ผลของการเสริมชีวนะ $Bacillus subtilis$ และ $Lactobacillus acidophilus$ ต่อประสิทธิภาพการใช้อาหารสัตว์กระต่ายหย่านม สมรรถภาพการเจริญเติบโต และดัชนีความคงที่ของอุจจาระของกระต่ายหย่านม

The effects of probiotics supplement ($Bacillus subtilis$ and $Lactobacillus acidophilus$) on feed efficiency, growth performance and fecal consistency index of weaning rabbits

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บทคัดย่อ

กระต่ายพันธุ์นิวซีแลนด์จำนวน 64 ตัว ถูกนำมาใช้ในการศึกษาผลของสารเสริมชีวนะต่อประสิทธิภาพการใช้อาหาร สมรรถภาพการเจริญเติบโต และดัชนีความคงที่ของอุจจาระ (fecal consistency index) แบ่งกระต่ายอายุ 28 วันออกเป็น 4 กลุ่ม และทำการให้อาหารตีด เป็นเวลา 6 สัปดาห์ กลุ่มควบคุม (ไม่ได้รับสารเสริมชีวนะ) กลุ่ม BS (ได้รับการเสริม $Bacillus subtilis$ ที่ $1 \times 10^6$ cfu/g) กลุ่ม LA (ได้รับการเสริม $Lactobacillus acidophilus$ ที่ $1 \times 10^7$ cfu/g) และกลุ่ม BL (ได้รับการเสริม $B. subtilis$ ที่ $0.5 \times 10^6$ cfu/g และ $L. acidophilus$ ที่ $0.5 \times 10^7$ cfu/g) ปรากฏว่าอัตราการเจริญเติบโต (ADG) เพิ่มขึ้นอย่างมีนัยสูงสุดทางสถิติ ($P<0.05$) จาก 24.0 กรัม/วัน ในกลุ่มควบคุม เป็น 28.1 และ 27.9 กรัม/วัน ในกลุ่ม LA และ BL ตามลำดับ อัตราการเปลี่ยนอาหารเป็นน้ำอ่อนลดลงอย่างมีนัยสูงสุดทางสถิติ ($P<0.05$) จาก 2.89 ในกลุ่มควบคุม เหลือ 2.55 และ 2.56 ในกลุ่ม LA และ BL ตามลำดับ ดัชนีความคงที่ของอุจจาระลดลงอย่างมีนัยสูงสุดทางสถิติ ($P<0.01$) จาก 0.59 ในกลุ่มควบคุม เหลือ 0.46 และ 0.44 ในกลุ่ม LA และ BL ตามลำดับ ไม่พบความแตกต่างใดเมื่อทำการเสริมเฉพาะ $B. subtilis$ เพียงชนิดเดียว โดยสรุป การใช้สารเสริมชีวนะผสมในอาหารประเภท $L. acidophilus$ ที่ $1 \times 10^7$ cfu/g สามารถช่วยปรับปรุงประสิทธิภาพการใช้อาหาร สมรรถภาพการเจริญเติบโต และช่วยลดค่าดัชนีความคงที่ของอุจจาระของกระต่ายหย่านม.

คำสำคัญ: $B. subtilis$, $L. acidophilus$, กระต่ายหย่านม, สมรรถภาพการเจริญเติบโต

ABSTRACT

Sixty-four weaning New Zealand White rabbits were used to investigate the effects of probiotics supplement on feed efficiency, growth performance, and fecal consistency index. At 28 days old, the animals were randomly distributed into 4 groups fed four diets for 6 weeks. The treatments consisted of basal diet with no probiotic supplement (control), $1 \times 10^6$ cfu/g $B. subtilis$ (BS), $1 \times 10^7$ cfu/g $L. acidophilus$ (LA), and $0.5 \times 10^6$ cfu/g $B. subtilis$ plus $0.5 \times 10^7$ cfu/g $L. acidophilus$ (BL). The results showed that the average daily gain significantly increased from 24.0 g/day in the control group to 28.1 and 27.9 g/day in the LA and BL groups, respectively. Feed conversion ratio decreased significantly compared with the control group ($P<0.05$). Fecal consistency index decreased significantly from 0.59 in the control group to 0.46 and 0.44 in the LA and BL groups, respectively. No significant difference was found when only $B. subtilis$ was supplemented. In summary, the use of probiotics mixture containing $L. acidophilus$ at $1 \times 10^7$ cfu/g in the diet improved feed efficiency, growth performance, and fecal consistency index of weaned rabbits.

Keywords: $B. subtilis$, $L. acidophilus$, weaning rabbit, growth performance

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control group to 28.1 and 27.9 g/day in the LA and BL groups (P<0.05). Feed conversion rate was significantly reduced to 2.55 and 2.56 in the LA and BL diets as compared to 2.89 in the control diet (P<0.05). Fecal consistency index was significantly reduced to 0.46 and 0.44 in the LA and BL groups as compared to 0.59 in the control group (P<0.01). No different effects were observed on performance of weaning rabbits when supplement only B. subtilis. In conclusion, dietary supplementation of probiotic such as $1 \times 10^7$ cfu/g L. acidophilus could improve feed efficiency, growth performance, and reduce fecal consistency index of weaning rabbits.

**INTRODUCTION**

Raising rabbits in an intensive system can cause many physiological and environmental stresses, especially during weaning period. These stresses result in spreading of enteric diseases such as E. coli, coccidiosis and epizootic rabbit enteropathy which have negative effect on growth performance, feed efficiency, and animal health status. Incorporation of antibiotics in feedstuff can reduce digestive disorders and improve growth performance of farm animals (Barton, 2000). However, bacterial resistance to antibiotics has urged scientists to find new alternatives to the use of antibiotics in animal production (Smith et al., 2002). In addition, on the base of consumer demand, European legislation is banning the antibiotics use as growth promoters for all livestock production in order to avoid crossed resistance in humans. Probiotics appear to be possible alternative feed additives to antibiotic products. However, there are fewer studies of probiotics supplement in rabbits. Furthermore, studies showing the effects of probiotics on feed efficiency, growth performance, and fecal consistency of weaning rabbits are still limited.

Objectives of this study were to investigate the effects of single and double strains probiotics supplement on feed efficiency, growth performance, and fecal consistency index of weaning rabbits. Hypothesis of this study is that feeding probiotics supplement to weaning rabbits can improve gut microbiota fermentation and digestion resulting in enhances feed efficiency, body weight gain and performance index.

**MATERIALS AND METHODS**

1. Animals management and experimental design

Healthy sixy-four weaning New Zealand White rabbits age 28 ±1 day were used in this experiment. The animals were randomly distributed into 4 groups with balance in weaning weight and sex. Each group included 4 cages of male and 4 cages of female (2 rabbits /
The rabbits were fed 4 diets for the next 6 weeks. During the entire feeding period, all rabbits were housed under ambient temperature with the temperature at about 30.7 ± 1.25°C (X ± SD) and humidity at about 66.7 ± 7.77% (X ± SD).

A randomized complete block design was used with 4 treatments and 8 replicates per each treatment. A commercial diet with no probiotic was used as a basal diet (control). Three probiotic diets were basal diet mixed with either: 1) B. subtilis (BS) at 10⁶ cfu/g feed, 2) L. acidophilus (LA) at 10⁷ cfu/g feed, or 3) B. subtilis and L. acidophilus (BL) complex at 0.5x10⁶ cfu/g feed and 0.5x10⁷ cfu/g feed, respectively.

2. Feed and feeding

From 28 to 70 days of age, the weaning rabbits were offered either the basal diet or the basal diet mixed with the probiotics. The basal diet was daily ground to pass through a 5-mm sieve. All treatment diets were daily prepared by mixing the basal diet with the probiotics powder (KMP Biotech Co., Ltd., Thailand). The cages for fattening rabbits equipped with feeders and automatic nipple drinkers to provide ad libitum feeds and water to the animals during the entire period. Roughage was not offer to the animals in this experiment.

3. Data records and calculation

Feed samples were collected once a week throughout the experiment to determine DM content (AOAC, 2000). Feed offered and refused were daily weighed to calculate average daily feed intake. The rabbits were weighed at the 28-day (weaning), 42-day, and 70-day to calculate average daily gain. Performance index was calculated as live body weight (kg) x 100/ feed conversion ratio (Amber et al., 2004).

Fecal consistency was daily evaluated every morning for 14 days after weaning period. Fecal scores were assigned from 1 to 4, where score 1: normal, hard pellets; score 2: soft; score 3: mixed soft and liquid; and score 4: completely liquid (Agin et al., 2005). Fecal consistency index (FCI) was calculated by following equation: \( FC = [(dE1 \times 1) + (dE2 \times 2) + (dE3 \times 3) + (dE4 \times 4)] / (Td \times 4) \), where Td is total days of the experiment; dE1, dE2, dE3 and dE4 are the number of days with fecal consistency scoring = 1, 2, 3, and 4, respectively (Meyer et al., 2001).

For the calculation of morbidity, the sick rabbits were daily counted only once during the experiment. The health risk index was calculated as a sum of morbidity and mortality (Baronegad et al., 2000).
4. Statistical analysis

Statistical analysis of the data was carried out as randomized complete block design. The effects of treatments were analyzed by General Linear Model. The significant differences between treatment means were tested by the Least Significant Difference method at $\alpha = 0.05$. The Logistic Regression was used to compare the morbidity and mortality means between the treatments.

RESULTS AND DISCUSSION

1. Effects of probiotics supplement on growth performance and feed efficiency

The growth performance and feed efficiency of weaning rabbits are shown in table 1. Average daily gain (ADG) significantly increased to 29.8 and 30.3 g/day in the LA and BL groups as compared to 25.0 g/day in the control group (P<0.05). Feed conversion ratio (FCR) was significantly reduced to 0.29 and 0.28 in the LA and BL groups when compared to the control group (P<0.05). For the last four weeks, the ADG was greater in the LA group (27.3 g/day) than the control group (23.5 g/day) (P<0.01). However, there was no effect of probiotics supplement on average daily feed intake (ADFI) and FCR of weaning rabbits. Overall, probiotics supplement could significantly improved ADG of weaning rabbit (P<0.05), the greatest value in the LA group (28.1 g/day) and lowest value in the control group (24.0 g/day). The effect of probiotics supplement on ADFI was not observed in the entire period of feeding (P>0.05). As the result, FCR was significantly decreased from 2.89 in the control group to 2.55 and 2.56 in the LA and BL groups (P<0.05). Trocino et al. (2005) reported that supplementation of Bacillus cereus var. toyoi at $2\times10^5$ cfu/g feed in weaning rabbit from 35 d until 70 d significantly increased ADG from 38.2 g/day (control group) to 40.0 g/day (probiotic group). This is in agreement with the ADG of weaning rabbits in the current study. Even though temperature-humidity index during the current study was about 29.0 (29.0-30.0: severe heat stress for animals, Marai et al., 2000), which could make the ADG lower than usual. Performance index of weaning rabbits was significantly increased to 65.8 and 65.2 % in the LA and BL groups as compared to 52.1 % in the control group (P<0.05). Amber et al. (2004) reported that L. acidophilus supplement at $4\times10^5$ cfu/g diet in the rabbits from 5 to 13 weeks of age significantly improved PI to 65.9 % as compared to the control group (56.9 %). An increased gut lactobacilli population by probiotics supplement could be beneficial for the host in terms of nutrient utilization as it would potentially increase the activity of their useful enzymes (Fuller, 1989) resulting in enhances feed efficiency, growth performance and performance index.
Table 1. Effects of probiotics supplement on average daily feed intake (ADFI, g/rabbit/day), average daily gain (ADG, g/rabbit/day), feed conversion ratio (FCR, g DM feed/g gain), and performance index (PI) (means±SE)

<table>
<thead>
<tr>
<th>Items</th>
<th>Treatments</th>
<th>Controls</th>
<th>BS</th>
<th>LA</th>
<th>BL</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW at weaning, g</td>
<td></td>
<td>498±44.3</td>
<td>498±41.6</td>
<td>497±42.1</td>
<td>498±45.4</td>
<td>0.999</td>
</tr>
<tr>
<td>BW at final, g</td>
<td>1.50±80.2</td>
<td>1.50±81</td>
<td>1.50±81.6</td>
<td>1.69±71.9</td>
<td>1.66±73.7</td>
<td>0.023</td>
</tr>
<tr>
<td>ADFI</td>
<td>47.7±3.0</td>
<td>48.2±2.6</td>
<td>48.3±3.1</td>
<td>48.2±2.7</td>
<td>0.711</td>
<td></td>
</tr>
<tr>
<td>ADG</td>
<td>25.0±1.7</td>
<td>27.8±1.3</td>
<td>29.8±1.5</td>
<td>30.3±1.3</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>FCR</td>
<td>1.91±0.1</td>
<td>1.72±0.7</td>
<td>1.62±0.8</td>
<td>1.63±0.6</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>PI, %</td>
<td>52.1±3.8</td>
<td>60.1±2.8</td>
<td>65.8±3.1</td>
<td>65.2±3.1</td>
<td>0.025</td>
<td></td>
</tr>
</tbody>
</table>


 básed on data from different superscripts are significantly different (P<0.05), n = 8

2. Effects of probiotics supplement on fecal consistency index

Table 2 shows that fecal score (FC) was 1.91 vs 1.79 in the LA and BL groups, while this was 2.36 in the control group (P=0.057) after one week feeding. Fecal consistency index (FCI) was reduced to 0.48 vs 0.45 in the LA and BL groups when compared to 0.59 in the control group (P=0.057). For the second week of feeding, probiotics supplement had significantly reduced FC to 1.66, 1.45, and 1.38 in the BS, LA, and BL groups, respectively, as compared to 2.02 in the control group (P<0.01). FCI significantly decreased by 0.08, 0.14, and 0.145 in the BS, LA, and BL, respectively, when compared to the control group (P<0.01). Overall, FC was significantly reduced to 1.68 vs 1.58 in the LA and BL groups, while this was
2.19 in the control group after two week of feeding (P<0.01). FCI was significantly reduced to 0.46 and 0.44 in LA and BL groups when compared with 0.59 in the control group (P<0.01). The lower fecal score and reduced FCI in weaning rabbits fed diets supplemented with LA and BL suggested a better healthy gut resulting in reducing morbidity rate (table 3). The two week period after weaning was a period of adaptation and potential stress, regardless of whether the animals were weaned at 2 or 6 weeks of age (McCracken and Kelly, 1993). The efficacy of probiotics was greater when the animals were confronted with challenge or stress (Estienne et al., 2005). This might explain the clearly positive effects of the probiotics supplement during the two weeks post-weaning in the present study.

**Table 2**: Effects of probiotics supplement on fecal consistency index (FCI) (means±SE)

<table>
<thead>
<tr>
<th>Items</th>
<th>Treatments</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>BS</td>
</tr>
<tr>
<td>28-35 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal score</td>
<td>23±0.15</td>
<td>20±0.12</td>
</tr>
<tr>
<td>FCI</td>
<td>0.59±0.04</td>
<td>0.52±0.03</td>
</tr>
<tr>
<td>35-42 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal score</td>
<td>20±0.15</td>
<td>16±0.15</td>
</tr>
<tr>
<td>FCI</td>
<td>0.5±0.05</td>
<td>0.4±0.04</td>
</tr>
<tr>
<td>Overall, 28-42 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal score</td>
<td>21±0.15</td>
<td>18±0.12</td>
</tr>
<tr>
<td>FCI</td>
<td>0.5±0.04</td>
<td>0.5±0.03</td>
</tr>
</tbody>
</table>


Means in a row with different superscripts are significantly different (P<0.05), n=8

3. Effects of probiotics supplement on morbidity, mortality, and health risk index (HRI)

The morbidity, mortality and health risk index (HRI) of weaning rabbits are shown in table 3. Morbidity rate was significantly reduced to 0% in the LA group while this value was 31.3% in the control group (P=0.096). None of animals in any groups died during the study. HRI was zero in the LA group. HRI of the control, BS, and BL groups was 31.3, 18.8, and 12.5%, respectively (P=0.096). Trocino et al. (2005) reported that supplementation of Bacillus cereus var. toyoi significantly decreased HRI to 25.0% as compared with 33.3% in the control group. Regionea et al. (2001) concluded that B. subtilis spores could significantly reduce colonization by pathogen.
in the cecum and the deep tissues leading reduce morbidity rate and HRI. According to the results of this study, the supplementation with 1x10⁷ cfu L. acidophilus/g feed or the mixture of 0.5x10⁶ cfu B. subtilis cfu/g feed and 0.5x10⁷ cfu L. acidophilus/g feed could significantly decreased the FCI, morbidity, and HRI. However, no significant effect was observed when supplementation with only B. subtilis at 1x10⁶ cfu/g feed. In current study, the gut lactic acid bacteria population significantly increased to 2.40x10⁷ CFU/g in the LA group as compared to 1.42, 2.52, and 13.7x10⁵ CFU/g in the control, BS, and BL groups, respectively (P<0.001) (data not shown). In contrast, the gut coliform population significantly reduced to 0.72x10³ MPN/g in the LA group when compared to 56.9, 33.7, and 17.2x10³ MPN/g in the control, BS, and BL groups, respectively (P<0.01) (data not shown).

Table 3  Effects of probiotics supplement on morbidity, mortality and HRI (means±SE)

<table>
<thead>
<tr>
<th>Items</th>
<th>Treatments</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>BS</td>
</tr>
<tr>
<td>Morbidity, %</td>
<td>31.3±13.2</td>
<td>18.8±13.2</td>
</tr>
<tr>
<td>Mortality, %</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HRI, %</td>
<td>31.3±13.2</td>
<td>18.8±13.2</td>
</tr>
</tbody>
</table>

a b Means in a row with different superscripts are significantly different (P<0.05), n=16

CONCLUSION

Supplementation of L. acidophilus alone at 1x10⁷ cfu/g feed or mixture of B. subtilis at 0.5x10⁶ cfu/g feed and L. acidophilus at 0.5x10⁷ cfu/g feed could improve growth performance, feed efficiency and performance index. In addition, fecal consistency index and health risk index were reduced in the animals supplemented with only L. acidophilus or mixture of B. subtilis and L. acidophilus. Supplementation of B. subtilis alone at 1x10⁶ cfu/g feed could not enhance growth performance, feed efficiency or health status of weaning rabbits.

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REFERENCES

Agin TS, C Zhu, LA Johnson, TE Thate, Z Yang, EC Boedeker. 2005 Protection against hemorrhagic colitis in an animal model by oral immunization with isogenic rabbit enteropathogenic Escherichia coli attenuated by truncating intimin in Infection and immunity. 73: 663-6619


