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Effects of Adding Metabolites of *lactococcus lactis* RW18 Isolated from Fermented Tapioca in Drinking Water of Rats

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Abstract: The effects of addition of different levels of *Lactococcus lactis* RW18 (RW18) metabolites in drinking water on growth performance, total cholesterol and faecal LAB, *Enterobacteriaceae* and pH in rats were investigated. The metabolites were prepared by inoculated and incubated the bacteria culture into MRS broth anaerobically. A total of 30 rats were used in this study. The rats were treated on control (without metabolite), 35% RW18 metabolites (v/v, 35% RW18) and 70% RW18 metabolites (v/v, 70% RW18) in drinking water. The initial and final body weights, growth rate, feed intake and feed conversion ratio were not significantly different among the treatment groups. The water consumption for RW18 rats was significantly lower than the control rats. Plasma total cholesterol levels were not significantly among the treatment groups. Faecal LAB count was only found to be significant at the end of experiment. RW18 rats had significant higher faecal LAB counts than the control rats. Reduction of faecal *Enterobacteriaceae* counts was found after one week of experiment. Faecal pH of rats treated with RW18 was not significantly reduced as compared with the control rats. The results suggest that addition of metabolites in drinking water may reduce and increase the faecal *Enterobacteriaceae* and LAB counts, respectively.

Key words: Rat, *Lactococcus lactis* RW18 metabolite, LAB, *Enterobacteriaceae*, plasma cholesterol concentration

Introduction

In modern animal farming, various methods have been explored to improve animal health and growth performance. These include better husbandry management, nutrition and utilisation of feed additive. The common feed additives used are antibiotic, probitics, enzymes and organic acids (Bernardeau *et al.*, 2002). Growth promoting antibiotic is the most commnon one among fed additives, mainly due to their positive effects in growth or feed conversion efficiency and also reduction of incidence of certain diseases. However, the extensive use of antibiotic may cause animals to develop resistance in a number of pathogenic bacteria species (Mikkelsen and Jensen, 2000). Likewise, cross-resistance may occur to therapeutic antibiotic belongs to the same class of drug, particularly those with close relationships with human antimicrobial therapies. Some countries already imposed restrictions or prohibitions on the use of antibiotics as growth promotants and this have drawn attention to possible alternatives (Wierup, 2000). Lactic Acid Bacteria (LAB) as probiotic is often suggested as alternative for replacing antibiotic. LAB widely used as starter cultures in meat and meat-products, play a very important role in ensuring the safety of different foods through the production of metabolites such as bacteriocins. Bacteriocins are proteinaceous compounds which has antimicrobial properties able to inhibit many different bacterial species, especially pathogenic bacteria (De Vuyst and Vandamme, 1994). This compound has received a great attention because they are produced by beneficial to human health bacteria and also often used as natural food preservatives.

It has been shown that administration of bacteriocins influences the bacterial ecology of the gastrointestinal tract and reduces the levels of pathogenic bacteria in different parts of gastrointestinal tract (Gaenzle *et al.*, 1999 and Winsen *et al.*, 2001). Gaenzle *et al.* (1999) showed that bacteriocin curvacin prouduced by *Lactobacillus curvatus* inhibited *E. coli* and *Listeria* inoculate in the stomach. It also has been shown that bacteriocins produced by *Lactococcus lactis* subspecies *lactis* have antibacterial properties (Mishra and Lambert, 1996). However, the effects of direct feeding of metabolites from *Lactococcus lactis* isolated from local *tapai ubi* (fermented sweet potato) have not been studied. The objectives of this experiment were to study the effect of adding different levels of *Lactococcus lactis* subspecies *lactis* metabolites in drinking water on growth performance, plasma cholesterol concentrations and faecal pH, LAB and *Enterobacteriaceae* counts in postweaning rats.

Materials and Methods

Culture Condition and Preparation of Metabolite: *Lactococcus lactis* subsps. *lactis* was obtained from our own collection, Department of Biotechnology, Universiti Putra Malaysia. This bacteria was isolated from *tapai ubi* (fermented tapioca). The bacteria was

kept in Man Rogosa Sharpe (MRS) broth containing 20% (v/v) glycerol at -20°C and was revived twice in MRS broth and incubated anaerobically at 30°C before preparing the metabolite.

2% (v/v) of overnight culture was inoculated into 1L MRS broth and incubated anaerobically for overnight at 30°C. The metabolite was collected by separating the bacterial cells with centrifugation at 8000 rpm for 10 min. The metabolite was then kept at 4°C. The pH of the metabolite was 4.3.

Experimental Animals and Experimental Protocol: The feeding experiment was carried out at Department of Animal Science, Universiti Putra Malaysia. A total of 15 male and 15 female post weaning rats, *Sprague dawley*, 4 week-old with the average body weight of 61g were used in this study. The rats were randomly assigned to 3 treatment groups of 10 each (5 male and 5 female). The treatments were: i) control (100% drinking water), ii) 35% + 65% drinking water (35% RW18) and iii) 70% + 30% drinking water (70% RW18). All the rats were acclimatised to the respective treatments for a week. They were housed individually in a 24-hour lit room with well-ventilated air-conditioned environment at 24-26°C and relative humidity of 60-64%. The rats were given basal feed *ad libitum* for 28 days. The compositions of basal diet are shown in Table 1. Daily feed, daily water intake and weekly body weight gain were measured throughout the experiment.

Table 1:The compositions of basal diet

Ingredients	Basal Diet
Broken rice	20.00
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 *	2.12
Palm oil	1.60
Fish meal	8.00

* The vitamin premix provides the following amounts per kilogram of diet: vitamin A, 5200 IU; cholescalciferol, 1000 IU; vitamin E, 10 IU; vitamin K, 1.3 mg; riboflavin, 8.0 mg; niacin, 25 mg; D-calcium pantothenic acid, 10 mg; choline chloride, 210 mg and vitamin B_{12}, 0.01 mg

Fresh faecal samples were collected directly from the rectum of each rat every week. The pH of the faeces was measured directly with a pH meter. The faecal (10% w/v) was suspended in sterile peptone water and incubated for an hour before further 10-fold dilutions (v/v) were made with peptone water for *Enterobacteriaceae* and total LAB counts. Total LAB counts were spread plated on MRS-agar (Merck®) and incubated at 30°C for 48 h whereas *Enterobacteriaceae* were plated on EMB-agar (Merck®) and incubated at 37°C for 24 h (Foo *et al.*, 2001). Numbers of colony forming units (CFU) are expressed as $\log_{10}$ CFU per gram.

At the end of experiment, the rats were fasted for 12 hours before blood collection. The rats were anaesthetized with diethyl ether and blood was collected by cardiac puncture into tube containing EDTA (vacutainer®, USA). Plasma was obtained after centrifuging the whole blood at 3000 rpm for 10 min for total cholesterol concentration analysis. Total cholesterol levels were determined through the Enzymatic Endpoint Method using a commercial diagnostic kit (Randox®, UK), as described by Loh *et al.* (2002).

Statistical Analyses: Results were expressed as mean ± standard of mean (SEM). The data was analysed by two-way analysis of variance (ANOVA). Duncan Multiple Range Test was used to compare the differences of means in growth performance, CFU of LAB and *Enterobacteriaceae*, faecal pH and plasma total cholesterol concentration among treatment groups. The statistical analysis were done using SAS program (1998) at differences of $p<0.05$.

Results

Table 2 shows the growth performance for the different treatment groups. There was no significantly differently ($P>0.05$) in the initial and final body weights, growth rate, feed intake and feed conversion ratio. However, the rats treated with RW18 had significant lower ($P<0.05$) water intake compared with control rats.

Fig. 1 shows the plasma total cholesterol concentration for the control, 35% and 70% RW18. There was no significantly different ($P>0.05$) among the treatment groups for the total plasma cholesterol concentration.

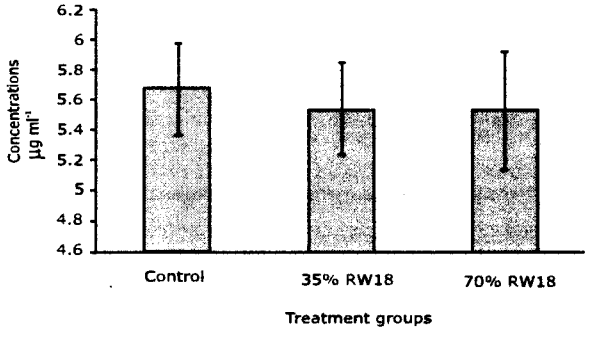
Fig. 2 shows the faecal LAB counts for the control, 35% and 70% RW18. The faecal LAB counts were not significantly different ($p>0.05$) between the control and treated groups from weeks 0 to 3 of the experiment. However, in the last week of experiment, the RW18 rats had significant higher ($P<0.05$) counts than the control rats.

The faecal *Enterobacteriaceae* counts for the control, 35% and 70% RW18 are shown in Fig. 3. The faecal *Enterobacteriaceae* counts for the control and RW18 rats were not significantly different ($P>0.05$) for the first week of experiment. However, the 35% RW18

Table 2: Effects of different levels of *Lactobacillus plantarum* I-RW18 (RW18) on growth performance in postweaning rats

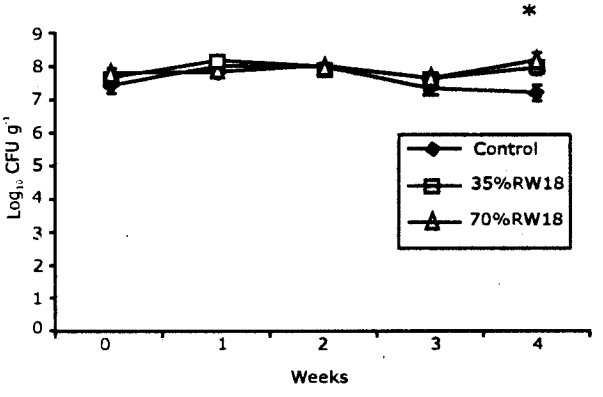
Treatments	Control	35% RW18	70% RW18
Initial body weight, g	60.67 ± 1.48	61.33 ± 2.50	59.33 ± 2.49
Final body weight, g	222.5 ± 21.58	225.83 ± 23.74	223.33 ± 22.37
Growth rate, g day^{-1}	5.78 ± 0.77	5.88 ± 1.98	5.86 ± 0.82
Total feed intake, g	432.17 ± 35.1	433.00 ± 37.30	431.00 ± 29.80
Total water intake, ml	742.83 ± 39.80^{a}	687.33 ± 51.30^{b}	665.33 ± 17.70^{b}
Feed conversion ratio	2.77 ± 0.19	2.73 ± 0.19	2.79 ± 0.24

The results are presented as mean values ± SEM. Values with different superscripts within row differ significantly at $p = 0.05$.



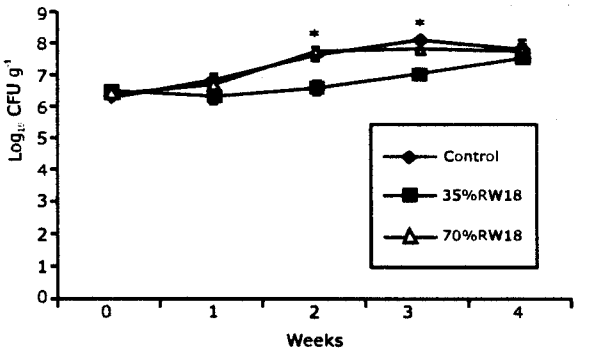
Values with different alphabets differ significantly at $P = 0.05$.

Fig. 1: Effect of different levels of RW18 metabolies on plasma cholesterol concentration in postweaning rat. Error bar indicates standard error of mean



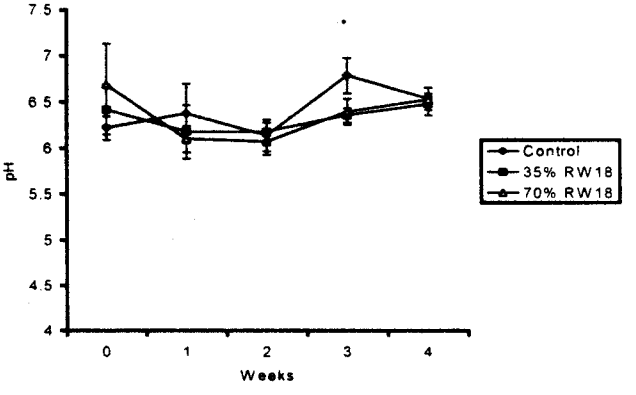
*indicates significant difference at $P < 0.05$

Fig. 2: Effect of different levels of RW18 metabolites on faecal LAB counts in postweaning rats. Error bar indicates standard error of mean



*indicates significant difference at $P < 0.05$

Fig. 3: Effect of different levels of RW18 metabolites on faecal Enterobacteriaceae counts in postweaning rats. Error bar indicates standard error of mean



* indicates significant difference at $P < 0.05$

Fig. 4: Effect of different levels of RW18 metabolites on faecal pH in postweaning rats. Error bar indicates standard error of mean

rats had the lowest faecal *Enterobacteriaceae* counts in the second and third week of experiment. There was no significant different ($P>0.05$) for faecal *Enterobacteriaceae* counts between 70% RW18 and control rats in the second week of experiment. However, the 70% RW18 rats had a lower ($P<0.05$) faecal *Enterobacteriaceae* counts than control rats in the third week of experiment. In the last week of experiment, no difference was observed ($P>0.05$) among the treatment groups.

Fig. 4 shows the faecal pH for the control, 35% and 70% RW18. The faecal pH was found to be significantly different ($p<0.05$) between the control and RW18 treated rats after two weeks of experiment. The rats treated with RW18 had lower faecal pH ($p<0.05$) than the control rats. However, no difference was observed ($P>0.05$) between 35% and 70% RW18. At the end of experiment, there were no differences between RW18 and control groups.

Discussion

The initial and final body weights, growth rate and feed intake were not significantly different among the treatment groups. However, the water consumption for control rats was higher than the RW18 rats. This might be associated with poorer taste of drinking water in RW18 treatments. *Lactococcus lactis* RW18 used in the present study is a homcfermentative LAB (Pot *et al.*, 1994), which capable to metabolise glucose primarily to form lactic acid and result in a reduction of pH. Thus, the sour taste of metabolite may not encourage the rats to drink.

No significant difference was observed for the plasma cholesterol concentration between treatment groups. The result indicates that the metabolites produced by *Lactococcus lactis* or the strain had no lowering effect on plasma cholesterol levels in rats. However, in other study show arotic acid and hydroxymethyl glutamic acid produced by LAB reduce serum cholesterol levels (Jaspers *et al.*, 1984). It was shown that utilisation of a selected *L. acidophilus* strain reduces serum cholesterol (Gilliland *et al.*, 1985 and Danielson *et al.*, 1989).

The rats treated with RW18 had a higher faecal LAB counts than the control rats at the end of experiment. Additionally, there was a slight increment of faecal LAB counts from the initial until the end of experiment for those rats treated with RW18. These results suggest that the addition of RW18 in the drinking water encourage and increase the growth and population of indigenous LAB in the gastrointestinal tract. Similar results in increasing faecal LAB counts have been obtained by the provision of dry fermented product to the rats (Loh *et al.*, 2003). In contrast, Demecková *et al.* (2002) reported no significant effect of fermented liquied feed when fed to the sow on the faecal LAB counts. In these studies, fermented feed is usually characterised by low pH, high numbers of LAB and high concentration of metabolites. These characteristics are very similar to that of metabolites produced in present study.

A significant reduced in faecal *Enterobacteriaceae* counts one week after commencement of metabolites feeding. 35% RW18 rats had the lowest count compared to other treatments. The results show similar trends to those reported elsewhere for pigs (Mikkelsen and Jensen, 1998; Winsen *et al.*, 2001 and Moran, 2001) and for rats (Loh *et al.*, 2003). Despite offering higher concentrations of metabolites to the 70% RW18 rats, faecal *Enterobacteriaceae* count was not lower than 35% RW18 rats. This result may be associated with lesser amount of metabolites consumed. This explanation could be supported by the results of lesser total water intake in 70% RW18 rats compared to the 35% RW18 rats. Mahadeo and Tatini (1994) applied a bacteriocin in poultry processing and demonstrated that nisin reduced the number of *Listeria* added to scald water. In a study on milk by Shahani *et al.* (1977), acidpophilin produced by *L. acidophilus* inhibits a wide range of genera including both Gram-negative and Gram-positive types when cultured in milk.

We believe that metabolites used in present study contain high concentration of organic acids particularly lactic acid. However, the faecal pH of rats treated with RW18 was not significantly reduced as compared with the control rats. This result indicates that the amount of metabolite intake may not be sufficient to modify the pH of faeces. A study by Moran (2001) showed no significant effect of fermented liquid feed on the pH of the pig lower gastrointestinal tract. In contrast, Canibe and Jensen (2000) reported significantly lower pH in the stomach of growing pigs fed fermented liquid feed. In conclusion, this study shows insight into the effects of metabolites produced from *Lactococcus lactis*. The data are strongly indicative of a change in faecal LAB and *Enterobacteriaceae* counts through high concentrations of metabolites. However, the taste of metabolites may not be favourable by the rats, this directly may affect the actual intake and effects of metabolites. The future involvement of a lower concentration of metabolites in drinking water and transformation of liquid metabolites to solid form are suggested as this may serve to confirm the trend observed in *Enterobacteriaceae* reduction and LAB increment during the treatment period.

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