Influence of Spices on the Antimicrobial Activity of Probiotics in Minced Chicken

By
Ms. Sabiha Shakil
ID. 4425324

A special project submitted to the Faculty of Biotechnology.
Assumption University in part fulfillment of the requirement
for the degree of Bachelor of Science

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Title	:	Influence of Spices on the Antimicrobial Activity of Probiotics in Minced Chicken
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Abstract

Trend in food preservation technique is trying to use natural antimicrobial substance instead of using artificial substance to inhibit food poisoning bacteria. Probiotics and spices are the good sources for natural antimicrobial substances. Two probiotic bacteria, *Lactobacillus acidophilus* (LA) and *L. casei* (LC) and five spices (coriander seed, garlic, ginger, turmeric and black pepper) were studied for antimicrobial activity using disc diffusion method. The mixture of LA and LC exhibited the highest inhibitory activity against *Salmonella typhimurium*, the second most common food borne illness. In addition, among five spices tested, turmeric showed the highest antimicrobial activity followed by garlic and ginger. Then the antimicrobial activity of the combination of the mixture of LA & LC and three spices (garlic, ginger and turmeric) were studied in minced chicken stored at 37°C for 2 days. Although the mixture of probiotic bacterial cultures and spices exhibited the antimicrobial activity against *S. typhimurium in vitro*, the combination of probiotics and spices has no effect on this microorganism in the minced chicken.

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Influence of Spices on the Antimicrobial Activity of Probiotics in Minced Chicken

Introduction

In recent years, there has been a growing need to control growth of pathogens in food. One of the most common illnesses caused by a pathogen is Salmonellosis. Salmonellosis is a food borne illness caused by the enteric infection of *Salmonella*. It is parasitic in domestic animals, rodents, reptiles, birds and humans. In the United States, over 40,000 cases of the illness are reported every year. It is the second most common food borne illness and causes 31% of food-related deaths. Therefore, the food and agricultural industry need to look for better methods of preserving food by finding better methods of controlling the growth of *Salmonella* in food. (Anonymous, 1999)

Probiotics are friendly microorganisms that make up the gastrointestinal tract and have many beneficial biological functions. These friendly microorganisms have been isolated and used as supplements due to their positive health effects. The health benefits are paramount and include helping with digestion, stimulating immune systems, and most importantly, inhibiting growth of pathogens.

Lactobacillus acidophilus and *Lactobacillus casei* are two common probiotics used in foods, especially dairy products, as health supplements. Recently, these beneficial microorganisms are used as biopreservatives due

to the use of chemicals have been proven to cause adverse health effects. The reason they can be used as biopreservatives, is that under Lactobacilli fermentation, antimicrobial metabolites are produced, which can inhibit pathogens.

Spices are mainly used in meat products for flavor and aroma enhancement. In recent years, they have also been proven to possess antimicrobial activity. However, the small doses of these spices sprinkled on food are not enough to completely inhibit microorganisms. Spices have been used in combination with other preservation techniques for better effectiveness.

Recent studies have shown that spices do not inhibit probiotic growth. Therefore, the combination of spices and probiotics could be a useful means of food preservation. This study was focused on determining whether or not Lactobacilli, in the presence of spices, can effectively inhibit the growth of *Salmonella typhimurium* in raw minced chicken.

Literature Review

Chemical preservatives are dangerous

For many decades, chemical preservatives and salt have been widely used to preserve food due to their antimicrobial properties. Chemicals and varying salt concentrations can be toxic to microbial growth. Although these chemicals are effective in decreasing microbial load, they are highly regulated by the government due to their adverse health effects on humans. According to the Australian Academy of Sciences, nitrates and nitrites that are used as preservatives can by converted into nitrosamines when these react with amino acids. Nitrosamines are cancer-causing substances. If these chemicals reach a certain level in the body, they cause vomiting, asthma, nausea and headaches. Moreover, high salts may lead to heart disease as it increases the blood pressure. Nowadays, researchers are looking for natural ways of preserving food by using natural remedies.

Probiotics and their benefits

Hippocrates once said, "Let food be thy medicine and medicine be thy food." This concept is becoming increasingly popular in the world today. Consumers are becoming more aware of the health benefits of the components in their foods. For example, there is an exponential rise in Green Tea consumption, due to the health benefits it has. People are taking better care of themselves by eating nutritious and beneficial food.

Very recent trends in the nutritious food market are foods containing probiotics. Currently, products available in the market are mainly dairy products such as fermented/cultured milk (Yakult), yoghurt, drinking yoghurt, etc. The health benefits of probiotics have been shown in Table 1.

Table 1 Alleged health effects of probiotics

Intestinal effects
• Relieve effects, promotes recovery from diarrhea, rotavirus, travelers' and antibiotic induced • Produce lactase, alleviate symptoms of lactose intolerance and malabsorption • Relieve constipation • Treat colitis
Immune system effects
• Enhance specific and nonspecific immune response • Inhibit pathogen growth and translocation • Stimulate gastrointestinal immunity • Reduce chances of infection from common pathogens like Salmonella, Shigella
Other effects
• Reduce certain cancers like colon and bladder • Detoxify carcinogen • Suppress tumors • Lower serum cholesterol concentrations • Reduce blood pressure in hypersensitives • Treat food allergies • Synthesize nutrients like folic acid, niacin, riboflavin, vitamins B6 and B12 • Increase nutrient bioavailability • Improve urogenital health • Optimize effect of vaccines e.g. rotavirus vaccines, typhoid fever vaccines.

Adapted from Dairy Council of California 2000, *Probiotics-Friendly Bacteria with a Host of Benefits*

Antimicrobial activity of probiotics

Probiotics can be used as biopreservatives agents because during Lactobacilli metabolism, antimicrobials such as lactic acid, other organic acids, hydrogen peroxide and bacteriocins are produced that play an important role in eliminating pathogens.

An undissociated form of lactic acid is produced which lowers the pH to acidic conditions and inhibits growth of certain pathogens (Oyetayo, 2004). It also interferes with metabolisms as it interferes with the oxidative phosphorylation process (Kumar, 2005). Bacteriocins, produced by probioitcs, are proteins or protein-particulate complexes that are antagonistic to pathogens (Kumar, 2005). The toxins affect cell membranes, membrane associated replication, and syntheses of DNA and proteins of harmful microorganisms.

Putrefactive microorganisms are inhibited by the production of hydrogen peroxides, carbon dioxides and diacetyl. Carbon dioxide prevents decarboxylation reactions and reduces membrane permeability while the oxidation of proteins by Lactobacilli, interact with arginine binding proteins. (Kumar, 2005)

In a recent study, Oh *et. al* (2000) isolated and studied the bacteriocins produced by *L. acidophilus* 30SC, which include acidocin 8912, lactacin, acidophilin, acidolin, and lactocidine. In another study, Oyetayo (2004), inhibitory potential of various lactobacillus species on pathogens and indicator microorganisms were studied. As a result, it was found that

L.acidophilus was a good inhibitor of *Escherichia coli* and *Lactobacillus casei* a good inhibitor of *Salmonella*.

Antimicrobial activity of spices

For centuries in Asia, spices have been used for flavor, aroma and sensory enhancement of food. Scientific studies have proven that spices and their components have antimicrobial affects. They inhibit gram positive and gram negative food borne microorganisms. However, they are required in high dosages if they are to be effective alone (Shelef, 1983).

The active ingredients against microbes in spices are generally essential oils. For instance, garlic contains allicin, which is a sulfur-containing essential that inhibits gram negative and gram positive bacteria depending on the concentration present in the food (Shelef, 1983).

In another study by Ceylan *et. al* (1998), spices reduced *E. coli* O157:H7 in meat. It concluded that cloves, cinnamon and garlic were the most effective in reducing the pathogen.

Effect of probiotics and spice combination in food preservation

In a recent study by Ibrahim *et. al* (2001), antimicrobial activity of *Bifidobacterium longum* in the presence of spices was examined. Their results indicated that the use of both *bifidobacterium* and spice had greater inhibitory affect on *E. coli* O157:H7 than using either spice or the probiotics alone in ground meat.

The spices, however, did not inhibit the growth of the probiotics. Although spices inhibit growth of many bacteria, for some reason, it stimulates the growth of starter cultures. Manganese (Mn^{2+}) is found in high concentrations in most spices and contributes to the growth of starter cultures (Zaika and Kissinger, 1984; Kang and Fung, 2000). The mechanism by which the stimulant affects growth of starter cultures has not been studied extensively and there is very little information available regarding it.

Natural herbs also contribute to the growth of dairy starter cultures. An increase in lactic acid production by the starter cultures *L. delbruekii* ssp. *bulgaricus* and *Streptococcus thermophilus*, indicated an increase in growth of the starter cultures. The herbs used were *Allium* (garlic, onions, etc.), *Thymus* (thyme), *Anthriscus* (chervil), and *Ferule* (asafetida) (Bakirci, 1999).

In this study, *L. acidophilus* and *L. casei* were used in combination with spices (garlic, black pepper, coriander seeds, ginger, and turmeric) to inhibit *Salmonella typhimurium* in raw minced chicken. Since both spices and probiotics have antimicrobial activity, it was expected that this microorganism should be inhibited.

Materials and Method

I. To study bacterial culture conditions

All aseptic tools were autoclaved overnight before use. Three cultures were obtained from the Microbiological Resource Center (MIRCEN) of Thailand's Institute of Scientific and Technological Research. Twenty four hours slants of *Lactobacillus acidophilus 450* (LA) and *L. casei 390* (LC) were inoculated into MRS broth and then incubated for 24 and 48 hours to obtain at least 10^8 CFU/ml. Each culture was plated on MRS agar to determine the CFU/ml for each period of incubation.

For enumeration of *Salmonella typhimurium 290*, a 24 hour Nutrient Agar (NA) slant of the culture was inoculated into Nutrient Broth and incubated for 24 hours at 37°C. To determine the bacterial count, the culture was spread on Bismuth Sulphite Agar and incubated for 24 to 48 hours. The incubation period for *S. typhimurium* 290 was 24 hours to achieve a count of at least 10^3 CFU/ml so that there would be 10^2 CFU/g in the minced chicken.

The cultures were then centrifuged at 3000 rpm and added to the samples.

II. To study antimicrobial activity of spices and probiotics using disc diffusion method

The spices used in the study were garlic, black pepper, coriander seeds, ginger and turmeric powder. The spices were blended with sterile

water using the ratio of 1:1. The spice mixtures were then shaken at 150 rpm for 7 days. After that, the mixtures were filtered and the filtrates (the spice extracts) were kept for antimicrobial testing against *S. typhimurium* using disc diffusion method as well as the 24 hour cultures of LA and LC and mixture of LA and LC. The plates were incubated for 24 hours at 37°C and clear zones were measured.

III. To study antimicrobial activity of the combination of spices and probiotics in minced chicken

Minced chicken, which is bought from the local fresh market, was stored in refrigerator and was used within 24 hours of purchase. The minced chicken was mixed with 2% of spices (garlic, ginger and turmeric), 10^3 cfu/ml of *S. typhimurium* and 10^5 cfu/ml of LA-LC mixture. The samples were incubated at 37°C for 0, 1 and 2 days. For each incubation period, the sample was collected and determined for the survival of

Results and Discussion

Pars I. Determination of Bacterial and Culture Incubation Conditions

The incubation periods for the probiotics and *Salmonella typhimurium* was found to be 24 hrs at 37°C. Two techniques were used for inoculation: one in which the cells were transferred using a loop; and the second, pouring of the broth into the culture slants. It was found that the pouring method allowed more bacteria to be cultured at a lesser time. Therefore, 24 hours at 37C was sufficient time to generate 8 Log cycles (Table 2) and 5 Log cycles (Table 3) for LAB and *S. typhimurium* respectively were obtained after incubation. (See Appendix for data).

Table 2: Number (CFU/mL) of *Lactobacillus acidophilus* and *L. casei* incubated at 37°C for 24 and 48 hrs

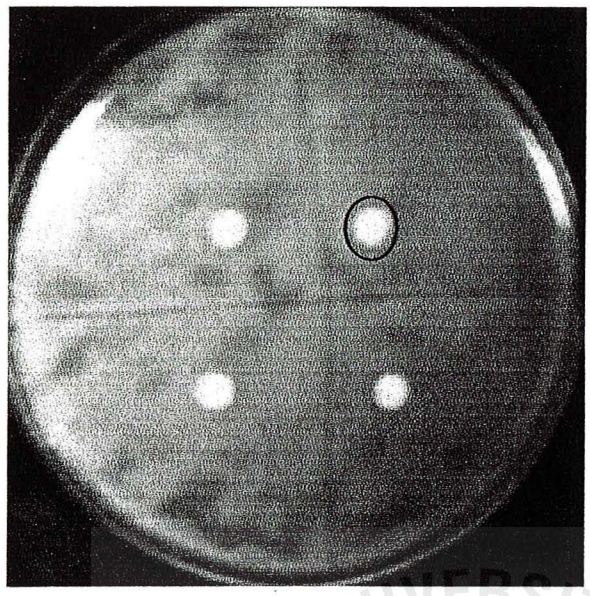
Microorganism	24 hrs (cfu/ml)	48 hrs
Lactobacillus acidophilus	$7.5x10^7$	TNTC ($>10^9$)
Lactobacillus casei	$1.715 x10^9$	TNTC ($>10^9$)

Table 3: Number (CFU/mL) of *Salmonella typhimurium* incubated at 37°C for 24 and 48 hrs

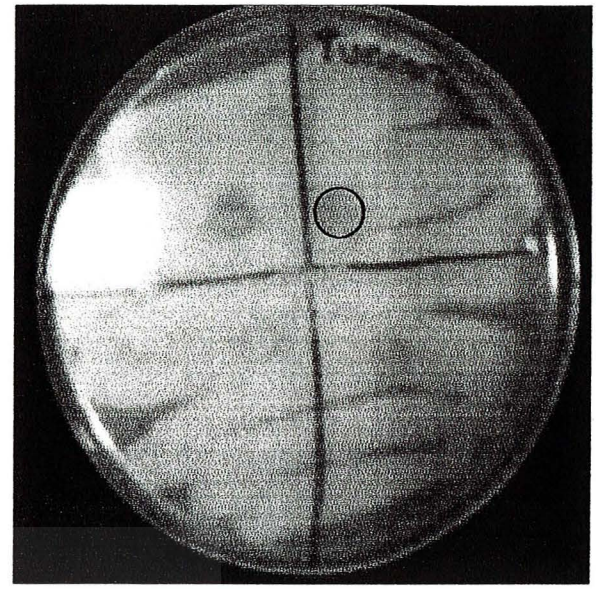
24 hrs (cfu/ml)	48 hrs
2.3×10^7	TNTC ($>10^9$)

Part III. Antimicrobial Assay activity of probiotic and spices using disc diffusion method

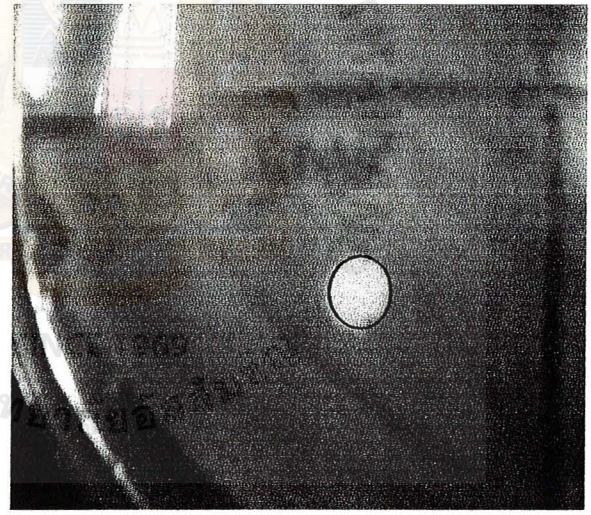
Agar diffusion assay was used to determine the antimicrobial activity of the spices and LAB. The following treatments were tested: *Lactobacillus acidophilus* (LA), *L. casei* (LC), LA & LC, garlic, ginger, black pepper, coriander seeds, and turmeric powder. The aforementioned spices were chosen because these are the most commonly used spices in Asian cooking (Katzer, 2006). The result has shown in Figure 3.



(a) Garlic

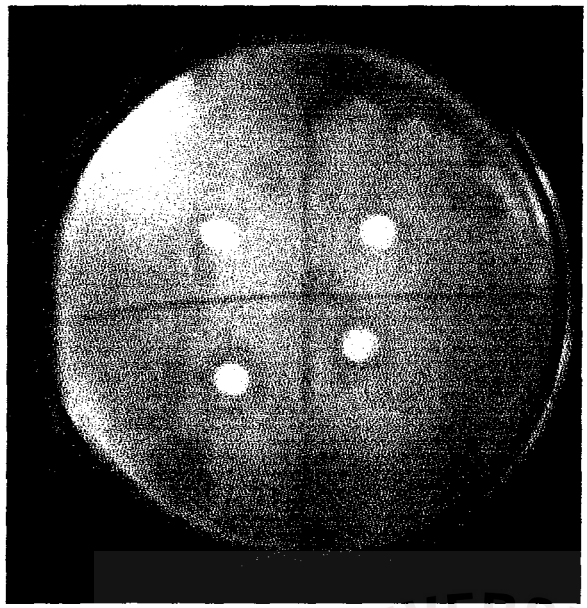


(b) Turmeric



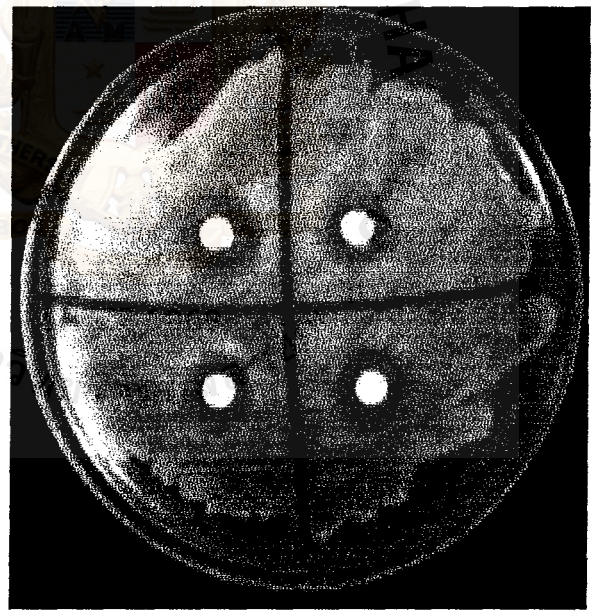
(c) Ginger

Figure 1: Agar Diffusion Assay of Spices. The figures show the antimicrobial activity of (a) garlic, (b) Turmeric, and (c) ginger.



(a) LC

(b) LA



(c)LA&LC Combined

Figure 2: Agar Diffusion Assay of Probiotics. The figure shows antimicrobial activity of (a) LA, (b) LC, and (c) LA & LC combined.

It was recognized that there were significant ($p>0.1$) differences among the probiotics and spices tested. The mixture of LA & LC provided the highest antimicrobial activity against *S. typhimurium* with the clear zone of 0.9 cm followed by LC, LA, turmeric, garlic and ginger, whereas, coriander seeds and pepper showed the lowest activities (approximately 0.55 cm). To study the synergistic effect of probiotic bacteria and spices on the antimicrobial activity in minced chicken, therefore, the mixture of LA & LC and three spices (garlic, ginger and turmeric) were chosen for the further experiment (Figure 3, see Appendix Table 4).

From doing statistical analysis, the treatments that showed most significant difference were LA&LC mixture, garlic, ginger, and turmeric powder. Figure 1 shows the degree of antimicrobial activity for each of the treatments. Therefore, LA&LC with each of these spices were used to test the raw infected minced chicken.

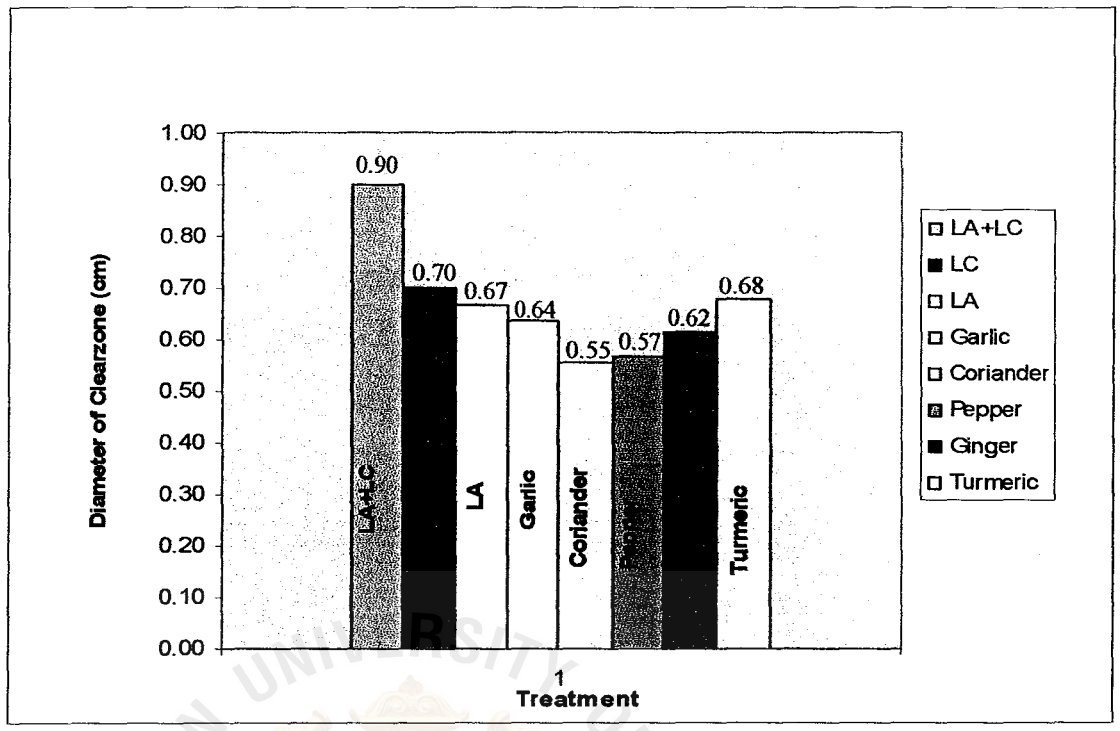


Figure 3: Degree of Antimicrobial Effect of Probiotics and Spices on *Salmonella typhimurium*

Part III. Antimicrobial activity of probiotic and spices in Test of Probiotics and Spices on Salmonella-infected minced chicken

The results for this part of the experiment were unexpected. The results showed that the number CFU of *S. typhimurium* were two times higher than the numbers that were mixed into the chicken initially. This means that the pathogen continued to grow without any major inhibition by the probiotics and/or spices even with large numbers of probiotics mixed or the raw minced chicken contained high load of *Salmonella sp.* (Table 2)

Table 4: Number of *Salmonella typhimurium* in minced chicken with probiotics mixture and incubated at 37°C for 0, 1 and 2 days.

Treatment	Number of microorganisms (cfu/g)		
	Day 0	Day 1	Day 2
Ginger	$>10^6$ EST	$>10^6$ EST	$>10^6$ EST
Turmeric	$>10^6$ EST	$>10^6$ EST	$>10^6$ EST
Galric	$>10^6$ EST	$>10^6$ EST	$>10^6$ EST

There are quite a few reasons as to why the pathogen was not inhibited by the probiotic. Tthe source of nutrients may not have been the ideal growth promoters for the probiotics. Lactic acid bacteria, especially, *L. acidophilus* and *L. casei* grow optimally in presence of carbohydrates, preferably lactose (Kumar, 2005). The probiotic cultures were grown in MRS broth and then transferred immediately to the minced chicken. So, its

source of nutrients changed from prominently a carbohydrate source to a protein source. Therefore, the cells would need longer time to adapt to the change in nutrients available and may result in a longer loag phase.

However, since the growth conditions arewere ideal suitable for *Salmonella*, its growth was probably not hindered in anyway because the antimicrobial agents of the probiotics arewere released only in the stationary phase of their growth cycle (Kumar, 2005). So, while the probiotics were adapting to the environment, the *Salmonella* was able to grow optimally to numbers so large that the spice alone could not inhibit its growth and the bacteriocins could not be produced in time to prevent its growth.

This is why for all three days of incubation, the same results were obtained.

It was suggested to add growth factor that stimulate or enhance the growth of probiotics in minced chicken that these organisms can produce enough bacteriocin to inhibit the growth of *S. typhimurium*.

What could also have been done was to prepare a culture medium that was similar in composition to the raw minced chicken composition. This could have prevented a long log phase for the probiotics. The amount of spices could not be increased as the chicken would become too spicy. Due to the lack of spice to stimulate growth of the probiotics, the spice could not contribute significantly to inhibiting the growth of the pathogen.

Conclusion

The mixture of *Lactobacillus acidophilus* and *L. casei* had the highest inhibitory effect against *Salmonella typhimurium* in *vivo* followed by the extract from some spices such as ginger, turmeric and garlic.

Although probiotic bacterial cultures and spices exhibited the antimicrobial activity against *S. typyimurium* in *vivo*, the combination of probiotics and spices has no effect on this microorganism in the minced chicken.

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Appendix

Part I. Determination of Probiotic and Salmonella typhimurium Incubation Period

Appendix Table 1: Number of CFU of LA and LC after 24 hour Incubation of culture on MRS Agar after 24 hours.

	$x10^6$	$x10^7$	$x10^8$
LA 1	7.5	12.5	0.0
LA2	241.0	37.5	8.0
Average	128	25	4
LC1	171.5	28.5	6.5
LC2	159.5	31.5	5.0
Average	165.5	30	5.75

After 48 hours the number of colonies was Too Numerous To Count and also the required CFU/mL was reached by 24 hours. To calculate CFU/mL for each probiotics the middle dilution result was used (10^7)

Calculation: CFU/mL = (number of colonies x dilution factor)/ 0.1 mL
(See Table 1 for results)

Appendix Table 2: CFU Number of *S. typhimurium* on Bismuth Sulphite Agar after 24 hour culture incubation period

	$x10^3$	$x10^4$	$x10^5$
Replication 1	11,8	3,0	0,20

Replication 2	8,25	89,4	26,0
Average	**13**	**32**	**23**

CFU/mL was calculated the same way as done so previously. Since the results for 10^5 dilution are more precise, these values were used to calculated CFU/mL.

Part II. Antimicrobial Activity of Probiotics and Spices

Appendix Table 3: Diameter of Clear Zones (cm) ofor antimicrobial activity test of probiotics and spices each treatment.using disc diffusion technique

Trial 1					
	1	**2**	**3**	**4**	**Average**
La+LC	0.900	0.600	0.700	0.800	0.750
LC	1.500	1.100	1.600	1.200	1.350
LA	1.300	0.800	0.800	0.700	0.900
Garlic	0.600	0.600	0.600	0.600	0.600
Coriander	0.500	0.600	0.500	0.500	0.525
Pepper	0.500	0.500	0.700	0.500	0.550
Ginger	0.500	0.600	0.600	0.500	0.550
Turmeric	0.700	0.600	0.600	0.600	0.625
Trial 2					
	1	**2**	**3**	**4**	**Average**
LA+LC	0.700	0.600	0.600	0.600	0.625
LC	0.800	0.600	0.700	0.700	0.700

LA	0.700	0.700	0.800	0.500	0.675
Garlic	0.65	0.6	0.65	0.6	0.625
Coriander	0.55	0.5	0.55	0.55	0.5375
Pepper	0.500	0.700	0.500	0.500	0.550
Ginger	0.600	0.600	0.600	0.600	0.600
Turmeric	0.700	0.600	0.600	0.600	0.625

Trial 3

	1	2	3	4	Average
LA+LC	0.600	0.500	0	0	0.550
LC	0.800	0.600	0.700	0.700	0.700
LA	1.100	0.700	0.800	0.500	0.775
Garlic	0.600	0.600	0.600	0.600	0.600
Coriander	0.5	0.5	0.5	0.5	0.5
Pepper	1.400	1.100	0.800	0.800	1.025
Ginger	0.600	0.600	0.600	0.600	0.600
Turmeric	0.65	0.7	0.7	0.7	0.6875

Trial 4

	1	2	3	4	Average
LA+LC	0.800	0.600	0.700	0.700	0.700
LC	0.700	0.900	0.700	0.700	0.750
LA	0.600	0.600	0.800	0.700	0.675
Garlic	0.600	0.600	0.600	0.600	0.600
	0.600	0.600	0.600	0.600	
Coriander	0.700	0.700	0.600	0.600	0.650

	0.600	0.600	0.700	0.700	
Pepper	0.600	0.600	0.600	0.600	0.600
Ginger	0.600	0.600	0.600	0.600	0.650
	0.600	0.600	0.600	0.600	
Turmeric	0.700	0.700	0.700	0.800	0.700
	0.700	0.650	0.700	0.650	

Trial 5

	1	**2**	**3**	**4**	Average
LA+LC	1.300	0.900	1.100	1.100	1.100
LC	0.600	0.600	0.600	0.800	0.650
LA	0.600	0.650	0.700	0.650	0.650
Garlic	0.600	0.700	0.700	0.600	0.688
	0.800	0.800	0.600	0.700	
Coriander	0.600	0.600	0.600	0.600	0.600
	0.600	0.600	0.700	0.600	
Pepper	0.600	0.600	0.600	0.600	0.600
	0.600	0.600	0.600	0.600	
Ginger	0.600	0.600	0.600	0.600	0.600
	0.600	0.600	0.600	0.600	
Turmeric	0.650	0.650	0.650	0.750	0.713
	0.600	0.800	0.800	0.800	

Appendix Table 4: Average Diameter of Clear Zones for Each of the Treatments (Raw Data for Figure 1)

	Treatment							
Replication	**LA+LC**	**LC**	**LA**	**Garlic**	**Coriander**	**Pepper**	**Ginger**	**Turmeri**
1	0.9	0.7	0.675	0.625	0.525	0.55	0.6	0.625
2	0.7	0.75	0.675	0.6	0.538	0.55	0.65	0.7
3	1.1	0.65	0.65	0.688	0.6	0.6	0.6	0.713
Average	0.90	0.70	0.67	0.64	0.55	0.57	0.62	0.68

Appendix Table 5: Statistical analysis to determine which treatment had most significant antimicrobial propertyantimicrobial testing of probiotics and spices using disc diffusion technique

Anova: Single Factor

SUMMARY

Groups	*Treatment*	*Count*	*Sum*	*Average*	*Variance*
Column 1	LA+LC	3	2.700	0.900	0.0400
Column 2	LC	3	2.100	0.700	0.0025
Column 3	LA	3	2.000	0.667	0.0002
Column 4	Garlic	3	1.913	0.638	0.0021
Column 5	Coriander	3	1.663	0.554	0.0016
Column 6	Pepper	3	1.700	0.567	0.0008
Column 7	Ginger	3	1.850	0.617	0.0008
Column 8	Turmeric	3	2.0380	0.6793	0.0023

ANOVA Table

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.2449	7	0.0349	5.5667	0.0022	2.6572
Within Groups	0.1006	16	0.0063			
Total	0.3456	23				

Multiple Comparison of Means

Part III. Antimicrobial Activity of LAB and spice mixture on Salmonella-infected Chicken

Appendix Table 6: CFU Number of *S. typhimurium* after sampling fromin minced chicken at 0, 1, and 2 days of incubation at 37°C.

Day 0 **Replication**

SS Agar	1	2	3
Garlic	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Ginger	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Turmuric	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Raw (control)	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
BSA	1	2	3
Garlic	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Ginger	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Turmuric	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Raw	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
BGA	1	2	3
Garlic	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Ginger	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Turmuric	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Raw	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU

Day 1

SS Agar	1	2	3
Garlic	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Ginger	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Turmuric	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
BSA	1	2	3
Garlic	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Ginger	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Turmuric	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
BGA	1	2	3
Garlic	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Ginger	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Turmuric	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU

Day 2

SS Agar	1	2	3
Garlic	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Ginger	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Turmuric	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
BSA	1	2	3

Garlic	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Ginger	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Turmuric	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
BGA	1	2	3
Garlic	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Ginger	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Turmuric	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU
Turmuric	$>10^6$ CFU	$>10^6$ CFU	$>10^6$ CFU