

## Effects of Resistant Potato Starch on Odor Emission from Feces in Swine Production Units

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Odor emission from swine facilities is determined by microbial breakdown of amino acids or carbohydrates in the pig colon. It was the aim to influence apoptosis and thus amino acid availability for odor formation by feeding resistant starch (300 g kg<sup>-1</sup> feed) over the whole fattening period to 40 pigs. Concentrations of 12 key components (indoles, volatile fatty acids, methanethiol) were measured in feces and headspace over the slurry duct and compared to 40 normally fed controls in a separate compartment. Concentrations of substances resulting from amino acids were reduced in feces by 70% (indoles) and 8% (branched chain fatty acids) and in the headspace by 72% and 20%. Resistant starch only led to minor increases of straight chain fatty acid concentration. Maximal reduction occurred for 3-methyl-1*H*-indole (skatole) which is the main determinant of malodor so that the results point to promising strategies for reducing pig odor emission.

**KEYWORDS:** *Pig; odorous compounds; feces; air; resistant potato starch*

### INTRODUCTION

Odor of manure from the monogastric pig is particularly unpleasant to the human nose, so that odor emissions from swine housing are a major environmental concern. More than 160 different compounds may contribute to this unpleasant odor (1, 2) but most of the offensiveness can be explained by comparatively few substances (3–6).

Several investigations (7, 8) confirm that 1*H*-indole (indole), 3-methyl-1*H*-indole (skatole), and 4-methylphenol (*p*-cresol) are the main determinants for odor offensiveness (3). Mixtures containing 1*H*-indole, 3-methyl-1*H*-indole, and 4-methylphenol are usually described as “pungent, nauseating, and fecal” by olfactory panels (9). 1*H*-Indole and 3-methyl-1*H*-indole result from microbial degradation of the amino acid tryptophan. 4-Methylphenol is the main end product of the fermentation of tyrosine (10). A less pronounced, nevertheless important, contribution is caused by volatile fatty acids (VFA) such as butanoic, pentanoic, hexanoic, and heptanoic acid as well as the branched chain VFA 2-methylpropanoic and 2/3-methylbutanoic acid. Except for the branched chain VFA, other VFA mainly result from carbohydrate fermentation by colonic microbes, but butanoic acid was shown to be also the result of fermentation of glutamine (11). Amino acid digestion of valine, leucine, and isoleucine in contrast leads to the branched chain VFA 2-methylpropanoic, 3-methylbutanoic, and 2-methylbutanoic acid, respectively. Methanethiol is a sulfur-containing product of microbial fermentation of methionine which also contributes to the malodor. Moreover, sulfates in the diet also support the synthesis of methanethiol. Only a minor contribution

was attributed to other VFAs such as ethanoic and propanoic acid which also result from carbohydrate degradation. These 12 substances were selected for the development of our method for the analysis of key compounds of odor from swine facilities (12).

Generally, small peptides or free amino acids in the colon are used to synthesize bacterial protein provided that energy is available (13). Modern breeds of pigs with a high potential to synthesize protein are fed with rations with a high digestibility specifically in the small intestine. In consequence, energy and amino acid availability for microbes in the colon are generally limited, so that a part of the remaining amino acids is fermented by microbes to obtain energy. The resulting substances are key compounds for odor offensiveness. In addition, carbohydrates with a low prececal digestibility are substrates for VFA synthesis. So far, strategies to reduce odor intensity in the exhaust air concentrated on technical approaches such as biofilters (14, 15). In addition, it was attempted to inhibit microbial activity in stored slurry by adding chemicals to counteract ongoing formation of malodorous compounds (16, 17). It might be more promising, however, to inhibit the formation of odor substances in vivo either by modifying the composition of the intestinal microflora, e.g. by probiotics, or by limiting substrate availability for odor-producing genera of microbes. Because altering microbial composition was not stable enough during prolonged periods (18, 19), other attempts concentrated on the feeding of carbohydrates with low prececal digestibility (“fiber”), so that the remaining amino acids in the colon are predominantly used for bacterial protein synthesis and are not degraded. Fructo-oligosaccharides such as inulin led to a decrease of 3-methyl-1*H*-indole formation (20). In a more recent trial about 5% of chicory inulin in the ration successfully reduced the 3-methyl-

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**Table 1.** Composition of the Rations for the Control and the RS Group in the Three Phases of the Fattening Period<sup>a</sup>

component	phase 1 (30–60 kg) %		phase 2 (60–85 kg) %		phase 3 (85 kg-end) %	
	controls	RS	controls	RS	controls	RS
barley	39.17	5.75	40.90	7.46	50.00	12.80
maize	-	19.73	-	20.40	-	10.00
potato starch	-	30.0	-	30.00	-	30.00
wheat	38.50	-	40.50	3.90	37.70	12.98
wheat bran	-	12.00	-	11.00	-	10.00
soya bean (extracted), 44% CP	-	16.00	-	13.00	-	11.00
soya bean (extracted), 49% CP	16.00	-	12.30	-	8.00	-
rape seed oil	-	2.50	-	2.00	-	2.00
potato protein	-	1.75	-	1.00	-	-
wheat gluten	-	5.00	-	4.00	-	4.00
wheat flour	3.00	3.80	3.00	3.80	1.00	3.80
DL-methionine	0.30	0.17	-	0.14	-	0.12
L-lysine HCl	0.30	0.30	0.30	0.30	0.30	0.30
minerals	3.00	3.00	3.00	3.00	3.00	3.00
energy content, MJ kg <sup>-1</sup> ME	12.95	12.74	12.91	12.68	12.78	12.62
CP content, g kg <sup>-1</sup>	172.20	176.80	158.10	158.20	139.70	146.50
CF content, g kg <sup>-1</sup>	34.00	33.60	34.10	32.30	35.70	32.20

<sup>a</sup> CF = crude fiber, CP = crude protein, ME = metabolizable energy.

1*H*-indole contents in feces but had no effect on other odorous compounds (21). Careful adaptation of amino acid content and composition of the diet to the actual demands of growing pigs did not alter odor formation and emission (22, 23). It was concluded, therefore, that those amino acids which are used by microbes for odor formation mainly result from apoptosis and thus from shedded cells in the gut mucosa (20, 24). In consequence, feeding strategies are developed which reduce gut cell apoptosis.

Those carbohydrates in the diet which are preferentially metabolized to butyrate in the large intestine (resistant starches such as raw potato starch) are candidate feed components to inhibit malodor formation because butyrate was shown to be an inhibitor of apoptosis both in the calf rumen (25) and the colon of the pig (26). Reduced apoptosis in turn leads to a lower amino acid availability for microbes. The resistance of raw potato starch to prececale digestibility results primarily from a high content of phosphate residues and larger starch granules with a lower surface (27, 28) and partly from a different crystalline structure (29). Thus, it is one of the carbohydrates which specifically increased butyrate formation by microbes and thus led to a remarkable reduction of 3-methyl-1*H*-indole formation (30). It is likely that some types of resistant starch (RS) additionally alter the microbial population in the gut and thus counteract the formation of malodorous substances by other mechanisms (31).

Systematic studies to reduce odor intensity based on such principles are yet to be performed, partly because they require objective analytical procedures to monitor the effect in the exhaust air. In a previous paper (12) we reported analytical developments for monitoring key substances leading to a highly practicable method which was based partly on the information which had been published previously (8, 32). Therefore, it was the aim of the study to monitor the effects of feeding raw potato starch on odor compounds in feces and air under field conditions along the fattening period.

## MATERIALS AND METHODS

**Experimental Groups and Housing.** The experiment was performed at the progeny testing and experimental station for pig breeding in the state of Baden-Württemberg, Forchheim, Germany (LSZ, Landesanstalt für Schweinezücht in Forchheim). For the experiments, 80 growing pigs (40 barrows and 40 gilts, Hybrid x Pietrain) were used. They were randomly allocated either to the control group or the RS group which

were characterized by different feeding regimes. The pig fattening unit had a size of 160 m<sup>2</sup> and a room height of 2.9 m, so that the air volume was 5.3 m<sup>3</sup> per pig as it is normally for pigs under fattening conditions (33). Fresh air was provided by forced ventilation, and room air was exhausted through chimneys. Prior to the onset of investigation the pig house and slurry ducts were airtight separated into two identical compartments. Each compartment contained four pens, and each pen held 10 pigs.

**Feeding.** The overall fattening period lasted from 30 kg till 105 kg (barrows) and 115 kg (gilts), respectively, corresponding to a duration of 99 days for barrows and 118 days for gilts (overall mean: 109 days). The average daily weight gain was 791 g.

Feeding was separated into three phases (Table 1). It was the aim of the experiment that the different rations for control and RS group were similar in nutrient contents. Minor differences between the control and RS diets are explained by a lower content in metabolizable energy of resistant potato starch due to energy losses during bacterial fermentation in the hind gut. The feed was provided twice daily as a semi ad libitum feeding regime which was adapted continuously to the actual demands, so that the mean supply increased from 1.6 to 2.5 kg per day in the first phase, from 2.5 to 2.8 kg per day in the second phase, and from 2.9 to 3.1 kg per day in the finishing phase.

**Sampling of Air and Feces.** To exclude effects of the gradual adaptation to the environment and feeding conditions, sampling started 28 days after the onset of the trial and was finished when more than 50% of the animals had reached the slaughter weight and were removed from the group. Thus, the sampling period was limited to 87 days (12 weeks). Within the sampling period standardized air samples were taken 3 days a week between 8.00 and 11.00 a.m. Samples were collected in the headspace of the slurry duct. Therefore, cartridges were positioned about 0.2–0.3 m under the slatted floor. Parallel to the air sampling fresh feces were taken randomly from three to four animals per pen on the same day. Because it is normal behavior of pigs that defecation occurs usually after feeding, an aliquot portion of feces was immediately taken in a standardized amount. Feces sampling thus occurred during air sampling. Feces samples of the same feeding group were pooled and kept deep frozen (–20 °C) till assayed. Parallel determinations of the key substances in feces and air were performed. Thus, a correlation between the source of odor and the resulting air concentrations could be determined.

**Analytical Evaluation. Selection and Analytical Determination of Key Compounds.** As described earlier (12), methods were developed for the determination of 12 odor substances. Their contribution to three substance categories and their analytical characteristics are given in Table 2.

**Determination in Feces.** The contents of 3-methyl-1*H*-indole, 1*H*-indole, and 4-methylphenol (indoles) in feces were determined by a validated method which had been published earlier (34). Samples were

**Table 2.** Selected Odor Compounds, Principles of Measurement for Air Determinations, and Sensitivity of Analytical Procedures<sup>a</sup>

substance category	substance	principle of measurement	sorbent material	lower limit of detection [ng L <sup>-1</sup> ]
indoles	1 <i>H</i> -indole	RP-HPLC	STRATA C 18-E	0.01
	3-methyl-1 <i>H</i> -indole			0.01
	4-methylphenol			0.28
VFA	ethanoic acid	GC	STRATA X	0.50
	propanoic acid			0.63
	2-methylpropanoic acid			4.21
	butanoic acid			4.80
	3-methylbutanoic acid			4.38
	pentanoic acid			4.27
	hexanoic acid			5.35
heptanoic acid	51.60			
VSC	methanethiol	RP-HPLC	STRATA FL-PR	1.37

<sup>a</sup> VSC = volatile sulfur compound.

extracted with methanol and purified on Amberlite XAD-7. Dried residues from methanol-extracted feces were taken for dry matter (DM) determination as a basis for indoles and VFA contents in feces. The chromatographic separation was performed by reversed-phase HPLC, and the chromatographic conditions chosen were the same as described (12) for the determination of indoles in air. The detection wavelength was 280 nm. The lower limit of detection was 0.2  $\mu\text{g g}^{-1}$  DM for both 3-methyl-1*H*-indole and 1*H*-indole. Because 4-methylphenol had not been quantified earlier by this method, the quality parameters were determined for sensitivity, accuracy, and reliability. The accuracy was determined by adding known amounts of 4-methylphenol to a fecal sample with an endogenous amount of 66  $\mu\text{g g}^{-1}$  DM. The spiking amounts of 4-methylphenol added were 70 and 150  $\mu\text{g g}^{-1}$  DM ( $n = 4$ ). Repeatability was determined by repeated measurement of a sample on the same day (intraassay repeatability,  $n = 6$ ) or on consecutive days (interassay repeatability,  $n = 8$ ). The sensitivity was defined as 3-fold the background of the detector.

The amounts of VFA were determined as described earlier (30). The samples were brought up to a mass of 0.5 g with bidistilled water and 0.25 M sulfuric acid was added. Thereafter, the samples were centrifuged and the supernatant was used for determination of VFA by GC and flame ionization detection. The sensitivity of the method was 0.5  $\mu\text{mol g}^{-1}$  DM. The recoveries of VFA from spiked samples were all between 78% and 85%.

Because a high number of sulfur-containing substances cross-reacts with fluorescence labeling agents, the specific determination of methanethiol would require excessive purification of feces samples and thus was avoided.

**Determination in Air.** Air concentrations were carried out as described (12). Briefly, air sampling was performed on cartridges filled with solid-phase extraction (SPE) material as shown in Table 2. For the determination of methanethiol an in situ derivatization was performed within sampling on the cartridge. Therefore, the FL-PR cartridges were prepared with 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-CL) while C18E and STRATA X cartridges (for sampling of indoles and VFA, respectively) were only preconditioned with methanol. After individual sampling times the retained substances were eluted with solvents. Indoles and VSC were analyzed by RP-HPLC, whereas VFA were determined by GC combined with flame ionization detection (see Table 2). All operation procedures are explained in detail in our earlier paper (12). For all substances, identity conformation was performed by retention times in comparison to an external standard solution as well as by spiked samples (data not shown). Beyond this, a few substances, especially 4-methylphenol and hexanoic and heptanoic acid, were identified by GC-MS using external standards as well as by spiked samples (data not shown). The lower limits of detection for each substance are given in Table 2. The quantification of substances

**Table 3.** Concentrations (mg g<sup>-1</sup> DM) of Selected Odor Compounds in Feces ( $n = 36$ ) Sampled over the Whole Fattening Period in Controls and RS Group

substance	control		RS		alteration RS group versus control (%)
	mean	SD	mean	SD	
1 <i>H</i> -indole	0.05 a	0.01	0.02 c	0.01	-64
3-methyl-1 <i>H</i> -indole	0.10 a	0.02	0.03 c	0.01	-74
4-methylphenol	0.37 a	0.10	0.37 a	0.15	±0
2-methylpropanoic acid	0.75 a	0.08	0.69 b	0.13	-8
3/2-methylbutanoic acid <sup>c</sup>	1.34 a	0.14	1.23 b	0.29	-8
ethanoic acid	16.30 a	2.06	18.93 c	2.70	+16
propanoic acid	7.42 a	0.82	8.51 c	0.81	+15
butanoic acid	6.81 a	0.90	6.31 b	1.60	-7
pentanoic acid	1.83 a	0.39	2.70 c	0.59	+50
hexanoic acid	0.30 a	0.16	0.66 c	0.27	+120
heptanoic acid	0.20 a	0.20	0.73 c	0.41	+250

<sup>a</sup> a, b, c indicates significance of values within a row (a/b,  $P \leq 0.05$ ; a/c,  $P \leq 0.001$ ). <sup>b</sup> Contents of methanethiol in feces were not determined. <sup>c</sup> 3-Methylbutanoic acid will be determined together with its isomer 2-methylbutanoic acid resulting from isoleucine degradation which could not be separated under the applied GC conditions.

was done following the internal standard method. Individual internal standards for each substance category were dropped onto the cartridges after sampling before the elution step was started. These internal standards were added in equal amounts to external standard solutions covering the range of linearity.

**Statistical Evaluation.** In the tables results are presented as mean values with their standard deviations (SD). The Statistical Package for the Social Sciences (SPSS for Windows, version 11.5; SPSS inc., Chicago, IL) was used to determine whether variables differed among the feeding groups. Data were checked for normal distribution with the Kolmogorov–Smirnov test before statistical analysis was performed. The independent samples *t*-test was applied to demonstrate significant differences. Differences were considered to be significant at  $P \leq 0.05$  and highly significant at  $P \leq 0.001$ . Correlations between concentrations in feces and air over the whole fattening period were determined by Pearson's test (35).

## RESULTS

**Quality Criteria for 4-Methylphenol Determinations in Feces.** Quality criteria for the determination of 1*H*-indole and 3-methyl-1*H*-indole were published already (34). The determination of 4-methylphenol could be easily combined with this method. Under the chosen chromatographic conditions, the three substances could be clearly separated. The retention times were 6.7, 9.5, and 14.3 min for 4-methylphenol, 1*H*-indole, and 3-methyl-1*H*-indole, respectively. For 4-methylphenol determination, the mean intraassay coefficient of variation was 7% ( $n = 6$ ) and the mean interassay coefficient of variation was 15% ( $n = 8$ ). Lower limit of detection was 1.0  $\mu\text{g g}^{-1}$  DM. The mean precision (recovery) was 94%.

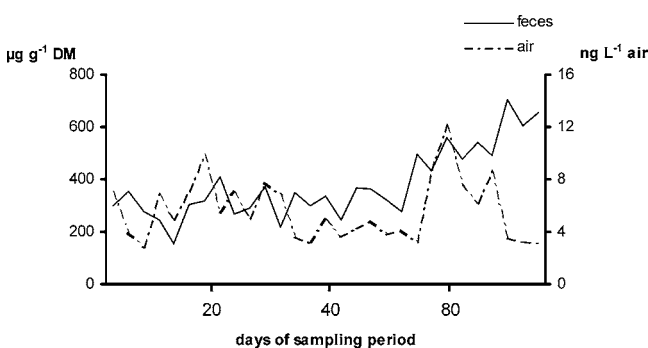
**Screening of Relevant Substances.** Mean values of the concentrations of the 12 substances in air and feces are listed in Table 3 (feces) and Table 4 (air). These tables also contain the significant differences between the RS and control group. Additionally, the correlation for each compound between the two matrices were calculated to give an impression of the dependence of the air contents on the values in feces.

As shown in Table 3, feeding of RS led to substance specific reactions in feces. 1*H*-Indole and 3-methyl-1*H*-indole were significantly decreased, whereas 4-methylphenol was not altered by RS. The branched chain fatty acids 2-methylpropanoic and 2/3-methylbutanoic acid result from amino acid fermentation.

**Table 4.** <sup>a,b</sup>Concentrations ( $\mu\text{g L}^{-1}$  Air) of Selected Odor Compounds in the Headspace of the Slurry Duct ( $n = 36$ ) Sampled over the Whole Fattening Period in Controls and RS Group

substance	control		RS		alteration RS group versus control (%)
	mean	SD	mean	SD	
1 <i>H</i> -indole	0.0034 a	0.0014	0.0012 c	0.0007	-65
3-methyl-1 <i>H</i> -indole	0.0109 a	0.0056	0.0023 c	0.0018	-79
4-methylphenol	0.0099 a	0.0035	0.0055 c	0.0025	-44
2-methylpropanoic acid	0.10 a	0.07	0.08 a	0.04	-20
3/2-methylbutanoic acid <sup>c</sup>	0.26 a	0.15	0.21 b	0.06	-19
ethanoic acid	2.60 a	1.58	3.08 a	1.61	+19
propanoic acid	0.80 a	0.51	1.00 a	0.65	+25
butanoic acid	0.56 a	0.25	0.70 b	0.34	+25
pentanoic acid	0.14 a	0.05	0.24 c	0.10	+71
hexanoic acid	0.04 a	0.04	0.08 c	0.06	+100
methanethiol	0.16 a	0.15	0.17 a	0.14	+6

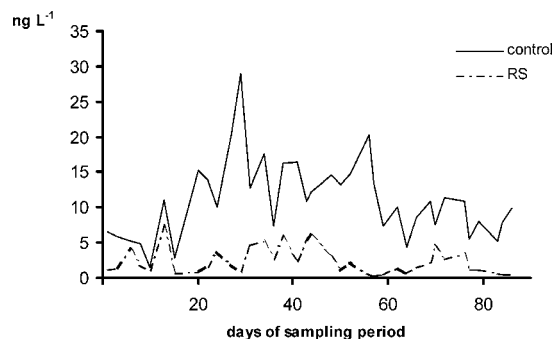
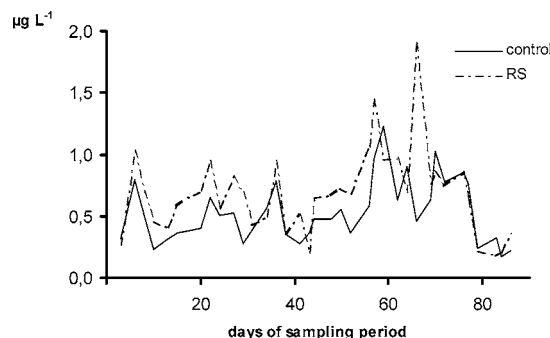
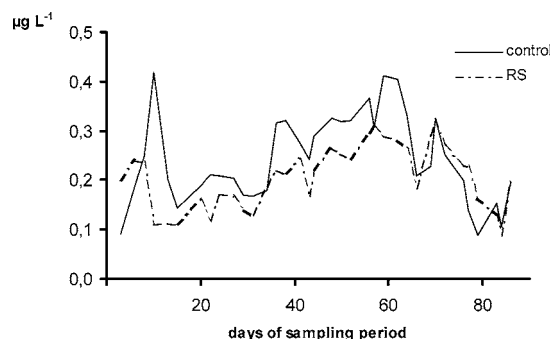
<sup>a</sup> a, b, c indicates significance of values within a row (a/b,  $P \leq 0.05$ ; a/c,  $P \leq 0.001$ ). <sup>b</sup> Contents of enanthic acid in air were below the detection limit. <sup>c</sup> 3-methylbutanoic acid and 2-methylbutanoic acid were determined together.

**Figure 1.** Profile of 4-methylphenol contents in air and feces of the control group along the fattening period. Sampling was done three times a week during the whole fattening period.

They revealed a weak but still significant decrease when compared to the control group. All other VFA (ethanoic, propanoic, pentanoic, hexanoic, and heptanoic acid) were significantly ( $P \leq 0.001$ ) increased, except for butanoic acid which was decreased by 7% but the level of significance was very low ( $P = 0.048$ ).

In the headspace of the slurry duct (**Table 4**), the contents of 4-methylphenol, 3-methyl-1*H*-indole, and 1*H*-indole were also considerably ( $P \leq 0.001$ ) decreased, so that concentrations in feces and the headspace were also correlated (1*H*-indole:  $r = 0.584$ ,  $P \leq 0.001$ ; 3-methyl-1*H*-indole:  $r = 0.670$ ,  $P \leq 0.001$  and 4-methylphenol:  $r = 0.776$ ,  $P \leq 0.001$  for controls,  $n = 35$ ; 1*H*-indole:  $r = 0.449$ ,  $P \leq 0.001$ ; 3-methyl-1*H*-indole:  $r = 0.531$ ,  $P \leq 0.001$  and 4-methylphenol:  $r = 0.614$ ,  $P \leq 0.001$  for RS group,  $n = 35$ ). In contrast to the feeding-dependent decrease of 4-methylphenol in the headspace, the concentrations in feces of the two feeding groups did not differ. For VFA and methanethiol no significant correlation was found between feces and headspace concentrations, except 2/3-methylbutanoic and pentanoic acid (2/3-methylbutanoic acid:  $r = 0.247$ ,  $P \leq 0.05$  and pentanoic acid:  $r = 0.434$ ,  $P \leq 0.001$ , for controls,  $n = 35$ ; for RS group no significant correlation was determined).

**Profiles of Selected Substances.** The high correlation of the air and feces contents in case of 4-methylphenol are shown in **Figure 1**. This figure gives the data from the control group but the RS group reacted identically (data not shown). The concentrations determined in air were correlated with the values

**Figure 2.** Profile of the 3-methyl-1*H*-indole content in the headspace of the slurry duct for control and RS group along the fattening period.**Figure 3.** Profile of the butanoic acid content in the headspace of the slurry duct for control and RS group along the fattening period.**Figure 4.** Profile of the 2/3-methylbutanoic acid content in the headspace of the slurry duct for control and RS group along the fattening period.

determined in feces along the whole fattening period ( $r = 0.776$ ,  $P \leq 0.001$ ). The decrease of concentrations in air but not in feces at the end of the fattening period is caused by the decreasing number of animals which reached their slaughtering weight at different ages.

**Figures 2, 3, and 4** compare the air concentrations of 3-methyl-1*H*-indole, butanoic acid, and 2/3-methylbutanoic acid for the two feeding groups along the fattening period. 3-methyl-1*H*-indole was chosen because it is the main determinant of malodor. It is formed out of the amino acid tryptophan. Among VFA, butanoic acid also has an unpleasant odor. It represents VFA which are formed out of carbohydrates whereas 2/3-methylbutanoic acid represents branched chain VFA originating from amino acids.

As also indicated by the data in **Table 4**, the course of 3-methyl-1*H*-indole (**Figure 2**) has the most pronounced feeding dependent difference which was found throughout the feeding period without exception, thus also suggesting that the effect of RS is maintained and not altered due to microbial adaptation. Butanoic acid (**Figure 3**) was increased in the air of the RS group except for a few sampling days where the concentrations

resulting from the controls were slightly higher. Concentrations in the feces, however, did not explain the elevated air values in the RS pigs.

Branched chain VFA (2/3-methylbutanoic acid) was lower again in the RS group when compared to the controls over the whole fattening period with the exception of only a few values.

## DISCUSSION

It is well-known that amino acids are precursor molecules for a variety of malodorous substances formed by microbial activity in the colon. Due to genetic selection for daily gain and protein synthesis, the digestibility of fattening rations for pigs was concomitantly increased and the pattern of amino acids adapted to the actual demands. Consequently, they are absorbed and do not reach the colon. Therefore, formation of malodorous substances which depend on amino acids require cell debris due to apoptosis in the distal parts of the digestive tract. It was also shown earlier that feeding of resistant starch leads to an increased formation of butyrate which in turn counteracts apoptosis (26).

The present data confirm that those compounds which depend on amino acid precursors were reduced both in feces and in the headspace when compared to the controls as shown for 1H-indole, 3-methyl-1H-indole, 2-methylpropanoic, and 2/3-methylbutanoic acid. Thus, these data provide for the first time experimental evidence that malodor from piggeries can be remarkably reduced by feeding strategies without reducing the overall daily weight gain of the pigs. It is obvious, however, that the identical concentration of 4-methylphenol in the feces of the controls and RS pigs seem to contradict the assumption. The fact that no significant difference was found in feces whereas differences in the air concentrations were apparent is partly explained by the route of excretion. 4-Methylphenol is efficiently absorbed from the intestinal tract, so that the main part is excreted as glucuronide via urine. After microbial hydrolysis, 4-methylphenol contributes to odor formation in the air. In consequence, the maintenance of the concentration in feces and the decrease in the air support the assumption that potato starch was able to decrease 4-methylphenol formation which, however, is not reflected by determination in feces. Routine determinations of urine under field conditions are not possible.

Additionally, 4-methylphenol is formed out of lignin by specialized microorganisms of the genus *Enterobacteriaceae* (36, 37). Because wheat bran contains lignin and was a component in the RS ration but not in the control ration (see **Table 1**), it might have been used for 4-methylphenol synthesis although amino acids were diminished in the RS group. The reduced air concentrations in the RS group are probably explained by an ongoing microbial formation of 4-methylphenol out of amino acids in the slurry of the controls. RS pigs are largely devoid of fecal amino acids, and microbial lignin fermentation is inhibited in slurry (19).

Similarly, methanethiol synthesis occurs by using methionine as a precursor amino acid, so that inhibition of apoptosis again should have led to a reduction in feces. It is known, however, that it can be formed as well from a variety of sulfates in the chyme (10).

The major VFA which are formed by degradation of carbohydrates with a low prececal digestibility reveal higher amounts in the RS group compared to the controls. The generally elevated air concentrations of VFA, which in part (especially in case of butyric acid) are not supported by the feces concentrations, again might be explained by ongoing microbial

degradation of carbohydrates in the slurry. It cannot be excluded that remaining amounts of raw potato starch excreted with the feces are used as substrate for microbial activity and thus explain the increased concentrations in the RS group.

It is surprising at a first look that a diet which was chosen to specifically elevate butyrate formation in the colon is not paralleled by a rise of butyrate in the feces. It must be regarded, however, that by far the highest amount of butyrate in the colon is used by colonocytes as a valuable and specific form of energy (24). A high amount of the remaining energy is additionally absorbed and used as energy for the overall metabolism of the pig (13).

The minor, although significantly decreased, butyrate levels in the feces of the treated pigs are thus rather explained by differences in the colonic passage of a feed which is rich in fiber (39). Differences in passage duration modify butyrate formation by microbes but also utilization by colonocytes.

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