did not differ significantly between treatment groups (Table 4). In wk 1, no significant differences in ADG, ADFI, and G:F were observed. In wk 2, ADG and G:F tended to differ between treatment groups. Greatest values for ADG in wk 2 were observed in group HY1 followed by groups HY3, CON, HY5, and NHY3 ($P = 0.056$). Gain:feed was greatest in group NHY3 followed by groups HY1, CON, HY5, and HY3 ($P = 0.067$). There was no difference in ADG, ADFI, or G:F between treatment groups in Period 2 or in overall trial period.

Averaged fecal scores showed no health relevant effects (data not shown). None of the pigs did exhibit diarrhea according to score 2 or 1. Treatment effects were not evident ($P = 0.474$).

Experiment 2

Overall performance was not different between treatment groups (Table 5). In the first week after weaning pigs gained almost no weight. In the second

week after weaning, total weight gain averaged to 1.06 ± 0.84 kg per pig. During the total trial period, the feed intake tended to be increased in group HY1 compared with the other groups ($P = 0.093$). The comparison of the single groups with each other showed that there was a higher feed intake in group HY1 compared with NHY1 ($P = 0.038$) and a trend for a higher feed intake compared with CON ($P = 0.076$) in total period. No differences or trends between CON and NHY were found ($P = 0.808$). In

Table 4. Effects of supplemented yeast prototypes on zootechnical performance of piglets during different feeding periods in experiment 1

	Dietary treatment					P-value
	CON	HY1	HY3	HY5	NHY3	
Replicates, <i>n</i>	4	4	5	4	5	
Piglets per replicate/pen	25	25	25	25	25	
Yeast dosage (% as fed)	0	1	3	5	3	
BW (kg)						
d 1	6.53 ± 0.22	6.39 ± 0.33	6.24 ± 0.45	6.63 ± 0.95	6.58 ± 0.59	0.607
d 7	7.27 ± 0.52	7.60 ± 0.30	7.29 ± 0.63	7.31 ± 0.84	7.36 ± 0.56	0.922
d 14	9.33 ± 0.59	9.84 ± 0.29	9.04 ± 0.77	9.19 ± 0.65	9.48 ± 0.48	0.356
d 42	22.37 ± 0.85	23.14 ± 0.71	21.27 ± 2.19	21.59 ± 1.60	21.80 ± 1.28	0.385
ADG (g)						
d 1–7	123 ± 52	202 ± 81	175 ± 70	113 ± 36	130 ± 78	0.218
d 8–14	294 ± 14	313 ± 37	302 ± 35	286 ± 28	271 ± 55	0.056
d 15–42	466 ± 16	475 ± 16	437 ± 52	443 ± 41	440 ± 35	0.419
d 1–42	386 ± 16	409 ± 24	367 ± 44	365 ± 25	371 ± 28	0.171
ADFI (g)						
d 1–7	218 ± 60	277 ± 79	252 ± 50	205 ± 49	257 ± 85	0.490
d 8–14	419 ± 57	447 ± 44	432 ± 64	409 ± 58	387 ± 27	0.397
d 15–42	802 ± 25	798 ± 22	769 ± 40	760 ± 36	752 ± 62	0.223
d 1–42	651 ± 19	662 ± 31	636 ± 32	619 ± 30	617 ± 51	0.188
G:F						
d 1–7	0.55 ± 0.10	0.72 ± 0.15	0.70 ± 0.22	0.57 ± 0.19	0.49 ± 0.22	0.297
d 8–14	0.71 ± 0.12	0.72 ± 0.07	0.58 ± 0.04	0.66 ± 0.07	0.79 ± 0.19	0.067
d 15–42	0.58 ± 0.03	0.60 ± 0.02	0.57 ± 0.05	0.58 ± 0.06	0.59 ± 0.04	0.857
d 1–42	0.59 ± 0.04	0.62 ± 0.03	0.58 ± 0.05	0.59 ± 0.05	0.60 ± 0.06	0.685

CON, control; HY1, 1% HY; HY3, 3% HY; HY5, 5% HY; NHY3, 3% NHY.

Table 5. Effects of dietary treatments on zootechnical performance in experiment 2 ($n = 8$)

	Dietary treatment			P-value
	CON	HY1	NHY1	
BW (kg)				
d 1	6.16 ± 0.35	6.16 ± 0.35	6.19 ± 0.29	0.977
d 14	7.09 ± 1.41	7.47 ± 1.06	7.06 ± 1.34	0.781
ADG (g)				
d 1–14	67 ± 84	94 ± 59	63 ± 94	0.706
ADFI (g)				
d 1–7	65 ± 60	73 ± 48	42 ± 33	0.431
d 8–14	354 ^b ± 78	440 ^a ± 53	349 ^b ± 90	0.041
d 1–14	220 ± 64	271 ± 46	207 ± 62	0.093

CON, control; HY1, 1% HY; NHY1, 1% NHY yeast.

^{a,b}Means with different superscripts within a row indicate significant differences ($P < 0.05$).

the second week after weaning, the feed intake of group HY1 was improved compared with groups CON and NHY1 ($P = 0.041$).

Table 6 summarizes results on small intestinal length, gut architecture, and AID of CP and EE. Small intestinal length did not differ between treatment groups. Jejunal villi were higher in group HY1 compared with groups CON and NHY1 ($P = 0.029$) and jejunal crypt depth was almost similar in all treatment groups. Jejunal villus/crypt ratio increased in group HY1 compared with group CON ($P = 0.016$). Colonal crypts were deeper in group HY1 compared with groups CON and NHY1 ($P = 0.006$). Average AID of CP and EE were 69.1% and 90.1%, respectively. AID of both CP and EE tended to differ between treatment groups ($P = 0.099$ and $P = 0.086$, respectively). The comparison of the single groups with each other showed a trend for a higher AID of

CP ($P = 0.058$) and EE ($P = 0.080$) in group HY1 compared with CON. No effect of different treatments on parameters measured in Ussing chambers was observed (**Table 7**). Basal electrophysiological parameters and the reaction to carbachol and L-glutamine (ΔI_{sc} , ΔG_t) were comparable.

Correlation analysis revealed a significant positive correlation between feed intake and performance (BW, ADG), gut architecture, and AID of CP and EE (**Table 8**). **Figure 1** depicts the relationship between total feed intake (d 1 to 14) and body weight at d 14 as well as jejunal villus height at d 14. Parameters were correlated with feed intake.

DISCUSSION

This study evaluated the effect of HY and NHY yeast products derived from *K. fragilis* on feed

Table 6. Effects of dietary treatments on length of small intestine, gut architecture in small and large intestine, and AID of CP and EE in 38-d-old piglets in experiment 2

	Dietary treatment			P-value
	CON	HY1	NHY1	
Length of small intestine ¹ (m)	10.11 ± 1.37	10.63 ± 1.03	9.96 ± 1.22	0.542
Morphological characteristics ¹				
Jejunal villus height (μm)	366 ^b ± 109	447 ^a ± 83	349 ^b ± 99	0.029
Jejunal crypt depth (μm)	184 ± 47	172 ± 37	159 ± 45	0.386
Jejunal villus/crypt ratio	1.98 ^b ± 0.32	2.68 ^a ± 0.58	2.20 ^{ab} ± 0.36	0.016
Colonal crypt depth (μm)	306 ^b ± 53	370 ^a ± 35	315 ^b ± 53	0.006
AID of nutrients ² (%)				
CP	64.2 ± 10.4	75.3 ± 5.6	67.8 ± 11.4	0.099
EE	86.3 ± 9.4	93.9 ± 2.7	90.2 ± 4.2	0.086

CON, control; HY1, 1% HY; NHY1, 1% NHY yeast.

^{a,b}Means with different superscripts within a row indicate significant differences ($P < 0.05$).

¹ $n = 8$.

² $n = 8$ for CON and $n = 7$ for groups HY1 and NHY.

Table 7. Effects of dietary treatments on the basal short-circuit current (I_{sc}) and tissue conductance (G_t) and on the change of short-circuit current (ΔI_{sc}) and the change of tissue conductance (ΔG_t) on isolated jejunal tissue from 38-d-old piglets in experiment 2 ($n = 6$)

	Dietary treatment			P-value
	CON	HY1	NHY1	
I_{sc} (μA/cm ²)				
Basal I_{sc}	-149 ± 49.4	-138 ± 54.9	-106 ± 66.3	0.428
ΔI_{sc} glutamine ¹	40.5 ± 11.2	30.9 ± 18.0	39.0 ± 20.2	0.420
ΔI_{sc} carbachol ²	36.3 ± 34.5	47.9 ± 38.0	28.0 ± 18.5	0.559
G_t (mS/cm ²)				
Basal G_t	27.1 ± 5.44	29.4 ± 2.19	28.2 ± 7.13	0.817
ΔG_t glutamine ¹	0.84 ± 2.15	-0.06 ± 1.07	0.20 ± 0.64	0.555
ΔG_t carbachol ²	-1.88 ± 1.20	-0.80 ± 1.74	-2.39 ± 2.76	0.397

CON, control; HY1, 1% HY; NHY1, 1% NHY yeast.

¹Difference between values obtained 6 s before and 30 s after addition of L-glutamine.

²Difference between values obtained 3 min before and 3 min after addition of carbachol.

Table 8. Pearson correlation coefficients between feed intake in different time periods and performance, gut architecture, AID, and electrophysiological measurements in Ussing chambers (I_{sc} , G_t)¹

	Feed intake		
	Week 1	Week 2	Total
BW, d 14 ²	0.818	0.720	0.816
ADG, d 1–14 ²	0.761	0.743	0.813
Length of small intestine ²	0.569	0.732	0.736
Jejunal villus height ²	0.511	0.826	0.784
Jejunal crypt depth ²	–	–	–
Jejunal villus/crypt ratio ²	0.433	0.638	0.619
Colonial crypt depth ²	0.448	0.712	0.678
AID of CP ³	–	0.677	0.570
AID of EE ³	–	0.571	0.542
I_{sc} ; G_t (L-glutamine, carbachol) ⁴	–	–	–

¹Only significant correlations are given ($P < 0.05$).

² $n = 24$.

³ $n = 21$.

⁴ $n = 18$.

Figure 1. (A) Relationship between feed intake in first 2 wk after weaning (d 1–14) and BW at d 14 after weaning (Pearson coefficient: 0.816, $P < 0.001$). (B) Relationship between feed intake in first 2 wk after weaning (d 1–14) and jejunal villus height at d 14 after weaning in experiment 2 (Pearson coefficient: 0.784, $P < 0.001$). HY1, 1% HY; NHY1, 1% NHY yeast.

intake and intestinal physiology in the postweaning period of pigs. In experiment 1, 1% HY (group HY1) seemed to be the most promising dosage to

improve ADG and G:F after weaning; however, the different yeast concentrations in the diet did not influence feed intake. It should be considered that experiment 1 had low statistical power due to limited number of pens/replicates per treatment. In main experiment 2, addition of 1% HY significantly improved feed intake in the second week after weaning and tended to improve feed intake for the total period.

To our best knowledge, HY based on *K. fragilis* was not tested before in pigs. In trials examining acidic HY products from *S. cerevisiae* at dosages of 0.2%, these supplements did not influence feed intake and performance parameters in weaned pigs (Šperanda et al., 2008, 2013; Molist et al., 2014). To improve palatability in humans, dietary levels of yeast extracts, which represent the soluble part of the yeast cell and 70% of the total yeast cell mass, amount usually 0.2% to 2.5% (Foster, 2011). Thus, it can be assumed that the applied concentrations of 0.2% of total cell mass (Šperanda et al., 2008, 2013; Molist et al., 2014) delivering approximately 0.14% yeast extract were too low to have effects on feed intake in pigs. On the other hand, yeast extracts from *S. cerevisiae* at higher inclusion rates of 2% to 4% improved feed intake in weaned pigs, which was comparable to the addition of blood plasma (Carlson et al., 2005; Pereira et al., 2012; Rigueira et al., 2013). Very little is known about effects of higher dosages of HY on feed intake. In a study of Rivera et al. (2016), descending dietary levels of an enzymatic HY based on *S. cerevisiae* (6%, 4%, 2%, 0% in wk 1, 2, 3, 4 after weaning, respectively) were tested in pigs weaned at 21 days of age in partial or total replacement of blood plasma. In the second

week after weaning, feed intake was improved by feeding diets containing a combination of blood plasma and HY or HY alone (by 26.3% or 13.7%, respectively). The authors concluded that at commercial farm conditions, the replacement of blood plasma by HY is a viable alternative, and may improve ADFI, ADG, and G:F and reduce mortality. In our study, HY improved feed intake in experiment 2 and seemed to be a suitable product to improve feed palatability in pigs. However, no effects on feed intake in experiment 1 were obvious, but it should be considered that statistical power of the experiment was low. NHY yeast products had no effect on feed intake either in experiment 1 or in experiment 2. It can be hypothesized that to ensure taste-enhancing properties of yeast products, the processing should be optimized to release the flavor components such as umami amino acids or nucleotides. Yeast extracts can be obtained by autolysis or hydrolysis. These degradation processes are carried out to break down the yeast cell wall and solubilize the cell components found within the cell (Chae et al., 2001; Tanguler and Erten, 2008; Foster, 2011).

Other than yeast extracts and yeast auto-/hydrolysates, dried yeasts are used for humans and animals as protein source. When replacing different levels of soybean meal by dried yeast (*K. fragilis*) in diets for weaned pigs (4% to 16% yeast in final feed), yeast improved performance and feed efficiency and seemed to be a superior protein source compared with soybean meal. The yeast stands out because of its high protein quality, which is also reflected by a high CP and lysine digestibility (Ajeani et al., 1979; Spark et al., 2005).

Next to the positive effects on feed intake, it can be assumed that 1% HY can improve intestinal architecture. The effect of weaning on intestinal micro-architecture has been well described. In the acute phase after weaning, from d 1 to 5 after weaning, villus atrophy can be often observed (Pluske et al., 1996; Lallès et al., 2007; Montagne et al., 2007). This is caused by either an increased rate of cell loss, for example, as a reaction toward altered microbial colonization or immunological reaction to ingested feed antigens, or reduced rate of cell renewal, resulting from low feed intakes. Both processes can lead to a reduced villus height/crypt depth ratio (Pluske et al., 1997). In experiment 2, pigs from group HY had a higher feed intake in wk 2 compared with groups CON and NHY. Furthermore, pigs from group HY had longer villi and an increased villus/crypt ratio, whereby crypt depth in jejunum was not affected.

As gut morphology was analyzed only at d 14 after weaning, it is not clear, if the higher feed intake in group HY1 either resulted in a reduced degree of villus atrophy after weaning or supported a faster recovery from villus atrophy. The gut morphology in the jejunum was related to the higher feed intake after weaning, which is underlined by significant correlations to villus height and villus/crypt ratio. No correlation between feed intake and jejunal crypt depth was observed. It could be hypothesized that next to the effects on feed intake, other functional and released components of the HY, like AA or nucleotides, may have additionally influenced the intestinal morphology (Kelly et al., 1991; Heo et al., 2013). Also, Martinez-Puig et al. (2007) have shown that yeast-derived nucleotides and AA (arginine and glutamine) protect pigs from villus atrophy after weaning. In contrast, other authors observed no effects of nucleotides on villus height and crypt depth in different parts of small intestine when fed at 0.1% to 0.2% (Lee et al., 2007; Moore et al., 2011). However, the weight of small intestine was increased by nucleotide supplementation. It was hypothesized that the increase in intestinal tissue growth could be due to the presence of nucleotides in the diet as nucleotides are the building blocks of nucleic acids that are required for growth of new tissue (Lee et al., 2007).

Deeper crypts in the colon may be attributable in part to the increased production of VFA from fermentation of dietary fiber reaching the large intestine that, in turn, stimulates the crypt-cell production rate in the small intestine (Pluske et al., 1997). In the present trial (experiment 2), deeper colonic crypts in group HY1 and a positive correlation between feed intake and colonic crypt depth were found. One possible explanation could be that yeast cell walls are representing approximately 30% of the cell dry weight of different yeast strains and mainly consist of a mixture of mannan-oligosaccharides (MOS), glucans, and small amounts of chitin and protein (Nguyen et al., 1998; Kogan and Kocher, 2007). Mannan-oligosaccharides can be considered as non-digestible and fermentable carbohydrates (NDO) and can modulate the intestinal microbiota (Metzler et al., 2005). The presence of NDO may result in a higher production of VFA, especially in large intestine. If specific components of HY have the potential to improve intestinal morphology in the large intestine is not clear yet and warrants further research.

In experiment 2, especially the AID of CP, but also the AID of EE, seemed to be on low levels with average values of 70% and 90%, respectively, with a

tendency of higher values in group HY compared with the CON. A possible explanation could be that endogenous losses through epithelial cell renewal are higher in the immediate postweaning period compared with the situation in older pigs. On the other hand, it is well established that the acute phase of weaning (d 1 to 14) is generally associated with a lower activity of pancreatic enzymes (e.g., lipase, trypsin, amylase) and brush-border enzymes (e.g., aminopeptidase N, dipeptidyl peptidase-4) in the jejunum and ileum (Montagne et al., 2007). Brush-border enzymes are released by enterocytes in the crypt and villus area. Their activity can therefore decline with villus atrophy (Heo et al., 2013). A lower activity of enzymes results in a reduced ileal digestibility of nutrients and both can be used as indicators for gut development. Literature data on AID of CP or EE in pigs 14 d after weaning are limited. AID of CP and EE seems particularly low in first 2 wk after weaning and is strongly influenced by diet composition. Average values for AID of CP of 66% to 67% early after weaning and of 79% at end of weaning phase (d 35) were reported (Giang et al., 2010; Jha et al., 2010). Supplementation of 0.1% to 0.4% MOS derived from yeast cell wall (*S. cerevisiae*) improved the AID of CP 2 wk after weaning (Nochta et al., 2010; Zhao et al., 2012), but had no effect on AID of CP at the end of the weaning period (Zhao et al., 2012). In pigs, no effects of MOS supplementation on AID of EE were observed and the AID of EE amounted to 94% (Nochta et al., 2010). Effects of whole yeast-based products on the AID of CP are inconsistent. Yeast (*S. cerevisiae*) at an inclusion rate of 6% had no impact on AID of CP in weaned pigs (Moehn et al., 2010), whereby Spark et al. (2005) reported positive effects on N-digestibility after supplementation of different yeast strains (*S. cerevisiae*, *Kluyveromyces lactis*, *K. fragilis*) replacing soybean meal at different levels (20%, 40%, 60%). The authors concluded that the quality of *K. fragilis* compared with *S. cerevisiae* and *K. lactis* stands out because of its high N-digestibility (92.2% vs. 87.2% and 82.8%) and higher N-retention (65.8% vs. 61.4% and 58.5%). In the presented study 1% HY tended to improve AID of CP and EE and might be influenced also by different indirect factors like improved feed intake and villus height.

The change of short-circuit current gives an indirect measure of electrolyte-dependent nutrient absorption such as glucose or AA (e.g., L-glutamine). Weaning induces transient modifications of intestinal physiology. For example, an increase of I_{sc} and a decrease of the transepithelial resistance were

observed in the jejunum of pigs 2 d but not 5 d after weaning (Boudry, 2005). In the presented study, after addition of either carbachol or L-glutamine, no differences for ΔI_{sc} or ΔG_t were found between the three different dietary treatments. Also, other authors did not find the effects of diet composition on parameters measured by Ussing chamber technique. The used diets differed either in protein sources (soya vs. egg powder or whey powder) or in protein concentration varying from 15% to 44% CP (Miller and Skadhauge, 1997; Spreeuwenberg et al., 2001; Boudry, 2005). However, supplementation of feed additives (e.g., zinc, pre- and probiotics) may influence transport properties and barrier function in weaned pigs (Boudry, 2005). The effect of yeast-derived prebiotic (MOS) was investigated in growing pigs fed MOS for 3 wk. Basal or forskolin stimulated I_{sc} were not altered by MOS, whereas G_t tended to be higher. MOS supplementation increased mucosal-to-serosal fluxes of mannitol in the distal jejunum (Breves et al., 2001). Probiotics (*Enterococcus faecium*, *S. boulardii*, *Bacillus cereus*) increased glucose adsorption in weaned pigs and numerically increased L-glutamine adsorption in *E. faecium*-fed pigs (Breves et al., 2000; Lodemann et al., 2006). In general, alterations of absorptive and secretory properties of the jejunal epithelium seemed to be more age-dependent than treatment-dependent.

In conclusion, the two tested yeast products based on *K. fragilis* had minor effects on performance and intestinal physiology. NHY yeast had no effect on feed intake and gut physiology, whereby HY at a dosage of 1% in the diet-stimulated feed intake and improved gut morphology in the early postweaning period (d 1 to 14).

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