Received: 23 November 2020 | Accepted: 15 March 2021

DOI: 10.1111/jpn.13549

SUPPLEMENT ARTICLE

Journal of Animal Physiology and Animal Nutrition WILEY

Effects of increasing dietary rye levels on physicochemical characteristics of digesta and its impact on stomach emptying as well as the formation of 'doughballs' in stomachs of young pigs

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Funding information
Federal Ministry of Food and Agriculture (BMEL)

Abstract

Despite quite similar contents of starch and crude fibre of wheat and rye, the unique non-starch-polysaccharide fraction of rye (e.g. high levels of arabinoxylans and fructans) might have an impact on physicochemical properties of the digesta in pigs. Forty pigs (age: 46.8 ± 5.28 days; bodyweight: 16.1 ± 4.13 kg) were divided into four treatment groups. During four weeks, the pigs received diets consisting of wheat and/or rye, barley, soy, potato protein and a mineral supplement. The sum of wheat and rye was 69% in all diets, whereby the compound feed of each group was characterized by a different ratio (%) of wheat/rye (69/0; 46/23; 23/46; 0/69, respectively). In the stomach, 'doughballs' occurred more frequently with increasing dietary rye levels (9/10; 69% rye). With higher DM content and extract-viscosity of gastric digesta, the stomach emptying tended to be retarded in rye groups. Compared to the control group (69% wheat), maximum dietary rye levels (69%) resulted in significantly higher concentrations of lactic acid in digesta of the stomach and small intestine. With increasing lactic acid concentrations, the pH tended to be lower in small intestinal digesta. With an intensified formation of lactic acid, effects against Gram-negative bacteria, for example *Salmonella*, can be expected. Moreover, because of higher viscosity and the retarded stomach emptying, there could be advantages of including rye in compound feeds when a longer lasting satiety is intended, for example when feeding pregnant sows (regularly fed restrictively).

KEYWORDS
dietary fibre, digesta, doughballs, lactic acid, pigs, rye, stomach

1 | INTRODUCTION

Modern animal husbandry in Germany is particularly confronted with challenges that are decisively influenced or determined by the consumer. The current Nutrition Report of the Federal Ministry of Food and Agriculture provides information on consumer expectations regarding animal production (BMEL, 2019). According to this report, animal welfare is of greatest importance, featuring even before resource efficiency and the quality of agricultural products. Animal nutrition has the task of developing appropriate solutions that take animal welfare, resource efficiency and food quality into account. In recent decades, there has been a clear trend in Europe towards including higher proportions of wheat in compound feeds (CF) for pigs, while those of barley and especially of rye have decreased (Kamphues et al., 2019). On the one hand, this was due to the higher energy and protein yields of wheat per ha (Rodehutscord et al., 2016); on the other hand, there are also certain reservations regarding higher dietary rye levels in compound feeds for pigs (Kamphues et al., 2019). However, due to success in rye breeding ('hybrid rye'), certain varieties now achieve higher yields and lower levels of ergot contamination (Kamphues et al., 2019). Inevitably, this will lead to an increased use of rye in feeding. Due to its specific non-starch polysaccharides (NSP), rye can be characterized as a cereal with a very high 'dietary fiber' content, affecting the physical and chemical characteristics of the digesta (Bach Knudsen & Lærke, 2010; Rodehutscord et al., 2016). Moreover, high amounts of soluble carbohydrates might lead to a forced formation of lactic acid in the front alimentary tract, which is beneficial for improving 'gut health' (Bunte, Grone, et al., 2020; Bunte, Keller, et al., 2020; Willamil et al., 2011). Therefore, the present study aimed at investigating changes in the physicochemical characteristics of digesta in young pigs fed increasing rye levels in the diet, considering potential benefits in terms of animal health, animal welfare and food safety.

2 | MATERIALS AND METHODS

2.1 | Experimental design, animals and housing

In total 40 young pigs (age: 46.8 ± 5.28 days; bodyweight (BW): 16.1 ± 4.13 kg) were transferred to the stables of the Institute for Animal Nutrition of the University of Veterinary Medicine Hannover and were allotted to two consecutive trials. On the basis of body weight, sex ratio (female/male castrated) and the sow (siblings distributed to the groups), the pigs were selected for the formation of four comparable treatment groups in each trial. The cross-bred pigs were housed individually in 1 m × 3 m pens with a concrete floor, equipped with a nipple drinker (permanently free access to water) and manipulable material as enrichment. Visual, olfactory and tactile contact to other pigs was possible at all times. In both trials, a single, that is one identical test protocol was used.

After four weeks of dietary treatment, the animals were anaesthetized via an intramuscular injection of ketamine (Ketamidor 100 mg/ml®, Richter Pharma; dosage: 20 mg ketamine/kg BW) and azaperone (Stresnil 40 mg/ml®, Lilly Deutschland; dosage: 2 mg azaperone/kg BW). The anaesthetized animals were euthanized with an intracardial application of T61® (Intervet; dosage: 0.4 ml/kg BW).

2.2 | Diets, feed analysis and feeding regime

The different groups were fed a dry and pelleted diet consisting of wheat and/or rye, barley, soy, potato protein and a mineral supplement, generally ad libitum (Table 1). Wheat and rye were ground with a hammer mill (3 mm sieve), followed by mixing and then pelleting. Diets were analysed for their chemical composition by standard procedures in accordance with the official methods of the Association of Agricultural Inspection and Research Institute (Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten; VDLUFA) as described by Naumann and Bassler (2012). The standardized methods at the Department of Animal Science, Aarhus University, Denmark, were used to measure the NSP in diets I and IV. Feed particle size distribution was assessed by the wet-sieve method in accordance with Wolf et al., (2012). Extract viscosity was measured as described by Grone (2018). The chemical composition, the particle size distribution and the extract viscosity of the diets are summarized in Table 2.

To determine the stomach-emptying rate, at the day before dissection at 17:00 the diets were taken away from the animals. After a period of 12 h without any feed intake, the diets were offered again ad libitum for a four-hour period. At the moment the first pig had been anaesthetized, the feed was taken away from all animals (to ensure identically end of feed intake). The pigs were then euthanized and dissected in such an order that the average times after feeding were comparable in all groups.

TABLE 1 Botanical composition of the diets (%)

Diet	I	II	III	IV
Group	3/3 Wheat	1/3 Rye	2/3 Rye	3/3 Rye
Ingredients				
Wheat	69.0	46.0	23.0	0
Rye	0	23.0	46.0	69.0
Soybean meal[a]	11.5	11.5	11.5	11.5
Barley	10.0	10.0	10.0	10.0
Potato protein	5.10	4.95	4.90	4.90
Calcium carbonate	1.00	1.00	0.95	0.90
Monocalcium phosphate	0.90	0.90	0.95	1.00
Fat (soybean oil)[a]	0.50	0.50	0.50	0.50
Sodium chloride	0.35	0.40	0.40	0.40
Feed additives[b]	1.65	1.75	1.80	1.80

[a]Soybean meal (steam heated, with soapstock) made from genetically modified soybeans.

[b]Additives (per kg as fed); nutritional additives: vitamin A (12,000 IU), vitamin D3 (2000 IU), vitamin E (150 mg), copper from copper-(II)-glycinate chelate hydrate (4 mg), copper from copper-(II)-sulphate pentahydrate (110 mg), manganese from manganese glycine manganese chelate hydrate (35 mg), manganese from manganese-(II)-oxide (45 mg), zinc from glycine zinc chelate hydrate (40 mg), zinc from zinc oxide (80 mg), iron from iron-(II)-sulphate monohydrate (200 mg), iodine from calcium iodate anhydrous (2.0 mg), and selenium from sodium selenite (0.40 mg).

2.3 | Analyses of digesta

In dissection, the different parts of the alimentary tract were separated. In the representative digesta samples, dry matter (DM) content and pH values were determined before freeze-drying. Digesta samples were then ground for further analysis (using centrifugal

TABLE 2 Analysed nutrient composition of compound feeds (g/kg DM)

Diet	I	II	III	IV
Group	3/3 Wheat	1/3 Rye	2/3 Rye	3/3 Rye
DM content (g/kg as fed)	897	897	894	899
Crude ash	48.4	53.2	46.2	51.3
Crude protein	205	205	198	198
Crude fat	27.4	28.1	32.6	24.5
Crude fibre	26.2	24.9	29.9	22.0
NfE	625	622	624	637
Starch	530	514	493	491
Sugar	41.3	46.5	52.1	60.0
NSP (total)	123	-	-	140
NSP (insoluble)	88	-	-	93
Arabinoxylans (total)	63	-	-	74
Arabinoxylans (soluble)	18	-	-	27
Dietary fibre	143	-	-	156
Cys	3.97	3.56	3.54	3.34
Met	5.24	5.22	4.64	5.60
Lys	14.7	14.7	14.4	15.0
ME (MJ/kg DM)[a]	15.8	15.8	15.7	15.7
Particle size (%)				
>1 mm	27.5	28.2	23.6	22.4
>0,2 mm	42.0	42.8	41.1	45.4
Extract viscosity (mpA*s)	1.47	1.81	1.83	2.78

[a]Calculated from nutrient composition.

mill/0.5 mm sieve). Digesta samples were analysed by standard procedures in accordance with the official methods of the VDLUFA (Naumann & Bassler, 2012). Moreover, extract viscosity in digesta was measured in accordance with Grone (2018).

2.4 | Statistical analyses

Statistical analyses were performed using the computer programmes Statistical Analyses System for Windows, SAS® 9.4 by means of Enterprise Guide Client Version 7.1 (SAS Institute Inc.) and Excel® 2016 (Microsoft Corp.). A test for normal distribution was performed by distribution analysis using the Shapiro–Wilk test for analytical evaluation. Normally distributed model residuals were tested by using a multiple range test (Ryan–Einot–Gabriel–Welsch test). For non-normally distributed data, the Kruskal–Wallis test with post hoc test for multiple two-sided paired comparisons tests in accordance with Dwass, Steel and Critchlow-Flinger was applied accordingly. The significance level was determined to be alpha = 5% ($p < 0.05$).

3 | RESULTS

The feed intake before dissection, the postprandial distance (time) and the digesta mass (DM) in the stomach at the time of dissection were taken into account to calculate stomach emptying. Therefore, stomach emptying matches the mass of digesta (DM) that had left the stomach from the beginning of feeding per hour. In comparison with the control group, the animals, fed rye, showed a reduced 'outflow' of digesta by about 13%. This means that 13% less DM mass left the stomach per hour towards the small intestine (Table 3).

TABLE 3 Feed intake during four-hour feed supply (ad libitum) before the dissections and time interval (hours) between feeding and dissection (time) as well as stomach-emptying rate

Group	Feed intake (DM)	Time (h)	Stomach empying[a] g DM/h
3/3 Wheat	559 ± 110	5.50 ± 1.29	80.9 ± 17.3
1/3 Rye	518 ± 80.4	5.60 ± 1.35	68.7 ± 16.6
2/3 Rye	531 ± 108	5.60 ± 0.651	70.0 ± 12.0
3/3 Rye	529 ± 90.7	5.50 ± 1.33	70.1 ± 20.1

[a]Calculated: feed intake (g/dry matter) minus amounts of digesta in the stomachs (g/dry matter) divided by time (hours after the diet was offered)

With higher dietary rye levels, 'doughballs' (compacted mass of ingested diet; Figure 1) were found more frequently in the otherwise liquid stomach contents. In summary, 90% of all animals in group 3/3 rye showed described differences in stomach content (Table 4).

With increasing proportions of rye in the diets, the extract viscosity increased in contents of the stomach and small intestine, whereas no significant differences occurred in caecal and colonic contents (Table 5).

FIGURE 1 'Doughball' found in stomach content of young pigs during dissection

TABLE 4 Occurrence of 'doughballs' in the stomach contents of the young pigs

Group	Occurence of doughballs (*n/n*)[a]
3/3 Wheat	1/10
1/3 Rye	2/10
2/3 Rye	5/10
3/3 Rye	9/10

[a]Observed/tested.

With an increasing dietary rye level, DM contents in digesta of stomachs rose significantly. No significant differences occurred in the digesta of the other alimentary tract sections (Table 6).

The pH values increased in gastric digesta in all groups with higher dietary shares of rye in the diet (Table 7). In digesta of the small intestine and of the colon, with higher dietary rye levels, the pH values tended to be lower.

With higher shares of rye in the diet, L-lactate concentrations (g/kg DM) rose significantly in the digesta of the stomach and small intestine. With higher standard deviations, the content tended to be higher in caecal digesta. At high concentrations of L-lactate, notable concentrations of D-lactate occurred (Tables 8 and 9).

4 | DISCUSSION

In spite of a similar composition of the diets, when rye was substituted for wheat interesting changes in the gastrointestinal tract (GIT) occurred, especially in the cranial GIT. Grone (2018) determined a 2.5 times higher extract viscosity for rye meal compared to wheat meal. Between the diets of group 3/3 wheat and 3/3 rye, this factor could be given as 1.9. The viscosity is influenced by the fibre components (Burton-Freeman, 2000). Molecules, which affect the extract viscosity, are generally referred to as soluble 'Dietary Fiber' (Weickert & Pfeiffer, 2008). According to findings by Bach Knudsen and Lærke (2010) and Grone (2018), the high amount of soluble arabinoxylans is responsible for the particularly high extract viscosity of rye—in diet of group 3/3 rye, this content of arabinoxylans was 50% higher than in control diet. Moreover, the extract viscosity is influenced by the time of soaking; the highest values were determined for rye meal after five hours of soaking (Grone, 2018). The increase in extract viscosity in the gastric digesta, compared to the values for the CF, was therefore expected. According to Grone (2018), there is also a positive correlation between DM content in digesta and

TABLE 5 Extract viscosity in the digesta of stomach, small intestine, caecum and colon (mpA*s)

Group	Stomach	Small intestine	Caecum	Colon
3/3 Wheat	$2.03^{a} \pm 0.628$	$2.33^{a} \pm 0.959$	2.32 ± 0.429	12.2 ± 8.90
1/3 Rye	$8.14^{ab} \pm 6.62$	$3.23^{ab} \pm 1.65$	2.15 ± 0.433	12.1 ± 9.45
2/3 Rye	$16.8^{b} \pm 15.6$	$4.06^{ab} \pm 2.78$	2.35 ± 0.285	11.7 ± 5.11
3/3 Rye	$51.8^{c} \pm 12.5$	$6.45^{b} \pm 4.75$	2.61 ± 1.09	7.72 ± 5.56

[a,b,c]Significant differences between the groups ($p < 0.05$).

TABLE 6 Dry matter contents (g/kg) in the digesta of stomach, small intestine, caecum and colon

Group	Stomach	Small intestine	Caecum	Colon
3/3 Wheat	$197^{b} \pm 49.2$	117 ± 32.6	106 ± 20.8	181 ± 47.7
1/3 Rye	$236^{b} \pm 108$	102 ± 38.9	95.7 ± 21.3	176 ± 26.5
2/3 Rye	$265^{b} \pm 81.8$	106 ± 30.0	119 ± 26.6	187 ± 30.2
3/3 Rye	$353^{a} \pm 66.1$	91.9 ± 36.1	98.8 ± 28.4	173 ± 29.3

[a,b,c]Significant differences between the groups ($p < 0.05$).

extract viscosity. Thus, with an increasing rye content in the diet, a significant increase in the DM content in the digesta of the stomach was also determined in the present study. In comparison with stomach digesta, DM content and extract viscosity were lower in the digesta of the small intestine, although significant differences in extract viscosity remained between the groups. According to Bach Knudsen and Lærke (2010), a large amount of soluble arabinoxylans is already degraded between the ileum and the caecum, which can explain a generally reduced viscosity in caecal digesta without evident differences between the groups. Generally, a higher viscosity in the digesta of the small intestine is said to be associated with reduced digestibility rates (Hooda et al., 2010), which might therefore explain a lower praececal digestibility of crude protein of rye (McGhee & Stein, 2018). However, a significantly higher viscosity in digesta of the small intenstine, achieved by adding carboxymethyl cellulose, did not negatively influence the praececal digestibility of crude protein (Hooda et al., 2010). The authors explained this with a prolonged retention time in the stomach and a slower digesta passage in the small intestine, due to the higher viscosity. According to Weickert and Pfeiffer (2008), a higher extract viscosity of small intestinal digesta could result in a slower/delayed glucose-absorption and thus a more moderate insulin response (Ellis et al., 1995). Due to the fact, that probably no postprandial hypoglycaemia occurs, this could result in a longer lasting satiety (Rosén et al., 2011).

Inevitably, the formation of 'doughballs' influenced the characteristics of the digesta and might have had an impact on stomach emptying. The highest values for stomach emptying were found in the animals of the control group (wheat only). Already Bolduan et al., (1988) noted a slower, rather delayed gastric emptying in rye rich feeds. Rainbird and Low (1986) reported a delayed gastric emptying when the viscosity in the gastric digesta increased. According to Kamphues (1988), gastric emptying is significantly influenced by feed intake and stomach fill and therefore the highest gastric emptying rates occur in the period immediately after feed intake or in the period when a high mass of filling is present.

The 'doughballs' led to higher DM contents and thus higher extract viscosity in gastric digesta, which is likely to result in delayed emptying. Besides this, 'doughballs' may result in a higher and longer degree of stomach filling, which, in turn, according to Kamphues (1988), should at least accelerate the passage of the liquid phase. It must be emphasized, however, that it took several hours (up to 7 hours after beginning of feed intake) until the 'doughballs' had disappeared. In conclusion, a reducing effect of the 'doughballs' on the stomach-emptying rate is more likely. In our own experiments, an effect of the forced feed intake on the mass and quantity of 'doughballs' was possible. However, it is known that 'doughballs' also occur under conditions of continuous ad libitum feeding, especially when pelleted or extruded diets are used (Liermann et al., 2015). According to these authors, formation of 'doughballs' occurs as a result of heat exposure during pelleting/extrusion (changes in the structure of starch). According to Lundblad et al., (2011), higher temperatures and physical forces (pressure/shear forces) result in higher extract viscosity of a pelleted or extruded CF. In the present study, the increasing occurrence of 'doughballs' with higher dietary rye levels cannot be explained by the production process (all diets were pelleted at identical conditions). In summary, a correlation between extract viscosity in stomach contents and the occurrence of 'doughballs' can be assumed. This can be favoured both by processing of the CF and, to a particular extent, by certain constituents in rye, forcing the development/formation of 'doughballs' in stomach digesta.

For rye, in comparison with wheat, a generally lower praececal digestibility of crude protein is known. The pH level within the

TABLE 7 pH values in the digesta of stomach, small intestine and colon

Group	Stomach	Small intestine	Colon
3/3 Wheat	3.68[b] ± 0.850	6.46 ± 0.739	6.01 ± 0.341
1/3 Rye	4.79[a] ± 0.888	6.16 ± 0.602	6.01 ± 0.299
2/3 Rye	4.79[a] ± 0.541	6.16 ± 0.481	5.98 ± 0.461
3/3 Rye	5.25[a] ± 0.600	5.76 ± 0.679	5.87 ± 0.344

[a,b,c]Significant differences between the groups ($p < 0.05$).

TABLE 8 L-lactate contents (g/kg DM) in the digesta of the stomach, small intestine, caecum and colon

Group	Stomach	Small intestine	Caecum	Colon
3/3 Wheat	1.19[b] ± 0.889	32.4[b] ± 24.6	12.0 ± 16.6	2.15 ± 5.82
1/3 Rye	3.11[ab] ± 2.42	47.7[ab] ± 21.9	15.4 ± 14.9	1.03 ± 3.04
2/3 Rye	2.79[ab] ± 1.57	42.9[b] ± 18.7	14.6 ± 18.9	2.33 ± 4.42
3/3 Rye	4.81[a] ± 3.45	75.8[a] ± 44.7	29.9 ± 30.2	3.28 ± 7.50

[a,b,c]Significant differences between the groups ($p < 0.05$).

TABLE 9 D-lactate content (g/kg DM) in the digesta of stomach, small intestine, caecum and colon

Group	Stomach	Small intestine	Caecum	Colon
3/3 Wheat	0.305 ± 0.372	0.741 ± 0.684	4.24 ± 5.71	0.873 ± 1.81
1/3 Rye	0.626 ± 0.898	0.795 ± 0.763	5.38 ± 5.97	0.378 ± 1.01
2/3 Rye	0.659 ± 0.453	1.44 ± 2.20	5.07 ± 5.09	0.877 ± 1.63
3/3 Rye	0.896 ± 0.855	1.37 ± 1.66	10.2 ± 9.01	3.10 ± 5.59

'doughballs' was approximately six, which clearly proves an only insufficient acidification. According to Kamphues et al., (2019), an impaired activity of pepsin can be assumed within the 'doughballs'. Negative effects on the protein digestibility in the small intestine can be expected if, on the one hand, denaturation of the proteins in the stomach is reduced by a less acidic milieu and, on the other hand, pepsin-related proteolysis is also delayed and less efficient (pH optimum of pepsin: 1–3.5; Simon, 2008; Wolffram, 2015).

An accumulation of lactic acid can have positive effects on intestinal health. For example, an antimicrobial effect against Gram-negative bacteria such as *Salmonella* can be assumed (Bunte, Grone, et al., 2020; Bunte, Keller, et al., 2020; Papenbrock et al., 2005). Kamphues (1988) reported considerable contents of lactic acid in the stomach and small intestine during a forced feed intake; the lactic acid contents varied in the stomach digesta by up to 14.9 g/kg DM and even higher values of up to 61.7 g/kg DM in small intestines digesta. A fermented liquid diet (rye content: 50%), given to young pigs in a study by Bunte, Grone, et al. (2020) and Bunte, Keller, et al. (2020), contained a lactic acid content of 53.7 g/kg DM. After an ad libitum intake, the lactic acid content varied by 32.4 g/kg DM in gastric digesta and by 47 g/kg DM in small intestinal digesta. Despite these high lactic acid contents, neither any loss in the zootechnical performance of the animals nor any negative effects on digestibility occurred. However, there was a strong impact on the intestinal microbiome (Bunte, Grone, et al., 2020; Bunte, Keller, et al., 2020). In our experiments, significantly higher contents of lactic acid in digesta were found with an increasing rye content in the diet. In group 3/3 rye, the lowest pH values were found. An 'excessive fermentation' in the sense of a significant exceeding of the resorption and buffering capacities of the intestine can be assumed. Nevertheless, these high lactic acid contents can, however, lead to a reduction in potentially pathogenic bacteria (Bunte, Grone, et al., 2020; Bunte, Keller, et al., 2020). Moreover, with the high contents of lactic acid as well as low pH values in digesta of the small intestine, the positive effects of rye based diets on the prevalence of *Salmonella* (Chuppava et al., 2020) might be explained in part. With regard to the observations, made by Kamphues (1988), however, it can be assumed that the previous forced feed intake had an influence on the above-mentioned results. Under conditions of ad libitum feeding, more moderate effects could therefore be expected.

The marked differences in extract viscosity in the diet as well as stomach and small intestinal content disappeared in the caecum and colon, which means that majority of the viscosity-affecting constituents seem to be degraded mostly when entering the caecum.

In conclusion, with a higher DM content and extract viscosity, the stomach emptying tended to be retarded in the rye groups, leading to a longer lasting stomach fill. With high contents of lactic acid as well as low pH values in digesta of the small intestine favourable effects of rye based diets against pathogens, like *Salmonella*, are to be expected. Thus, there could be advantages including rye in compound feeds when a longer lasting satiety is intended, for example when feeding pregnant sows (regularly restrictive), or when effects against *Salmonella* are desirable.

ACKNOWLEDGMENTS

We would like to thank Professor K. E. Bach Knudsen and his staff for analysing the NSP content and Frances Sherwood-Brock for proofreading the manuscript to ensure correct English. The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page. The experiments were carried out in accordance with German regulations. These animal experiments required no approval in accordance with the Animal Protection Act (§ 7, paragraph 2, sentence 3). Interventions before dissection were not carried out. The animals were killed in accordance with § 4, paragraph 3 of the Animal Protection Act, exclusively to use their organs or tissues for scientific purposes. The experiments were approved by the Animal Welfare Officer of the University of Veterinary Medicine Hannover, Hanover, Germany (reference: TiHo-T-2018-24).

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How to cite this article: Wilke V, Grone R, von Felde A, Abd El-Wahab A, Wolf P, Kamphues J. Effects of increasing dietary rye levels on physicochemical characteristics of digesta and its impact on stomach emptying as well as the formation of 'doughballs' in stomachs of young pigs. *J Anim Physiol Anim Nutr*. 2021;00:1–7. https://doi.org/10.1111/jpn.13549