The effects of live yeast *Saccharomyces cerevisiae* on postweaning diarrhea, immune response, and growth performance in weaned piglets[1,2]

M. Trckova,* M. Faldyna,* P. Alexa,* Z. Sramkova Zajacova,*
E. Gopfert,* D. Kumprechtova,† E. Auclair,‡ and R. D'Inca‡[3]

*Veterinary Research Institute, Hudcova 70, Brno 62132, Czech Republic; †Institute of Animal Science Prague, Přátelství 815, 104 00 Praha, Czech Republic; and ‡Lesaffre Feed Additives, 137 rue Gabriel Péri, 59700 Marcq-en-Baroeul, France

ABSTRACT: The effects of live yeast *Saccharomyces cerevisiae* (strain CNCM I-4407, 10^{10} cfu/g; Actisaf; Lesaffre Feed Additives, Marcq-en-Baroeul, France) on the severity of diarrhea, immune response, and growth performance in weaned piglets orally challenged with enterotoxigenic *Escherichia coli* (ETEC) strain O149:K88 were investigated. Live yeast was fed to sows and their piglets in the late gestation, suckling, and postweaning periods. Sows were fed a basal diet without (Control; $n = 2$) or with (Supplemented; $n = 2$) 1 g/kg of live yeast from d 94 of gestation and during lactation until weaning of the piglets (d 28). Suckling piglets of the supplemented sows were orally treated with 1 g of live yeast in porridge carrier 3 times a week until weaning. Weaned piglets were fed a basal starter diet without (Control; $n = 19$) or with (Supplemented; $n = 15$) 5 g of live yeast/kg feed for 2 wk. Significantly lower daily diarrhea scores ($P < 0.05$), duration of diarrhea ($P < 0.01$), and shedding of pathogenic ETEC bacteria ($P < 0.05$) in feces was detected in the supplemented piglets. Administration of live yeast significantly increased ($P < 0.05$) IgA levels in the serum of piglets. Evidence indicates that decreased infection-related stress and severity of diarrhea in yeast-fed weaned piglets positively affected their growth capacity in the postweaning period ($P < 0.05$). The results suggest that dietary supplementation with live yeast *S. cerevisiae* to sows and piglets in the late gestation, suckling, and postweaning periods can be useful in the reduction of the duration and severity of postweaning diarrhea caused by ETEC.

Key words: diarrhea, enterotoxigenic *Escherichia coli*, probiotic live yeast, swine

INTRODUCTION

Postweaning diarrhea (**PWD**), caused by enterotoxigenic *Escherichia coli* (**ETEC**), is one of the most economically significant swine diseases. The ban on antibiotics as growth promoters in animal feed in the European Union (**EU**) in 2006 has increased the frequency of PWD followed by impaired growth performance and high mortality of piglets (Casewell et al., 2003). Therefore, alternatives are searched for to prevent infections on farms.

Live yeast (*Saccharomyces* spp.) has been used as a preventive and therapeutic agent for the treatment of a variety of intestinal diseases in humans and animals (Zanello et al., 2009). Recently, the ability of *Saccharomyces cerevisiae* CNCM I-4407 strain to exert the anti-inflammatory effect has been demonstrated in vitro using IPI-2I and IPEC-1 porcine epithelial cell culture challenged with ETEC K88 (Zanello et al., 2011a,b). The same in vitro model testing *S. cerevisiae* var. *boulardii* indicated that anti-inflammatory abilities may be a property of several yeasts, at least from *S. cerevisiae* gender (Badia et al., 2012). These results indicate that yeasts from *S. cerevisiae* spp. may reduce the direct impact of ETEC on intestinal epithelium during the course of the disease. *Saccharomyces cerevisiae* CNCM I-4407 has also been shown to increase antibody levels in colostrum and milk of sows and thus to increase piglet immunity during the postnatal period (Jurgens et al., 1997). However, to our knowledge,

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there are relatively few in vivo data regarding the effect of *S. cerevisiae* on the reduction of PWD, and none have shown a beneficial effect (Bekaert et al., 1996).

In the present study, we have hypothesized that feeding live yeast *S. cerevisiae* strain CNCM I-4407 to sows and their piglets in the nursing and postweaning periods could reduce both occurrence and severity of PWD induced by ETEC K88 by enhancing the piglet general and specific immunity, reducing intestinal colonization with pathogenic bacteria, and increasing the growth rate of piglets. This investigation was aimed to evaluate the effects of dietary live yeast on the course of diarrhea, quantitative and specific immune response, and growth performance of weaned piglets in an experimental model of ETEC infection.

MATERIALS AND METHODS

Animals and Housing

Animal handling followed the EU directive 86/609/EEC concerning animal care. The animal care protocol for this experiment followed the Czech guidelines for animal experimentation and was approved by the Branch Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic (permission number MZe 1103).

Four pregnant clinically healthy sows originated from a conventional farm with a good epizootiological history and their piglets ($n = 34$; Duroc × Pietrain × Landrace) were used in this trial. The sows were transported into the experimental animal facility of the Veterinary Research Institute, Brno, Czech Republic, at d 94 of gestation. Farrowing occurred at d 110 ± 1. Sows were housed in individual pens and kept with piglets until weaning. Piglets were weaned on d 28 and allocated into 4 pens next to their dam in a nursery room. The temperature in the farrowing and nursery rooms was 24 to 26°C and humidity 51 to 62%.

Dietary Treatments

Sows. Pregnant sows were allocated to 2 dietary treatments with 2 replicates (sows) for each treatment. They were fed a basal diet (Table 1) without (Control) or with (Supplemented) 1 g of live yeast/kg feed, as previous tests of immune modulation of the yeast strain showed positive effects between 0.5 and 5 g/kg of feed in sows (Zanello et al., 2013). The live yeast product (Actisaf; Lesaffre Feed Additives, Marcq-en-Baroeul, France) consisted of live yeast cells of *S. cerevisiae* CNCM I-4407 (Sc 47 strain) containing 10^{10} cfu/g. The same dietary treatments were maintained from d 94 of gestation and during lactation until weaning of piglets (d 28).

Table 1. Ingredient and chemical composition of diets (as-fed basis)

Item	Diet for sows	Diet for weaned piglets
Ingredient, g/kg		
Wheat	530.5	397.2
Barley	220.0	300.0
Soybean meal, 47% CP	160.0	180.0
Wheat bran	40.0	0.0
Rape oil	12.0	0.0
Soybean oil	0.0	20.0
Limestone, ground	13.0	12.0
Dicalcium phosphate	12.0	11.0
Salt	4.7	3.0
Sodium carbonate	0.0	1.0
L-Lysine HCl	0.8	0.2
L-Threonine	0.0	0.3
DL-Methionine	0.0	0.3
Vitamin and trace mineral premix[1]	7.0	0.0
Vitamin, amino acid, and trace mineral premix[2]	0.0	15.0
Biolac[3]	0.0	50.0
Formic acid	0.0	10.0
Actisaf[4]	±1.0	±5.0
Calculated chemical composition		
ME, MJ/kg	12.99	12.93
CP, g/kg	171.93	182.99
Lysine, g/kg	10.46	12.73
Threonine, g/kg	6.63	8.28
Methionine, g/kg	2.80	3.84
Tryptophan, g/kg	2.03	2.35
Ca, g/kg	8.79	8.16
P, g/kg	3.10	3.88

[1]The following was provided per kilogram of premix: vitamin A 1,400,000 IU; vitamin D$_3$ 250,000 IU; vitamin E 3,500 mg; vitamin K$_3$ 100 mg; vitamin B$_1$ 100 mg; vitamin B$_2$ 800 mg; vitamin B$_6$ 300 mg; vitamin B$_{12}$ 5 mg; biotin 10 mg; niacinamide 2,500 mg; calcium pantothenane 2,000 mg; cholinchloride 20,000 mg; betaine 5,000 mg; iron sulphate monohydrate (Fe) 16,500 mg; manganese oxide (Mn) 6,000 mg; zinc oxide (Zn) 18,200 mg; copper sulphate pentahydrate (Cu) 3,200 mg; potassium iodide (I) 80 mg; sodium selenite (Se) 40 mg; cobalt sulphate heptahydrate (Co) 100 mg; butylhydroxytoulen 3,400 mg; propyl gallate 1,400 mg; wheat flour as a carrier.

[2]The following was provided per kilogram of premix: vitamin A 1,330,000 IU; vitamin D$_3$ 130,000 IU; vitamin E 6,670 mg; vitamin K$_3$ 120 mg; vitamin B$_1$ 130 mg; vitamin B$_2$ 510 mg; vitamin B$_6$ 510 mg; vitamin B$_{12}$ 4 mg; biotin 10 mg; niacinamide 2,670 mg; calcium pantothenane 1,000 mg; folic acid 67 mg; cholinchloride 20,000 mg; betaine 5,000 mg; vitamin C 8,340 mg; L-lysine 250 g; L-threonine 107 g; DL-methionine 50 g; L-tryptophan 20 g; iron sulphate monohydrate (Fe) 21,350 mg; potassium iodide (I) 75 mg; manganese iodide (Mn) 4,000 mg; copper sulphate pentahydrate (Cu) 10,300 mg; zinc oxide (Zn) 7,300 mg; sodium selenite (Se) 26 mg; cobalt carbonate hydroxide (Co) 43 mg; wheat flour as a carrier.

[3]Dry whey and soy protein concentrate.

[4]Live yeast *Saccharomyces cerevisiae* strain Sc47 CNCM I-4407 (Actisaf; Lesaffre Feed Additives, Marcq-en-Baroeul, France).

Suckling Piglets. After farrowing, suckling piglets of the supplemented sows ($n = 15$) were orally treated with 1 g of live yeast in a porridge carrier 3 times a week

until weaning. Piglets of the control sows ($n = 19$) received porridge without any supplementation.

Weaned Piglets. Weaned piglets remained in the same treatment groups defined by their dams with 2 replicates (pens) for each treatment. Piglets were fed with a basal starter diet (Table 1). The starter diet for piglets delivered by supplemented sows was supplemented with 5 g of live yeast/kg feed (chosen as representing 5 times the recommended dose for healthy weaning pigs). The dietary treatments were maintained for 14 d (until d 42).

All diets for sows and piglets were in a meal form and free of antibiotics and zinc-based supplementation. They were fed twice a day ad libitum. Water was provided by automatic waterers.

Enterotoxigenic Escherichia coli *Challenge*

One day after weaning (d 29), all piglets were orally challenged by ETEC strain, serotype O149:K88, LT+, with a single dose of 1.5×10^{11} cfu/piglet. The challenge level (10^{11} cfu) is usually used in the experiments to induce the diarrhea disease. The ETEC strain intended for the infection was grown in medium containing 12.5 g of acid casein hydrolysate, 12.5 g of enzymatic casein hydrolysate, and 0.5 g of yeast extract (Oxoid, Basingstoke, United Kingdom) per 1 L and incubated at 37°C for 16 h. For individual administration, the culture was condensed by centrifugation and subsequently incorporated into semolina porridge paste.

Enterotoxigenic Escherichia coli *Shedding in Feces*

Bacteriological examination of fecal samples was performed on d 28, 30, 32, 34, 36, 38, 40, and 42 to evaluate the intestinal colonization by the challenged ETEC strain. The analyses were based on cultivation of the samples, characterization of the strain by a serological method, and confirmation by PCR. Fecal samples were plated on MacConkey and Columbia agar containing 5% of lamb blood (LabMediaServis, Jaromer, Czech Republic) and cultured at 37°C for 16 h. In prime cultures, the ratio of hemolytic colonies to the total colony count was recorded. Hemolytic colonies of bacterial cells (cfu < 5 per sample) were isolated and after incubation tested for virulence factors. O-serogroups were serologically detected using anti-O rabbit sera (Salajka et al., 1992). K88 adhesin and heat-labile enterotoxin analyses were performed by multiplex PCR as described previously (Alexa et al., 1997). The PCR reaction was performed in a 20 µL volume using the PCR Master Mix Kit (Qiagen, Hilden, Germany). The volume of 10 µL of the PCR products was analyzed and visualized by standard gel electrophoresis on 2% agarose gel.

Severity of Diarrhea

After the challenge infection, piglets were daily observed for signs of diarrhea. The severity of diarrhea was assessed visually and evaluated by individual scoring the consistency of the feces: 0 = normal feces, 1 = pasty feces, 2 = mushy feces, 3 = liquid feces, and 4 = diarrhea with blood. The mean daily diarrhea score (**DDS**) was calculated as a sum per group divided by the number of piglets in the group. The duration of diarrhea was recorded individually and the mean duration per group was calculated. Mortality rates were recorded throughout the monitoring period.

Total Immunoglobulin and IgA Levels

The estimation of immunomodulatory effects of live yeast was performed via quantification of total immunoglobulin and IgA levels in colostrum and serum from sows and piglets. Colostrum and blood samples from sows were taken 1 h after farrowing. Piglet blood samples were taken at d 2, 28, 34, and 42. Total immunoglobulin levels were determined spectrophotometrically measuring the turbidity resulting from the addition of zinc sulfate to the samples as previously described (McEwan et al., 1970). Levels of IgA were detected using pig IgA ELISA quantitation kit (Bethyl Laboratories, Inc., Montgomery, TX) according to manufacturer's recommendations.

Detection of Enterotoxigenic Escherichia coli *K88-Specific Antibodies*

Enterotoxigenic *Escherichia coli* K88-specific antibodies were detected using the agglutination test. The challenge ETEC strain was incubated with K88 fimbrial antigen in nutrient broth at 37°C for 18 h. For better visualization of the reaction, the culture was stained red by adding 25 µL of 4% triphenyltetrazolium chloride to 5 mL of the culture and incubated at 37°C for 1 h. After staining, the culture was inactivated by adding 0.25% phenol.

The examined sera were geometrically diluted in 0.85% NaCl in U-shaped microtiter plates. Then, the bacteria were added to each well, mixed, and incubated at 37°C for 12 h. The results were read with the naked eye on the subsequent day. The agglutination titer is the reciprocal of the highest dilution that gave complete agglutination.

Growth Performance

The piglets were individually weighed at farrowing (d 0), weaning (d 28), and the end of the trial (d 42).

Statistical Analyses

All data were obtained individually. For statistical analyses, Generalized Linear Model for repeated measures under SPSS v11.5 (IBM, Armonk, NY) was used. Treatment effect, replication effect, and interaction were tested, and statistically significant differences were considered at $P < 0.05$. Except for duration of diarrhea ($P < 0.05$), no significant interaction was observed ($P > 0.05$) between treatment and replication. Data in the tables and figures are presented as mean ± SEM.

RESULTS AND DISCUSSION

The primary objective of this study was to investigate the effect of dietary live yeast *S. cerevisiae* strain CNCM I-4407 on the reduction of PWD in weaned piglets. To imitate common conditions on conventional farms, we performed our study using sows originating from a conventional herd. The piglets were challenged with a pathogenic ETEC O149:K88 strain, which is commonly present in the environment and frequently causes diarrheal infection in weaned piglets (Fairbrother et al., 2005). Live yeast was fed to sows and their piglets in the late gestation, suckling, and postweaning periods. We assumed that feeding live yeast to sows would affect their immune system and humoral antibody production in colostrum thus stimulating the immune system of piglets and finally protecting them from PWD. An adequate supply of good quality colostrum to newborns decreases the risk of disease, especially bacterial infections, in the subsequent period. Complementarily to the stimulation of immune response, we assumed that direct-fed live yeast to piglets would also prevent pathogenic bacteria from binding to intestinal epithelial cells.

Severity of Diarrhea

The beneficial effects of live yeast on the challenging ETEC infection and on the sanitary status of piglets were observed in the present study. Piglets of both groups began scouring on the first day after challenge, but the severity as expressed by the DDS and the duration of diarrhea was significantly different (Fig. 1). The supplemented piglets presented a significantly ($P < 0.05$) lower DDS in comparison with the control group. The mean DDS of control and supplemented groups for the entire scouring period were 1.44 ± 0.43 and 0.77 ± 0.45, respectively. Diarrhea in the control piglets was observed for a 7.8 d on average whereas this was significantly ($P < 0.01$) shorter in the supplemented group (mean duration 4.8 d).

On the first day after challenge (d 30), 58 and 20% of control and supplemented piglets, respectively, were scouring with predominating pasty feces. The difference in scouring was even greater on d 31, when 84 and 33%

of control and supplemented piglets, respectively, had pasty or mushy feces. On d 32, all piglets were scouring, but diarrhea was more severe in the control group. Liquid and mushy feces predominated in the control group whilst no liquid feces were observed in the supplemented group. The DDS peaks were observed 4 d after challenge (d 33). Diarrhea score of the groups returned to normal after 11 d from challenge. No mortality was observed throughout the monitoring period.

Significantly less severe and shorter episodes of diarrhea were detected in piglets supplemented with live yeast. The observation that live yeast *S. cerevisiae* strain CNCM I-4407 was able to reduce diarrhea in piglets can be related to its ability to induce anti-inflammatory activity in different swine intestinal epithelial cells challenged with ETEC K88, as was demonstrated in vitro (Zanello et al., 2011a,b), and also to its capacity to interfere with pathogenic *E. coli* reaching the intestinal epithelium and thus preventing disease. This above assumption was confirmed by bacterial analyses of fecal samples.

Enterotoxigenic Escherichia coli *Shedding in Feces*

Results of bacteriological analyses of fecal samples are expressed as the percentage of the challenged ETEC O149:K88 strain from all hemolytic *E. coli* in the samples (Fig. 2). Significantly lower ($P < 0.05$) shedding of the challenge ETEC strain by the supplemented group was observed in comparison with control group although there were individual differences among piglets within each group.

No pathogenic ETEC O149:K88 were detected in feces of piglets before challenge. Already on the first day after challenge (d 30), 95 and 85% of control and supplemented piglets, respectively, shed the challenge strain ETEC O149:K88. A marked decrease of shedding in the supplemented group was detected on d 32 when 80% piglets shed less than 10% of the challenge ETEC strain in the feces. In the control group, ETEC shedding remained high (higher than 90% in 48% piglets). On d 36, ETEC O149:K88 was still detected in the feces of 26% control piglets whilst in none of the supplemented piglet feces. Shedding of the pathogenic challenge ETEC strain in the feces of control and supplemented group persisted for 9 and 7 d, respectively.

Significantly lower ETEC shedding in feces of piglets receiving live yeast was measured in the present study. Reduction of bacterial shedding suggests a similar diminishment of pathogenic ETEC adhesion and colonization in the small intestine, as was previously described using a different strain of *S. cerevisiae* (Daudelin et al., 2011). Although the use of fecal samples has obvious limitations when describing colonization of the anterior gastrointes-
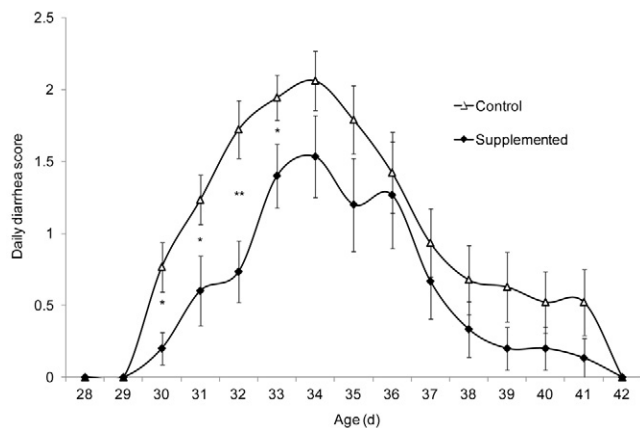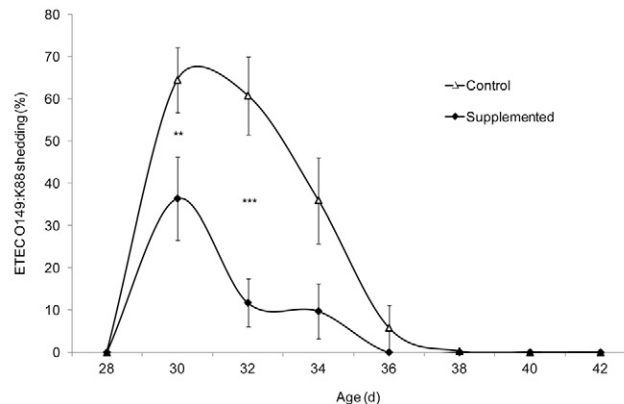
Figure 1. Daily diarrhea score of the enterotoxigenic *Escherichia coli* O149:K88 challenged (d 29) weaned piglets fed without (Control; *n* = 19) or with (Supplemented; *n* = 15) supplementation of live yeast *Saccharomyces cerevisiae* strain CNCM I-4407 (Actisaf; Lesaffre Feed Additives, Marcq-en-Baroeul, France). Scale used was 0 = normal feces, 1 = pasty feces, 2 = mushy feces, 3 = liquid feces, and 4 = diarrhea with blood. Values represent mean ± SEM. Statistically significant difference was observed between treatments (*P* < 0.05). Differences indicated for each time point are **P < 0.05 and **P < 0.01. Replication effect was not significant (*P* > 0.05).

Figure 2. Shedding of the pathogenic strain enterotoxigenic *Escherichia coli* (ETEC) O149:K88 in the feces of challenged (d 29) weaned piglets fed without (Control; *n* = 19) or with (Supplemented; *n* = 15) supplementation of live yeast *Saccharomyces cerevisiae* strain CNCM I-4407 (Actisaf; Lesaffre Feed Additives, Marcq-en-Baroeul, France). Values indicate the percentage of the challenged ETEC O149:K88 strain from all hemolytic *Escherichia coli* present in the feces. Values represent the mean ± SEM. Statistically significant difference was observed between treatments (*P* < 0.05). Differences indicated for each time point are **P < 0.01 and ***P < 0.001. Replication effect was not significant (*P* > 0.05).

tinal tract, fecal samples are excellent indicators of shedding of potential pathogens (White et al., 2002).

The ability of *S. cerevisiae* spp. yeasts and separated mannan oligosaccharides (MOS) to block the adherence of potentially pathogenic bacteria such as *E. coli* or *Salmonella* spp. has been previously observed in vitro (Gedek, 1999; White et al., 2002; Pérez-Sotelo et al., 2005; Jensen et al., 2008; Badia et al., 2012). In a field trial, separated MOS decreased the enterobacteria population in the jejunum and improved the mean fecal score value in weaned piglets (Castillo et al., 2008). White et al. (2002) showed that dried yeast as a source of MOS reduced colonization of total coliforms and their shedding in the feces, but it did not have a consistent effect on *E. coli* K88. A significant decrease in fecal ETEC shedding and milder diarrhea was also observed when β-glucans extracted from yeast cell walls were fed to weaned piglets (Stuyven et al., 2009). Therefore, the use of *S. cerevisiae* may allow us to provide the piglets with both MOS and β-glucans sources. Combined with anti-inflammatory capacities of live yeast mediated by several secreted molecules (Zanello et al., 2011a), it has contributed to the efficient protection of the piglets observed during the challenge period.

There are relatively few reports dealing with the live yeast effect on the protection of piglets against ETEC infection and PWD, and none have specified the beneficial effect. Bekaert et al. (1996) reported that feeding the yeast culture of *S. cerevisiae* to piglets in the postweaning period did not significantly affect diarrhea or performance. A limited effect of live yeast supplementation on the intestinal microflora, including *E. coli* and fecal coliform count, was confirmed by Mathew et al. (1998) and van Heugten

et al. (2003). However, results of Mathew et al. (1998) were not obtained under field conditions but in fistulated animals. Conditions of assessment are essential to observe benefits coming from yeast supplementation in piglets as postulated by van Heugten et al. (2003). Likewise, a direct comparison between experiments cannot be made because of different levels of live yeast supplementation to diets ranging from 0.5 to 5% and because of using different *S. cerevisiae* strains. In the present study, long-term supplementation (including the suckling period) and the use of high doses of live yeast during the challenge period may have induced greater protection of the animals than what is generally reported (Bekaert et al., 1996; Mathew et al., 1998; van Heugten et al., 2003).

Our results are in agreement with Kiarie et al. (2011) who investigated the effect of *S. cerevisiae* fermentation products fed to weaned piglets challenged with ETEC K88. Pigs receiving a yeast fermentation product showed a smaller number of ileal mucosa adherent ETEC and a tendency to a smaller fecal score. A positive effect of dietary live yeast on the severity of diarrhea was also reported by Galvão et al. (2005) in weaned calves.

Immune Response

Total immunoglobulin concentrations in serum and colostrum did not differ among sows during farrowing. The concentrations 15.88 and 15.54 mg/mL in serum and 114.40 and 104.60 mg/mL in colostrum of control and supplemented sows, respectively, were detected.

Furthermore, there was no difference in IgA concentrations in colostrum of control and supplemented sows (3.25
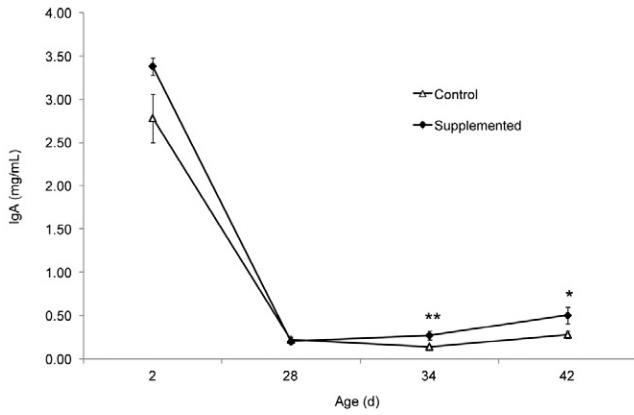
Figure 3. Serum IgA content (mg/mL) in piglets fed without (Control; $n = 19$) or with (Supplemented; $n = 15$) supplementation of live yeast *Saccharomyces cerevisiae* strain CNCM I-4407 (Actisaf; Lesaffre Feed Additives, Marcq-en-Baroeul, France). Values represent the mean ± SEM. A statistically significant difference was observed between treatments ($P < 0.05$). Differences indicated for each time point are *$P < 0.05$ and **$P < 0.01$. Replication effect was not significant ($P > 0.05$).

Table 2. Piglet serum specific antibodies against the challenge strain enterotoxigenic *Escherichia coli* O149:K88 in piglets fed without (Control; $n = 19$) or with (Supplemented; $n = 15$) supplementation of live yeast *Saccharomyces cerevisiae* strain CNCM I-4407 (Actisaf; Lesaffre Feed Additives, Marcq-en-Baroeul, France) challenged at d 29. Values represent mean ± SEM of titer. Statistically significant difference was observed between treatments ($P < 0.05$). Differences indicated for each time point are †$P < 0.10$, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. Replication effect was not significant ($P > 0.05$)

| Age, d | Treatment | | Statistical significance |
	Control	Supplemented	
1	646.7 ± 88.5	183.5 ± 19.3	***
28	152.4 ± 36.2	33.1 ± 3.8	**
34	105.3 ± 15.6	59.7 ± 6.4	*
42	65.7 ± 9.8	40.5 ± 7.9	†

and 3.40 mg/mL, respectively). However, greater (nonsignificantly) IgA level was detected in serum of the supplemented sows (1.54 vs. 0.76 mg/mL in control sows).

In piglets, no significant differences ($P > 0.05$) were observed in the total immunoglobulin concentration in serum of control and supplemented piglets over the entire experimental period (mean concentration was 12.19 mg/mL). However, serum of supplemented piglets contained significantly higher ($P < 0.05$) IgA concentrations (Fig. 3).

Jurgens et al. (1997) and Zanello et al. (2013) demonstrated increased IgG in colostrum and milk of sows fed live yeast *S. cerevisiae* during the later stage of gestation. As IgG constitutes the majority of total immunoglobulins in the periparturient period in the blood and mammary secretions (Klobasa et al., 1987), we could expect increased total immunoglobulin levels in serum or colostrum of sows in the present study. However, the trial was designed to focus on piglet health and growth and not on the sows, and therefore, the use of 2 sows in each group precluded any extrapolation of this aspect. On the contrary, in piglets, a significant improvement of the response to the challenge was observed with a significant increase in IgA production, mainly observed during the challenge and at the end of it.

Agglutination tests showed that serum samples taken from control piglets contained greater amounts of K88-specific antibodies when compared to serum samples from supplemented piglets (Table 2). It correlated well with the fact that serum of control sows and mainly their colostrum contained more K88-specific antibodies than those of the supplemented sows. The titers of K88-specific antibodies in colostrum of control and supplemented sows were 1,024 and 4,096 vs. 512

and 1,024, respectively. The amount of K88-specific antibodies was decreasing in control piglets during the whole experimental period. In contrast, levels of specific antibodies increased after the experimental infection in supplemented piglets (Table 2).

The finding may be explained by the fact that supplementation of sows with live yeasts led to lower antigenic pressure in the gut and thus, the gut-associated immune system produced a lower amount of K88-specific antibodies. Subsequently, a lower amount of K88-specific antibodies was transferred to piglets via the colostrum (Salmon et al., 2009; Nechvatalova et al., 2011). The fact that less K88-specific antibodies were detected in the supplemented group may indicate that the gut mucosa-associated immune tissue of the piglets was less stimulated by the pathogen.

Growth Performance

Growth performance of piglets is summarized in Fig. 4. Piglet BW did not differ significantly among treatments before and during the challenge period. However, significantly greater BW ($P < 0.05$) was observed in the supplemented group after challenge at d 42. As our results showed, the growth rate during the suckling prechallenge period was not significantly affected by feeding live yeast to piglets and sows. We suppose that increased growth rate in yeast-fed piglets in the postweaning period was positively affected by less severe and shorter episodes of diarrhea in this period.

The improvement of growth performance in weaned piglets after live yeast or yeast culture supplementation has been reported previously in healthy animals (Jurgens et al., 1997; Mathew et al., 1998; Bontempo et al., 2006;
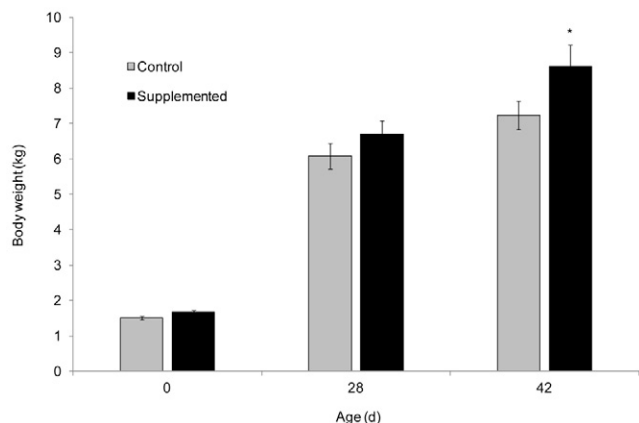
Figure 4. Body weight of the piglets fed without (Control; $n = 19$) or with (Supplemented; $n = 15$) supplementation of live yeast *Saccharomyces cerevisiae* strain CNCM I-4407 (Actisaf; Lesaffre Feed Additives, Marcq-en-Baroeul, France). Data present the mean ± SEM. A statistically significant difference was observed between treatments ($P < 0.05$). Differences indicated for each time point are *$P < 0.05$. Significant interaction between time and replication was observed ($P < 0.05$). Replication effect was not significant ($P > 0.05$).

Li et al., 2006; van der Peet-Schwering et al., 2007; Shen et al., 2009). In contrast, some studies have reported no benefit of yeast products on growth performance (Kornegay et al., 1995; White et al., 2002; van Heugten et al., 2003). These discrepant findings may be due to differences between yeast products, doses and duration of yeast dietary supplementation, composition of diets, and the sanitary conditions of the animals. The exact level of yeast required to improve pig performance has not been clearly defined. It seems that 5 g of live yeast/kg feed administered to piglets during the weaning period can be very efficient, similar to the results obtained by Shen et al. (2009). Most of the demonstrated mechanisms of action of the present probiotic yeast strain such as improvement of fiber digestibility in vitro (Pinloche et al., 2012) and nonspecific immune stimulation in vivo (Zanello et al., 2013) in healthy animals, so as to protection against pathogen-induced inflammation in vitro (Zanello et al., 2011b), are proportional to the probiotic dose provided. Therefore, preventive use of higher doses around weaning in conditions of high contamination may therefore abolish diarrhea due to *E. coli* K88.

In conclusion, our results demonstrate that dietary supplementation with live yeast *S. cerevisiae* to sows and piglets in the late stage of gestation, suckling, and postweaning periods can be useful for reducing the duration and severity of PWD caused by ETEC. Decreased infection-related stress and severity of diarrhea in yeast fed weaned piglets can positively affect the growth performance in the preweaning period. These results suggest that live yeast *S. cerevisiae* strain CNCM I-4407 could be an alternative for prevention and treatment of PWD.

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