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Effects of probiotic supplement (*Bacillus subtilis* and *Lactobacillus acidophilus*) on feed efficiency, growth performance, and microbial population of weaning rabbits

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Objective: This study aimed to investigate the effects of single or/and double strains of probiotic supplement on feed efficiency, growth performance, and microbial population in distal gastrointestinal tract (GIT) of weaning rabbits.
Methods: Sixty-four weaning (28 days old) New Zealand White rabbits were randomly distributed into four groups with treatments including: basal diet without probiotic supplement (control) or supplemented as follows: 1×10^6 cfu/g *B. subtilis* (BS group), 1×10^7 cfu/g *L. acidophilus* (LA group), or 0.5×10^6 cfu/g *B. subtilis* plus 0.5×10^7 cfu/g *L. acidophilus* (BL group). During the research, the male and female rabbits were fed separately. Body weight of the rabbits was recorded at 28, 42, and 70 d of age.
Results: There was an increase ($p<0.05$) in body weight gain for the LA group at 42 d. Rabbits fed BL responsed with a greater growth ($p<0.05$) and better feed conversion ratio ($p<0.05$) than those fed with no probiotic. Digestibility coefficients of dry matter, organic matter, crude protein, neutral detergent fiber, and gross energy were higher ($p<0.05$) in LA and BL groups than those in the control group. Male rabbits had higher ($p<0.05$) *Bacilli* spp. and *Coliformis* spp. in the ileum than female rabbits. Rabbits supplemented with BS had greater ($p<0.05$) numbers of bacilli in all intestinal segments than those receiving no probiotic, whereas intestinal Lactobacilli populations were greater ($p<0.001$) in the LA and BL diets compared to control. Average intestinal coliform populations were lowest ($p<0.05$) in the rabbits supplemented with LA as compared to those fed the control and BS.
Conclusion: Supplementation of *L. acidophilus* alone or in combination with *B. subtilis* at a half of dose could enhance number of gut beneficial bacteria populations, nutrient digestibility, cecal fermentation, feed efficiency, and growth performance, but rabbits receiving only *B. subtilis* alone were not different from the controls without probiotic.

Keywords: *Bacillus Subtilis*, *Lactobacillus Acidophilus*, Probiotic, Rabbit

INTRODUCTION

An intensive system of rabbit production, especially during weaning period, can cause many physiological and environmental stresses. These problems result in concentrating and spreading of enteric pathogens such as *Escherichia coli* (*E. coli*), coccidian, and epizootic rabbit enteropathy which have negative effects on growth performance, feed efficiency, and rabbit health status [1,2]. Use of antibiotics as growth promoter in livestock farms has been banned by European legislation regarding bacterial resistance [3]. For decades, researches have been conducted to identify alternative substances used for animal production [4]. Supplementation of beneficial normal flora, such as *Bacillus subtilis* and *Lactobacillus acidophilus*, in order to improve gut environment, as probiotics appear to be possible alternative feed additives.

Various modes of action of probiotics have been proposed and proved [5]. Currently, there are still few studies of probiotic supplementation in rabbits. Information regarding the effects of probiotics on feed efficiency, growth performance, and microbial population of weaning rabbits are still limited. It is hypothesized that probiotic supplement to weaning rabbits could possibly improve gut-microbial population and digestion resulting in enhanced feed efficiency, body weight gain (BWG), and performance index. This study aimed to investigate the effects of single or/and double strains of probiotic supplement on feed efficiency, growth performance, and microbial population in distal gastrointestinal tract (GIT) of weaning rabbits.

MATERIALS AND METHODS

Animals and diets

Animal used and protocol were approved by Institutional Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University, Thailand. Sixty-four healthy weaning New Zealand White rabbits (32 males and 32 females) aged 28±1 day were used in this experiment. These rabbits were randomly allocated into four groups with balance in weaning weight and sex as a 2 (male vs female)×4 (4 diets) factorial design. The male and female rabbits were fed separately in this experiment. Two rabbits were kept in each cage, and there were 32 experimental units in this study. Feeding period lasted for six weeks. All rabbits were housed under natural light and ambient temperature.

A commercial diet for growing rabbits was used as a basal diet (Table 1). Four dietary treatments composed of basal diet without probiotic supplement (control), *B. subtilis* at 1×10^6 cfu/g feed (BS), ii) *L. acidophilus* at 1×10^7 cfu/g feed (LA), or iii) *B. subtilis* and *L. acidophilus* complex at 0.5×10^6 cfu/g feed and 0.5×10^7 cfu/g feed, respectively (BL). Doses of *B. subtilis* and *L. acidophilus* used in this experiment followed the recommendation by SCAN [6,7]. From 28 to 70 days of age (d), the rabbits were offered either the basal diet or the basal diet mixed with 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 probiotic powders (K.M.P Biotech Co., Ltd., Chonburi, Thailand). Feeds and clean water were provided *ad libitum*.

Table 1. Nutrient composition of basal diet used in the experiment

Chemical composition (g/kg of DM unless otherwise noted)	
Dry matter	891
Organic matter	924
Crude protein	174
Neutral detergent fiber	390
Acid detergent fiber	203
Crude fiber	155
Ether extract	31.4
Ash	75.6
Nitrogen free extract	564
Non fiber carbohydrate	329
Gross energy (kcal/kg)	4,398
Digestible energy (kcal/kg)	2,747
Metabolizable energy (kcal/kg)	2,580
Digestible crude protein	118

DM, dry matter.

Sample collection and determination

Offered and refused feeds were daily weighed to calculate average daily feed intake (ADFI). Feed samples were collected once a week for proximate analyses [8]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using the method of [9]. The rabbits were weighed at 28, 42, and 70 d in order to calculate average daily gain. At 63 d, total fecal and urine output of all rabbits in this study were collected daily for a consecutive 5-day period to determine apparent total tract apparent digestibility coefficient of nutrients and nitrogen retention [10]. The urine samples were kept in 10% sulfuric acid, and placed at –20°C for analysis of nitrogen concentration. Gross energy (GE) in feed and fecal samples was determined by an automatic adiabatic oxygen bomb calorimeter. At 70 d, 32 rabbits were sacrificed by an overdose injection of pentobarbital sodium at 60 to 70 mg/kg live weight to immediately obtain the intestine. Intestinal contents were subjected for enumeration of *Bacillus* spp., *Lactobacillus* spp., and coliform populations. The bacilli were cultured on MYP (Mannitol Egg York Polymyxin-B) agar, under aerobic conditions for 24 h at 37°C [11]. The lactobacilli were cultured in MRS (DeMan, Rogosa, Sharpe) agar, under anaerobic conditions for 48 h at 37°C [12]. Total intestinal coliform was cultured in lauryl triptose broth (LTB), under aerobic conditions for 24 h at 37°C [13]. The pH of representative digesta contents from fresh cecum was determined using pH meter (PB 10 model, Mettler Toledo, Goettingen, Germany) and kept at –80°C for measurement of volatile fatty acids (VFA) concentration using gas chromatography.

Calculations

Nitrogen free extract (NFE) and non fiber carbohydrate (NFC) were calculated by following equations: NFE = organic matter (OM) – crude protein (CP) – ether extract (EE) – crude fiber (CF) and NFC = OM – CP – EE – NDF. Metabolizable energy (ME) content was estimated as following equation [14]: ME (MJ/kg dry matter [DM]) = DE (digestible energy) (0.995 – 0.048 digestible crude protein [DCP]/DE), where DE (MJ/kg DM) = 15.3 – 0.19ADF [15], DCP (g/kg DM) = –34.67+0.876 CP [16]. The ME and DE after estimating were converted from MJ/kg DM to Kcal/kg DM. Total digestible nutrients (%/DM) = DCP+digestible nitrogen free extract (DNFE)+digestible crude fiber (DCF)+2.25 digestible ether extract (DEE) [17]. Performance index (PI) was calculated as live body weight (kg)×100/feed conversion ratio (FCR) [18]. Fecal consistency was daily evaluated in the morning. Fecal scores were assigned from 1 to 4, where score 1: normal; 2: soft; 3: mixed soft and liquid; and 4: completely liquid [19]. Fecal consistency index (FCI) was calculated by the following equation: FCI = ([dE1×1]+[dE2×2]+[dE3×3]+[dE4×4])/(Td×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 [20]. Daily temperature and humidity were recorded at 9:00 am and 3:00 pm. Temperature-humidity index (THI) was calculated according to [21]. The THI values were classified as following: <27.8 = absence of heat stress; 27.8 to 28.9 = moderate heat stress; 29.0 to 30.0 = severe heat stress; and >30.0 = very severe heat stress.

Statistical analysis

Experimental data were analyzed as a randomized complete block design, with 2×4 factorial arrangement of sex and dietary treatments, using the General Linear Model procedures of SAS [22]. Treatment, sex, and interaction between treatment and sex were defined as sources of variation. Significant differences between means were evaluated by Tukey's multiple comparison tests after a significant F-test. These differences were considered to be significant as $p \leq 0.05$, meanwhile a tendency toward significance was declared at $0.10 > p > 0.05$. Data were expressed as mean±standard error of the mean (SEM), which represents the pooled SEM for the model.

RESULTS

In this experiment, the average daily temperature and relative humidity were recorded between 26.6°C to 33.8°C (30.7°C ±1.25°C) and 50% to 90% (66.7%±7.77%), respectively. As the result, the THI was ranged from 26.2 to 30.8. The average THI value of the experiment was about 29.0±0.79.

Feed efficiency and growth performance

No significant differences were observed on BWG, FCR, and PI when compared between male and female groups (Table 2). However, ADFI from d28 to d42 was higher ($p<0.01$) and d28 to d70 tended to be greater ($p = 0.05$) in the female group. Between 42 and 70 d, the rabbits received LA and BL had greater ($p<0.01$) BW than those fed the control diet. There was a significant increase in BWG ($p<0.05$) for the LA group than in the control group at 42 days old. Rabbits supplemented with BL responded with a greater growth ($p<0.05$) and better FCR ($p<0.05$) than rabbits supplemented with no probiotic. No differences were found between the groups at 70 days old for ADFI, BWG, and FCR. An increased BWG ($p<0.05$) was obtained in rabbits supplemented with either LA or BL when compared to those rabbits in the control from 28 to 70 days old. The BL supplemented group had greater ($p<0.05$) PI than the control group. No significant differences were observed when compared between the BS, LA, and BL groups for ADFI, BWG, FCR, and PI at all age. Overall, rabbits supplemented with LA showed the best growth performance among dietary

Table 2. Effects of probiotic supplement on body weight (BW), average daily feed intake (ADFI, g DM/d), body weight gain (BWG, g), feed conversion ratio (FCR, g DM/g gain), and performance index (PI, %) of weaning rabbits

Items	Sex[1)]		Treatments[2)]				SEM	p-value[3)]		
	M	F	Control	BS	LA	BL		S	T	S×T
BW (g)										
d 28	476.72	518.59	497.81	497.50	496.88	498.44	22.58	<0.001	0.999	0.821
d 42	864.06	921.41	847.81[b]	886.56[ab]	913.44[a]	923.13[a]	40.34	<0.001	0.006	0.225
d 70	1,566.00	1,659.44	1,506.31[b]	1,598.19[ab]	1,677.75[a]	1,668.63[ab]	119.17	0.038	0.032	0.746
d 28-d 42										
ADFI	46.42	49.51	46.83	48.42	48.11	48.50	3.06	0.009	0.679	0.667
BWG	387.34	402.81	350.00[b]	389.06[ab]	416.56[a]	424.69[a]	44.62	0.338	0.013	0.299
FCR	1.68	1.72	1.87[a]	1.74[ab]	1.62[ab]	1.60[b]	0.20	0.711	0.029	0.117
d 42-d 70										
ADFI	79.09	82.57	79.16	79.86	82.58	81.72	6.16	0.126	0.664	0.540
BWG	701.94	738.03	658.50	711.63	764.31	745.50	91.52	0.277	0.136	0.940
FCR	3.15	3.13	3.37	3.14	3.02	3.07	0.39	0.890	0.213	0.641
d 28-d 70										
ADFI	68.20	71.55	68.39	69.38	71.09	70.64	4.55	0.050	0.632	0.587
BWG	1,089.28	1,140.84	1,008.50[b]	1,100.69[ab]	1,180.88[a]	1,170.19[a]	124.10	0.253	0.041	0.768
FCR	2.63	2.63	2.85	2.65	2.53	2.54	0.27	0.995	0.052	0.446
PI	60.44	63.50	53.43[b]	60.74[ab]	66.75[ab]	66.96[a]	9.62	0.379	0.033	0.508

SEM, standard error of the mean.
1) M: male; F: female. 2) BS, control diet+*B. subtilis*; LA, control diet+*L. acidophilus*; BL, control diet+*B. subtilis*+*L. acidophilus*.
3) S, sex; T, treatment; S×T, sex and treatment interaction.
a,b Means in a row with different superscripts are significantly different ($p<0.05$), n = 8.

Table 3. Effects of probiotic supplement on fecal consistency index of weaning rabbits

Items	Sex[1)]		Treatments[2)]				SEM	p-value[3)]		
	M	F	Control	BS	LA	BL		S	T	S×T
Fecal score										
d 28-d 35	1.89	2.17	2.36	2.07	1.91	1.79	0.41	0.073	0.063	0.480
d 35-d 42	1.46	1.79	2.02[a]	1.66[ab]	1.46[b]	1.38[b]	0.34	0.011	0.005	0.438
d 28-d 42	1.67	1.98	2.19[a]	1.87[ab]	1.69[b]	1.58[b]	0.33	0.017	0.008	0.575
Fecal consistency index (FCI)										
d 28-d 35	0.47	0.54	0.59	0.52	0.48	0.45	0.10	0.073	0.063	0.480
d 35-d 42	0.37	0.45	0.50[a]	0.42[ab]	0.36[b]	0.35[b]	0.08	0.006	0.013	0.307
d 28-d 42	0.46	0.54	0.59[a]	0.51[ab]	0.46[b]	0.44[b]	0.09	0.013	0.009	0.524

SEM, standard error of the mean.
1) M, male; F, female. 2) BS, control diet+*B. subtilis*; LA, control diet+*L. acidophilus*; BL, control diet+*B. subtilis*+*L. acidophilus*.
3) S, sex; T, treatment; S × T, sex and treatment interaction.
a,b Means in a row with different superscripts are significantly different (p < 0.05), n = 8.

treatments.

Fecal score and fecal consistency index

The sex and dietary treatment affected fecal score and FCI (Table 3). Sex appeared to have such effects since female rabbits had higher (p<0.05) fecal score and FCI than male rabbits at age 35 to 42 and 28 to 42 days. Reduced fecal score and FCI (p<0.05) were obtained in the rabbits receiving either LA or BL supplement when compared to those receiving commercial diet alone at 35 to 42 and 28 to 42 days old. Rabbits supplemented with BS didn't show any significant effects on fecal score and FCI as compared to the rabbits in other treatments.

Digestibility and nitrogen retention

No differences were observed on digestibilities between male and female groups (Table 4). The results showed that the digestibility coefficients of DM, OM, CP, NDF, and GE were higher (p<0.05) in the rabbits supplemented with either LA or BL than in rabbits received with no probiotic supplement. The digestibility of CF in rabbits received no probiotic was the lowest (p<0.05) when compared to rabbits supplemented with BS, LA, or BL. No differences were found among the treatment groups on nitrogen balance, but retention nitrogen showed a greater tendency (p = 0.055) in the rabbits fed LA compared to the control rabbits.

pH and volatile fatty acid production of cecal content

There were no significant shifts in cecal pH, total VFA concentration, and molar proportions of individual VFA among treatments (Table 5). There was an interactive effect (p = 0.01)

Table 4. Effects of probiotic supplement on coefficient of total tract apparent digestibility and nitrogen retention of growing rabbits at 9 weeks of age

Items	Sex[1)]		Treatments[2)]				SEM	p-value[3)]		
	M	F	Control	BS	LA	BL		S	T	S×T
Coefficient of total tract apparent digestibility										
DM	0.67	0.67	0.65[b]	0.67[ab]	0.68[a]	0.68[a]	0.016	0.435	0.016	0.928
OM	0.68	0.68	0.66[b]	0.68[ab]	069[a]	0.69[a]	0.015	0.504	0.012	0.945
CP	0.70	0.70	0.68[b]	0.69[ab]	0.72[a]	0.71[a]	0.024	0.695	0.008	0.995
NDF	0.44	0.43	0.40[b]	0.44[ab]	0.46[a]	0.45[a]	0.027	0.811	0.003	0.959
CF	0.32	0.31	0.28[b]	0.31[a]	0.33[a]	0.32[a]	0.023	0.229	0.001	0.575
EE	0.80	0.80	0.78	0.80	0.81	0.81	0.025	0.675	0.051	0.464
NFE	0.77	0.77	076	0.77	0.77	0.77	0.021	0.757	0.533	0.736
TDN	0.54	0.55	0.52	0.54	0.57	0.56	0.039	0.400	0.057	0.727
GE	0.64	0.64	0.62[b]	0.64[ab]	0.65[a]	0.65[a]	0.017	0.369	0.014	0.914
Nitrogen balance (g/d)										
NI	2.38	2.37	2.31	2.36	2.44	2.40	0.18	0.831	0.539	0.250
FN	0.70	0.71	0.74	0.73	0.68	0.69	0.08	0.731	0.381	0.534
UN	0.33	0.33	0.36	0.34	0.31	0.33	0.09	0.941	0.776	0.597
RN	1.68	1.65	1.57	1.63	1.76	1.71	0.14	0.657	0.055	0.340

SEM, standard error of the mean; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; CF, crude fiber; EE, ether extract; NFE, nitrogen-free extract; TDN, total digestible nutrients; GE, gross energy; NI, nitrogen intake; FN, fecal nitrogen; UN, urinary nitrogen; RN, retention nitrogen.
1) M, male; F, female. 2) BS, control diet+*B. subtilis*; LA, control diet+*L. acidophilus*; BL, control diet+*B. subtilis*+*L. acidophilus*.
3) S, sex; T, treatment; S × T, sex and treatment interaction.
a,b Means in a row with different superscripts are significantly different (p < 0.05), n = 8.

Table 5. Effects of probiotic supplement on cecal pH and volatile fatty acids (VFA) of growing rabbits at 10 weeks of age

Items	Sex[1)]		Treatments[2)]				SEM	p-value[3)]		
	M	F	Control	BS	LA	BL		S	T	S×T
pH	6.47	6.53	6.51	6.52	6.48	6.50	0.08	0.059	0.763	0.094
Cecal VFA concentration (mM)										
Total	42.48	41.44	39.05	41.93	44.52	42.35	3.70	0.435	0.056	0.426
Acetate	34.77	34.64	32.92	34.50	34.58	36.82	2.67	0.888	0.060	0.252
Propionate	2.31	2.12	1.86	2.27	2.39	2.35	0.52	0.297	0.177	0.732
Butyrate	5.39	4.68	4.26	5.16	5.31	5.41	1.02	0.062	0.130	0.058
Cecal VFA proportions (%)										
Acetate	82.44	83.70	84.39	83.11	83.03	81.76	1.97	0.085	0.099	0.066
Propionate	5.27	5.11	4.75	5.16	5.30	5.54	1.02	0.656	0.483	0.377
Butyrate	12.29	11.19	10.86	11.73	11.67	12.70	1.57	0.061	0.173	0.010

SEM, standard error of the mean.
1) M, male; F, female. 2) BS, control diet+*B. subtilis*; LA, control diet+*L. acidophilus*; BL, control diet+*B. subtilis*+*L. acidophilus*.
3) S, sex; T, treatment; S × T, sex and treatment interaction.

of sex and treatment on molar proportion of cecal butyrate, the higher value (p = 0.06) was found in the male rabbits.

Gut microbial populations

The effect of sex and treatment interaction on bacilli, lactobacilli, and coliform population in different segments of intestine was not found in this study (Table 6). The male rabbits had higher (p<0.05) bacilli and coliforms in the ileum than those in the female rabbits. The female rabbits appeared to have lower numbers of bacilli in ileum (p<0.01) than in cecum and colon. The rabbits supplemented with BS had greater (p<0.05) numbers of bacilli in ileum and colon than the rabbits received no

Table 6. Effects of probiotic supplement on microbial populations ($\log_{10}$ cfu/g) in different intestinal segments of growing rabbits at 10 weeks of age

Items[1)]	Sex[2)]		Treatments[3)]				SEM	p-value[4)]		
	M	F	Control	BS	LA	BL		S	T	S×T
Bacilli										
Ileum	5.89	5.54^{B}	5.50^{b}	6.07^{a}	5.54^{ab}	5.75^{ab}	0.40	0.022	0.039	0.287
Cecum	6.15	5.95^{A}	5.64^{b}	6.34^{a}	6.01^{ab}	6.21^{a}	0.31	0.076	0.001	0.844
Colon	5.80	6.00^{A}	5.55^{b}	6.25^{a}	5.78^{ab}	6.01^{ab}	0.36	0.122	0.005	0.754
Average	5.95	5.83	5.56^{c}	6.22^{a}	5.77^{bc}	5.99^{ab}	0.23	0.149	<0.001	0.367
SEM	0.41	0.42	0.43	0.25	0.36	0.40				
p-value	0.051	0.006	0.812	0.103	0.057	0.099				
Lactobacilli										
Ileum	5.33	5.38	4.69^{b}	4.76^{b}	6.13^{a}	5.85^{a}	0.63	0.814	<0.001	0.300
Cecum	5.58	5.85	5.09^{b}	5.37^{b}	6.36^{a}	6.03^{a}	0.42	0.074	<0.001	0.422
Colon	5.57	5.66	5.01^{c}	5.33^{bc}	6.12^{a}	6.00^{ab}	0.51	0.627	<0.001	0.897
Average	5.49	5.63	4.93^{b}	5.16^{b}	6.20^{a}	5.96^{a}	0.38	0.315	<0.001	0.625
SEM	0.80	0.61	0.46	0.59	0.52	0.45				
p-value	0.618	0.100	0.218	0.097	0.586	0.699				
Coliform										
Ileum	3.53	2.79	3.68	3.24	2.59	3.13	0.89	0.029	0.140	0.409
Cecum	3.75	3.37	4.30^{a}	3.83^{a}	2.70^{b}	3.42^{ab}	0.80	0.190	0.005	0.259
Colon	3.98	3.42	4.26^{a}	4.09^{a}	2.70^{b}	3.75^{ab}	0.85	0.077	0.006	0.589
Average	3.75	3.19	4.08^{a}	3.72^{a}	2.66^{b}	3.43^{ab}	0.70	0.034	0.043	0.283
SEM	1.03	0.84	0.86	0.72	0.35	0.96				
p-value	0.471	0.073	0.299	0.083	0.773	0.450				
Total G^+	6.20	6.17	5.69	6.29	6.41	6.36	0.24	0.689	<0.001	0.836
G^+/G^-	1.75	2.06	1.48^{b}	1.80^{b}	2.43^{a}	1.93^{b}	0.35	0.020	<0.001	0.428

SEM, standard error of the mean.
1) G^+ (gram positive) = total gut bacilli and lactobacilli populations; G^- (gram negative) = total gut coliform population.
2) M, male; F, female. 3) BS, control diet+*B. subtilis*; LA, control diet+*L. acidophilus*; BL, control diet+*B. subtilis*+*L. acidophilus*.
4) S, sex; T, treatment; S × T, sex and treatment interaction.
a-c Means in a row with different superscripts are significantly different (p < 0.05), n = 8.
A,B Means in a column with different superscripts are significantly different (p < 0.05), n = 16.

probiotic. Numbers of bacilli in cecum of rabbits supplemented with BS or BL were greater (p<0.01) than those received no probiotic. The greatest average numbers of bacilli in all segments were observed in the rabbits supplemented with either BS or BL (p<0.05). Numbers of lactobacilli in ileum, cecum, and average number in all intestinal segments of the rabbits supplemented with LA or BL were greater (p<0.001) when compared to the rabbits supplemented with no probiotic or BS. Rabbits supplemented with LA had greater number of lactobacilli in colon compared to those in control and BS. No difference was observed for coliform number in ileum among the treatments, but the rabbits supplemented with LA had lower (p<0.05) coliform number in cecum, colon, and average number in all segments than the rabbits received no probiotic or BS. As the result of increasing beneficial bacteria, mainly lactobacilli, and decreasing of coliform bacteria populations in the intestine, the ratio of Gram-possitive (G^+) to Gram-negative (G^-) bacteria was the highest (p<0.001) value in the rabbits received LA treatment compared to other treatments.

DISCUSSION

The THI at 29.0 as found in this study was the starting point of heat stress for the growing rabbits [21]. There was a strong negative correlation between feed efficiency, BWG, and thermal comfort level of the habitat in the rabbits, whereas there was a positive correlation between THI and rabbit respiration rate [21]. An increase environmental temperature resulted in low BWG, feed efficiency, and high respiratory rate in the animals [21]. The adverse effect of high ambient temperature on rabbit performance might relate to a decrease in feed consumption, animal dehydration, and tissue catabolism [23]. In addition, more energy could be consumed by the increase respiratory frequency. Therefore, low ME left for growth [24].

The BWG was greater and FCR was lower in the rabbits fed diets supplemented with *L. acidophilus* alone or the complex of *B. subtilis* and *L. acidophilus* compared to the control group demonstrated that *L. acidophilus* had enhanced growth performance of weaning rabbits. These could be due to the greater nutrient digestibility and nitrogen retention in the LA- and BL-supplemented rabbits. The positive effects of probiotic supplement on growth performance and feed efficiency of weaning rabbits were clearly showed in the first two weeks after feeding, while no significant difference was observed in the last four weeks. In the researches of pig, the intestinal microflora becomes stable [25], and normal gut functions have been re-established [26] during two to three weeks of post-weaning period. Therefore, the effect of probiotic supplement on animal growth performance could be expected to be less importance after weaning for 3 weeks [27]. There might be the synergistic effects between *B. subtilis* and *L. acidophilus* since supplementation of mixing *B. subtilis* and *L. acidophilus* at half dose of each (0.5×10^6 and 0.5×10^7 cfu/g feed) showed similar results in almost all parameters as *L. acidophilus* supplement alone at full dose. Survival *L. acidophilus* was able to release peptides, which could help the growth of typically weakly proteolytic probiotics as *Bacillus* spp. [28]. Moreover, bacterial activities of Lactobacillus strains were increased after co-culture these bacteria with *Bacillus* spp.. For this reason, *Bacillus* spp. could stimulate biosynthetic capacities of L*actobacillus* strains [29]. Further investigation should be performed in order to confirm their activities.

The fecal score in current study was used as one parameter to determine fecal status of the weaning rabbits. The lower fecal score in the rabbits supplemented with *L. acidophilus* or combination of *B. subtilis* and *L. acidophilus* might relate to the greater total VFA concentration in the intestine of these rabbits (Table 5). VFA provide a powerful driving force for the movement of water and sodium out of the colonic lumen, which leads to reduce moisture content in the feces and therefore a lower fecal score [30]. However, the supplementation of *B. subtilis* alone did not result in any improvement in the fecal score of weaning rabbits in this study.

The rabbits fed diets supplemented with *L. acidophilus* had greater number of intestinal lactobacilli, which could enhance intestinal hydrolytic enzyme activity in these rabbits resulting in an increase of nutrient digestibility and feed efficiency utilization [31]. Moreover, gut function might have been improved by feeding diet supplemented with *L. acidophilus* due to the increase of lactase and sucrase activities in the small intestinal mucosa [32]. In addition, the lower fecal score and greater intestinal VFA concentration in the rabbits fed diets containing *L. acidophilus* could contribute to the improvement of nutrient digestibility [33]. The higher organic acid concentration in the intestine should be expected to reduce pH [34], and a low gut pH has been shown to have a beneficial effect on nutrient digestibility [35,36]. However, the diet supplemented with *B. subtilis* alone did not improve nutrient digestibility and nitrogen retention of weaning rabbits.

The improved tendency of total cecal VFA concentration of the weaning rabbits receiving LA could be due to the greater lactobacilli activity in the cecum [30]. An increase of the total cecal VFA concentration in the rabbits supplemented with *L. acidophilus* was expected to reduce cecal pH, which might exert adverse effect to the intestinal pathogens [34]. In addition, a low gut pH has been shown to have a beneficial effect on nutrient digestibility [36]. The rabbits fed basal diet supplemented with LA had greater acetic acid concentration in the cecal content than the rabbits fed only basal diet. At the same time, no significant differences were found on cecal concentrations of propionic and butyric acids. This might be due to heterofermentative *Lactobacilli* strains under strictly anaerobic condition of rabbit cecum leading to produce mainly acetic acid [28].

Lactobacilli are generally absent in GIT of normal adult rabbits [37] due to highly acidic environment in the stomach. An increase of the cecal lactobacilli population in the rabbits supplemented with *L. acidophilus* lead to an increase the cecal acetic acid and total VFA concentration and a reduced intestinal coliform population [38]. Acetic acid has been shown to penetrate into the bacterial cytoplasm resulting in a reduced internal bacterial pH and collapse the electrochemical proton gradient, leading a bacteriostasis and death of susceptible bacteria such as cecal coliforms [39]. The decrease of the intestinal coliform population could contribute to a reduction of the gastrointestinal problems in the weaning animals [40]. An increase of gram positive and gram negative bacteria ratio in the intestine of LA-fed rabbits suggested that the intestine was predominantly colonized by non-pathogenic bacteria, and toxic substances of intestinal pathogens were inhibited by gut beneficial bacteria.

CONCLUSION

Supplementation of *L. acidophilus* alone at 1×10^7 cfu/g feed or mixture of *B. subtilis* at 0.5×10^6 cfu/g feed and *L. acidophilus* at 0.5×10^7 cfu/g feed could enhance number of gut beneficial bacteria populations, nutrient digestibility, cecal fermentation, feed efficiency, and growth performance. In addition, gut coliform population and fecal score were reduced in the rabbits supplemented with only *L. acidophilus*. However, there were no significant effect on growth performance and feed efficiency in the weaning rabbits supplemented with *B. subtilis* alone at 1×10^6 cfu/g feed. The combination of *B. subtilis* and *L. acidophilus* at half dose of each showed similar results as the supplementation of *L. acidophilus* alone at the full dose. These results suggest that the L. acidophilus has potential benefits in terms of probiotic effects in the weaning rabbits.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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