Influence of dietary β-glucan on growth performance, lymphocyte proliferation, specific immune response and haptoglobin plasma concentrations in pigs

By S. Hiss and H. Sauerwein

Summary
Immunomodulatory feed additives might offer alternatives to anti-microbial growth promoters in swine production. The present study was conducted to assess the effects of β-1,3/1,6 glucans, i.e. of specific yeast cell wall components, on immune function and growth performance in pigs. After weaning at 4 weeks of age, 75 piglets were allocated to 3 different groups for 4 weeks, the diet was supplemented with 0, 0.015 or 0.03% of β-glucan, respectively. All animals were vaccinated against porcine reproductive and respiratory syndrome (PRRS). After 4 weeks, average daily gains (ADG) of β-glucan treated pigs were not different from the controls. Feed intake was tendentiously (p < 0.1) increased at 0.03% β-glucan, without alteration of feed efficiency. Serum haptoglobin concentrations at the end of the 4 week treatment were increased in all groups when compared to the initial levels (p < 0.001), without differences between the groups (p > 0.05). Haptoglobin levels were inversely related to ADG. Lymphocyte proliferation indices were not different in control and treatment groups. Specific vaccination responses, as quantified by the PRRS antibody titres occurred in all animals, but no relation with β-glucan feeding was observed. Our results indicate marginal benefits of β-glucan supplements for growth performance and no effect on the immune parameters tested. The observed trend towards increased feed intake needs further elucidation.

Introduction
Public concerns about potential risks involved in the use of anti-microbial growth promoters in animal feeds have led to an increased disapproval of such substances. A general ban seems reasonable and desirable to consumers (Witte, 1998; Berend et al., 2001). However, experiences from Sweden and Switzerland where such a ban has been realised, demonstrate that there are problems arising; among others, increased losses during pig weaning and rearing are reported (Robertsson and Lundehem, 1994; Althaus, 1998). In order to minimise these problems and to secure animal health, several approaches have to be considered, one of the most important aspects being the optimal adaptation of housing systems in terms of animal welfare and of hygiene. Modulating immune function is also discussed as a potential means to decrease disease susceptibility in farm animals (Blecha and Charley, 1990). There is a wide spectrum of potential immune modulators (for review see Mashi 2000), e.g. probiotic bacteria (Matsuzaki and Chin 2000), vitamin E (Tengerdy, 1989) and phytosterols (Greiner et al., 2001). A variety of polysaccharides from various natural sources have the ability to modulate the immune system. The most active appear to be branched (1 → 3)-β-D-glucans from fungi and are also referred to as (1 → 3) (1 → 6)-β-D-glucans; in the following text the abbreviated...
term β-glucan stands for these compounds. Their common structure is a main chain consisting of \((1 \rightarrow 3)\)-linked β-D-glucopyranosyl units along which randomly dispersed single β-D-glucopyranosyl units are attached by \(1 \rightarrow 6\) linkages (for review see BOHN and BEMILLER, 1995). For the yeast-derived β-D-glucans, stimulatory effects on both specific and nonspecific immune responses have been demonstrated in mammals (WILLIAMS et al., 1989; SUZUKI et al., 1990) and also in fish (ROBERTSEN et al., 1990; JENEPY AND ANDERSON, 1993). Owing to their oral activity and to sporadic reports about beneficial effects on growth performance in pigs (SCHOENHERR et al., 1994; DRITZ et al., 1995), these glucans are of particular interest for pig fattening. At weaning, young pigs can be stressed by the change of feed and of social environment. Enhanced glucocorticoid secretion then leads to impaired immune function and, in consequence, to increased susceptibility towards infections. In this situation, immune stimulators might have specific benefits. We, therefore, aimed to determine the effects of dietary β-glucans at two different doses on growth performance and on specific and nonspecific immunity in weaned pigs.

Materials and methods

Animals and experimental design
At weaning at 4 weeks of age, 75 crossbred piglets were randomly allocated to three different feeding groups. The three treatment groups (n = 25 per group) were as follows:

i. controls which only received the basal diet (RWZ-STart M, Raiffeisen, Köln, Germany): 19.5% Crude protein; 5.1% Crude fat; 4.5% Crude fibre; 6.2% Ash; 1.3% Lysine; 0.8% Calcium; 0.6% Phosphorus; 0.25% Sodium; 14.0 MJ ME/kg; 16 000 IU of vitamin A, 2000 IU of vitamin D3, 100 mg of vitamin E, 40 mg of Avilamycin, 165 mg Cu, propionic acid, 500 ITU 3–Phytase, formic acid, 1*10⁹ cfu/kg Bacillus cereus, Ethoxyquin.

ii. Basal diet with 0.015% β-1,3/1,6-glucan (Antaferm MG, Dr. Eckel GmbH, Niederzissen, Germany).

iii. Basal diet with 0.03% β-glucan.

The pigs were kept in pens with partly slatted floors, with 8–9 pigs per pen and 3 replicate pens per group. Each pen contained two nipple waterers and feed was offered in troughs to provide \(\text{ad libitum}\) access to feed and water. Feed intake was calculated per pen. Body weights were determined at weaning and at the end of the 4 week trial period. To evaluate the effects of β-glucan supplements on specific immune response, all animals were vaccinated against porcine reproductive and respiratory syndrome virus (PRRSV; Ingelva®PRRS MLV, Boehringer, Ingelheim, Germany) on day 1 after weaning.

Blood collection and analyses
Blood was obtained from each pig by venipuncture of the vena cava cranialis at weaning and also at the end of the experimental period, i.e. 4 weeks after the start of the experiment. In addition, blood samples were drawn from 4 pigs of each treatment group in weekly intervals to evaluate lymphocyte proliferative activity. Serum was collected and analysed for the acute phase protein, haptoglobin (all samples collected), and for PRRS antibody titres (samples from the beginning and the end of the experiment).

Lymphocyte proliferation assay
Lymphocytes were isolated from blood according to WATERS et al., (2000) with minor modifications and were grown in microtitre plates in presence of 10% foetal calf serum.
Six 50,000 cell aliquots from each sample were incubated in presence or absence of 5 μg Concanavalin A (Sigma-Aldrich GmbH, Deisenhofen, Germany) per ml for 4 days at 37°C and 5% CO₂. Cell proliferation was then quantified with a colorimetric test kit (Cell proliferation ELISA, BrdU, Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturers instructions. The lymphocyte proliferation index was then calculated as the ratio between the Concanavalin A stimulated cells and the unstimulated cells.

**Haptoglobin**

A competitive and species homologous enzyme immunoassay (Biofocus, GmbH, Recklinghausen, Germany) developed and validated by Hiss et al., (2001) was applied to measure haptoglobin concentrations in serum obtained from all animals at each blood sampling date.

**PRRS titre determination**

PRRS titre determination was carried out in a commercial veterinary diagnostic laboratory (IVD GmbH, Hannover, Germany) by ELISA. Titres are given as the ratio between the individual sample and a positive control sample. Values above 0.4 are classified as positive.

**Statistical comparisons**

All results are given as means ± standard deviations (SD) unless SEMs are stated (Table 1). Comparisons between groups were made by using the Kruskal–Wallis test or by one-way-analysis of variance. Haptoglobin serum concentrations and lymphocyte proliferation index were tested via Spearman and Pearson correlation analysis, respectively, for potential relationships between both parameters.

Effects were considered significant at p < 0.05 and probability values between p > 0.05 and p < 0.10 were considered as trends.

**Results**

**Growth performance**

Average daily gains (ADG), average daily feed intake (ADFI) and feed efficiency (FE, feed:gain) of the pigs from the three different feeding groups during the 4 week experimental period are summarised in Table 1. ADFI was tendentiously increased in

<table>
<thead>
<tr>
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<th>Control</th>
<th>0.015% β-glucan</th>
<th>0.03% β-glucan</th>
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<tr>
<td>ADG (g/day)</td>
<td>265 ± 19</td>
<td>293 ± 20</td>
<td>307 ± 23</td>
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<tr>
<td></td>
<td>p = 0.51</td>
<td></td>
<td>p = 0.17</td>
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<tr>
<td>ADFI (g/day)</td>
<td>434.5 ± 24.5</td>
<td>466.0 ± 6.2</td>
<td>501.0 ± 13.5</td>
</tr>
<tr>
<td></td>
<td>p = 0.33</td>
<td></td>
<td>p = 0.076</td>
</tr>
<tr>
<td>FE (g/g)</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>p = 0.8</td>
<td></td>
<td>p = 0.97</td>
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Groups were compared pairwise using the non-parametric Kruskal–Wallis approach. The P-values given refer to the comparison with the control group.
the 0.03% β-glucan fed group in comparison with the controls, but FE was equal in all three groups.

**Haptoglobin serum concentrations**

Fig. 1a shows the haptoglobin concentrations from all pigs at the beginning and at the end of the experiment. The initial mean values before the vaccination were four to ten-fold lower than those at the end of the experiment, i.e. 4 weeks after weaning and vaccination, in all groups. At neither the first nor the last blood sample were differences between the groups observed.

![Graph](image-url)

Fig. 1. (a) Haptoglobin blood concentrations in weaned pigs fed with or without β-glucan (25 animals per group) at the beginning (left columns) and at the end (right columns) of the experiment (means ± SD). Mean values sharing the same letter are not different (p > 0.05). Fig. 1 (b) Weekly recordings of haptoglobin blood concentrations from weaned pigs fed with or without β-glucan (4 animals per group, means ± SD). Mean values sharing the same letter are not different (p > 0.05)
Following the haptoglobin concentrations in weekly intervals (4 animals per group, Fig. 1b), an increase from the first to the second week was observed in the control group and in the 0.015% β-glucan fed group. In the 0.03% β-glucan fed group, this increase did not reach the level of significance. The further course of the haptoglobin serum concentrations shows slight decreases, but the low levels observed at weaning and before the vaccination were not reached within the following 4 weeks.

**Lymphocyte proliferation index (LPI)**

LPI was evaluated in 4 animals from each group at weekly intervals beginning in the first week after the vaccination (Fig. 2). No significant group differences could be established at any of the sampling dates. Concerning the course of LPI during the experiment, no distinct changes were seen.

**PRRS titres**

Before and 4 weeks after the PRRS vaccination, serum samples from all pigs were tested for specific PRRS antibodies. In all groups, PRRS titres were increased at the last blood collection day compared to pre-vaccination levels (Fig. 3). The titre heights reached were not different between the groups. Positive titres were detectable in 27 out of the total 75 pigs before vaccination. In those animals the increase of PRRS titres until the 4th week was less compared to the other pigs that initially had no positive titres. The distribution of PRRS titre positive animals over the different groups showed no obvious bias, i.e. 11, 10 and 6 animals were demonstrated to be PRRS positive in the control group, the 0.015 and the 0.03% β-glucan group, respectively.

**Relationships between the different parameters recorded**

To test for potential relationships between ADG and haptoglobin, at the end of the experiment all 75 pigs were classified into three groups according to their ADG, (regardless of feeding), and their haptoglobin concentrations were compared. As shown in Fig. 4, the highest haptoglobin concentrations were found in pigs with inferior growth rates and vice
versa. In general, haptoglobin concentrations and individual growth rates were negatively correlated (Spearman coefficient of correlation $r = -0.31; p = 0.008$).

Comparing pigs that were PRRS titre positive or negative before the actual vaccination also showed relations to ADG: the growth of those pigs with positive titres at the beginning of the study was slower than the titre negative ones.

There was no relation between haptoglobin serum concentrations and LPI when comparing all sampling dates from the 4 pigs of each group as tested by analysis of correlation. Subdividing the individual sampling days, yielded the same result with one exception: in the blood samples taken 3 weeks after weaning and vaccination, LPI and
haptoglobin were negatively correlated (Spearman correlation coefficient $r = -1$, $p < 0.05$).

**Discussion**

The present study evaluates the efficiency of β-glucan application in pigs with regard to growth performance (ADG, ADFI, FE) and to specific (PRRS titres and LPI) and non-specific (haptoglobin) immune response. Immuno-modulatory effects of β-glucan have been demonstrated on immune cells *in vitro* (Abel et al., 1989; Fruehauf et al., 1982; Hamuro et al., 1978) and are at least partly explained by the stimulation of cytokine production in specific immune cells. Poutsika et al., (1993) showed that stimulation of human macrophages *in vitro* with a *saccharomyces cerevisiae* derived β-glucan lead to increased synthesis of the interleukin-1 (IL-1) receptor antagonist, whereas the pro-inflammatory IL-1β was not increased unless very high β-glucan doses were applied. For other cytokines no relationships with β-glucans have been established so far. Numerous *in vivo* studies with β-glucans have been conducted in fish and have unanimously demonstrated beneficial effects on disease resistance (Raa, 1996; Engstad and Robertsen, 1995; Robertsen et al., 1990). In addition, increased ADG has been reported in aquaculture trials, as reviewed by Engstad and Raa, (1999). In domestic animals comparable trials are limited to monogastric species, i.e. to horses (Krákowski et al., 1999) and to pigs (Schoenherr et al., 1994; Dritz et al., 1995; Decuypere et al., 1998). In horses, oral application has not been tested so far. The product Antaferm (Dr. Eckel GmbH) used in the study presented herein, is qualitatively similar to the β-glucan preparation tested in the three pig studies quoted above (MakroGard®, Biotec-ASA, Tromso, Norway). Both products are *saccharomyces cerevisiae* derived and are water insoluble, but different degrees of purity are reached. An improvement of growth performance by use of β-glucans as indicated in our study is partly in line with the data reported by Dritz et al., (1995). From one out of their three trials, they reported increased growth rates in weaned pigs. However, in the two other trials conducted, they found no effects or even reduced ADG, respectively. As a possible explanation for these divergences, the authors hypothesized that different hygienic status, i.e. different pathogen loads present in the different housing systems investigated, might have effects on the animal’s response to β-glucan. Decuypere et al., (1998) substantiated this by comparing the growth rates of glucan-fed pigs vs. controls kept on two farms with different hygienic status. In their experiment, only the pigs at the farm with an inferior hygienic status responded with increased ADGs, whereas no β-glucan related benefits on growth rate were detectable in those pigs with decreased pathogen loads. Based on the haptoglobin serum concentrations which are, in general, elevated during immune challenges and also under poor hygienic conditions in pigs (Eurell et al., 1992; Gymnich et al., 2001; Rekitt et al., 2001), we can conclude that the pigs studied herein were not exposed to such stressors at weaning. Mean haptoglobin concentrations were between 0.15 and 0.43 mg/ml in the three groups compared at weaning and were well within the range characterised for healthy pigs of comparable age (Lipperheide et al., 2000). Increased haptoglobin levels at the beginning of the experiment, which might be attributed to either remaining colostral antibodies or earlier individual antigen contact, was not accompanied by the presence of positive PRRS titres prior to vaccination. Nevertheless, the growth of these animals was markedly slower. The relationship between ADG and haptoglobin found in pigs regardless of feeding agrees with Eurell et al., (1992) who reported that increased haptoglobin concentrations indicate higher inflammatory cytokine production and are correlated with decreased weight gain in pigs. In contrast to our study, Dritz et al., (1995) demonstrated reduced haptoglobin levels in β-glucan treated pigs vs. controls in one trial using a biochemical assay system. Neutrophil and macrophage function were unchanged in the study of Dritz et al.,
We speculate that in our study, the vaccination-stimulated haptoglobin release (Rekitt et al. 2001) might have superimposed more subtle changes induced by the β-glucan application. However, our data do not support the notion that β-glucan treatment affects nonspecific immune defence. Considering the parameters of specific immune response, neither LPI nor PRRS titre development were affected by β-glucan treatments in our study. Taking together the results from the literature and from our study, the effects of β-glucans to the immune system can so far be not regarded as truly established. Reduced haptoglobin concentrations, and more critical, increased mortality rates after Streptococcus suis challenge in β-glucan fed pigs (Dritz et al., 1995), indicate that β-glucan treatment might not have exclusively stimulating actions on immune functions, but might also comprise inhibitory effects. It is generally accepted that mounting immune response likely requires using resources that could otherwise be allocated to other biological functions, e.g. to reproduction, lactation or growth (Sheldon and Verhulst, 1996; Demas et al., 1997). Reduced immune function during β-glucan treatment might thus explain for the partly observed beneficial effects on growth performance. In view of the fact that all reports about improved ADGs in pigs are attributable to increased ADFIs with unaltered FE, mechanisms other than a pure substrate sparing effect might account for the elevated gains. To qualify our study we should emphasise that the increased FI observed in our study is based on a relatively small number of observations, i.e. 3 pens per group. However, if pro-inflammatory cytokines are reduced in response to β-glucan stimulation, their systemic effects, mediated by the central nervous system, might contribute to increased feed intake (for review see Langhans and Hrupka, 1999).

In conclusion, β-glucan immune modulatory actions as well as the mode of action of beneficial growth effects need further investigation. In view of the negative relationship between haptoglobin blood concentrations and growth rates, and reports on decreased defence abilities in β-glucan treated pigs, amelioration of hygienic status in general may have more profound and sustained effects on animal health and are thus more favourable than aiming to alter immune status via supplements.

Acknowledgements
Appreciation is expressed to Dr. Eckel GmbH for partial financial support and Raiffeisenhof Rheinland for use of animals and facilities.

References
Althaus, F. R., 1998: Das Verbot von antimikrobiellen Leistungsförderern ist problematisch. DLZ-Agrarmagazin 1, VII.


Jeney, G.; Anderson, D. P., 1993: Glucan injection or bath exposure given alone or in combination with a bacterin enhance the non-specific defence mechanisms in rainbow trout (Oncorhynchus mykiss). Aquaculture 116, 315.


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