ABSTRACT

This experiment was conducted to investigate the effects of yeast extract (YE) replacing spray dried plasma protein (SDPP) on growth performance, plasma biochemical indices and intestinal morphology of early-weaned piglets. One hundred and forty seven piglets (Duroc×Landrace×Yorkshire) from 15 pens (average pen weight 6.22±0.16 kg; weaned at 21±1 d) were assigned into 3 treatment groups. The pigs were fed one of the following diets for 14 days: a control diet, a SDPP diet (SDPP, 30 g/kg control diet), and a YE diet (YE, 30 g/kg control diet). On the 15th day of the experiment, five piglets per treatment were randomly selected from each replicate for plasma sample, and then slaughtered for jejunum and ileum collections. The results showed that no significant difference was observed in ADG and FI among three groups. Compared with the SDPP group, plasma phosphorous was lower in the YE group (P<0.05). Compared with the control group, plasma lysine (P=0.052) and methionine (P=0.082) tended to decrease in the SDPP and YE groups, but plasma isoleucine increased significantly both in the SDPP and YE groups (P<0.01), and plasma valine only increased in the YE group (P<0.05), and leucine tended to increase in the SDPP and YE groups; plasma glutamate and glutamine concentrations decreased by 20.5% (P=0.07) and 19.02% (P=0.08) in the weanling piglets from YE group. No difference was observed in the villus height and crypt depth of jejunum. These results indicated that SDPP could be replaced by yeast extract in diet without any detrimental effect on growth performance in early-weaned pigs.

Keywords: Yeast extract; Spray dried plasma protein; Intestinal morphology; Early-weaned piglets.

INTRODUCTION

During weaning period, piglets experience significant physiological, environmental, and social challenges including a different food source, social hierarchy stress, co-mingling with pigs from other litters (Campbell et al., 2013). Weaning stress is one of the most stressful events in the pig’s life, which may cause intestinal and immune system dysfunctions that result in reduced pig health, growth, and feed intake, particularly during the first week after weaning (Hampson, 1986; Gu et al., 2002). These physiological changes encountered by the piglet at weaning times can be countered by correct nutrition using dietary proteins, which are essential for the piglets. Amino acids, normally supplied by dietary protein, are necessary for the growth and repair of tissue, red blood cells, enzymes, and other materials in the body to take part in metabolic reactions, including the biosynthesis of polypeptides and proteins, and the synthesis of nucleotides. Spray Dried Plasma Protein (SDPP) is an effective protein source for use in the Phase I (d 0 to 14 post-weaning) diet for the early-weaned pig (Moreto and Perez-Bosque, 2009). However, SDPP may be a potential danger of infection as a protein source, which are now coming under public scrutiny (Lalles et al., 2009). This is in part due to a lack of public confidence stemming from appearance of BSE and other disease scaring along with salmonella and E. coli contamination of animal food products.

An introductory series of trials exploring the potential of products containing YE and peptides to replace plasma has been previously summarized that equal or better performance when uniformly balanced diets were fed (Tibbetts, 2000). YE is a vegetable protein source derived from yeast cell contents and rich in glutamate and nucleotides, which offer important nutritional implications and has a beneficial impact on growth of piglets (Gallois et al., 2009). Additionally, nucleotides have potential beneficial effects upon the immune system, small intestine growth and development, lipid metabolism and hepatic function (Kulkarni et al., 1994; Walker, 1994; Yu, 1998). Dietary nucleotide before weaning can improve the adaptive capabilities of weaned piglets to the stressors, and enhance the growth performance (Superchi et al., 2012).

Moreover, it has been showed that SDPP and partially substituting SDPP for YE are beneficial for growth performance of piglets, which may be ascribed to...
the improved metabolic status and humoral immune response (Hu et al, 2014). Subsequently, the aim of this experiment was conducted to investigate the effects of replacing SDPP with YE on growth performance, plasma biochemical indices, amino acids and intestinal mucosa morphology of early-weaned piglets.

MATERIALS AND METHODS

Experimental Animal and Management: One hundred and forty seven Duroc × Landrace × Yorkshire piglets (aged at 21 d and weighing 6.22 ± 0.29 kg) were chosen for the experiment. Piglets were randomly divided into three groups: control group (a basal diet), SDPP group (SDPP, 30 g/kg basal diet), and a YE diet (YE, 30 g/kg basal diet). Each group was divided into five pens, with n=9 or 10 piglets in each replicate. The basal diet formulation and nutrient content included: crude protein (CP) 21-22%, Ca, 0.85%, P 0.68%, and Lys 1.40% (NRC, 1998) and supported by Shenzhen Premix INVE Nutrition Co. Ltd (Shenzhen, China). The experimental diets based diet supplemented with the experimental material composition, the different parts of the protein level by adding alanine to regulate protein balance. YE used in the experiments was supplied by the Angel Yeast Co., Ltd (Yichang, 443003 China). Nucleotides and glutamate contents in YE are 10.5% and 17.0%, respectively, which were determined by High Performance Liquid Chromatography (HPLC) in Hubei Provincial Key Laboratory of Yeast Function (Yichang, 443003).

Samples collection: On the 15th day of the experiment (at day 35 of age), five piglets per treatment were randomly selected for blood sample and tissue collection after fasting for 12h. Blood samples (10 mL) were obtained into heparinized tubes and centrifuged at 3,000×g for 10 min at 4°C. The supernatant (plasma) was collected and immediately stored at -20°C for amino acids analyses.

After plasma sampling, piglets were immediately anaesthetised with an i.v injection of sodium pentobarbital (50 mg/kg BW) and bled by exsanguination. The entire intestine was rapidly removed, thoroughly flushed with sterile saline to remove intestinal digesta, and then dissected free mesenteric attachments, weighed, and placed on a smooth, cold surface tray. Duodenum, jejunum and ileum samples were obtained as described by Wu et al. (2010).

Samples of intestinal segments were taken for the assessment of intestinal morphology. Formalin-fixed jejunum and ileum samples were embedded in paraffin; Cross-sections of the segments were cut approximately 5 μm thick with a microtome and stained with haematoxylin and eosin. In each section, the villus height and the associated crypt depth were measured using a light microscope with a computer-assisted morphometric system. Villus height is defined as the distance from the villus tip to crypt mouth and crypt depth from crypt mouth to base (Wu et al, 2010).

This study was performed in accordance with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences.

Growth performance and diarrhea: Average daily gain (ADG) and average daily feed intake (ADFI) were determined for all piglets.

Number of piglets with diarrhea was recorded daily throughout the study. The severity of diarrhea was evaluated using the fecal consistency score system (Marquardt et al, 1999). Fecal consistency scores used was as: 0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea, were determined by two trained persons with no prior knowledge of dietary treatment allocation. The incidence of diarrhea was calculated as the total number of piglets with diarrhea/the total number of all experimental piglets × 100%.

Determination of amino acids in plasma: Plasma (0.5 mL) was deproteinized with 0.5 ml of 1.5 mM HClO₄, followed by addition of 0.25 ml of 2 M K₂CO₃. The neutralized extract was analyzed for amino acids using high-performance liquid chromatography. This method involved the precolumn derivatization of amino acids with o-phthalaldehyde and fluorescence detection. Amino acids in samples were quantified on the basis of known amounts of standards (Sigma Chemicals, St. Louis, MO, USA).

Statistical analysis: A one-way analysis of variance (ANOVA) was used to determine differences among the treatment groups. Results were statistically analyzed using followed the Student-Newman-Keuls multiple comparison test (SAS Institute, NC, USA), with the pen as the experimental unit for growth performance, diarrhea score and other variables, respectively. Values are presented as the mean±SEM, and probability values ≤0.05 were considered statistically significant, and 0.05<P<0.10 was considered as a trend.

RESULTS

Growth performance and diarrhea: SDPP and YE protein source treatments presented similar growth performance in ADG and ADFI (Table 2). No difference was observed (P>0.05) in the incidence of diarrhea or the diarrhea index between the YE and SDPP groups.

Effects of SDPP and Yeast extract on plasma amino acids concentrations: The result of plasma essential amino acids in the jugular artery is shown in Figure 1. Compared with the control group, plasma lysine (P=0.052) and methionine (P=0.082) tended to decrease
in the SDPP and YE groups, whereas plasma isoleucine increased significantly in the SDPP and YE groups (P<0.01), plasma valine increased in the YE group (P<0.05), and leucine tended (0.05<P<0.1) to increase in the SDPP and YE groups (Figure 1). Additionally, compared with the SDPP group, essential amino acids including lysine and methionine in the YE groups had a downtrend (0.05<P<0.10). A decreasing trend (0.05<P<0.10) was reported for essential AA including lysine, methionine, tryptophan in the YE treatment, when compared with other two groups.

In non-essential amino acids (Figure 2), compared with the control group, plasma serine decreased (P=0.0036) in the SDPP and YE groups, whereas plasma aspartic acid tended to decrease (P=0.079) in the YE group compared to the control and SDPP groups. Compared with the SDPP group, plasma Glutamine and Glutamate decreased by 20.5% (P=0.07) and 19.02% (P=0.08) in the weanling piglets from YE group, respectively. Moreover, plasma cysteine increased in the SDPP group (P<0.05) and presented an uptrend in the YE groups (0.05<P<0.10), and plasma carcine (P<0.01) and 5M-Histidine (P=0.038) increased both in the SDPP and YE groups compared with that in the control group (Figure 3).

Effect of SDPP and YE on intestinal morphology: The jejunum and ileum morphology are summarized in Figure 4. There were no differences in jejunum villus height and ileum villus height, as well as ileum crypt depth between the YE and SDPP groups (P>0.05). The ratio of villus height to crypt depth did not differ (P>0.05) between two groups. However, when compared with the SDPP group, there was a decreasing trend (0.05<P<0.10) in ileum villus height and crept depth in the piglets from the YE group.

Table 1. Composition of the diets for pigs (as-fed basis).

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Control group</th>
<th>SDPP group</th>
<th>YE group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn grain</td>
<td>51.55</td>
<td>51.55</td>
<td>51.55</td>
</tr>
<tr>
<td>Soybean expanded</td>
<td>24.2</td>
<td>24.2</td>
<td>24.2</td>
</tr>
<tr>
<td>Fish meal</td>
<td>6.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>SDPP</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
</tr>
<tr>
<td>Whey</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Cream (50% fat)</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Flavor</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>L-Lysine·HCl</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Mineral-vitamin premix</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Nutritional levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP (%)</td>
<td>19.35</td>
<td>19.45</td>
<td>19.40</td>
</tr>
<tr>
<td>DE (Kcal)</td>
<td>3600</td>
<td>3585</td>
<td>3585</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.85</td>
<td>0.83</td>
<td>0.82</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.68</td>
<td>0.65</td>
<td>0.67</td>
</tr>
<tr>
<td>Digestable P (%)</td>
<td>0.50</td>
<td>0.48</td>
<td>0.49</td>
</tr>
<tr>
<td>NaCl (%)</td>
<td>0.83</td>
<td>0.79</td>
<td>0.79</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.40</td>
<td>1.41</td>
<td>1.41</td>
</tr>
</tbody>
</table>

1 Providing the following per kg diet: CuSO4·5H2O, 19.8 mg; KI, 0.20 mg; FeSO4·7H2O, 400 mg; NaSeO3, 0.56 mg; ZnSO4·7H2O, 359 mg; MnSO4·H2O, 10.2 mg; vitamin K (menadione), 5 mg; vitamin B1, 2 mg; vitamin B2, 15 mg; vitamin B12, 30 µg; vitamin A, 5,400 IU; vitamin D3, 110 IU; vitamin E, 18 IU; choline chloride, 80 mg; antioxidants, 20 mg; Fungicide 100 mg.

Table 2. Effect of replacing SDPP with YE on growth performance, feed intake and fecal scours in weanling piglets.

<table>
<thead>
<tr>
<th>Items</th>
<th>Control group</th>
<th>SDPP group</th>
<th>YE group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (kg)</td>
<td>6.24±0.30</td>
<td>6.23±0.30</td>
<td>6.20±0.28</td>
<td>0.996</td>
</tr>
<tr>
<td>Average daily gain (g)</td>
<td>142.12±5.46</td>
<td>159.58±11.06</td>
<td>150.48±19.46</td>
<td>0.660</td>
</tr>
<tr>
<td>ADFI (g)</td>
<td>316.03±10.08</td>
<td>324.45±8.43</td>
<td>304.38±12.33</td>
<td>0.689</td>
</tr>
<tr>
<td>Diarrhea rate (%)</td>
<td>6.21±1.03a</td>
<td>2.66±0.36b</td>
<td>3.03±0.69b</td>
<td>0.042</td>
</tr>
</tbody>
</table>

Values are presented as the mean±SEM. n=5. a,b Means within the same row with different letters differ significantly (P<0.05).
Fig. 1. Effects of SDPP and YE on plasma essential amino acids in the jugular artery of piglets. Values are presented as mean±SEM. n=5. * means differ from the control group (P<0.05), and 0.05<P<0.10 was considered as a trend.

Fig. 2. Effects of SDPP and YE on non-essential plasma amino acids in the jugular artery of piglets. Values are presented as the mean±SEM. n=5.
Fig. 3. Effects of SDPP and YE on other plasma amino acids in the jugular artery of pigs. Values are presented as the mean±SEM. n=5. *Means differ significantly from the control group (P<0.05).

Fig. 4. Effects of SDPP and Yeast extract on jejum (A) and ileum (B) mucosal morphology in weanling piglets (n=5). Representative staining of ileum mucosal morphology of piglets. Original magnification, 200×.
**DISCUSSION**

SDPP and YE are two kinds of high quality feed ingredients, containing functional nutrients, and easy to digest. SDPP is an effective protein source for use in the Phase I (d 0 to 14 post-weaning) diet for the early-weaned pigs. Our findings supported the fact that plasma proteins derived from cattle or pigs are a very effective intake enhancer in most trials. However, performance in terms of growth and feed efficiency did not always follow the improved intake, especially if piglets were disease-challenged (Tibbetts, 2000).

Plasma amino acids are largely dependent on the food ingested (Fernstrom et al, 1979; Nasset et al, 1979). It is well known that plasma free amino acids balance are altered in subjects with various diseases and malnutrition. Protein digestibility, amino acids balance and palatability should be considered for early weaned piglets. Lysine is the first limiting amino acid for early weaned pigs. It has been reported that piglets’ growth rate and feed conversion rate increased with lysine content increasing. However, the content of some essential amino acids, including lysine, methionine and isoleucine, is relatively low in SDPP group. Dietary supplementation with methionine improved piglets’ ADG, ADFI, and G/F from d 0 to 14 post-weaning. Inflection point analysis projected maximum ADG at the methionine:lysine ratio of 27% and 27.5% for pigs fed 1.4% and 1.8% lysine, respectively (Owen et al, 1995). However, in the present study, compared with the SDPP group, essential amino acids including lysine and methionine had a downtrend in the YE group, which may indicate that lysine, methionine and tryptophan may also are relatively low in the YE group.

Branched-chain amino acid (BCAA), including leucine, isoleucine, and valine are of special importance because they help to inhibit protein breakdown and enhance protein synthesis (Buse and Reid, 1975). In the present study, it was interesting that BCAA increased significantly or tended to increase in the SDPP and YE groups, which indicated that SDPP and YE are contributed to the protein synthesis of the piglets.

As a precursor for the synthesis of amino acids and carbon receptor of other amino acid’s catabolism or protein, glutamate maintains the nitrogen balance within body (Santokh G, 2005). Glutamate is necessary for the repair of intestinal epithelial cell and plays a major role in regulation of intestinal blood flow, secretion of the enzyme and growth of epithelial cell (Rhoads et al, 1991). Recently, Rezaei et al. (2013) found that dietary supplementation with 1%, 2% and 4% MSG dose-dependently increased plasma concentrations of glutamate, glutamine, and other amino acids (including lysine, methionine, phenylalanine and leucine), daily weight gain, and feed efficiency in pigs during the post-weaning period (Rezaei et al, 2013). It was interesting that plasma glutamate, glutamine, aspartic acid, and proline tended to decrease in the piglets from the YE group, which might be an indication of their efficient utilization from the diet.

Weaning causes intestinal mucosa dysfunction (Hampson, 1986; Lalles et al, 2007b). An increased villus height and shorter crypt depth may prevent diarrhea after weaning because higher villi and shorter crypts have greater absorption capacity and a lower rate of secretion from secretory cells (Nabuurs et al, 1993). Nutritional management can improve gut health of piglets around weaning (Lalles et al, 2007a). Researchers has reported that SDPP improved intestinal health in post-weaning piglets by increasing villus height and villus height:crypt depth ratio regardless of feed intake (Boren et al, 2001). This was thought to be similar to improved pig performance seen with plasma inclusion in early-weaned piglet diets. Boren et al. (2001) followed a series of nursery feeding trials with extensive intestinal morphology measurements to investigate effects of vegetable proteins containing YE and peptides in piglets, and found that similar improvement in villus height:crypt depth ratio was observed. Cross-sectional area of the lamina propria and villus width were less than the plasma treatment (Boren et al., 2001). Dietary nucleotides may have positive effects on intestinal morphology and function, intestinal microbiota, immune function, nutrient metabolism, hepatic morphology and function as well as growth performance (Sauer et al, 2011). In our previous study, it has been found that glutamic acid affected intestinal epithelium cell proliferation (Wu et al, 2012). In the present study, no significant similar difference was observed in piglets. Moreover, it has been shown that piglets fed the YE diet showed an improved duodenal villous height and the YE is likely attributable to glutamic acid and nucleotides in the yeast extract (Moore et al, 2011). However, it also been found no effect of the addition of plasma or yeast extract on duodenal or jejunal villus height in piglets slaughtered at 28 day of age (Carlson and Veum, 2000).

Cysteine and carnosine have a number of antioxidant properties (Carlson et al, 2002; Levonen et al, 2004). The results from the present study also showed that cysteine was increased in the SDPP and presented an up trend in the YE group, and plasma carnosine increased both in the SDPP and YE groups, which indicated that SDPP and YE might attenuate oxidative stress in weaned piglets.

**Conclusion:** Results from the present study indicated that YE could replace SDPP without any detrimental effect on growth performance in early-weaned pigs, although YE decreased the villus height and crypt depth in ileum to some extent. Collectively, our findings indicated that YE could be extensively supplied in early weaned piglet diet,
and this substitute would reduce the risk of disease transmission through the protein source SDPP.

**Conflict of interest statement:** There is no conflict of interest.

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### REFERENCES


