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The Effect of *Bacillus subtilis* on The Bacterial Content in Rabbits Caeci

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ABSTRACT

In the present study, the effect of *B. subtilis* (10^7cfu/gm) supplemented to the basal diet of two groups of New Zealand rabbit of 28 days (n=15/group) on the count of commensal microflora (*Bifidobacteria* and *Lactobacilli* spp.) and some opportunistic pathogens (*E. coli* and *C. perfringens* type A) in the rabbit caeci was investigated. Results indicated that the count of *Bifidobacteria* and *Lactobacilli* spp. are significantly increased at all intervals (7, 14, 28 and 40 days post supplementation) in the supplemented group compared with non-supplemented group with P< 0.001. On the other hand, there is a significant decrease in the count of *E. coli* and *C. perfringens* specially at 40 days post supplementation from (5.8 log 10 cfu ±0.06) to (3.1 log 10 cfu ±0.07) and from (4.8 log 10 cfu ±0.09) to (2.3 log 10 cfu ±0.09) respectively with P< 0.001. *B. subtilis* proved to be tolerant to the simulated gastric and intestinal juice for one hour (100% tolerance). However, after two hours, the tolerance to the gastric juice decreased to 70%, with no effect of the artificial intestinal juice on the viability of the organism. In vitro, the antimicrobial effect of *B. subtilis* on *C. perfringens* type A by well diffusion method showed an inhibition zone of 10 mm. Results of in vitro effect of *B. subtilis* on *C. perfringens* count showed that at 10^3 dilutions of *C. perfringens*, its count was reduced 2.1×10^5 to 3×10^4 after adding *B. subtilis*, while at dilution of 10^2, the count was reduced from 2.4×10^5 to 7×10^3cfu/ml after adding *B. subtilis*. It could be concluded that *B. subtilis* can be used as a probiotic in rabbits ration due to its ability to increase the commensal microflora count beside its antibacterial effect against some opportunistic pathogens.

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INTRODUCTION

In developing countries, livestock production provides food and play an important role in economic improvement and is considered as a significant income source for many family farms. In the past, the use of antibiotic growth promoters (AGPs) in large scale to animal feed has contributed to the increase in livestock production. However, the over-use of AGPs resulted in the emergence of multidrug-resistant microorganisms and consequently prohibited by the European Union which introduced a total ban on AGPs in 2006 **(Castanon, 2007).**

The prohibition on the sub-therapeutic use of antibiotics in animal feed resulted in decreased animal production **(Cheng *et al.*, 2014)** due to higher rates of infections in livestock and has also increased the risk of food-borne diseases in consumers **(Hao *et al.*, 2014)**. Several replacements/alternatives have been proposed to overcome the problems associated with the ban of AGPs on livestock production **(Cheng *et al.*, 2014)**. The use of probiotics is considered as one strategy that has proven effective.

In the last decade, several strains of some *Bacillus spp.* are currently used as probiotic dietary supplements in animal feeds. Bacillus is a genus of Gram-positive, aerobic or facultatively anaerobic, endospore-forming bacteria. *Bacillus spp.* are saprophytes commonly associated with soil, water, dust and air In addition to an intestinal origin, Bacillus probiotics can also be isolated from other sources, including food, plants, marine algae, aquatic habitats and soil **(Mingmongkolchai and Panbangred, 2018)**.

Spore-forming-Bacillus spp. Among a variety of bacterial species used as probiotics has been identified as an appropriate probiotic because its spores are resistant to harsh conditions and long-term storage

at ambient temperature as well as an inhibition for pathogenic bacterial growth **(Chen *et al*., 2013)**. Compared with other probiotic microorganisms, *Bacillus spp*. have many advantages as probiotic supplements in animal feed because of their ability to produce spores. In addition to heat stability, Bacillus sp. spores have the ability to survive the low pH of the gastric barrier. They can reach the small intestine to exert their probiotic properties **(Cutting, 2011).**

It was found that Bacillus spores germination occur in the small intestine **(Cartman *et al*., 2008)**, and may induce their protective effect in the host through metabolically active mechanisms, such as secretion of antimicrobial substances and/or competition with pathogenic bacteria for essential nutrients **(Duc *et al*., 2004)**. In addition to stimulating positive changes in the intestinal microflora, diarrhea recovery, increased body weight gain and feed efficiency were observed in the host when the animals were fed B. Subtle **(Jiraphocakul *et al*., 1990)**.

Probiotics have been used widely in poultry production. Several studies were conducted to investigate the beneficiary effect of *B. subtilis* probiotics against *S.* Enteritidis or *Clostridium perfringens* (**La Ragione and Woodward 2003)**, *C. perfringens* and *Enterobacteriaceae* **(Jeong and Kim 2014)**, *Campylobacter* (**Guyard-Nicodeme *et al*., 2016**) and *Salmonella* colonization in broilers **(Knap *et al*., 2011).** An intensive system of rabbit production, especially during the weaning period, can cause many physiological and environmental stresses. These problems result in concentration and spreading of pathogens such as *E. coli*, coccidian and epizootic rabbit enteropathy which have adverse effects on growth performance, feed efficiency and rabbit health status **(Bovera *et al*., 2010 and Licois *et al*., 2000)**.

Therefore, our study was conducted to investigate the beneficiary effect of *B. subtilis* as a feed supplement in rabbit feeding, and its effect on some commensal and opportunistic pathogens such as *E. Coli and C. perfringes* in rabbit *caeci (in vivo*) and to study its effect on *C. perfringens* type A *(in vitro*).

MATERIALS AND METHODS

Bacterial strains and Media

1.Bacillus *subtilis* strain

A reference commercial strain of *B. subtilis* (ATCC 10876) was kindly obtained from Food and Meat Hygiene Department in Animal Health Research Institute. It was aerobically cultured in trypton soya broth (TSB) supplemented with 1% (w/v) yeast extract (TSBYE) and incubated for18 hours at 37ºC aerobically.

2. Clostridium perfringens type A strain

It was isolated from a case of enteritis in rabbits, the pathogenicity and toxicity of this isolate were tested for lethality and dermonecrotic reaction in Swiss mice and guinea pigs respectively **(willis, 1977)**. *C. perfringens* isolate was anaerobically cultured in thioglycolate broth and incubated for 18 hours at 37º C. Both *B. subtilis* and *C. perfringens* broth cultures were centrifuged (3000xg for 10 min) and washed using sterile phosphate buffer saline (PBS) pH 7.4. In cell culture medium a final concentration of 107cfu / ml was re-suspended for further testing

Experimental design

Study the effect of B. *subtilis* on bacterial content count in rabbit caeci:

To study the effect of *B. subtilis* on bacterial content in rabbit caeci *in vivo*, 30 healthy weaned New Zealand White male rabbits (28 day-old) were divided into 2 groups consisting of 15 rabbits each. Group 1 were fed with a basal diet supplemented with 10^7cfu/gm basal diet of *B. subtilis*, while rabbits of group 2 were fed with a basal diet only and served as a negative control group. All groups ran contemporaneously in a battery with sufficient room temperature, feed, and ventilation until the end of the experiment (6 weeks).

Intestinal samples were collected at 7, 14, 28 and 40 days post supplementation (3 samples/time) from each group for enumeration of some commensal and opportunistic pathogenic microorganisms as described by **(Guo *et al*., 2017)** as follows: The intestinal contents were serially diluted in 0.85% sterile saline solution and each dilution was plated in duplicate onto selective agar for each microorganism (eosin methylene blue for enumeration of *E. coli*, tryptose sulphate cycloserine agar for *C. perfringens*, MRS agar for *Lactobacillus spp*., and Bifidobacterium agar medium for *Bifidobacterium spp*.)

Survival of *Bacillus subtilis* in a simulated gastro-intestinal juice

A simulated gastrointestinal juice was made to investigate the survival of *B. subtilis* in these juices. The suspension of 2.3×10^7cfu/ ml of *B. subtilis* were inoculated into Trypton Soya Broth (TSB) and pH was adjusted to 2.5 by adding a hydrochloric acid solution with 1% pepsin (as gastric juice) or to 7.2 with 1% trypsin (as intestinal juice) and incubated at 37º C for up to two hours. At 0,1 and 2 hours of incubation, 100 µl of the culture were removed and spread onto trypton soya agar plates in duplicate for cell number estimation as described by **(Guo *et al*., 2017)**.

Antimicrobial activity of *B. subtilis*

The well diffusion method was performed on agar using cultured broth. The target strain *C. perfringens* in a concentration of (10^7cfu/ml) was incorporated into agar (1% w/v) plates, mixed for uniformity and poured onto plates to solidify. Overnight culture of *B. subtilis* (10^8cfu/ml) was transferred to holes (5mm in diameter) punched into

the agar plates. The plates were then incubated anaerobically at 37º C for 24 hours and the antimicrobial inhibition zone was recorded as described by **(Zhang *et al.*, 2012)**.

Effect of *B. subtilis* on *C. perfringens* count (*in vitro*)

Clostridium *perfringens* suspension in PBS was prepared as 10^5cfu/ml using McFarland tube from which tenfold dilution was made, and 1 ml of each dilution was dispensed in sterile Petri dishes then 10 ml of molten tryptose sulphate cycloserine agar (TSC) was poured, mixed well and left to solidify before another layer was poured. All *C. perfringens* suspension dilutions were seed with 0.1 ml of overnight culture of *B. subtilis* (10^7 cfu/ml) and incubated for 1 hour at 37º C then cultured onto TSC agar as mentioned before. After solidification, the plates were incubated anaerobically at 37º C for 24 hours then enumerated as described by **(Guo *et al*., 2017)**.

Statistical Analysis

Experimental data were assessed by one-way analysis of variance (ANOVA)-Duncan test using SPSS software statistical program (windows version 20.0, USA) to observe mean differences **(SPSS 20.0, 2014)**. Data were expressed as mean ± SE when (n=5) and were regarded as significant when ($P \leq 0.05$).

RESULTS

Detection of *B. subtilis* effect on bacterial content in rabbit caeci

It was found that the beneficial microflora e.g. *Bifidobacterium* and *Lactobacillus spp*. obviously increased in group treated with *B. subtilis* and the opportunistic pathogenic organisms count were decreased in this group. On the other hand, the control group nearly showed stability in the count of all microorganisms as shown in Table (1).

Survival of *Bacillus subtilis* in simulated gastric and intestinal juices

Bacillus subtilis ATCC 10876 exhibited a good probiotic property in vitro including artificial gastric juice and intestinal juice. The strain can tolerate the alkalinity of the intestinal juice till 2 hours; however, it may show less tolerance with the acidity of gastric juice after 2 hours as shown in Table (2).

Effect of *B. subtilis* on *C. perfringens* type A count (*in vitro*)

It was observed that, at 10^3 dilutions, the count of *C. perfringens* culture was 2.1×10^5cfu/ml, however, when *B. subtilis* was added, the count of *C. perfringens* decreased to 3×10^4cfu/ml (Figs. 1 and 2). On the other hand; when *C. perfringens* culture was at 10^2 dilutions, its number reached 2.4×10^5cfu/ml and decreased to 7×10^3cfu/ml after treated with *B. subtilis* (Figs.3 & 4).

Antimicrobial activity of *B. subtilis*

The sensitivity of *C. perfringens* against *B. subtilis* on tryptose sulphate cycloserine agar (TSC) was made and a zone of inhibition of about 10 mm has been formed. A sterile Trypricase soya yeast extract (TSY) broth was used as control negative in another well (Fig.5)

Fig. 1: count of *C.perfringens* diluted 10^3 times without adding *B. subtilis* culture (2.1×10^5cfu/ml).

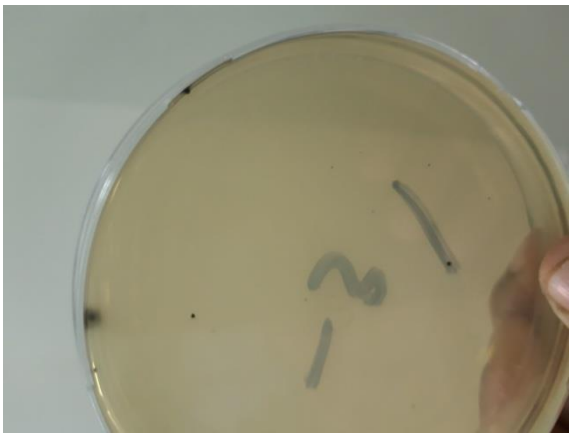

Fig.2: count of *C.perfringens* diluted 10^3 times after adding *B.subtlis* culture (3×10^4cfu/ml)

Fig.3: The count of *C. perfringens* diluted 10^2 times without adding *B. subtlis* culture(2.4×10^5cfu/ml).

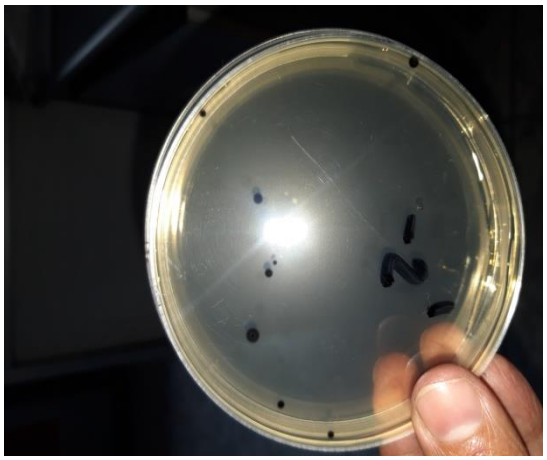

Fig.4: The count of *C. perfringens* diluted 10^2 times after adding *B. subtlis* culture(7×10^3cfu/ml).

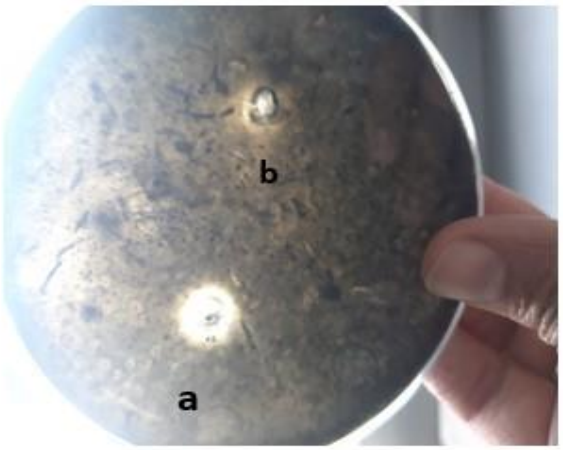

Fig.5: The sensitivity of *C. perfringens* against *B. subtlis* on TSC agar. (a) showing a zone of inhibition of 10 mm. (b) Control negative using sterile TSB broth

Table 1: Enumeration of commensal and pathogenic bacteria in caeci of rabbits.

Groups / Microorganisms	Count of commensal and pathogenic opportunistic microorganisms in (log_{10})							
	Group 1 (*B. subtilis*-supplemented)				Group2 (Negative control)			
day post supplementation	7	14	28	40	7	14	28	40
E. coli	5.4± 0.06	5.3± 0.09	4.4± 0.03***	3.1± 0.07***	5.5± 0.06	5.2± 0.09	5.9± 0.03***	5.8± 0.06***
C. perfringens	4.5± 0.06*	3.5± 0.07***	2.2± 0.06***	2.3± 0.09***	4.3± 0.03*	5.7± 0.1***	4.7± 0.09***	4.8± 0.09***
Bifidobacteriumspp	7.8± 0.06***	8.1± 0.06***	9.0± 0.06***	9.4± 0.09***	7.1± 0.06***	7.4± 0.03***	7.6± 0.06***	7.7± 0.06***
Lactobacillus spp.	9.0± 0.03***	8.9± 0.03***	9.6± 0.09***	9.7± 0.06***	7.6± 0.09***	7.8± 0.03***	6.7± 0.03***	7.8± 0.03***

*p<0.05, **p<0.01, ***p<0.001

Table 2: Survival rate of *B. subtilis* at artificial gastric juice and intestinal juice (%) (The mean of count).

Juice / Time	0 hour	1 hour	2 hours
Gastric juice	2.3×10^7	100% (1.8×10^7)*	70% (1.6×10^6)**
Intestinal juice	2.3×10^7	100% (1.9×10^7)*	100% (1.7×10^7)*

* count not changed (complete tolerance)

** count changed (partial tolerance)

DISCUSSION

Probiotics are safe alternative to antibiotics which will improve growth performance and act as therapeutically or prophylactically drugs, as antibiotics disturbs the normal microbial balance and beneficial bacteria **(Simoneit *et al.*, 2014)** and **(Sullivan *et al.*, 2001)**. *B. subtilis* are considered to be suitable probiotics as they produce essential nutrients such as amino acids and vitamin K and B_{12}, promoting growth performance and proliferation of beneficial bacteria as well **(Cutting, 2011**and **Zhou *et al.*, 2013)**. The effectiveness of probiotics depends largely on the dose ingested and bacterial strains, therefore, it is essential to determine the minimal effective dosage of probiotic strains **(Mingmongkolchai and Panbangred, 2018)**.

In this study, the role of *B. subtilis* as a dietary probiotic in rabbit ration was investigated to determine its effect on some opportunistic pathogens as well as some beneficial commensal bacteria in the gut of rabbits. It was found that *B. subtilis* had a significant inhibitory effect on the count of pathogenic bacteria in the gut of rabbits as the count of *E. coli* as well as *C. perfringens* were decreased significantly throughout the experiment. These results are on line with **Phuoc and Jamikorn (2017)** who investigated the role of *B. subtilis* supplement on feed efficiency, growth performance and microbial population in the distal gastrointestinal tract of weaning rabbits and found that the average intestinal coliform populations were lowest ($P<0.05$) in the rabbits supplemented with *B. subtilis* than control.

Additionally; suppression of colonization and persistence of *S* Enteritidis or *C. perfringens* was demonstrated in pre-dosing newly hatched specific pathogen-free chicks with a suspension of 1×10^9 spores of *B. subtilis* $PY79^{hr}$. Moreover, *S.* Enteritidis shedding was also reduced significantly in the pre-dosed birds for the 36-days duration of the experiment **(La Ragione and Woodward 2003)**. Similarly, **Jeong and Kim (2014)** showed that the number of *C. perfringens* and *Enterobacteriaceae* in the excreta is reduced when birds were fed *B. subtilis* C-3102.

Reduction in shedding of *Campylobacter* is observed in broilers fed *B. subtilis* C-3102, (**Guyard-Nicodeme *et al.*, 2016)** and a significant reduction in Salmonella colonization in broilers fed with either *B. cereus var toyoi* (Toyocerin) **(Vila *et al.*, 2009)** or *B. subtilis* DSM 17299**(Knap *et al.*, 2011)**. Supplementation of *B. subtilis* PB6 to broilers also resulted in a reduction in intestinal *C. perfringens* counts **(Jayaraman *et al*., 2013)**.

It was found that the count of beneficial commensal bacteria (*Bifidobacterium spp*. and *Lactobacillus spp*.) significantly increased in *B. subtilis* supplemented group ($P<0.001$). **Guo *et al.* (2017)** found that the survival rate of the rabbits fed with *B. subtilis* was significantly higher than those in the control groups post infected with *E. coli*. It is worth to say that *B. subtilis* strains that do not have any probiotic activity or killed cells cannot produce digestive enzymes, vitamins and antibacterial substances, which were produced by probiotic. For that, it is speculated that strains without probiotic activity have no significant effects on growth performance, intestinal flora and disease resistance. The ability of *B. subtilis* to inhibit the adhesion of enterotoxic *E. coli* to the surface of intestinal epithelial cells and *S.* Enteritidis through competitive exclusion (CE) mechanism was reported by other investigators (**Thirabunyanon and Thongwittaya, 2012; Ye *et al.*, 2013)**.

Probiotics should also be excellent in adapting to acidic conditions in the stomach and alkalinity in the intestine to influence the gastrointestinal tract. The ability of the spores to survive at low pH of the gastric barrier and reach the small intestine to produce the probiotic effects was documented by **Barbosa *et al.* (2005)** and **Cutting (2011)** that accord with our findings as it was found that *B.subtilis* can tolerate the alkalinity of the simulated intestinal juice in a rate of 100% after 1 and 2 hours incubation. Meanwhile, the organism can tolerate the acidity of the artificial gastric juice in a rate of 100% after 1 hour and the rate decreased after 2 hours to 70%. Germination of Bacillus spores in the small intestine has been reported in several studies **(Cartman *et al.*, 2008)**. Their beneficiary effect in the animal host may be attributed to the active metabolic mechanism, such as competition with pathogenic bacteria for essential nutrients and/or secretion of antimicrobial substances **(Duc *et al.*, 2003)**. It is worthy to mention that before using a probiotic strain to demonstrate its beneficial effect on animal host; the in *vivo* and in *vitro* studies must be investigated **(Mingmongkolchai and Panbangred, 2018)**.

In the present study; *in vitro* study at dilution 10^3, the count of *C. perfringens* type A reached 2.1×10^5 cfu/ml, however, when *B. subtilis* was added, the count reduced to 3.0×10^4 cfu/ml. On the other hand, when *C. perfringens* culture was at 10^2 dilutions, the count reached 2.4×10^5 cfu/ml and reduced to 7.0×10^3 cfu/ml after treated with *B. subtilis*. Several antimicrobial compounds such as bacteriocins, lipopeptides, bacteriocins like inhibitory substances and surfactin are known to be produced by genus Bacillus members. These compounds are typically active against Gram-positive bacterial pathogens, but some display activity against Gram-negative bacterial pathogens as well as fungal pathogens **(Kerr, 1999; Teo and Tan, 2005; Khochamit *et al.*, 2015)**. Moreover, by conducting

antimicrobial activity of *B. subtilis* against *C. perfringens*, a 10 mm inhibition zone was produced indicating the antimicrobial efficacy of *B. subtilis*. These results are in agreement with Teo and Tan, (2005) who examined the antimicrobial activity of *B. subtilis* against *C. perfringens* ATCC 13124.

CONCLUSIONS

Bacillus subtilis is safe and reliable animal probiotic with great potential to be used as an alternative to antimicrobial drugs which is significant in the context of antibiotic abuse in food and animal production. Dietary supplementation of *B. subtilis* in rabbit ration will decrease the bacterial load of pathogens and subsequently improves the growth rate of animals.

Declaration of Competing interest

On behalf of all authors, I hereby declare that no conflict of interest may interfere with the publication of the manuscript.

REFERENCES

BARBOSA, T. M., SERRA, C. R., LA RAGIONE, R. M., WOODWARD, M. J. AND HENRIQUES, A. O., 2005. Screening for Bacillus isolates in the broiler gastrointestinal tract. *Appl. Environ. Microbial*.71:968-978.

BOVERA, F., NIZZA, S., AND MARONO, S. K., 2010. Effect of mannan oligosaccharides on rabbit performance, digestibility and rectal bacterial anaerobic populations during an episode of epizootic rabbit enteropathy. *World Rabbit Sci*., 18:9-16.

CARTMAN, S.T., LA RAGIONE, R.M., AND WOODWARD, M.J., 2008. *Bacillus subtilis* spores germinate in the chicken gastrointestinal tract. *Appl. Environ.Microbial*.74:5254-5258.

CASTANON, J., 2007. History of the use of antibiotics as growth promoters in European poultry feeds. *Poult. Sci.,* 86 2466-2471.

CHEN, W., WANG, J. P., YAN, L. AND HUANG, Y.Q., 2013. Evaluation of probiotics in diets with different nutrient densities on growth performance characteristics in broilers. *British Poultry Science*, 54, 635-41.

CHENG, G., HAO, H., XIE, S., WANG, X., DAI, M., HUANG, L. AND YUAN, Z., 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry. *Front. Microbiol.,* 5:69-83.

CUTTING, S.M., 2011. Bacillus probiotics. *Food Microbiol.,* 28: 214-220.

DUC, L. H., HONG, H. A., BARBOSA, T. M., HENRIQUES, A. O. AND CUTTING, S. M., 2004. Characterization of Bacillus probiotics available for human use. *Appl. Environ. Microbiol.,* 70: 2161-2171.

DUC, L.H., HONG, H. A., FAIRWEATHER, N., RICCA, E. AND CUTTING, S.M. 2003. Bacterial spores as vaccine vehicles. ,71:2810-2818.

GUO, M., WU, F., HAO, G., QI, Q., LI, N., WEI, L. AND CHAI, T., 2017. *Bacillus subtilis* improves immunity and resistance in rabbits. *Frontiers in Immunity*, 8:1-13.

GUYARD-NICODEME, M., KEITA, A., QUESNE, S., AMELOT, M., POEZEVARA, T., LEBERRE, B., SANCHEZ, J. AND VESSEUR, P., 2016. Efficacy of feed additives against *Campylobacter* in live broilers during the entire rearing period. *Poult. Sci.*, 95: 298-305.

HAO, H., CHENG, G., IQBAL, Z., AI, X., HUSSAIN, H. I., HUANG, L., DAI, M. AND WANG, Y., 2014. Benefits and risks of antimicrobial use in food-producing animals. *Front. Microbiol*., 5:288.

JAYARAMAN, S., THANGAVEL, G., KURIAN, H., MANI, R., MUKKALIL, R. AND CHIRAKKAL, H., 2013. Bacillus subtilis PB6 improves intestinal health of broiler chickens challenged with Clostridium perfringens-induced necrotic enteritis. *Poult. Sci.*, 92:370-374.

JEONG, J. S., AND KIM, I. H., 2014. Effect Of *Bacillus Subtilis* C-3102. spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers. *Poult. Sci.*,93:3097-3103.

JIRAPHOCAKUL, S., SULLIVAN, T.W., AND SHAHANI, K.M., 1990. Influence of a dried *Bacillus subtilis* culture and antibiotics on performance and intestinal microflora in turkeys. *Poult. Sci*.69:1966-1973.

KERR, J. R., 1999. Bacterial inhibition of fungal growth and pathogenicity. *Microbiol. Ecol. Health Dis.*, 11: 129.

KHOCHAMIT, N., SIRIPORNADULSIL, S., SUKON, P. AND SIRIPORNADULSIL, W., 2015. Antibacterial activity and genotyping-phenotyping characteristics of bacteriocin producing *Bacillus subtilis* KKU213; potential as a probiotic strain. *Microbiol. Res*., 170: 36.

KNAP, I., KEHLET, A., BENNEDSEN, M., MATHIS, G., HOFACRE, C., LUMPKINS, B., JENSEN, M. AND RAUN, M., 2011. *Bacillus subtilis* DSM17299 significantly reduces Salmonella in broilers. *Poult. Sci.*, 90: 1690-1694.

LA RAGIONE, R. M., AND WOODWARD, M. J., 2003. Competitive exclusion by *Bacillus subtilis* spores on *Salmonella enteric serotype Enteritidis* and *Clostridium perfringens* in young chickens. *Poult. Sci.*, 94:245-256.

LICOIS, D., COUDERT, P, CERE, N. AND VAUTHEROT, J.F., 2000. Epizootic enterocolitis of the rabbits: a review of current research. Proceedings of the 7th World Rabbit Congress; Valencia, Spain, pp. 187-194.

MINGMONGKOLCHAI, S. AND PANBANGRED, W., 2018. *Bacillus* probiotics: an alternative to antibiotics for livestock production. *J. Appl. Microbiol.* 124: 1334-1346.

PHUOC, T. L. AND JAMIKOREN, U., 2017. Effect of probiotic supplement (*B. subtilis* and *Lactobacillus acidophilus*) on feed efficiency, growth performance, and microbial population of weaning rabbits. *Asian-Australas. J. Anim. Sci.*,30(2):198-205.

SIMONEIT, C., BUROW, E., TENHAGEN, B. A. AND KASBOHRER, A., 2014. Oral administration of antimicrobials increases antimicrobial resistance in *E.*

coli from chicken- a systematic review. *Prev. Vet. Med.*, 118: 1-7.

SPSS 20.0., 2014. Statistical package for social science, SPSS for windows release20.0, Standard version, SPSS Inc. Chicago, IL.

SULLIVAN, A., EDLUND, C. AND NORD, C.E., 2001. Effect of antimicrobial agent on the ecological balance of human microflora. *Lancet Infect. Dis.*, 1: 101-114.

TEO, A. Y. AND TAN, H., 2005. Inhibition of *Clostridium perfringens* by a novel strain of *Bacillus subtilis* isolated from the gastrointestinal tract of healthy chickens. *Appl. Environ. Microbiol.*, 71(8):4185-90.

THIRABUNYANON, M. AND THONGWITTAYA, N., 2012 Protection activity of a novel probiotic strain of *B. subtilis* against *Salmonella Entriditis* infection. *Res. Vet. Sci.*, 93:74-81.

VILA, B., FONTGIBELL, A., BADIOLA, I., ESTEVE-GARCIA, E., JIMENEZ, G., CASTILLO, M. AND BEUFAU, J., 2009. Reduction of *Salmonella enteric var. Enteriditis* colonization and invasion by *Bacillus cereus var. toyoi* inclusion in poultry feeds. *Poult. Sci.*, 88:975-979.

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WILLIS, A. T., 1977. Anaerobic Bacteriology. Clinical and Laboratory Practice.3rd ed., Butter Worth, London.

YE, X.; LI, P.; YU, Q. AND YANG, Q., 2013. *B. subtilis* inhibition of enterotoxic *Escherichia coli*-induced activation of MAPK signalling pathways in Caco-2 cells. Ann. Microbiol., 63: 577-581.

ZHANG, D., RUI, L. AND LI, J., 2012. *Lactobacillus reuteri* ATCC 55730 and L22 display probiotic potential in vitro and protect against Salmonella-induced pullorum disease in a chick model of infection. *Res. Vet. Sci.*, 93:366-73.

ZHOU, M., WING, H. AND ZE-YANG, L., 2013. Study on resistance to stress factors of *Bacillus subtilis* from swine intestinal juice. *J. Anhui. Agricultural Univer.*, 40: 519-522.