Prevention of post weaning diarrhoea by a *Saccharomyces cerevisiae*-derived product based on whole yeast

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**A B S T R A C T**

The aim of this study was to examine whether yeast derivate (YD) based on whole brewery yeast added to the creep feed of suckling and newly weaned piglets or to the creep feed of the piglets and the sow’s diet prevented post weaning diarrhoea (PWD) or affected performance. Thirty sows and their litters were randomly allocated to three treatment groups: PSP (1.5 g/kg of YD to the sows’ feed from 1 wk before expected farrowing to weaning; 3 g/kg or 2 g/kg of YD added to the piglets’ creep feed from 2 wk of age until 2 wk post weaning (PW) and from wk 2 to 5 PW, respectively); PP (YD added to the piglets’ creep feed as in PSP); or C (control, no YD added). At weaning (4 wk of age) 2 individually housed piglets from all litters were subjected to either experimental *Escherichia coli* (E. coli) challenge or placebo treatment on d 1 to 3 PW, whereas performance was measured on 3 group-housed piglets from each litter. In individually housed piglets the faecal consistency score (FCS) was affected by an interaction between days PW, treatment group, and challenge group (*P*<0.005). In general, FCS was lower in placebo than in *E. coli*-challenged piglets and in PSP and PP piglets than in C piglets. The PSP and PP piglets had lower risk of PWD, defined as FCS > 3, on d 2 to 6 PW compared to C piglets (*P*=0.014 and *P*=0.001, respectively). This effect was evident in both placebo and *E. coli*-challenged PP piglets (*P*<0.010 and *P*<0.038, respectively), whereas PSP piglets only differed from C in *E. coli*-challenged piglets (*P*=0.030). In *E. coli*-challenged piglets faecal shedding of haemolytic *E. coli* was lower in PP than in C piglets (*P*=0.026). In placebo piglets the latency time to first observation of PWD was longer in PP than in PSP and C piglets (*P*=0.048 and *P*=0.017, respectively). The specific antibody titre in piglets or sows was not affected by YD. In group-housed piglets the medical treatment against PWD tended to occur in fewer PP than PSP and C pens within the first 3 wk PW (*P*=0.078). The average daily gain did not differ between treatments, but PSP piglets had an improved gain to feed ratio (G:F) in wk 0 to 5 PW (*P*<0.01). In conclusion, YD may prevent PWD at weaning at 4 wk of age if added to the creep feed 2 wk before weaning and PW. Adding YD to the sow as well may, however, antagonize the effect on PWD at a low pathogenic *E. coli* load, but may improve the G:F compared to no YD supplementation.

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**Abbreviations:** ADFI, average daily feed intake; ADG, average daily gain; ADWI, average daily water intake; BA, blood agar plates; CFU, Colony Forming Units; *E. coli*, *Escherichia coli*; FCS, faecal consistency score; G:F, gain-to-feed ratio; MMA, Mastitis Metritis and Agalacti; PW, post weaning; PWD, post weaning diarrhoea; YD, yeast derivate based on *Saccharomyces cerevisiae*.

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1. Introduction

Post weaning diarrhoea (PWD), frequently associated with enterotoxigenic *Escherichia coli* (*E. coli*) infection (Frydendahl, 2002), is one of the major welfare and economic problems in pig production at weaning. Due to the ban of prophylactic use of antibiotics in the European Union, other actions that have the potential to reduce the risk of PWD have to be identified.

Yeast derivatives based on *Saccharomyces cerevisia* (YD) added to the feed may be such an alternative. Mannans from YD may be immunomodulatory and prevent colonization of pathogenic *E. coli* as *E. coli* agglutinates to mannans (e.g. White et al., 2002; Davis et al., 2004b), whereas YD β-glucans may improve the specific immunity and prevent an exaggerated innate immune response to *E. coli* (e.g. Li et al., 2005, 2006; Wang et al., 2008a, 2008b; Gallois et al., 2009; Ganner et al., 2010; Juul-Madsen et al., 2010). In addition, YD based on whole yeast may contain nucleotides improving intestinal development and immunity (e.g. Sauer et al., 2011).

Studies on the effect of YD on post weaning (PW) performance show conflicting results, but YD may improve the resistance to *E. coli* infection in piglets pre-treated with YD for 1–4 wk PW (White et al., 2002; Maiorano et al., 2007; Stuyven et al., 2009). Studies on the effect of YD on PW at the usual time of PWD outbreak (d 3–4 PW; Madec et al., 1998) combined with pre-treatment through supplementary creep feed to suckling piglets have not been reported. Furthermore, it has not been investigated whether adding YD to the sow during late pregnancy and lactation may enhance a potential preventive effect of YD on PWD. The aim of the present experiment was to study the effect of adding YD based on whole yeast to the creep feed for suckling piglets weaned at 4 wk of age on diarrhoea within 1 to 3 wk after weaning, specific antibody titre, and performance as well as whether the concurrent addition of the YD to the sow improved the effect.

2. Materials and methods

All procedures involving animals were approved by the Danish Animal Experiments Inspectorate in accordance with the Danish legislation.

2.1. Animals

The experiment included 30 sows and their litters (Landrace-Yorkshire or Landrace-(Yorkshire-Duroc) cross-bred litters), originating from the sow production unit at Aarhus University, Foulum. The herd had a high health status according to the Danish specific-pathogen-free scheme, and was declared free from toxigenic *Pasteurella multocida*, *Sarcopostes scabei var. suis*, *Haemophilus suis*, *Brachyspira hyodysenteriae*, and *Actinobacillus pleuropneumoniae* serotypes 1,2,3,4,5,7,8,9,10. The sows were multiparous (parity 2–7). Before entering the experiment, the sows had been DNA-tested (Jørgensen et al., 2004) and found to be homozygote carriers of the gene encoding for intestinal F4 fimbria receptors for *E. coli*: F4 adhesion. All their offspring therefore would also have intestinal F4 receptors.

The piglets were housed from birth until weaning in undisrupted litters in farrowing crates with the dimension 1 m × 2.5 m for the sow plus an additional 1 m × 2.5 m get-away-area for the piglets equipped with a heating lamp. The experimental buildings were insulated and thermostatically controlled to approximately 20°C. Straw was used for bedding. The piglets were weaned at 4 wk of age. At weaning 2 piglets from each litter were assigned to an *E. coli* challenge trial and 3 piglets from each litter continued in a performance trial, whereas the sow and the remaining piglets were returned to the production unit.

The piglets in the *E. coli* challenge trial were individually housed in 0.72 m × 1.55 m pens, whereas the piglets in the performance trial were group-housed in 1.28 m × 1.63 m pens. Sawdust was used for bedding. At weaning the room temperature was 28°C and thereafter it was decreased by 1°C per week. During the whole experiment the pig house was exposed to natural daylight (56° 29’ N, 9° 34’ E) from windows, combined with artificial lighting from 07:30 to 15:30 h.

2.2. Experimental design

The experiment included 10 repetitions of 3 sows and their litters randomly allocated to one of three treatment groups: (1) YD added to the feed of sow and piglets during lactation and post weaning (PSP); (2) YD added to the feed of only the piglets during lactation and post weaning (PP); and (3) a control group that did not get YD in the feed (C).

The YD used was ProgutTM (Suomen Rehu Oy, Helsinki, Finland), which, according to the manufacturer, is whole brewery yeast hydrolysed after a patented process, consisting of heat treatment (70°C) of the yeast for inactivation, storage below 10°C in a storage tank, evaporation of water with an evaporator rising the dry matter content of the yeast up to 300–400 g/kg, processing with acid-alkaline treatment, spray drying of the processed product, cooling, sieving, and bagging. The dietary composition and nutrient profile of the feed and YD are shown in Table 1. In treatment group PSP, 1.5 g/kg of YD was added to the sows’ feed from 1 wk before expected farrowing and during lactation. The piglets were offered a solid weaner diet from 2 wk of age until 2 wk after weaning and a starter diet from 2 wk after weaning until 5 wk after weaning. In the treatment groups PSP and PP the weaner diet were added 3 g/kg of YD and the starter diet were added 2 g/kg of YD. The sows were fed twice daily with a daily ration per 10 piglets of 2.6 kg, 4.5 kg, 6.0 kg, 7.0 kg, and 7.5 kg at d 2, 7, 14, 21, and 28 after farrowing, respectively. The piglets were fed ad libitum.
Table 1

Dietary composition and nutrient profile of the yeast derivate (YD) (g/kg on an as fed basis unless otherwise stated).

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Sow diet</th>
<th>Weaner diet/creep feed</th>
<th>Starter diet</th>
<th>Progut™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>400</td>
<td>319.5</td>
<td>311.7</td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td>222.5</td>
<td>140.3</td>
<td>217</td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>319</td>
<td>319.5</td>
<td>311.8</td>
<td></td>
</tr>
<tr>
<td>Vitamin and trace Mineral premix a</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>40% l-lysine</td>
<td>4</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>40% DL-methionine</td>
<td>0.5</td>
<td></td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Fish meal</td>
<td>120</td>
<td></td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Milk powder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>4</td>
<td>1.8</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>12</td>
<td>4.9</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>10.4</td>
<td>5.6</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>30</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>YD in PSP and PP treatments b</td>
<td>1.5</td>
<td>3</td>
<td>2</td>
<td>–</td>
</tr>
</tbody>
</table>

**Composition (g/kg of diet or Progut™) c**

| Moisture                  | 134.1    | 124.4                  | 126.2        | 50      |
| Crude protein             | 178.8    | 228.1                  | 224.5        | 380     |
| Crude fat                 | 51.1     | 79.2                   | 75.7         | 20      |
| Crude fibres              | 146.1    | 118.78                 | 127.95       | 2       |
| Ash                       | 53.5     | 56.1                   | 61.1         | 150     |
| Calcium                   | 7.52     | 9.27                   | 10.06        | 2       |
| Phosphorus                | 5.87     | 6.64                   | 6.81         | 37      |
| Sodium                    | 1.73     | 2.48                   | 2.24         | 13      |
| Lysine                    | 5.62     | 11.41                  | 10.64        | 25      |
| Methionine + cysteine     | 2.65     | 5.10                   | 4.68         | 6       |
| Threonine                 | 3.70     | 6.03                   | 5.78         | 16      |
| Mannose                   |          |                        |              |         |
| β-glucans                 |          |                        |              |         |
| Mononucleotides, µg/kg   |          |                        |              |         |

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a Vitamin and trace Mineral premix provided for sows, content per kg feed: 8800 IU vitamin A, 1000 IU vitamin D3, 66.98 mg vitamin E (acetate bound), 60 mg α-tocopherol, 2.2 mg vitamin K1, 2.2 mg vitamin B1, 5.5 mg vitamin B2, 16.5 mg D-pantothenic acid, 22 mg niacin, 0.22 mg biotin, 0.022 mg vitamin B12, 1.65 mg folic acid, 3.3 mg vitamin B6, 100 mg Fe, 20 mg Cu, 150 mg Zn, 27.72 mg Mn, 0.304 mg I, 0.300 mg Se, provided by Trouw Nutrition Denmark A/S (Vejen, Denmark). Vitamin and trace Mineral premix provided for piglets, content per kg feed: 10,000 IU vitamin A, 2000 IU vitamin D3, 93.49 IU vitamin E (acetate bound), 85 mg α-tocopherol, 2.4 mg vitamin K1, 2.4 mg vitamin B1, 4.8 mg vitamin B2, 12.14 mg D-pantothenic acid, 25.54 mg niacin, 0.24 mg biotin, 0.024 mg vitamin B12, 3.6 mg vitamin B6, 200 mg Fe, 165 mg Cu, 200 mg Zn, 55.44 mg Mn, 0.304 mg I, 0.3 mg Se, 55 mg Na, 100 mg BHT, 1.2 mg Lysine provided by Trouw Nutrition Denmark A/S (Vejen, Denmark).

b Progut™ was mixed in the feed of sows and piglets in treatment PSP and in piglets’ feed in treatment PP.

c The composition of the diets is calculated by the software, WinOpti (AgroSoft A/S, Tørring, Denmark) on basis of the Danish national average content of the feed ingredients. The composition of Progut™ is from the product sheet and is based on frequent analysis by the manufacturer of the active components of the product in the period where the study was performed.

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The experiment was blinded, i.e. the veterinarian and technicians performing the clinical diarrhoea scorings and samplings did not know which treatment the sows and litters belonged to.

2.3. *E. coli* challenge trial

In each repetition two piglets per treatment group were subjected to either experimental *E. coli* challenge or placebo treatment on d 1, 2 and 3 PW. At weaning two female piglets of median weight from each litter were allocated to the experimental *E. coli* challenge trial and randomly distributed to either *E. coli* challenge or placebo treatment. The piglets were individually housed with snout contact to neighbouring piglets outside the experiment via a small 20 cm × 20 cm barred hole in the pen wall. *E. coli*-challenged piglets and placebo piglets were housed in separate rooms. The placebo piglets were always handled before the *E. coli*-challenged piglets. Environmental conditions such as temperature, air change and bedding were the same in the two rooms.

The challenge piglets received 2 × 10⁸ CFU *E. coli* 0149 (strain 9910045-1) suspended in 20 ml 0.15 M sodium chloride on days 1, 2 and 3 PW. The *E. coli* challenge strain was isolated at the Technical University of Denmark, National Veterinary Institute, Copenhagen, Denmark, from the intestinal content of a pig with PWD. According to a polymerase chain reaction (PCR) analysis of virulence factor genes the bacterial strain holds genes for fimbriae F4ac and enterotoxins Stb, LT, EAST1 (Frydendahl et al., 2001). The *E. coli* challenge strain was stored at −80 °C in a Luria-Bertani (LB) medium (Merck KGaA, Darmstadt, Germany) with glycerol (1:1, v/v). For each inoculation a fresh culture was prepared as previously described in Sørensen et al. (2009). The *E. coli* challenge was given by oro-gastric tube, and the tube was flushed with 20 ml 1.19 M sodium bicarbonate (NaHCO₃) before and after the *E. coli* solution was given. The placebo piglet was given only the 2 × 20 ml 1.19 M NaHCO₃ via oro-gastric tube.

Faecal samples were collected from the piglets once daily from weaning until d 6 PW and placed in sterile jars. The d 1 sample was collected before inoculation with *E. coli*. Faecal samples were scored daily for consistency and at d 1 to 3 PW
analyses were made in order to measure the dry matter content and the quantitative faecal shedding of haemolytic *E. coli* bacteria. In addition, the piglets were weighted daily and the daily feed and water intake were measured. Furthermore, samples of unstabilized blood for determination of specific antibody level to *E. coli* were collected from the piglets by puncture of the *vena jugularis* at the day of weaning, d 7 PW and d 14 PW. In addition, samples of unstabilized blood for determination of specific antibody level to *E. coli* were collected from the sows by puncture of the *vena jugularis* at d 0 after farrowing, and all medical treatments of Mastitis, Metritis and Agalacti (MMA) in the sows were noted.

### 2.3.1. Faecal consistency scoring and dry matter analysis

Faecal consistency was scored at sampling according to the following definitions: Score 1: hard, dry and cloddy, 2: firm, 3: soft but able to retain some shape, 4: soft and able to retain any shape, 5: watery and dark, 6: watery and yellow, 7: foamy and yellow. The faecal samples were immediately transported to the laboratory for bacteriology. A minimum of 1 g faeces from each sample was weighed, dried at 103 °C ± 2 °C oven for 20 h, and weighed again to determine dry matter content.

### 2.3.2. Bacteriological examinations

For the bacteriological examinations, blood agar plates (BA) (Columbia agar (Oxoid Ltd., Basingstoke, United Kingdom) supplemented with 50 ml/l calf blood) and MacConkey agar plates (Merck KGaA, Damstadt, Germany) were used. Serial ten-fold dilutions on BA were incubated at 37 °C for 18 h and the quantification of the haemolytic *E. coli* faecal shedding was performed as described in Sørensen et al. (2009), with a detection range of 10^5 to 10^11 Colony Forming Units (CFU) of haemolytic bacteria per gram faeces. Whenever the quantitative faecal shedding of haemolytic *E. coli* was less than 1 × 10^5 CFU/g, the value in the dataset was set to 50,000 CFU/g.

### 2.3.3. Antibody level to *E. coli*

Blood samples were kept at room temperature until centrifugation. Serum was obtained by centrifugation at 1850 × g for 20 min and was stored at −20 °C until analysis for specific antibodies to *E. coli*. Anti-*E. coli* antibodies were determined in a whole cell-based indirect ELISA. The ELISA plates (Maxisorp 442404. Nalge Nunc International, Roskilde, Denmark) were coated with a whole cell suspension of *E. coli* in 50 mM Na_2CO_3, pH 9.6 (100 µl/well) and incubated overnight at 4 °C. After coating, the ELISA plates were washed and pre-incubated with phosphate buffered saline (2.3 mM KH_2PO_4, 7.7 mM Na_2HPO_4, 140 mM NaCl, pH 7.2) with 0.45 mM Tween-20 (Merck, Hohenbrunn, Germany) and 0.15 mM bovine serum albumin (Sigma–Aldrich, St. Louis, MO, USA) (200 µl/well). After application of pig serum samples (100 µl/well) in duplicates of two-fold dilution series and washing of the ELISA plates, the bound antibodies were detected using peroxidase-conjugated rabbit anti total swine antibodies (P0164, Dako, Glostrup, Denmark). A solution containing 670 µg/ml 6-phenylene diamine and 3.7 mM H_2O_2 dissolved in citric acid buffer pH 5.0 was used as substrate (50 µl/well). Colour development was stopped by addition of 0.5 M sulphuric acid (150 µl/well) and optical density was measured at 490 nm minus 600 nm by dual endpoint read in an ELISA plate reader. The titre was defined as the geometrical mean of the reciprocal value of the highest dilution of serum with an optical density value > 0.500. The ELISA could detect titres between 10 and 5120.

### 2.4. Performance trial

The piglet performance trial included 3 piglets from each litter housed in littermate groups and randomly selected according to sex and representing low, median and high piglet weights within the litter. Average daily weight gain (ADG), average daily feed intake (ADFI), average daily water intake (ADWI), and gain-to-feed ratio (G:F) were measured at pen level from weaning until 5 wk PW. The piglets were weighed and the feed consumption was measured on d 0, 14, and 35 PW. Diarrhoea was treated with antibiotics if FCS was 5 to 7 or if FCS was 4 and piglet wellbeing was reduced. Medical treated diarrhoea was noted.

### 2.5. Statistical analyses

Due to occurrence of pre weaning diarrhoea one litter on PSP was excluded in the data analysis. Furthermore, one *E. Coli* challenged piglet and one placebo piglet on PSP were excluded in the data analysis due to, respectively, pneumonia or death of an unknown cause. In the performance trial 2 piglets on PP from different pens died at d 10 and d 28 PW, respectively. The affected pens were excluded in the analyses of the performance data because data collection occurred at pen level. However, as the deaths were not linked to diarrhoea the pens were included in the analyses of medical treatment of diarrhoea.

The daily occurrence of diarrhoea during d 1–6 in individual *E. coli*-challenged/placebo piglets was analysed as outcome according to the recorded FCS using a generalized linear model with binomial distribution and logit link-function (GLIMMIX procedure in SAS, version 9.2, SAS Institute Inc., Cary, NC, USA). For the statistical analyses diarrhoea was used as a binomial variable, i.e. “no diarrhoea” was defined as FCS on 1, 2, or 3, and “diarrhoea” was defined as clinical observation of FCS on 4, 5, 6, or 7. The model included treatment group (PSP, PP, C), challenge group (*E. coli* challenge, placebo), time PW (d 1, 2, 3, 4, 5, 6) and significant interactions between these effects as fixed effects. The random effects were repetition, the interaction between repetition and treatment groups, and sow. The correlation between repeated measures of diarrhoea on d 1 to 6 for each piglet within litter was accounted for using an autoregressive correlation structure. The use of the logit link-function
in the analysis ensures that the results concerning differences between treatment groups are identical to the log of the odds ratios. Thus, the phrase, a lower risk of diarrhoea, is used in case of an odds ratio lower than one.

The remaining variables were subjected to analysis of variance using the Maximum Likelihood method in the mixed model methods (MIXED procedure) with multiple error terms in SAS (version 9.2., SAS Institute Inc., Cary, NC, USA). To obtain homogenous variance, the variables, faecal shedding of haemolytic E. coli and antibody titre in the E. coli challenge trial and ADWI in the performance trial, were transformed by the logarithmic transformation, the variables, ADPI and ADWI in the E. coli challenge trial were transformed by the square root transformation, and G:F were logit transformed. Individual measurements of individually housed pigs or pen average of group-housed pigs were used as experimental units. The model included the same fixed and random effects as in the above-mentioned model. In addition, linear and quadratic effect of weaning weight and the interaction between weaning weight and time PW were included in all analyses as covariates if significant. In the analysis of the antibody titre of the piglets the sow’s antibody titre and the interaction between the sow’s antibody titre and time PW were used as covariates. Time PW was treated as repeated measurements using a covariance matrix with compound symmetry and heterogeneous variances for each time. The exception from this was the analyses of FCS and ADWI, which used an autoregressive correlation structure. Based on the evaluation of the model fit the residual variance was assumed to differ according to challenge group (FCS) or treatment group (daily feed intake in the challenge experiment and G:F in the performance experiment) by using the repeated statement of the MIXED procedure with either challenge group or treatment group as a GROUP factor (SAS, version 9.2., SAS Institute Inc., Cary, NC, USA).

In addition, the sows’ antibody levels at farrowing were subjected to analysis of variance using the Maximum Likelihood method in the mixed model methods with multiple error terms in SAS (version 9.2., SAS Institute Inc., Cary, NC, USA). To obtain homogenous variance the antibody titre was transformed by the logarithmic transformation. This model included either treatment group (PSP, PP and C) or whether the sow got YD (PSP vs. PP and C) as fixed effects and repetition as random effect.

Due to unbalanced data material as a result of lack of observations, Kenward Roger’s approximation was used for calculation of degrees of freedom for all models. The results of the analysis with MIXED and GLIMMIX procedure are given in F-value and the degree of freedom for the investigated effect (df1) and of the error term in the denominator (df2), and the P value. Pair-wise comparisons belonging to effects are presented by the P value. Means presented are least square means ± standard error.

Differences in the ratio of piglets with no diarrhoea (individually housed piglets) or not medically treated (group-housed piglets) were expressed by the latency time to first observation of clinical diarrhoea or medical treatment and were analysed using the nonparametric survival analysis, the LIFETEST procedure in SAS (version 9.2., SAS Institute Inc., Cary, NC, USA). In case of no response, observations were treated as censored as is usual for survival analysis. Treatment group was treated as strata. The results are given by the $\chi^2$ value, degree of freedom, and P value.

The analysis for differences between treatment groups in the number of pens with group-housed piglets that were medically treated was done by Fischer’s exact probability test using the FREQ procedure in SAS, and the correlation between selected variables was analysed by Spearman Rank Correlation Coefficient test by using the SPEARMAN procedure in SAS (version 9.2., SAS Institute Inc., Cary, NC, USA).

All analyses were performed as two-tailed tests and a 5% significance level was used. P-values less than 0.10 were considered as tendencies.

3. Results

3.1. E. coli challenge trial

The feed intake from weaning to d 6 PW (Fig. 1) was influenced by an interaction between challenge group and days PW ($F_{5,124} = 2.49$, $P=0.035$) and increased from day 1 to 2 PW in E. coli challenge piglets ($P<0.001$). No difference between challenge groups was evident at d 1 PW, but the feed intake tended to be higher in E. coli challenge piglets compared to placebo piglets at d 3 ($P=0.066$) and was higher at d 2, 4, 5 and 6 PW ($P<0.032$). No differences were found between treatments groups ($F_{2,37} = 0.99$, $P=0.382$). The ADFI from weaning to d 6 PW was 141 ± 25 g in PSP, 153 ± 19 g in PP and 112 ± 21 g in C piglets.

Weight at weaning (8.5 ± 0.2 kg) and daily gain did not differ between treatment groups (data not shown, $F_{2,29} = 0.64$, $P=0.535$, and $F_{2,28} = 0.69$, $P=0.509$, respectively). However, daily gain was affected by an interaction between challenge group and d PW ($F_{2,127} = 2.62$, $P=0.027$) reflecting that the gain was positive at d 3 (235 ± 60 g/d; $P=0.0002$) in placebo piglets and higher than in challenge piglets (−46 ± 60 g/d; $P=0.0013$) that did not differed from zero at d 3 ($P=0.443$). Weight at weaning tended to differ between challenge groups (8.2 ± 0.3 kg in placebo piglets and 8.5 ± 0.3 kg in challenge piglets; $F_{1,27} = 3.31$, $P=0.08$). The water intake was affected by an interaction between treatment group and challenge group ($F_{2,69} = 3.86$, $P=0.027$) due to an increased ADWI in placebo piglets compared to challenge piglets in the PSP treatment ($P=0.012$). In addition, the water intake was affected by d PW ($F_{3,257} = 16.72$, $P<0.0001$). During the first day PW the average water intake was 1.0 ± 0.2 l, whereas it increased to 3.5 ± 0.4 l at d 2 PW ($P<0.0001$) and tended to decreased to 2.7 ± 0.3 l at d 5 PW ($P=0.07$).

The analysis of the risk of PWD (Fig. 2) revealed effects of treatment group ($F_{2,58} = 6.24$, $P=0.004$), challenge group ($F_{1,71} = 32.21$, $P<0.001$), and days PW ($F_{3,303} = 8.16$, $P<0.001$). The PSP and PP piglets had a lower risk of PWD on d 2 to 6 PW compared to the C piglets ($P=0.014$ and $P=0.001$, respectively). This effect was evident in both placebo and E. coli-challenged
Fig. 1. Feed intake in the individually housed, placebo piglets (black–white columns) and *E. coli*-challenged piglets (black–grey columns) in the treatment groups, PSP (YD added to sow and piglets; hatched columns; *n*=8 per challenge group), PP (YD added to the piglets; dotted columns; *n*=10 per challenge group), and C (no YD added; empty columns; *n*=10 per challenge group). The values are least square means ± SEM. Stars over the columns indicate significant differences (***P<0.001; **P<0.01; *P<0.05; (*)P<0.1). No differences were found between treatment groups. Challenge with *E. coli* or placebo treatment was performed at d 1, 2, and 3 PW.

Fig. 2. Ratio of piglets with diarrhoea in relation to the treatment groups, PSP (YD added to sow and piglets; diamonds; *n*=8 per challenge group), PP (YD added to the piglets; boxes; *n*=10 per challenge group), and C (no YD added; triangles; *n*=10 per challenge group). Solid marks and thick lines indicate the occurrence of diarrhoea in experimentally *E. coli*-challenged piglets, and empty marks and thin lines indicate the occurrence of diarrhoea in placebo piglets. SEM ranged from 0 to 0.19. Challenge with *E. coli* or placebo treatment was performed at d 1, 2, and 3 PW.

PP piglets (*P*=0.010 and *P*=0.038, respectively), whereas PSP piglets only differed from C in *E. coli*-challenged piglets (*P*=0.030). The faecal shedding of haemolytic *E. coli* (Fig. 3) was affected by an interaction between challenge group and d PW (*F*<sub>1,36</sub> = 7.01; *P*=0.012). The shedding did not differ between d 2 and 3 PW in placebo piglets (*P*=0.856), whereas it increased from d 2 to 3 in challenged piglets (*P*=0.001). At both days the faecal shedding of haemolytic *E. coli* were higher in challenged piglets than in placebo piglets (*P*=0.004 and *P*=0.0001, respectively). In *E. coli*-challenged piglets the faecal

Fig. 3. Faecal shedding of haemolytic *E. coli* at d 2 and 3 post weaning in the individually housed, placebo piglets (black–white columns) and *E. coli*-challenged piglets (black–grey columns) in the treatment groups, PSP (YD added to sow and piglets; hatched columns; *n*=8 per challenge group), PP (YD added to the piglets; dotted columns; *n*=10 per challenge group), and C (no YD added; empty columns; *n*=10 per challenge group). The values are least square means ± SEM. Stars over the columns indicate significant differences (***P<0.001; **P<0.01; *P<0.05; (*)P<0.1). In *E. coli*-challenged piglets PP differed from C (*P*=0.026). Challenge with *E. coli* or placebo treatment was performed at d 1, 2, and 3 PW.
Fig. 4. Ratio of individually housed, placebo piglets (a) and *E. coli*-challenged piglets (b) with no diarrhoea from d 1 to 6 PW. The broken line represents treatment group, PSP (YD added to sow and piglets; · · ·; n = 8 per challenge group), the dotted line PP (YD added to the piglets; · · ·; n = 10 per challenge group), and the full-drawn line C (no YD added; —; n = 10 per challenge group). SEM ranged from 0 to 0.17. Challenge with *E. coli* or placebo treatment was performed at d 1, 2, and 3 PW.

shedding of haemolytic *E. coli* tended to be affected by treatment group (*F*<sub>2,16</sub> = 3.06; *P* = 0.075) due to a lower shedding in PP than C piglets (*P* = 0.026), whereas PSP piglets neither differed significantly from PP nor from C piglets.

In placebo piglets the latency time to first observation of clinical diarrhoea (Fig. 4a) was longer in PP than in PSP and C piglets (Life test, Wilcoxon; χ² = 3.91, DF = 1, *P* = 0.048 and χ² = 5.66, DF = 1, *P* = 0.017, respectively). In *E. coli*-challenged piglets, the latency time to first observation of clinical diarrhoea did not differ between treatment groups (Fig. 4b). However, when pooling the two treatment groups receiving YD (PP and PSP) a tendency for a longer latency time was found in YD than in C piglets (Life test, Wilcoxon; χ² = 3.6906, DF = 1, *P* = 0.055).

The FCS (Fig. 5) was highly correlated with the faecal dry matter content (*R* = −0.77, *P* < 0.001) and was affected by an interaction between day PW, treatment group and challenge group (*F*<sub>10,225</sub> = 2.64, *P* = 0.005). The *E. coli*-challenged piglets had a higher FCS than placebo piglets at d 3, 4, and 5 PW in the PSP group (*P* < 0.039), at d 4, 5, and 6 PW in the PP group (*P* < 0.020), and at d 3 and 4 PW in the C group (*P* < 0.006). The placebo PSP piglets had a lower FCS than C piglets at d 4 and 5 PW (*P* < 0.025), whereas PP piglets had a lower FCS than C piglets at d 5 and 6 PW (*P* < 0.007). At d 3 PW the FCS was lower in placebo PSP piglets than in placebo PP piglets (*P* = 0.016). In the *E. coli*-challenged piglets the FCS was lower in PSP than in C piglets at d 4 PW (*P* = 0.049), whereas PP piglets had a lower FCS than C and PSP piglets at d 3 PW (*P* < 0.005).

The antibody titre was influenced by day PW (*F*<sub>2,44</sub> = 11.01, *P* = 0.0001) and was higher on d 7 and 14 PW than at weaning in *E. coli*-challenged piglets (*P* = 0.012 and *P* = 0.002, respectively), whereas the level did not change during the first 14 days PW.

Fig. 5. Faecal consistency score (FCS) in relation to treatment groups, PSP (YD added to sow and piglets; diamonds; *n* = 8 per challenge group), PP (YD added to the piglets; boxes; *n* = 10 per challenge group), and C (no YD added; triangles; *n* = 10 per challenge group). Solid marks and thick lines indicate faecal score in *E. coli*-challenged piglets, and empty marks and thin lines indicate faecal score in placebo piglets. SEM ranged from 0.30 to 0.44. Challenge with *E. coli* or placebo treatment was performed at d 1, 2, and 3 PW.
in placebo piglets. The antibody titre was not affected by treatment group either in piglets or in sows ($F_{2,23} = 0.59$, $P=0.563$ and $F_{2,12} = 0.22$, $P=0.80$, respectively). Likewise, the antibody titre of the sows was not affected by whether the sow got YD or not ($F_{1,11} = 0.11$, $P=0.75$). The mean antibody titres in the piglets were $300 \pm 47$ in the PSP, $272 \pm 53$ in the PP and $232 \pm 40$ in the C treatment group, whereas the mean antibody titres in the sows were $616 \pm 201$ in the PSP, $644 \pm 252$ in the PP and $495 \pm 171$ in the C treatment group.

The occurrence of MMA in the sows requiring medical treatment was not affected by YD. Four of 10 sows fed with YD and 6 of 19 sows fed with control feed were treated for MMA (data not shown).

### 3.2. Performance trial

In group-housed piglets the G:F was affected by treatment group ($F_{2,10} = 9.59$; $P=0.005$) due an improved G:F in PSP compared to C (Table 2; $P<0.01$). In addition, the ADWI tended to be affected by an interaction between time PW and treatment group ($F_{2,39} = 2.91$, $P=0.066$) reflecting a lower ADWI in PSP piglets than in PP and C piglets from wk 0 to 2 PW ($P=0.046$ and $P=0.019$, respectively) and a lower ADWI in PP than in C in wk 2 to 5 PW ($P=0.031$) The ADFI and ADG did not differ between treatments group at any time.

The latency to first occurrence of diarrhoea in the pen requiring medical treatment was not significantly influenced by treatment group (Fig. 6). However, within the first 3 wk PW medical treatment against diarrhoea tended to occur in fewer PP pens than in PSP and C pens pooled ($P=0.078$; 3 of 10 PP pens as opposed to 12 of 19 PSP and C pens).
4. Discussion

The present investigation showed that YD has the potential to reduce the risk of diarrhoea in the first week PW in piglets weaned at 4 weeks of age if added to the piglets’ creep feed from 2 weeks before weaning. However, at least in non-challenged piglets, feeding YD to both sow and piglets (PSP) compared to giving it to the piglets only (PP) weakened the preventive effect on PW. Thus, in placebo piglets only PP piglets had reduced risk and delayed time of onset of PW compared to C piglets, and in group-housed piglets the medical treatment against PW tended to be lower in PP pens than in the remaining pens. In the E. coli-challenged piglets the risk of PW was reduced if the piglets got YD, irrespective of whether the sow got it as well, but the faecal shedding of haemolytic E. coli at day 2 and 3 PW was only significantly reduced in PP compared to C piglets. Adding YD to the feed of sows and piglets, however, improved the G:F from 2 to 5 weeks PW and overall, whereas YD for the piglets only did not affect performance. This suggests that YD has additional mechanisms of action working independently of disease resistance. The improving effect on the mechanisms preventing PW and on performance may occur at different doses of the bioactive components of YD.

To the best of our knowledge it has not previously been reported that adding YD to the supplementary creep feed during lactation has the potential to improve the resistance to an E. coli challenge immediately after weaning. However, signs of an increased resistance to an E. coli challenge have been shown 2–5 wk PW if the piglets were pre-treated with YD for one (Maiorano et al., 2007), two (Stuyven et al., 2009), or 4 weeks PW (White et al., 2002). The preventive effect of YD on PW in the present study was, however, not restricted to a high pathogen load. If YD was added to the piglets’ feed only (treatment PP), it also reduced the FCS, risk of PW, and time of onset of diarrhoea in the placebo piglets, and it tended to reduce the number of pens with diarrhoea requiring medical treatment during the first 3 wk PW in group-housed piglets. Previous studies did not show any effects of YD on PW in unchallenged piglets (Kim et al., 2000; Rozeboom et al., 2005; Wang et al., 2008a), but this may have been due to a generally low incidence of spontaneous diarrhoea. In addition, product specific differences may exist in effect. As opposed to previous studies, the present YD consisted of hydrolysed whole brewery yeast.

This means that, besides mannans and β-glucans from the cell wall, it contained a small amount of nucleotides, which at least at higher doses may improve intestinal growth and development as well as the immune competence (Lee et al., 2007; Martinez-Puig et al., 2007; Sauer et al., 2011). Thus, the present results may be specific for the product used. Anyway, the results indicate that the intake of supplementary creep feed during lactation is sufficient to induce the PW preventive mechanisms of YD in 2 to 4-wk-old piglets, or alternatively that some important preventive mechanisms triggered by YD only require pre-treatment of a very short duration.

The specific antibody titre to E. Coli, which may be a PWd preventive mechanism of YD that depends on pre-treatment for some time, did not differ according to whether the animals got YD in the present study, neither in the piglets nor in the sows. Previous studies have shown that yeast cell wall derivatives may improve humoral immunity as indicated by increased serum level of either IgA or IgG in response to vaccination, weaning, or pathogen challenge (White et al., 2002; Li et al., 2005; Han et al., 2007; Sauerwein et al., 2007; Wang et al., 2008a, 2008b), but some studies using a very low dose of YD failed to show this (Hiss and Sauerwein, 2003; Hahn et al., 2006). In the present study the intended dosage of YD was relatively high with respect to β-glucans. Thus, the lack of effect of YD on the antibody titre of the piglets indicates that the intake of creep feed during lactation may have been too low to improve humoral immunity. Furthermore, the unchanged antibody titre in the sows that got YD may reflect that pre-treatment with YD for one week is too short to cause an increase in humoral immunity.

In the present study the antagonizing effect of adding YD also to the feed of the sow was less marked in E. Coli challenged piglets than in unchallenged piglets. As the E. coli-challenged piglets, irrespective of treatment group, ate more than the placebo piglets and therefore had more YD in the intestine per se, this result indicates that at a high pathogen load the PW preventive effect mainly depended on the newly consumed level. One of the previously documented mechanisms, which is likely to be involved in a PWd preventive effect is agglutination between E. coli and YD (e.g. White et al., 2002; Kogan and Kocher, 2007). Furthermore, previous studies have shown that the present product may increase the counts of potential health promoting intestinal bacteria, such as bifidobacteria and Faecalibacterium prausnitzii-group bacteria or lactobacilli, in relation to potential harmful bacteria, such as the Bacteroides–Porphyromonas–Prevotella-group bacteria and the enteric group bacteria (Vahtovuo et al., 2007).

The antagonizing effect on PWd of adding YD to the feed of the sow as well (PSP) at the low pathogen load in placebo and group-housed piglets was surprising. We expected that the PSP treatment would lead to more robust and resistant pigs at weaning due the improved quality or quantity of colostrums or milk in sows fed YD in previous studies (Decuyper et al., 1998; Newman and Newman, 2001; O’Quinn et al., 2001; Le Dividich et al., 2009). However, in the present study none of the suggested indicators of the quality or quantity of colostrum or milk, such as the occurrence of MMA, and the E. coli-specific serum antibody level of the sows at delivery and of the piglets at weaning, differed between treatment groups, but these measure may be too coarse to be indicative as separate measures (e.g. Foisnet et al., 2010). Although the PWd preventive effect of YD was weaker if both sow and piglets got YD compared to only the piglets getting YD, the reverse was the case with respect to G:F, which was only significantly improved if both sow and piglets received YD. This result indicates that YD-induced improved performance is not simply a consequence of improved health. The improved G:F did not result in an improved ADG although ADG was unaffected. Several previous studies have investigated the effect of YD on performance with varying results, probably due to differences between studies in experimental factors (e.g. Gallois et al., 2009). In most studies the investigated YD have been stated to consist of either one or the other yeast cell wall
component, β-glucans or mannann oligosaccharides, but usually the purity was not informed or was relative low (250–300 g/kg); and probably most commercial yeast cell wall derivates contain both components in varying amounts although not disclosed. However, Li et al. (2006) and Wang et al. (2008a) documented that an almost pure β-glucan preparation from yeast (purity 861 g/kg and 915 g/kg, respectively) is able to affect the ADG PW in a quadratic manner and the weight gain was highest at a dose of approximately 43–46 mg/kg feed of pure β-glucan. Taking into account the significance of the product–specific carbohydrate structure and solubility and uninformed purity, the order of the effective ADG increasing dose of β-glucans seems to be confirmed by other studies of YD stated to be rich in β-glucans (Schoenherr et al., 1994; Dritz et al., 1995; Hahn et al., 2006). The increased ADG has been accompanied by either an increased ADFI (Dritz et al., 1995; Li et al., 2006), increased G:F (Schoenherr et al., 1994; Wang et al., 2008a), or increased digestibility (Hahn et al., 2006). In accordance with the quadratic effect documented by Li et al. (2006) and Wang et al. (2008a), studies with twice as large doses expressed as pure β-glucan or more did not result in changes in performance (Kim et al., 2000; Hiss and Sauerwein, 2003; Burkey et al., 2004; Li et al., 2006; Sauerwein et al., 2007; Wang et al., 2008a). Expressed as pure β-glucan the dose in the present study was 220–330 mg/kg feed indicating that the lack of improved ADG in piglets getting YD may be due to overdosing of β-glucan.

With respect to mannann oligosaccharides highly purified products have not been studied, for which reason an effect of this component on performance is not experimentally verified. Studies on YD stated as rich in mannann oligosaccharides have shown that 560–840 mg/kg feed expressed in pure mannann oligosaccharides may increase the ADG PW depending on the nutritional level of e.g. Zn and Cu and the use of concurrent in-feed antibiotics (Davis et al., 2002, 2004a, 2004b; LeMieux et al., 2003; Rozeboom et al., 2005), whereas higher doses (White et al., 2002) or lower doses comparable with the one in the present study (Kim et al., 2000; Hiss and Sauerwein, 2003; Burkey et al., 2004; Sauerwein et al., 2007) had no or the reverse effect on performance. The dose for the piglets in the present study was only 160–240 mg/kg feed expressed in pure mannann oligosaccharides and may have been too low to induce a potential mannann oligosaccharide effect on weight gain.

In conclusion, the present study shows that the hydrolysed whole brewery YD, ProgutTM, administered to piglets at a dosage of 3 g/kg creep feed from 2 wk before weaning at 4 wk of age until 2 wk after weaning followed by a dosage of 2 g/kg feed has the potential to reduce the risk of PWD without affecting performance. Adding of the product to the sow as well from 1 week before expected farrowing and during lactation may weaken the beneficial effect on PWD at a low pathogenic E. coli load, but it improved G:F compared to no use of YD in the feed. The present YD may be an alternative to therapeutic antibiotics in relation to PWD as it tended to reduce the number of pens medical treated with antibiotics in the group housed piglets. However, further research comparing the effect of YD with the effect of antibiotics and focussing on the exact composition and dosage of the bioactive components of YD is needed to verify this as well as to explain the mechanisms of action of YD and to optimize the dosage.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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