

INFLUENCE OF DRIED *Bacillus subtilis* AND LACTOBACILLI CULTURES ON INTESTINAL MICROFLORA AND PERFORMANCE IN BROILERS

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Summary

Two hundred 10-day-old, male Arbor Acres broiler chicks divided randomly into 4 groups of 50 chicks each were used. Different feeding treatment was carried out for each group. Chicks in treatment 1 were fed a basal diet (Starter feed) (control); treatment 2, a basal diet + 0.1% *B. subtilis* culture; treatment 3, a basal diet + 0.2% lactobacilli culture in the feed; and treatment 4, a basal diet + 5 g lactobacilli in the drinking water. The viable bacterial counts for each treatment were approximately 10⁹ cells/kg feed.

The weight gain in chickens given feeds incorporated with *B. subtilis* and lactobacilli was significantly ($p < 0.05$) higher than those of the control. With regard to feed efficiency, there was a definite tendency towards a higher feed : gain ratio in the control group and those fed lactobacilli in drinking water, but those fed lactobacilli in feed had a significantly lower ($p < 0.05$) feed : gain ratio. A significantly ($p < 0.05$) larger population of *Lactobacillus* was found in the small intestine of chickens fed with feed incorporated with *B. subtilis* at 21 and 28 days and with lactobacilli at 14, 21 and 28 days. Populations of intestinal *E. coli* in broilers given feed added with *B. subtilis* were not significantly ($p < 0.05$) different from those of the control, but in chickens fed lactobacilli-added feed, their populations were significantly lower ($p < 0.05$) at 14 and 21 days. No significant differences were found among the treatments and the control in the occurrence of *Salmonella* and *Campylobacter* during the whole experimental period.

(Key Words : Broiler, Performance, Pathogenic Bacteria, *Lactobacillus*, *B. subtilis*, Probiotic)

Introduction

The concept of using microbial preparation in poultry production has become an area of great interest because continued use of sub-therapeutic levels of antibiotics in animal feeds may result in the development of drug-resistant microorganisms in human. Brant et al. (1994) reported that, in America, 13,300 hospital patients had died from infections caused by bacteria which were resistant to the antibiotics prescribed by the doctors. In Malaysia, the antibiotic resistance situation is as acute as, if not worse than, that in US since antibiotics are freely available for use in animals and human. A possible

alternative to antibiotics for growth promotion and improvement of feed efficiency in domestic avian species is by feeding living microbial cultures to them (Damron et al., 1981; Miles et al., 1981a,b; Goodling et al., 1987; Lyons, 1987; Fuller, 1989).

The use of microbial cultures containing lactobacilli is well documented in the temperate region, but very little information is available in the hot and humid area. Viable cells of lactobacilli fed to chickens were found to compete with undesirable microorganisms like pathogenic *Escherichia coli* (Mitchell and Kenworthy, 1976; Muralidhara et al., 1977; Suzuki et al., 1989) and maintain favorable microbial populations in the digestive tract of chickens. Fuller and Turvey (1971) demonstrated that lactic acid producing bacteria such as lactobacilli and streptococci were common microorganisms which were fundamental in the efficient conversion of feed for growth and production of chickens. This equilibrium of the intestinal microflora could be upset at any time when the animal is put under stress, such as excessive heat or humidity.

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There have been very few reports on the use of *Bacillus subtilis* culture in poultry. It has been hypothesized that *B. subtilis* will associate with the gut wall and favor the balance of beneficial intestinal microflora (Saartchit and Sullivan, 1990). There is also a lack of information on the effects of living microbial cultures on anaerobic bacteria and pathogenic *Campylobacter* in the intestine; the latter has been found to cause diarrhea in chicken and human.

The objective of this study was to investigate the effects of dried *B. subtilis* and lactobacilli cultures on the intestinal microflora, including *Campylobacter* and anaerobes, and the performance of broilers in hot and humid climate.

Materials and Methods

Animals

Two hundred 10-day-old, male Arbor Acres broiler chicks were used. All the chicks were raised in wooden cages containing 10 birds each. Each cage was provided with wood shavings litter, a plastic feeder and a water fount.

Cultures used

The *B. subtilis* (CIP5832, Institut Pasteur) culture used was from Guyomarch Co. France, and it contained approximately 1×10^9 living microorganisms/g. The lactobacilli culture was from Kemin Industries, Inc., Iowa, USA, and it also contained $0.5-1 \times 10^9$ viable cells/g.

Feeding treatments

The antibiotic-free basal feed (Starter feed) was from Federal Flour Company, Malaysia. The composition and nutrient analysis of the basal feed are given in table 1.

Four feeding treatments, with 50 chicks for each treatment, were carried out. Chicks in treatment 1 were fed a basal diet and this treatment acted as the control. Chicks in treatment 2 were given a basal diet + 0.1% *B. subtilis* culture; in treatment 3, a basal diet + 0.2% lactobacilli culture in the feed; and in treatment 4, basal diet + 5 g lactobacilli in the drinking water per cage per day. The experimental period for all the treatments was 28 days. The feed and water were provided *ad libitum* throughout the experimental period. The basal feed and drinking water given to the chicks in all the treatments were checked for bacterial (*E. coli*, *Lactobacillus*, *Salmonella* and *Campylobacter*) contamination.

The chickens in each treatment were weighed in groups of ten at the beginning and end of the experiment. Feed consumed by the ten chickens in each cage was

TABLE 1. COMPOSITION AND NUTRITION ANALYSIS OF BASAL DIET

Composition	Starter (0-3 wk) (%)
Corn	59.00
Fishmeal	2.50
Soybean meal	28.00
Corn gluten meal	3.00
Palm oil	2.20
Dicalcium phosphate	1.30
Vitamin premix	0.50
Mineral premix	0.50
Limestone powder	1.00
DL-methionine	0.60
Choline chloride	1.40
Total	100.00
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Nutrient analysis	(%)
Crude protein	22.00
Crude fiber	4.42
ME (MJ/kg)	12.51
Ash	5.48
Ca	1.03
Available phosphorus	0.54
Lysine	1.23
Methionine & cysteine	0.93

(Source : Federal Flour Company, Selangor, Malaysia)

recorded, and feed efficiencies were calculated. Mortality of the chickens in each treatment was also recorded. Temperature and humidity of the surrounding environment were measured daily using a thermohydrometer.

Isolation and enumeration of intestinal microflora

Intestinal microflora of chickens fed with the four different feeding treatments was examined at 0, 7, 14, 21 and 28 days of the experiment. Three birds from each treatment were randomly selected and sacrificed by severing the jugular vein. Samples from the small intestine were used for isolation of *Lactobacillus* and *Enterobacter*, and samples from the cecum were used for *Campylobacter* and *Salmonella* isolation.

Approximately 1 g of intestinal content was mixed with 9 ml of sterile blank dilution solution (Bryant and Burkey, 1953), and homogenized (3 min) using a homogenizer. The mixture was diluted in a 10-fold dilution series to 10^{-7} for intestinal samples and 10^{-9} for cecal samples. For each dilution, 0.1 ml was overlaid with agar medium. Four media were used. They were, BCP Plate Counting Agar (Eiken Chemical Co., Ltd., Tokyo,

Japan) for *Lactobacillus*, MacConkey agar (Oxoid, England) for *Enterobacter*, XLD Medium agar (Oxoid, England) for *Salmonella*, and Campylobacter Blood-free Selective Agar Base (Oxoid, England) for *Campylobacter*. All the plates were then incubated at 37°C. For isolation of *Enterobacter*, the plates were incubated for 24 h, for *Campylobacter* and *Salmonella*, 48 h, and for *Lactobacillus*, 72 h. Petri dishes which had 30 to 300 colonies were counted using a colony counter (Funke Gerber, Germany). Duplicates were made for each dilution and each medium used.

For isolation and estimation of facultative anaerobic bacteria, a total of 12 chickens were selected at the end of the experimental period and anesthetized completely with chloroform. Methods for the isolation and estimation of the facultative anaerobes from the contents of the duodenum, ileum and cecum were the same as those described above, except the isolation was carried out under anaerobic conditions. FM98-5 medium (Jin et al., 1994) and roll tubes instead of petri dishes were used for isolating and counting of the facultative anaerobes (Hungate, 1969).

Statistics

Mean values between treatments were compared by Least Significance Difference (LSD) using Statistical Graphic System (STATIGRAPHIC, USA).

Results

The average maximum and minimum temperatures during the experimental period were 34.8°C and 24.8°C respectively, and the average relative humidity was 97%.

Bacterial populations present in the basal feed and drinking water are shown in table 2. The numbers of *E.*

TABLE 2. BACTERIAL POPULATION IN BASAL FEED AND DRINKING WATER USED IN THE EXPERIMENT

Bacteria	Bacterial population (Log CFU/g or ml)		
	Feed	Drinking water	Cultures
<i>E. coli</i>	2.98	2.08	NT
<i>Lactobacillus</i>	2.70	0	8.95
<i>Salmonella</i>	0	0	NT
<i>Campylobacter</i>	0	0	NT
<i>B. subtilis</i>	NT	NT	9.00

NT = Not tested.
CFU, colony-forming unit.

coli and *Lactobacillus* were too low to cause any effects on the animal performances. *Salmonella* and *Campylobacter* were not found in both the basal feed and drinking water.

The average weight gain, feed efficiency and percentage of mortality of the chickens in the four feeding treatments are shown in table 3. It can be seen that the average weight gain was significantly more ($p < 0.05$) in chicken given feeds incorporated with *B. subtilis* and lactobacilli. When lactobacilli were added to the drinking water, the weight gain was not significantly different ($p > 0.05$) from that of the control. With regard to feed efficiency, there was a definite tendency towards a higher feed:gain ratio in the control group and those fed lactobacilli in drinking water, but those fed lactobacilli in feed had significantly lower ($p < 0.05$) feed:gain ratio than the control. There were no significant differences in the percentage of mortality of chickens in all the four feed treatments.

TABLE 3. INFLUENCE OF FEEDS INCORPORATED WITH *B. SUBTILIS* AND LACTOBACILLI CULTURES ON BODY WEIGHT GAIN, FEED EFFICIENCY AND MORTALITY OF BROILERS

Treatment	Body weight (g)			Feed/Gain	Mortality (%)
	Initial wt.	Final wt.	Gain		
Control	283.04	1,643.33	1,360.29 ^b	1.88 ^a	6.0 ^a
<i>B. subtilis</i> (feeding)	277.61	1,714.33	1,436.72 ^a	1.80 ^{ab}	4.0 ^a
Lactobacilli (feeding)	276.09	1,734.00	1,457.91 ^a	1.72 ^b	2.0 ^a
Lactobacilli (drinking)	276.96	1,689.00	1,412.04 ^{ab}	1.85 ^a	4.0 ^a

^{ab} Means with different superscripts in the same column are significantly different at $p < 0.05$.

A significantly ($p < 0.05$) larger population of intestinal *Lactobacillus* was found in chickens fed with feed incorporated with *B. subtilis* after 21 and 28 days and with lactobacilli, after 14, 21 and 28 days (table 4). However, there were no significant differences between the populations of intestinal *Lactobacillus* of the control chickens and those which had lactobacilli added in their drinking water throughout the experimental period.

Populations of intestinal *E. coli* in broilers fed feed incorporated with *B. subtilis* were not significantly ($p > 0.05$) different from that of the control, but populations of intestinal *E. coli* in chickens fed lactobacilli added in feed were significantly lower ($p < 0.05$) at 14 and 21

TABLE 4. POPULATIONS OF INTESTINAL *LACTOBACILLUS* FROM BROILERS FED FEEDS INCORPORATED WITH *B. SUBTILIS* AND LACTOBACILLI CULTURES AT 0 TO 28 DAYS POST FEEDING

Treatment	Population of intestinal <i>Lactobacillus</i> (Log CFU / g)				
	0 d	7 d	14 d	21 d	28 d
Control	7.25 ± 0.30 ^a	7.14 ± 0.22 ^a	7.61 ± 0.05 ^b	7.50 ± 0.58 ^b	7.64 ± 0.13 ^b
<i>B. subtilis</i> (feeding)	7.44 ± 0.45 ^a	7.96 ± 0.42 ^a	8.20 ± 0.50 ^b	9.07 ± 0.07 ^a	8.91 ± 0.51 ^a
Lactobacilli (feeding)	7.29 ± 0.33 ^a	7.64 ± 0.40 ^a	9.22 ± 0.25 ^a	8.87 ± 0.46 ^a	9.27 ± 0.12 ^a
Lactobacilli (drinking)	7.14 ± 0.21 ^a	7.04 ± 0.45 ^a	7.98 ± 0.09 ^b	8.02 ± 0.16 ^{ab}	7.61 ± 0.24 ^b

Values represent Mean ± SE.

^{ab} Means with different superscripts in the same column are significantly different at $p < 0.05$.

CFU, colony-forming unit per gram intestinal content.

d, days postfeeding.

TABLE 5. POPULATIONS OF INTESTINAL *E. COLI* FROM BROILERS FED WITH OR WITHOUT *B. SUBTILIS* AND LACTOBACILLI CULTURES

Treatment	Population of intestinal <i>E. coli</i> (Log CFU / g)				
	0 d	7 d	14 d	21 d	28 d
Control	6.30 ± 0.21 ^a	6.67 ± 0.47 ^a	7.28 ± 0.15 ^a	7.45 ± 0.36 ^a	7.58 ± 0.09 ^a
<i>B. subtilis</i> (feeding)	6.48 ± 0.30 ^a	6.56 ± 0.17 ^a	6.96 ± 0.09 ^{ab}	6.91 ± 0.76 ^{ab}	6.99 ± 0.50 ^a
Lactobacilli (feeding)	6.18 ± 0.19 ^a	6.19 ± 0.59 ^a	6.47 ± 0.35 ^b	6.48 ± 0.18 ^b	7.00 ± 0.50 ^a
Lactobacilli (drinking)	6.43 ± 0.34 ^a	6.23 ± 0.44 ^a	6.61 ± 0.17 ^{ab}	6.69 ± 0.49 ^{ab}	7.07 ± 0.16 ^a

Values represent Mean ± SE.

^{ab} Means with different superscripts in the same column are significantly different at $p < 0.05$.

CFU, colony-forming unit per gram intestinal content.

d, days postfeeding.

days (though not significant at 28 days) (table 5). When lactobacilli were given to the chickens in drinking water, the population of intestinal *E. coli* was not significantly different ($p > 0.05$) from that of the control.

Table 6 shows the occurrence of cecal *Salmonella* and *Campylobacter* of chickens fed the four different dietary treatments. No significant differences were found among the treatments and the control in the occurrence of cecal

Salmonella and *Campylobacter* in chickens during the whole experimental period.

There were no significant differences in the populations of facultative anaerobic bacteria among the control and treatment groups of chicken (table 7). It can be seen that the addition of *B. subtilis* and lactobacilli to the feed did not affect significantly the populations of facultative anaerobic bacteria in the duodenum, ileum and

TABLE 6. INFLUENCE OF *B. SUBTILIS* AND LACTOBACILLI CULTURES ON THE OCCURRENCE OF CECAL *SALMONELLA* AND *CAMPYLOBACTER* AT 14 TO 28 DAYS POST FEEDING

Treatment	Occurrence of cecal <i>Salmonella</i>			Occurrence of cecal <i>Campylobacter</i>		
	No. of chickens examined	No. of positive birds	Log CFU	No. of chickens examined	No. of positive birds	Log CFU
Control	9	1	4.27	9	5	6.62-8.22
<i>B. subtilis</i> (feeding)	9	0	—	9	4	5.40-7.81
Lactobacilli (feeding)	9	1	4.06	9	4	5.93-8.36
Lactobacilli (drinking)	9	1	4.41	9	5	7.06-8.24

CFU, colony-forming unit per gram intestinal content.

TABLE 7. POPULATIONS OF FACULTATIVE ANAEROBIC BACTERIA IN THE DUODENUM, ILEUM AND CECUM OF BROILERS FED DIETS ADDED WITH OR WITHOUT *B. SUBTILIS* AND LACTOBACILLI CULTURES

Treatment	Population of facultative anaerobe (Log CFU / g)		
	Duodenum	Ileum	Cecum
Control	6.13 ± 0.36 ^a	7.75 ± 0.05 ^{ab}	11.01 ± 0.07 ^a
<i>B. subtilis</i> (feeding)	6.43 ± 0.47 ^a	7.20 ± 0.36 ^b	10.95 ± 0.09 ^a
Lactobacilli (feeding)	5.93 ± 0.39 ^a	8.24 ± 0.19 ^a	10.99 ± 0.09 ^a
Lactobacilli (drinking)	6.53 ± 0.08 ^a	7.87 ± 0.14 ^{ab}	10.82 ± 0.35 ^a

Values represent Mean ± SE.

^{ab} Means with different superscripts in the same column are significantly different at $p < 0.05$.

CFU, colony forming unit per gram intestinal content.

cecum of broilers throughout the experimental period.

Discussion

The results of this study showed that there was an increase ($p < 0.05$) in body weight gain of broilers and an improvement ($p < 0.05$) in feed efficiency when *B. subtilis* and lactobacilli cultures were added to their diets. Although there is a lack of available information on the use of *B. subtilis* culture as a growth promotant in chickens, it has been suggested that *B. subtilis* culture, when fed to poultry, would associate with the gut wall and favor an increase in the numbers of natural *Lactobacillus*, which, in turn, may suppress undesirable enteric microorganisms such as *E. coli*. The present results showed that an addition of 0.1% *B. subtilis* culture in the diet of chickens could increase their intestinal *Lactobacillus* population after 21 and 28 days of feeding; however, there was no decrease in the intestinal *E. coli* populations. These results support, in part, the above hypothesis. In contrast, Saartchit and Sullivan (1990) found that ingestion of *B. subtilis* culture had no significant effect on the populations of intestinal *Lactobacillus* and *E. coli* in turkeys.

The effects of lactobacilli cultures on the growth of domestic avian species have been studied extensively. Tortuero (1973) and Alder and DaMassa (1980) reported that feeding a *Lactobacillus* culture to chicks improved their body weight gain. Couch (1978) fed a probiotic to commercial broilers at a rate of 454 g/ton and found that

Lactobacillus-supplemented birds had an increase in average weight gain and feed conversion. Kalbande et al. (1992) also found that growing pullets fed with a diet added with 0.02% probiotic (*Lactobacillus sporogenes* spores) significantly increased body weight gain and feed efficiency. However, Watkins and Kratzer (1983a,b) observed that broilers raised in battery brooders and fed a diet or drinking water containing *Lactobacillus* culture did not perform as well as the control birds or those fed antibiotics. Lyons (1987) suggested that the effectiveness of probiotic addition was related to two factors, namely, correct numbers of living bacteria used and the presence of stress on the chicken. The results of the present experiment where there was an increase in body weight gain and feed efficiency when *Lactobacillus* cultures were added to the diets of broilers support this theory as the experimental broilers were reared in hot (average temp. 29.3°C) and humid (97%) climate which has been found to be stressful to the chickens (Andersson, 1977). It is not known, from the results of this study, why the addition of lactobacilli in the drinking water failed to increase significantly the performance of the broilers.

The present result which showed a decrease in the populations of the intestinal *E. coli* in broilers fed a diet added with lactobacilli agrees with the findings of Mitchell and Kenworthy (1976) and Suzuki et al. (1989) and supports the hypothesis that lactobacilli compete with coliform for sites of adherence on the intestinal surface. Muralidhara et al. (1977) also found that homogenates of washed intestinal tissues collected from piglets dosed with *L. lactis* had markedly higher numbers of attached *Lactobacillus* and lower numbers of *E. coli* than scouring or normal control pigs.

There has been a great deal of interest to prevent *Salmonella* infection in chickens because infected birds can serve as a source of infection for human, and human infection can result in serious illness or even death (Ziprin and Deloach, 1993). Rantala and Nurmi (1973) and Snoeyenbos et al. (1978) pioneered the use of a probiotic culture, and showed that the probiotic had the ability to prevent the establishment of *Salmonella infantis* in the ceca of chicks although, in this case, lactobacilli were not the only organism present. Shanhani et al. (1976, 1977) reported that lactobacilli could inhibit the growth of *Salmonella in vitro*. Goren et al. (1984) and Impey and Mead (1989) pointed out that microbial cultures should be given to chicks very early in life, preferably before the chicks were exposed to *Salmonella*. In the present study, the results indicates that both *Lactobacillus* and *B. subtilis* cultures fed to broilers do not have an effect on the occurrence of *Salmonella* in the ceca of the broilers

throughout the experimental period. Further study is needed to confirm this point.

Supplementation of lactobacilli in feed and drinking water or *B. subtilis* in feed did not seem to suppress infection by *Campylobacter* in the present experiment. Throughout the 28-day experimental period, the occurrence of *Campylobacter* among the broilers in the treatment groups and the control were not significantly different. Apart from Soerjadi et al. (1982, 1984) who reported that chicks colonized by native gut microflora were relatively resistant to subsequent colonization by some strains of *Campylobacter*, there is no other information on the effect of gut microflora on *Campylobacter*.

In conclusion, the present study demonstrates that the additions of *B. subtilis* and lactobacilli in the feeds of broilers improve the performance of the broilers, enhance their intestinal *Lactobacillus* population and suppress intestinal *E. coli* numbers, but they have no effect on the colonization of pathogenic cecal *Salmonella* and *Campylobacter* of broilers.

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