Effects of potato (*Solanum tuberosum* l. cv. golden valley) protein on performance, nutrient metabolizability, and cecal microflora in broilers

Einfluss von Kartoffelprotein (*Solanum tuberosum* l. cv. golden valley) auf die Leistung, die Nährstoffverdaulichkeit und die Mikroflora im Blinddarm von Broilern

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Introduction

Use of sub-therapeutic doses of antibiotics as growth promoting agents in swine and poultry diets has been a common practice since last five decades (Deinzer and Richards, 2005). However, their continuous use and misuse has led to the emergence of antibiotic resistance and residues of antibiotics in animal products (Schwarz et al., 2001), thus ultimately having a negative impact on human health (WHO, 1997). This has prompted the European Union (Castanon, 2007), US and several other countries to ban the use of antibiotics in animal feed, making it necessary to identify alternatives to replace antibiotics so as to maintain better animal productivity and health.

Recently, antimicrobial peptides which are small gene-encoded peptides having a broad range of activity against gram-negative and gram-positive bacteria, fungi and mycobacteria have been identified (Zasloff, 2002). Plants produce several types of such proteins that mediate defense against pathogens and invading microbes (Pouvreau et al., 2003). Potatoes (*Solanum tuberosum*) are commonly cultivated for human consumption and known to have antimicrobial (Has et al., 1996), and antifungal (Do et al., 2004) properties. A protein of 5.6 kDa, named as potide-G has been isolated from the potato tubers of Golden valley potatoes (Thirath et al., 2006). Potide-G was found to inhibit the growth of *Staphylococcus aureus*, *Listeria monocytogenes* and *E. coli* (Kim et al., 2006). Inclusion of 0.25 to 0.75% potato protein (PP) obtained from the tubers of Golden valley potatoes in the diet of weaning pigs improved feed efficiency and also reduced the population of coliforms in feces and contents of large intestine (Jin et al., 2008).

Thus it was hypothesized that PP obtained from the tubers of Golden valley potatoes could be used as an antimicrobial agent in the diet of broilers. Hence in the present study, PP were extracted from the tubers of Golden valley potatoes and included in the diet of broilers at different inclusion levels to study its effects on growth performance, apparent nutrient metabolizability, and microflora in the excreta and caecum.

Material and methods

Potato tubers (*Solanum tuberosum* l. cv. Golden valley) were procured from the Potato Valley Co. Ltd. (Samchiri, Kangwon-do, South Korea) and stored in the dark at 4°C (relative humidity 95 to 100%) for 6 months. The further processing of potato tubers to obtain PP was done as described previously by Jin et al. (2008). The analyzed crude protein and amino acid content of PP is presented in Table 1.

Six hundred unsexed day old Ross broiler chicks (average body weight 44.2 ± 2.11 g) were allotted to five dietary treatments, each comprising of 4 pens as replicates, with 30 chicks in each. Dietary treatments were: PC (Positive control; basal diets added with antibiotic, avilamycin), and basal diet added with 0.0, 0.25, 0.50 and 0.75% PP. Birds were fed in two phases: starter (0 to 3 wk) and finisher (4 to 6 wk) phases. The starter (Table 1) and finisher (Table 2) diets had 21.8 and 19.7% CP and 1.06 and 0.97% lysine, respectively. All diets were formulated to meet the National Research Council (1994) nutrient requirement recommendations.

Birds were housed in rice hull-covered floor pens. The house temperature was maintained at 34°C for the first 5 days and then gradually reduced according to normal management practices, until a temperature of 23°C was achieved. Lighting was provided for 23 hours/day. All the chicks had *ad libitum* access to feed and water. Day old birds were wing-banded and individually weighed and allotted to one of the five treatments. The body weights and pen feed intake were noted at the end of each phase to calculate body weight gain and feed conversion ratio (FCR) during starter and finisher phase. A metabolic trial was conducted in the last week of both the phases to determine metabolizability coefficient of dry matter (DM) and crude protein (CP). Two birds from each replicate (40 birds) were allocated to individual cages (one bird/cage) to facilitate collection of excreta samples. The starter and finisher diets containing 0.25% chromium oxide as an indigestible marker were fed from day 15 and 35 onwards, respectively. Excreta samples were collected from each bird on the fourth day after feeding the diets containing marker, dried.
in forced-air drying oven at 60°C for 3 days and stored for chemical analysis. The DM and CP analysis of feed and excreta samples were done according to the methods of AOAC (1990). Chromium was determined with an automatic spectrophotometer (Shimadzu, Japan) according to the procedure of FENTON and FENTON (1979). Amino acid analysis of PP and experimental diets was performed after acid hydrolysis (KNAIBE et al., 1989). Methionine and cystine were determined after oxidation with performic acid (MOORE, 1963).

In addition, excreta samples were collected on day 21 and 42 of the experiment to study the changes in microbial populations. At the end of the experiment, 12 birds per treatment (3 birds per replicate) were slaughtered to study the microflora of cecal contents. The excreta and cecal microflora was analyzed as described previously by Jia et al. (2008). The microbial groups analyzed were total bacteria (plate count agar, Difco laboratories), and coliform bacteria (violet red bile agar, Difco laboratories). The microbial populations were log transformed before statistical analysis.

All the data generated was analyzed by using SAS (1990) software. The data was subjected to analysis of variance test and when significant differences were noticed, the means were separated by using Duncan’s multiple range test. In addition, linear and quadratic trends were tested.
for comparing the effects of increasing dietary PP levels (0.0 to 0.75%). The replicate was the experimental unit for the analysis of all the parameters, while the P-values of less than 0.10 (P < 0.10) were considered to be significant.

Results

There were no differences in the growth performance of birds during starter, finisher and overall period except for greater body weight gain (P = 0.098) in birds fed PC diet when compared with birds fed 0.0 and 0.75% PP diets during finisher period (Table 3). Moreover, an increase in the inclusion level of PP in the diet of birds had no effect on the growth performance of birds, except for a quadratic (P = 0.051) increase in the feed intake of birds during starter period.

Metabolizability coefficient of DM during d 21 was greater (P = 0.037) in birds fed PC and 0.50% PP diets when compared with birds fed 0.0% PP diet (Table 4). However, the metabolizability coefficient of CP at d 21 and DM and CP at d 42 did not differ among the dietary treatments. In addition, an increase in the inclusion level of PP in the diet of birds did not show any linear or quadratic (P > 0.10) effect on the DM and CP metabolisability.

On d 21, the birds fed 0.0 and 0.25% PP diets had a higher number of coliforms (P = 0.055) in their excreta when compared with birds fed PC diet, while the population of total bacteria remained unaffected (Table 5). At d 42, the birds fed PC diet had less total bacteria (P < 0.001) in their excreta than birds fed 0.0 to 0.50% PP diets, while birds fed PC and 0.75% PP diets had fewer coliforms (P < 0.001) in their excreta when compared with birds fed 0.0 to 0.50% PP diets. In addition, linear decrease in the excreta total bacteria (d 42, P < 0.01) and coliform (d 21, P < 0.10 and d 42, P < 0.01) population was noticed with an increase in the inclusion level of PP in the diet of birds. Less number of total bacteria (P < 0.001) was noticed in the cecal contents of birds fed PC diets when compared with birds fed 0.0 to 0.75% PP diets, while birds fed 0.75% PP diet had fewer total bacteria in their cecal contents than birds fed 0.0 to 0.50% PP diets. Birds fed PC diet had less coliforms (P = 0.083) in the cecal content than birds fed 0.0% PP diet. Moreover, the populations of coliforms (P < 0.10) and total bacteria (P < 0.01) in the cecal contents decreased linearly as the inclusion level of PP was increased in the diet of birds.

Discussion

Potato proteins are by-product of potato starch processing industry. They are generally recovered by heat treatment of the waste water effluent and heat treatments results in irreversibly precipitated proteins which have lost all functionality (CLAUSSEN et al., 2007). In the present study protein extraction buffer was used for extraction of the PP followed by centrifugation. Further by freeze drying it was ensured that there were no changes in the structure and thus functionality of all the recovered proteins. Moreover, the tubers of potatoes used were of Golden valley potatoes that are known to possess antimicrobial peptide, potide-G (Kim et al., 2006).

In the present study birds fed antibiotics showed significantly higher body weight gain during finisher period and greater metabolisability of DM for starter diet. Nevertheless, birds fed antibiotics showed numerically higher overall body weight gain (2125 g) than birds fed 0.0, 0.25, 0.50 and 0.75% PP diets (2032, 2062, 2075 and 2053 g, respectively), and numerically higher DM and CP metabolizability during finisher period; however, none of these reached significant levels. The various benefits of feeding antibiotic growth promoters have been previously discussed by number of researchers (JUKES et al., 1950; STUTZ et al., 1983; HINTON, 1988; FERKET, 2004; DIBNER and RICHARDS, 2005).

Avilamycin is an orthosomycin antibiotic produced by Streptomyces viridochromogens. It is predominately ac-

Table 3. Effect of potato protein on the growth performance of broilers

<table>
<thead>
<tr>
<th>Item</th>
<th>PC2</th>
<th>PP2 (%)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>0.25</td>
<td>0.50</td>
<td>0.75</td>
</tr>
<tr>
<td>Starter (0 to 3 wk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>722</td>
<td>683</td>
<td>702</td>
<td>710</td>
</tr>
<tr>
<td>Feed intake, g</td>
<td>1195</td>
<td>1159</td>
<td>1179</td>
<td>1203</td>
</tr>
<tr>
<td>FCR</td>
<td>1.66</td>
<td>1.70</td>
<td>1.68</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finisher (4 to 6 wk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>1403</td>
<td>1349</td>
<td>1360</td>
<td>1365</td>
</tr>
<tr>
<td>Feed intake, g</td>
<td>2623</td>
<td>2661</td>
<td>2634</td>
<td>2668</td>
</tr>
<tr>
<td>FCR</td>
<td>1.87</td>
<td>1.97</td>
<td>1.93</td>
<td>1.96</td>
</tr>
<tr>
<td>Overall (0 to 6 wk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>2125</td>
<td>2032</td>
<td>2062</td>
<td>2075</td>
</tr>
<tr>
<td>Feed intake, g</td>
<td>3818</td>
<td>3820</td>
<td>3813</td>
<td>3871</td>
</tr>
<tr>
<td>FCR</td>
<td>1.80</td>
<td>1.88</td>
<td>1.85</td>
<td>1.87</td>
</tr>
</tbody>
</table>

Means within a row without a common superscript are significantly different.

Each treatment mean represents 4 pens.

PC: positive control; PP: potato protein.

Quadratic effect of increasing PP (P < 0.05).

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Avilamycin acts on the bacterial ribosome and inhibits the binding of formylmethionyl-tRNA to the 30 S ribosomal subunit, thus preventing the formation of 70 S initiation complex in bacterial protein synthesis (Wolf, 1973). In the present study, the impact of added avilamycin in reducing the populations of total bacteria, and coliforms in the excreta and cecal contents of birds was more evident. Improved performance of broilers fed avilamycin has been reported previously (Weltenreiter et al., 2000; Mallet et al., 2005; Denev, 2006) and these improved performances may be due to the benefits obtained from the antibacterial property of avilamycin. The gut microflora decrease nutrient absorption, cause inflammatory changes in the intestine resulting in increased thickness of intestinal tract, decrease the rate of digesta passage, and also increase nutrient requirements of the host by increasing turnover of the gut mucosae and by competing with the host for a portion of the dietary energy and protein (Ravindran et al., 1984; Apajalahti et al., 2004). However, in the present study even though there was reduction in the microbial populations, not any changes were observed in the morphology of different segments of the small intestine (data not presented).

Inclusion of PP at different levels also did not elicit any significant improvement in the body weight gain even though numerical increment was noticed up to 0.50% PP level. Also birds fed 0.50% PP diet had the highest feed intake (quadratic effect) and DM metabolisability during starter period. The population of coliforms and total bacteria in the excreta and cecal contents were decreased as the inclusion level of PP was increased from 0.0 to 0.75% in the diet of birds. These findings on reduced microbial populations are in agreement with our previous study in weaned pigs fed diets with increasing levels of PP (0.25 to 0.75%; Jin et al., 2008). In addition, Jin et al. (2008) had also noticed linearly improved feed efficiency with an increase in the inclusion of PP in the diet of weaned pigs which is in contradiction with the findings of the present study.

These results indicate that PP at higher levels has antimicrobial activity and both antibiotics and PP at higher levels were effective in reducing the microbial population in excreta and cecal contents. However, the changes in microbial population might be too small to be reflected in growth performance, nutrient metabolisability and intestinal morphology. Also the absence of any significant differences in the performance of birds in the present study

### Table 4. Effect of potato protein on the apparent nutrient digestibility (%) in broilers

<table>
<thead>
<tr>
<th>Item</th>
<th>PC&lt;sup&gt;2&lt;/sup&gt;</th>
<th>PP&lt;sup&gt;2&lt;/sup&gt; (%)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.776&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.745&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.003</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>0.657</td>
<td>0.620</td>
<td>0.006</td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td>0.766&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.756&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.004</td>
<td>0.423</td>
</tr>
<tr>
<td></td>
<td>0.697</td>
<td>0.626</td>
<td>0.006</td>
<td>0.164</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means within a row without a common superscript are significantly different.

<sup>1</sup> Each treatment mean represents 4 pens.

<sup>2</sup> PC: positive control; PP: potato protein.

### Table 5. Effect of potato protein on the excreta and caecal microbial populations (log<sub>10</sub> cfu/g) in broilers

<table>
<thead>
<tr>
<th>Item</th>
<th>Microbes (log&lt;sub&gt;10&lt;/sub&gt; cfu/g)</th>
<th>PC&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total bacteria</td>
<td>7.88</td>
<td>0.043</td>
<td>0.266</td>
</tr>
<tr>
<td></td>
<td>Coliforms&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.045</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>total bacteria (d 21)</td>
<td>8.17</td>
<td>0.069</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Coliforms&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.047</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>total bacteria (d 42)</td>
<td>8.08</td>
<td>0.052</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Coliforms&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.039</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>total bacteria (d 42)</td>
<td>8.10</td>
<td>0.039</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>Coliforms&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.039</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>total bacteria (d 42)</td>
<td>7.98</td>
<td>0.039</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>Coliforms&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.039</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>total bacteria (d 42)</td>
<td>0.003</td>
<td>0.039</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>Coliforms&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.004</td>
<td>0.039</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>total bacteria (d 42)</td>
<td>0.004</td>
<td>0.039</td>
<td>0.083</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means within a row without a common superscript are significantly different.

<sup>2</sup> Each treatment mean represents 4 pens.

<sup>1</sup> PC: positive control; PP: potato protein.

<sup>3</sup> Linear effect of increasing PP (P < 0.10).

<sup>4</sup> Linear effect of increasing PP (P < 0.01).
might be attributed to clean and less stressful environment in which these birds were reared. It is well known that the response to antibiotics is strongly influenced by the cleanliness of the environment and the disease load of the hosts involved. Thus the responses to antibiotics are minimal when tested in a clean and less stressful environment. In addition, the number of replicates (four per treatment) used in the present study might also be a constraint. Thus future studies using PP in birds under challenge conditions should be conducted to further reveal the potential of PP as an alternative to antibiotics.

In our previous study we reported that 1000 µg/ml PP from Golden valley potato was the minimum inhibitory concentration to be effective against Salmonella choleraesuis, Salmonella gallinarum and E. coli, while growth of Staphylococcus aureus was inhibited at 1250 µg/ml (Jin et al., 2008). In addition, Lim et al. (2004) had noticed that the ethanol-water extract of Golden valley potato inhibited the growth of C. perfringens and E. coli but had no effect on the growth of Bilobobacterium bifidum, B. breve, B. longum and Lactobacillus casei. This suggests that PP may have further potential advantage over antibiotics by selectively inhibiting the more pathogenic bacteria. The antimicrobial activity of Golden valley PP may be attributed to the presence of antimicrobial peptide potide-G. The exact mechanism by which potide G exhibits antimicrobial activity is not clear, however, mechanisms like pore formation and membrane depolarization, disruption of bacterial energy metabolism, and interference with biosynthetic pathways have been suggested (Kim et al., 2006).

Thus, from the results of our study it may be concluded that potato protein of Golden valley potato tubers has in-vivo antimicrobial activity and may replace antibiotics in broiler diet. Moreover, maximum in-vivo antimicrobial activity was noticed up to the inclusion of 0.75% PP, while, in-vivo antimicrobial activity and may replace antibiotics in the diet of birds. Hence potato protein has the potential to be used as an alternative to antibiotics in the diet of broilers.

**Summary**

In this study, 600 Ross broiler chicks (initially 44.2 ± 2.11 g) were used to investigate the use of potato protein (PP) as an alternative to antibiotics. The PP was obtained from the tubers of Solanum tuberosum L. cv. Golden valley potatoes known to have antimicrobial activity against gram positive and negative bacteria. Birds were randomly allotted to five dietary treatments, comprising 4 replicates in each with 30 birds per replicate. Dietary treatments were basal diet added with antibiotics (PC, Positive control), and basal diet added with 0.0, 0.25, 0.50 and 0.75% PP.

Birds fed PC diet had greater body weight gain (P = 0.098) during starter period than birds fed 0.0 and 0.75% PP diet, while the apparent metabolisability of DM was higher (P = 0.037) in birds fed 0.50% PP and PC diet when compared with birds fed 0.0% PP diet. In general the population of total bacteria and coliforms in excreta and cecal contents was lowest in birds fed PC diet and highest in birds fed 0.0% PP diet. An increase in dietary PP resulted in an increase (quadratic, P = 0.051) in feed intake of birds during starter period, wherein the highest feed intake was noticed in birds fed 0.50% PP diet. In addition, linear reduction in the number of coliforms (d 21, P < 0.10, and d 42, P < 0.01) and total bacteria (d 42, P < 0.01) in excreta, and total bacteria (P < 0.01) and coliforms (P < 0.10) in cecal contents were noticed as the level of PP inclusion was increased in the diet of birds. Thus, potato protein obtained from tubers of Golden valley potatoes showed in-vivo antimicrobial activity being more effective at 0.75% inclusion level, while the feed intake, body weight gain and nutrient metabolisability were maximized when PP were included at 0.50% level in the diet of birds. Hence potato protein has the potential to be used as an alternative to antibiotics in the diet of broilers.

**Key words**

Broiler, nutrition, potato protein, Solanum tuberosum L cv Golden valley, growth performance, nutrient metabolisability, cecal microflora

**Zusammenfassung**

Einfluss von Kartoffelprotein (Solanum tuberosum L. cv. golden valley) auf die Leistung, die Nährstoffverdaulichkeit und die Mikroflora im Blinddarm von Broilern

In der Studie wurden 600 Ross Broiler (Kükengewicht 44,2 ± 2,11 g) verwendet, um den Einsatz von Kartoffelprotein (PP) als Alternative zu Leistungsförderern mit Antibiotikacharakter in der Broilerfütterung zu untersuchen. Das Kartoffelprotein wurde aus den Knollen der Sorte Solanum tuberosum L. cv Golden Valley gewonnen, die für ihre antimikrobielle Wirkung gegen grampositive und gramnegative Bakterien bekannt ist. Die Broiler wurden zufällig auf fünf Behandlungsgruppen mit jeweils vier Wiederholungen zu 30 Tieren verteilt. Folgenden Behandlungen eingesetzt: Grundration mit Antibiotikum (PC, Positivkontrolle) sowie Grundration mit einer Zulage von 0,0, 0,25, 0,50 und 0,75% Kartoffelprotein (PP).

Die Broiler der Positivkontrolle erreichten ein höheres Lebendgewicht (P = 0,098) in der Starterperiode als die Tiere der Behandlungen 0,0 und 0,75% PP. Demgegenüber wiesen die Broiler der Behandlungen 0,50% PP und der Positivkontrolle eine höhere scheinbare Verdaulichkeit der Trockenmasse (P = 0,037) auf als die Tiere der Behandlung 0,0% PP. Die geringsten Gesamtkeimzahlen und die geringste Anzahl an coliformen Keimen in den Exkreten und im Blinddarm wurde bei der Positivkontrolle beobachtet, die höchsten bei der Behandlung 0,0% PP. Der Erhöhung des Gehaltes an PP in der Ration führte zu einer Zunahme der Futteraufnahme in der Starterphase (quadratisch, P = 0,051). Die höchste Futteraufnahme wurde bei der Behandlung 0,50% PP registriert. Mit der Erhöhung des Zusatzes von PP zu den Futterrationen nahm die Anzahl der coliformen Keime (d 21, P < 0,10; d 42, P < 0,01) und die Gesamtkeimzahl (d 42, P < 0,01) in den Exkreten sowie die Gesamtkeimzahl (P < 0,01) und die Anzahl an coliformen Keimen (P < 0,10) im Blinddarminhalt linear ab. Daraus kann der Schluss gezogen werden, dass zwar die antimikrobielle Wirkung des aus den Knollen der Sorte Golden Valley gewonnenen Kartoffelproteins bei einer Einsatzmenge von 0,75% effektiver ist, aber die Futteraufnahme, die Lebendmassezunahme und die Nährstoffverdaulichkeit bei einer Einsatzmenge von 0,50% besser ist. Generell scheint es so, dass Kartoffelprotein eine Alternative zu Leistungsförderern mit Antibiotikacharakter in der Broilerfütterung sein kann.
Stichworte
Broiler, Fütterung, Kartoffelprotein, Solanum tuberosum L cv Golden Valley, Nährstoffverdaulichkeit, Wachstum, Blinddarm mikroflora

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