Effects on growth performance, faecal microflora and plasma cholesterol after supplementation of spray-dried metabolite to postweaning rats

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ABSTRACT: A study was conducted to study the effects of feeding a spray-dried metabolite (SDM) produced by *Lb. plantarum* I-UL4 in the diets of postweaning rats on growth performance, faecal pH, *Enterobacteriaceae* bacterial and lactic acid bacteria (LAB) counts in the faeces and on plasma cholesterol concentrations. A total of 15 female 4-weeks-old *Sprague dawley* rats were randomly assigned to 3 groups of diets: basal diet (control), 0.25% SDM and 0.5% SDM diets. Daily feed intake, daily growth rate, blood plasma for total cholesterol concentration and faecal *Enterobacteriaceae* and LAB were measured. The growth rate, total feed intake, feed conversion ratio, and pH were not significantly different ($P > 0.05$) among all the treatment groups. The faecal *Enterobacteriaceae* counts in the 0.25% and 0.5% groups were significantly ($P < 0.05$) lower than in the control group. However, there was no significant difference ($P > 0.05$) in the LAB counts among all the treatment groups. The plasma cholesterol concentration was significantly reduced ($P < 0.05$) following the feeding of the metabolite. The control had the highest concentration of cholesterol. However, the 0.5% SDM group had the lowest plasma cholesterol concentration, followed by the 0.25% SDM group.

Keywords: metabolite; lactic acid bacteria (LAB); *Enterobacteriaceae*; plasma cholesterol; rats

The important role of the gastrointestinal microbiota in health and growth performance has been demonstrated in animals. Probiotics have been used extensively in animals as they are viable friendly bacterial cultures which are able to improve the balance of intestinal flora and exert beneficial effects by decreasing harmful bacterial metabolites such as amines and indoles (Benno and Mitsuoka, 1992). Lactic acid bacteria (LAB) have always been known as a probiotic and they are able to produce a wide variety of antibacterial substances and inhibitory primary metabolites such as acetic acid, lactic acid, propionic acid, ethanol, hydrogen peroxide, bacteriocins and antibiotic-like substances with activity against gram-negative bacteria (Earnshaw, 1992). These metabolites have been recognised for over a century but recently they have received a greater scientific attention due to broader applications in food industries, as well as feed additives in livestock industries. However, there is still very limited scientific information pertaining to the use of metabolites in livestock industries. Since the metabolites have the properties of antibacterial effects, these substances could be used to replace antibiotics in

the feed of animals. Antibiotics have been widely used to enhance the livestock growth efficiency. There is a growing concern about the continuous feeding of sub-therapeutic levels of antibiotics to livestock, which may result in the presence of antibiotic residues in animal products and in the development of antibiotic-resistant bacteria that are dangerous and potentially lethal when transmitted to humans. Such a negative impact leads to considerable interest to find a number of strategies for improvements in animal health, productivity, and microbial food safety without involving antibiotics (Aarestrup, 2000).

It has been demonstrated that the administration of LAB metabolites in drinking water of rats improves growth performance, reduces faecal *enterobacteria* counts and increases faecal LAB counts. However, Foo et al. (2003b) claimed that the undesirable taste of these metabolites may lead to a low consumption of water. Therefore, it has been suggested to offer these metabolites in the form of powder in order to remove the undesirable taste. However, the effects of feeding a spray-dried metabolite (SDM) in the diets of animals have not been documented yet. Therefore, the objectives of this study were to determine the effect of feeding SDM, which mainly contained bacteriocin from *Lb. plantarum* I-UL4, in the postweaning rats with respect to their growth, faecal pH, population of *Enterobacteriaceae* and LAB in the faeces and the plasma cholesterol concentration.

MATERIAL AND METHODS

The LAB, *Lb. plantarum* I-UL4 used in this study was isolated from 'tempeh' (Foo et al., 2003a). These bacteria were used to produce SDM, which mainly contained bacteriocin. The bacteria were stored at –20°C in Man Rogasa Sharpe (MRS) broth plus 20% glycerol in the freezer. The stock culture was revived twice by transferring into 10 ml MRS broth and incubated at 30°C anaerobically. The streak plate method was used for reviving *Lb. plantarum* I-UL4. It was kept in an incubator at 30°C. After 2 days, a single colony of the bacteria was picked and inoculated into 10 ml MRS broth for 24 h. An overnight culture consisting of 2% (v/v) was inoculated into 1 l MRS broth and incubated anaerobically for 24 h at 30°C. The metabolite was harvested through the separation of bacterial cells by centrifugation at 10.000 rpm for 20 min at 4°C. The cell pellet of the centrifugation was discarded and the supernatant was ultrafiltered with a 0.1 KD filtrate membrane for a desalting purpose. The retentate of the ultrafiltration was used for spray-drying at 60°C (Patent Filing No. PI2002296).

Fifteen postweaning rats, *Sprague dawley*, with an average initial body weight of 81 to 84 g were obtained from the Laboratory Animal Unit, Department of Animal Science. The rats were housed individually in cages in a temperature-controlled room (28 ± 2°C) with a 12-h light dark cycle and relative humidity of 70–80%. The body weights were weighed individually every week for four weeks. Water and feed were offered *ad libitum*. The compositions of the basal diet are shown in Table 1. The feed was provided daily in a mash form where the powder form of SDM was mixed with the basal diet. The animals were assigned into three groups of five rats whereas the body weight of each group was similar. The different groups of rats received different diets: (i) basal diet (as control); (ii) basal diet + 0.25% (w/w) SDM and (iii) basal diet + 0.5% (w/w) SDM. All the rats were acclimatized to the respective diets for one week before the experiment started.

Fresh faecal samples were collected directly from the rectum of the rats by rectal stimulation and transferred into sterile universal bottles and kept at 4°C. The faecal suspension (10% w/v) was made

Table 1. The composition of basal diet

Ingredients	Basal diet
Broken rice	31.70
Corn	30.88
Soybean meal (46% CP)	22.00
Dicalcium phosphate	1.40
Salt	0.70
Limestone	0.60
DL-methionine	0.50
L-lysine	0.50
Vitamin premix[1]	2.12
Palm oil	1.60
Fish meal	8.00

[1]the vitamin premix provides the following amounts per kilogram of diet: vitamin A 5 200 IU; cholecalciferol 1 000 IU; vitamin E 10 IU; vitamin K 1.3 mg; riboflavin 8.0 mg; niacin 25 mg; D-calcium pantothenate acid 10 mg; choline chloride 210 mg and vitamin B_{12} 0.01 mg

Table 2. Effects of feeding control diet, 0.25% and 0.5% spray-dried metabolites on the growth performance of rats after weaning

Treatments	Control	0.25% SDM	0.5% SDM
Initial body weight (g)	83.00 ± 3.69	81.60 ± 4.02	84.60 ± 2.71
Final body weight (g)	153.80 ± 6.70	150.20 ± 7.15	151.00 ± 3.29
Growth rate (g/day)	2.53 ± 0.22	2.45 ± 0.26	2.37 ± 0.21
Total feed intake (g)	366.80 ± 16.90	356.00 ± 9.14	369.40 ± 15.70
Feed conversion ratio	5.28 ± 0.29	5.38 ± 0.46	5.65 ± 0.27

The results are presented as mean values ± SEM

using peptone water and incubated for an hour. 10-fold dilutions (v/v) were prepared with peptone water for *Enterobacteriaceae* and total LAB counts. The spread plate was carried out on EMB (Eosin Methylene-blue Lactose Sucrose) agar. Plates were incubated at 35°C for 24 h aerobically. For LAB enumeration, the spread plate was conducted on MRS agar and incubated at 30°C for 48 h as described by Foo et al. (2001).

The faecal samples were mixed homogeneously at the ratio of 1 g faeces: 10 ml distilled water in a universal bottle. The pH of the faeces was measured with an electronic pH meter. At the end of the feeding trial, the rats were fasted for 12 h before the blood collection. The rats were anaesthetized with diethyl ether. Approximately 3 ml blood was sampled through a cardiac puncture using a 26G needle attached to a 5 ml syringe. The blood samples were collected in vacutainer tubes (Beckton Dickinson, UK) containing disodium EDTA as anticoagulant. The plasma was separated from the blood by centrifugation at 3.000 rpm for 10 min for the cholesterol concentration analysis. The plasma cholesterol concentration was determined by the diagnostic kit (Randox®, UK) as described by Loh et al. (2002).

The results were expressed as mean ± standard error of the mean. All the growth parameters, colony counts, faecal pH value and cholesterol concentration were compared using one-way analysis of variance (ANOVA). Least significant difference (LSD) was used to assess difference between means. The statistical software program of SAS (SAS, 1988) was used for the data analysis.

RESULTS

The growth performance of SDM-supplemented rats is shown in Table 2. There were no significant differences ($P > 0.05$) in growth rate, total feed intake and feed conversion ratio.

Figure 1 shows the number of faecal *Enterobacteriaceae* of rats fed control, 0.25% and 0.5% SDM di-

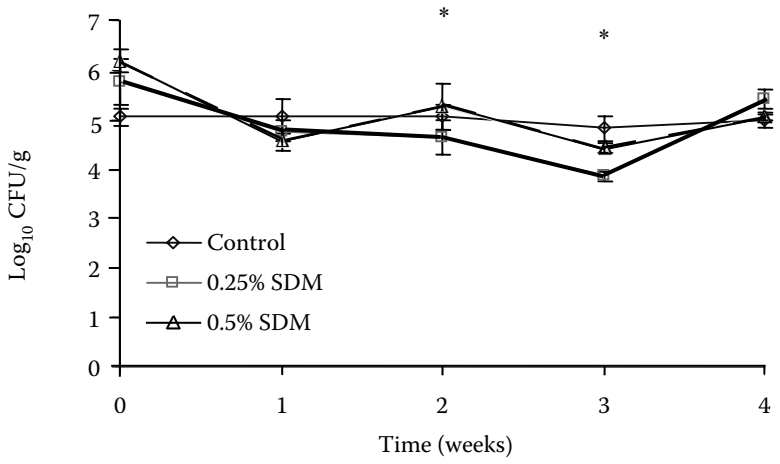

Figure 1. Effects of feeding control diet, 0.25% and 0.5% spray-dried metabolites on faecal counts of *Enterobacteriaceae* (log_{10} value of CFU per gram) of rats after weaning. Error bars indicate the standard error of the mean (*< 0.05)

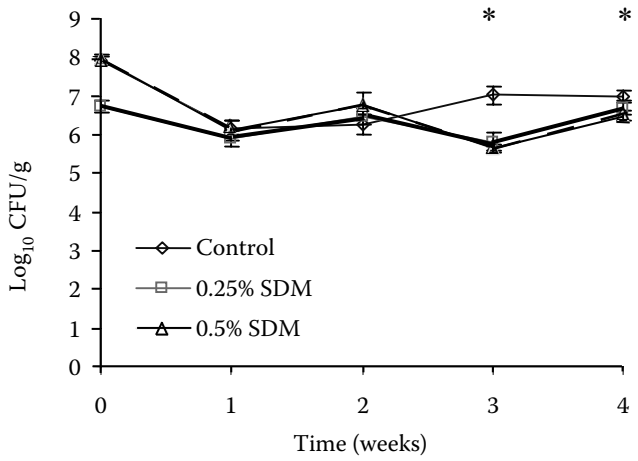

Figure 2. Effects of feeding control diet, 0.25% and 0.5% spray-dried metabolites on faecal counts of LAB (log_{10} value of CFU per gram) of rats after weaning. Error bars indicate the standard error of the mean (*< 0.05)

ets. At the beginning of the experiment, the number of *Enterobacteriaceae* in the control group was the lowest and in 0.5% SDM it was the highest. The faecal *Enterobacteriaceae* counts for 0.25% SDM decreased progressively throughout the experimental period except for the last week of treatment. After three weeks of the experiment, lower *Enterobacteriaceae* counts were found in the faeces obtained from rats receiving SDM diets compared to rats fed the control diet. At the end of the experiment, there was an increase in *Enterobacteriaceae* counts for all the groups. However, the results were not significantly different ($P > 0.05$) among the treatments.

Figure 2 shows the effects of feeding the control, 0.25% and 0.5% SDM diets on faecal LAB counts. The faecal LAB counts were not significantly different ($P > 0.05$) between the control and treated groups in the first two weeks. However, in weeks 3 and 4 of the experiment, the rats fed the control diet had significantly higher ($P < 0.05$) counts than the 0.5% SDM group.

The mean faecal pH in rats after feeding the control, 0.25% SDM and 0.5% SDM diets is shown in Figure 3. There was no significant difference ($P > 0.05$) in the pH among all the treatment groups throughout the experimental period.

Figure 4 shows the plasma total cholesterol concentration of rats receiving the control, 0.25% and 0.5% SDM diets. The rats supplemented with 0.25% and 0.5% SDM had a significant1y lower ($P < 0.05$) plasma cholesterol concentration than those of the control group. However, there was no significant difference ($P > 0.05$) in the plasma cholesterol concentration between the rats on diets with 0.25% and 0.5% SDM.

DISCUSSION

The results of this study demonstrated that the growth rate, feed intake and feed conversion ratio were not affected by the addition of SDM to the

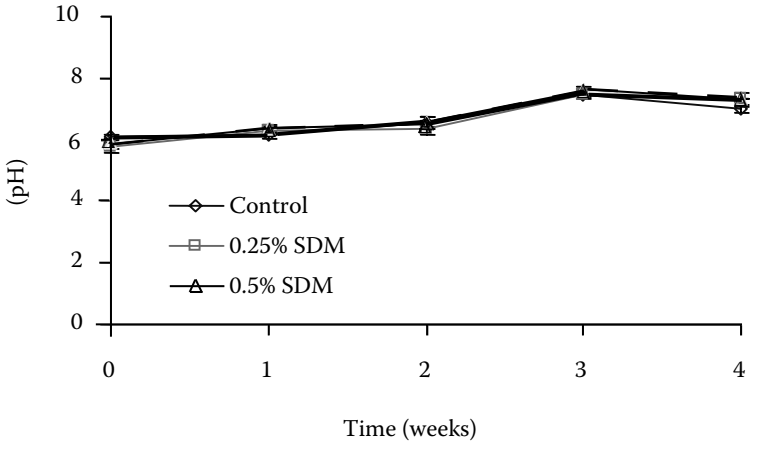

Figure 3. Effects of feeding control diet, 0.25% and 0.5% spray-dried metabolites on faecal pH in rats after weaning. Error bars indicate the standard error of the mean (*< 0.05)

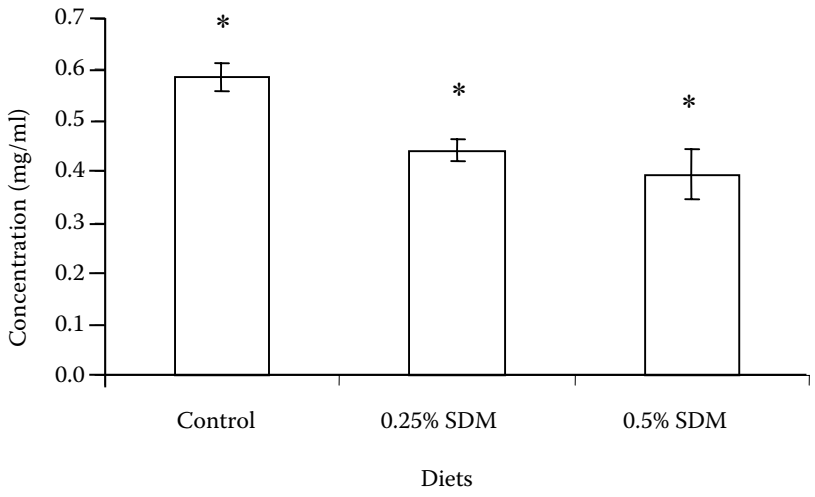

Figure 4. Effects of feeding control diet, 0.25% and 0.5% spray-dried metabolites on the total plasma cholesterol concentration in rats after weaning. Error bars indicate the standard error of the mean (*< 0.05)

diet. Similar results were obtained in mice and rats receiving different doses of the different LAB (Zhou et al., 2000; Foo et al., 2003a). Bernardeau et a1. (2002) also reported that the weight gain, feed intake and water intake of mice were not affected by the supplementation of *Lb. acidophilus* in drinking water. Watkins and Kratzer (1983, 1984) showed that there were no significant differences in weight gains of chickens given diets with or without *Lactobacillus* cultures. On the contrary, Mathew et al. (1998) claimed that weanling pigs fed a yeast culture had better growth performance. The contradictory results might be attributed to the variation of *Lactobacillus* sp. or other cultures used in the studies.

The addition of SDM to the diets of rats significantly decreased the *Enterobacteriaceae* counts in the faeces in the third week of the experiment. The results showed similar trends to those reported in pigs (Urlings et al., 1993) and rats (Loh et al., 2003). The reduced shedding of *Enterobacteriaceae* in the faeces was also found in studies using fermented feed containing *Lactobacillus* cultures (Mikkelsen and Jensen, 1998; Loh et al., 2003). Similarly, probiotics supplemented to piglets within a day of birth significantly reduced the numbers of *Salmonella choleraesuis* (Fedorka et al., 1999) and *E. coli* detected in gut tissues and faeces. The decreased *Enterobacteriaceae* population in the faeces of treated rats may be due to the ability of the metabolite to inhibit the growth of various gram-negative bacteria, particularly pathogenic *E. coli.* This is consistent with the study by Todorov and Dicks (2005), who found that bacteriocin produced by *Lb. plantarum* inhibited *E. coli, Staphylococcus aureus* and *Enterococcus faecalis*. At the end of the experiment, there was an increase in *Enterobacteriaceae* counts for all the treatment groups. It is known that stress due to rough handling can have an impact on the gastrointestinal tract, which also can create disturbances in the gastrointestinal microbe ecology. Furthermore, the risk for a *Salmonella* and/or *E. coli* infection would be increased (Tuchscherer et al., 1998).

The rats treated with SDM of *Lb. plantarum* I-UL4 did not show any significant difference in LAB counts in the faeces compared to the control rats. This is in contrast with the results obtained by Foo et al. (2003a,b) and Loh et al. (2003). However, Usman and Hosono (2000) reported that the rats fed with non-fermented milk produced from *Lb. gasseri* were maintaining a high level of faecal lactobacilli throughout the experimental period. Demeckova et al. (2002) showed no significant effect of fermented liquid feed when fed to the sows on the faecal LAB counts. This could be explained by the availability of the antimicrobial properties such as bacteriocin and their potential to inhibit a broad spectrum of antibacterial activity against other genera of lactic acid bacteria (Klaenhammer, 1988). *Lb. plantarum* I-UL4 used in this study has the ability to produce bacteriocin, which could inhibit the growth of *Listeria monocytones* and closely related LAB (Foo et al., 2003a).

The results of the present study showed that there was no significant difference ($P > 0.05$) in the faecal pH between the rats supplemented with SDM of *Lb. plantarum* I-UL4 and the control rats after four weeks of experiment. This is consistent with the study by Moran (2001), no significant effect of fermented liquid feed on the pH of the pig lower gastrointestinal tract. In contrast, Meredith (2003)

showed a reduction of faecal pH in pigs treated with a new probiotic feed supplement. Additionally, Foo et al. (2003a) also showed that the rats receiving *Lb. plantarum* I-UL4 in their diets had a lower faecal pH than the control rats after two weeks of experiment. The study also suggested that the pH of faeces cou1d be modified by the inclusion of *Lb. plantarum* in the diet.

A significant reduction in the plasma cholesterol concentration was observed in rats treated with SDM. The result indicated that the metabolite produced by *Lb. plantarum* I-UL4 had a lowering effect on plasma cholesterol levels in rats. Similar results of a reduction in the serum cholesterol concentration were obtained by the oral administration of live cells of *Lb. plantarum* in the diet of rats (Foo et al., 2003a). Usman and Hosono (2000) also reported that non-fermented milk produced from *Lb. gasseri* significantly reduced serum total cholesterol and low density lipoprotein (LDL) cholesterol, and bile acids of rats. These findings were in agreement with works published earlier (Harrison and Peat, 1975; Grunewald, 1982). A serum cholesterol reduction was also demonstrated in rats with the administration of a probiotic mixture (Fukushima and Nakano, 1996) and in pigs with the oral administration of *Lb. johnsonii* and *Lb. reuterii* (du Toil et al., 1998).

In conclusion, this study proves the importance and benefits of SDM of *Lb. plantarum* I-UL4 in the diet of rats with respect to a reduction in faecal *Enterobacteriaceae* counts and lowering effect on the plasma cholesterol concentration. The growth performance, faecal pH and faecal LAB counts of rats were not affected after supplementation of SDM of *Lb. plantarum* I-UL4 to the diets.

REFERENCES

Aarestrup F.M. (2000): Characterization of glycopeptide-resistant *Enterococcus faecium* (GRE) from broilers and pigs in Denmark: genetic evidence that persistence of GRE in pig herds is associated with co-selection by resistance to macrolides. Journal of Clinical Microbiology, 38, 2774–2777.

Benno Y., Mitsuoka T. (1992): Impact of *Bifidobacterium longum* on human. Microbiology and Immunology, 36, 683–694.

Bernardeau M., Vemoux J.P., Gueguen M. (2002): Safety and efficacy of probiotic lactobacilli in promoting growth in postweaning Swiss mice. International Journal of Food Microbiology, 77, 19–27.

Demeckova V., Moran C.A., Cavenay C., Campbell A.C., Kuri V., Brook P.H. (2002): The effect of fermentation and/or sanitation of liquid diets on the feeding preferences of newly weaned pigs. In: Lindberg J.E., Ogle B. (eds.): Digestive Physiology of Pigs. CABI Publishing, Wallingford, UK, 291–293.

du Toil M., Franz C.M., Dicks L.M. (1998): Characterisation and selection of probiotic *Lactobacilli* for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. International Journal of Food Microbiology, 40, 93–104.

Earnshaw R.G. (1992): The antimicrobial action of lactic acid bacteria: natural food preservation systems. In: Wood B.J.B. (ed.): The Lactic Acid Bacteria in Health and Disease. Chapman and Hall, London, UK, 211–232.

Fedorka C.P.J., Bailey J.S., Stern N.J., Cox N.A., Ladely S.R., Musgrove M. (1999): Mucosal competitive exclusion to reduce Salmonella in swine. Journal of Food Protection, 62, 1376–1380.

Foo H.L., Lim Y.Z., Rasul G. (2001): Isolation of bacteriocin producing lactic acid bacteria from Malaysian fermented food, Tapai. In: Proceeding of the 11th World Congress of Food Sciences and Technology, Seoul, Korea, 185 pp.

Foo H.L., Loh T.C., Law F.L., Lim Y.Z., Kufli C.N., Rusul G. (2003a): Effects of feeding *Lactobacillus plantarum* I-UL4 isolated from Malaysian Tempeh on growth performance, faecal flora and lactic acid bacteria and plasma cholesterol concentrations in postweaning rats. Food Science and Biotechnology, 4, 403–408.

Foo H.L., Loh T.C., Lai P.W., Lim Y.Z., Kufli C.N., Rusul G. (2003b): Effects of adding *Lactobacillus plantarum* I-UL4 metabolite in drinking water of rats. Pakistan Journal of Nutrition, 2, 283–288.

Fukushima M., Nakano M. (1996): Effect of a mixture of organisms *Lactobacillus acidophilus* of *Streptococcus faecalis* on cholesterol metabolism in rats fed on a fat and cholesterol enriched diet. British Journal of Nutrition, 76, 857–867.

Grunewald K.K. (1982): Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. Journal of Food Science, 47, 2078–2079.

Harrison V.C., Peat G. (1975): Serum cholesterol and bowel flora in the newborn. American Journal of Clinical Nutrition, 28, 1351–1355.

Klaenhammer T.R. (1988): Bacteriocins of lactic acid bacteria. Biochimie, 70, 337–49.

Loh T.C., Foo H.L., Tan B.K., Jelan Z.A. (2002): Effect of palm kernel cake on growth performance and blood

lipids in rats. Asian-Australasian Journal of Animal Sciences, 15, 1165–1169.

Loh T.C., Foo H.L., Tan S.H., Goh Y.M., Shukriyah M.H., Kufli C.N. (2003): Effects of fermented products on performance, faecal pH, *Enterobacteriaceae* and lactic acid bacteria counts and interrelationships and plasma cholesterol concentration in rats. Journal of Animal and Feed Sciences, 12, 633–644.

Mathew A.G., Chattin S.E., Robbins C.M., Golden D.A. (1998): Effects of a direct-fed yeast culture on enteric microbial populations, fermentation acids, and performance of weanling pigs. Journal of Animal Science, 76, 2138–2145.

Meredith M. (2003): New Probiotic Feed Supplement for Swine. United States Patent. USA, 6, 524–574.

Mikkelsen L.L., Jensen B.B. (1998): Performance and microbial activity in the gastrointestinal tract of piglets fed fermented liquid feed at weaning. Journal of Animal and Feed Sciences, 7, 211–215.

Moran C.A. (2001): Developments and benefits of liquid feeding for the postweaning pig. [PhD Thesis.] University of Plymouth, Plymouth, UK.

SAS (1988): SAS® User's Guide: Statistics. SAS Institute Inc., Cary, NC.

Todorov S.D., Dicks L.M.T. (2005): *Lactobacillus plantarum* isolated from molasses produces bactericins active against Gram-negative bacteria. Enzyme and Microbial Technology, 36, 318–326.

Tuchscherer M., Puppe B., Tuchscherer A., Kanitz E. (1998): Effects of social status after mixing on immune metabolic and endocrine responses in pigs. Physiology and Behavior, 64, 353–360.

Urlings H.A.P., Mul A.J., van't Klooster A.T., Bijker P.G., van Logtestijn J.G., van Gils L.G. (1993): Microbial and nutritional aspects of feeding fermented feed (poultry by-products) to pigs. Veterinary Quarterly, 15, 146–151.

Usman, Hosono A. (2000): Effect of administration of *Lactobacillus gasseri* on serum lipids and fecal steroids in hypercholesterolemic rats. Journal of Dairy Science, 83, 1705–1711.

Watkins B.A., Kratzer E.H. (1983): Effect of oral dosing of *Lactobacillus* strains on gut colonization and liver biotin in broiler chicks. Poultry Science, 62, 2088–2094.

Watkins B.A., Kratzer E.H. (1984): Drinking water treatment with commercial preparation of a concentrated *Lactobacillus* culture for broiler chickens. Poultry Science, 63, 1671–1673.

Zhou J.S., Shu Q., Rutherfurd K.J., Prasad J., Gopal P.K., Gill H.S. (2000): Acute oral toxicity and bacterial translocation studies on potentially probiotic strains of lactic acid bacteria. Food and Chemical Toxicology, 38, 153–161.

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