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In Vitro And In Vivo Studies On Survival Of Some Probiotic Bacteria In Gastrointestinal Tract

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Abstract: The potential probiotic characteristics of ***L. acidophilus*** ATCC 4356, ***L. johnsonii*** ATCC33200 and ***B. bifidum*** ATCC2203 were studied, with regard to acid / bile salt tolerance and survival in simulated gastric and intestinal juices. Furthermore, an in vivo feeding experimental was adopted to estimate the transit tolerance of potentially probiotics in the gastrointestinal tract of Japanese quail chicks and their antagonistic effect against some harmful organism (***E .coli***).

Results of in vitro experiments revealed that ***L. johnsonii*** was the most acid tolerant, and possessed the highest figures for viable cell counts, actually 7.61 and 7.88 log cfu / ml after 180 min. of incubation at pH 2.0 and 3.0respectiely. Also, it could be noticed that ***B.bifidum*** ATCC 2203 survived better than ***L. acidophilus*** ATCC 4356, either at pH 2.0 or 3.0.

Moreover, ***L.acidophilus*** ATCC 4356 was selected as the highest bile tolerance at 0.3 % (w/v) bile concentration, while at 0.5 and 1.0 (w/v) bile, ***L. johnsonii*** ATCC33200 was more resistance. In contrast, ***B. bifidum*** ATCC2203 was the most sensitive strain at different tested bile salt concentrations, and exhibited great reduction in its viable counts after 180 min. of incubation, being 1.95, 2.07 and 2.81 Log cfu / ml at 0.3, 0.5 and 1 % (w/v) bile concentrations, respectively.

Furthermore, ***L. johnsonii*** ATCC33200 was the most tolerance strain to either gastric or intestinal juices, followed by ***B. bifidum*** ATCC2203, the gave survival % of 100 % and 98%.45%, respectively after 60 min of exposure to gastric juice, while the corresponding figures were 102.19 % and 100 % after 360 min. of exposure to intestinal juice. Contrarily, ***L .acidophilus*** ATCC 4356 ranked slight decreases of 0.32 and 013 log cycles after 60 and 360 min. of exposure to gastric and intestinal juices, respectively.

The results of in vivo feeding experiment declared that the count of ***L .acidophilus*** ATCC 4356 recovered from the small intestine of Japanese quail chicks decreased by 1.23 log cycle after 2h of feeding,

while ***B. bifidum*** ATCC2203 showed less decrease being 1.09 log cycle. However, both tested strain were successfully transit through gastrointestinal tract with viable cell count attained 107cfu/g.

In order to investigate the antagonistic effect of both potential probiotic strains against ***E. coli***, three trails were conducted. Results obtained revealed that birds receiving basal diet for 5 days, led to increase in the count of ***E.coli*** by 3.87 log cycles and attained 7.75 log cfu/g in feces samples. While, feeding on traditional yoghurt, reduce the count of ***E.coli*** to 5.90 log cfu/g. Moreover, the highest reduction in ***E.coli*** counts, being 5.03 log cycles was detected in samples of birds administered acidophilus – bifidus – yoghurt for 5 days. This statement may be ascribed to antagonistic behavior of ***B. bifidum*** and ***L. acidophlus.***

Introduction

Probiotic have been recently defined as " live microorganisms administered in adequate amounts which confer a beneficial health effect on the host (FAW/ WHO, 2001). Indeed, probiotics have been reported to play therapeutic roles by modulating immunity , lowering cholesterol, improving lactose tolerance and preventing cancer (Kailasapathy and Chin, 2000).Therefore, therapeutic benefits have been led to an increase in the incorporation of probiotic bacteria such as ***lactobacilli*** and ***bifidobacteria*** in dairy products, especially youghrt (Lourens – Hattingh and Viljoen, 2001). In order to for these bacteria to exert positive health effects, thy have to reach their site of action alive and establish themselves in certain numbers (10^7 cfu/g). However, studies indicate that the bacteria may not survive in high enough numbers when incorporated into dairy products (Beal et al., 1999 and Gardini et al., 1999). Therefore, the important of in vitro selection criterion for probiotic microorganisms must be considerable. However, be considerable the majority of probiotic bacteria belong to two bacterial genera i.e. ***Lactobacillus*** and ***Bifidobacterium***. To be considered as probiotics, these bacteria should become a part of the normal microbial in the intestine, survive the gastrointestinal passage and be able to adhere and colonize the intestinal tract (Havennaar et al., 1992). Therefore, a stringent selection criteria for identification of probiotic strains is required in order to achieve consistent and positive probiotic effects. Recently, Collins et al., (1998) have compiled a list of 12 important criteria for selecting a potential probiotic strain. Essentially, these criteria suggested that the selected strains must be able to tolerance low pH, high bile concentration and survive gastric and intestinal juices.

Further, the survival of probiotic bacteria through their passage in the gastrointestinal tract is questionable. Many reports indicated that

there were poor survival of probiotic bacteria during transit through high acidic conditions of stomach and enzymes and bile salts in the small intestine. In this connection, Marteau et al, (1993) stated that the maximal survival rate of lactobacilli through the human n gastrointestinal tract has been estimated to be between 2 and 5 %, while, Bquhink et al., (1992) recorded a higher average for survival rate of bifidobacteria, actually 30 %.

Moreover, human gastrointestinal tract especially the large intestine is major colonization site of bacterial, viral and parasitic pathogens. e.g. Salmonella, Listeria and certain strains of ***E. coli***. However, probiotic can be used to balance or prevent such disturbances Salminen et al., (1998).

Although resistance to human gastric transit has been demonstrated in vitro for potentially probiotic bacteria and constitutes an important in vitro selection criterion for probiotic microorganisms a satisfactory studies in vivo gastric transit has not been carried out.

Therefore, the objective of the current investigation was to focus on two aspects: I- An in vitro studies on three tested ***L .acidophluus, L. johsonii*** and ***B. bifidum*** strains, for their tolerance to low pH, high bile concentrations and survival gastric and intestinal juices were followed. II. To evaluate – in vivo – the survival of ***L. acidophluus***, and ***B. bifidum*** through their passage in the gastrointestinal tract of Japanese quail chick. Also, another target was to study the antagonistic effect of probiotics against ***E.coli*** bacteria in gastrointestinal tract.

Materials And Methods

1. Milk: Fresh whole buffalo's milk was obtained from the herd of Mostorod experimental from Faculty of Agriculture, Al-Azhar University.
2. Bacterial cultures: ***L. acidophilus*** ATCC 4356 , ***B. bifodum*** ATCC 2203 and ***L. johnsonii*** ATCC 33200 were secured from American type culture collection , Manassas. While, ***L. delbrueckii subsp. Bulgaricus*** EMCC 11102 and ***S. thermophilus*** EMCC 11044 were obtained from Egyptian Microbial culture collection (EMCC) at Cairo Microbiological Resources center (Cairo MIRCEN), Faculty of Agriculture, Ain shams University. Bacterial cultures were propagated daily in 10% sterilized skim milk maintained in M-17 broth (Terzaghi and Sandine,1975) and stored at 4 ºC until used.
3. Birds (Japanese quail chicks): six male Japanese quail chicks, 6 weeks old, with mean body weights of 185.45±0.4 gm., and obtained from Poultry experimental station, Faculty of Agriculture, Al-Azhar University, were used in the present investigation.

4. Basal diet: the chemical composition of basal or control diet was as follows: ground yellow corn, 60.8%, soybean meal, 20.7%, corn gluten meal,10.8%, Dicalcium phosphate, 1.17%, Limestone, 5.76% , premix. 0.3%, NaCl, 0.3% and L-Lysine HCL,0.17%.
5. Acid tolerance: the tested strains were evaluated for their ability to grow in low pH values (2&3) according to Pereira and Gibson (2002) with some modifications: An overnight culture was inoculated into MRS broth previously adjusted to pH 2 or 3 with sterilized HCL. The mixtures were incubated at 37C for 3 h., one-milliter samples were taken at various times (0,60,120 and 180 min.), serially diluted , plated in triplicate onto MRS agar and the plates were incubated at 37C for under anaerobic conditions.

6 Bile tolerance: Overnight cultures were inoculated (1% vol/vol.) into m-MRS broth and m-MRS containing 0.3, 0.5 and 1.0% (wt/vol) oxagel and incubated anaerobically at 37°C for 4 h. Cultures were monitored at 0,60,120 and 180 min. by using general plate count technique. The plates were incubated under anaerobic conditions for 48h. at 37 °C.

7. Tolerance to gastric and pancreatic juices : gastric and pancreatic juices were prepared fresh by dissolving pepsin (sigma)from porcine stomach mucosa (3g/L) and pancreatin (sigma) from porcine pancreas (1g/L) in sterile saline (5g/L) according to Charteris et al.(1998). Subsequently, the pHs of the gastric and pancreatic preparation were, respectively, adjusted to 2.0 and 8.0 with 12M HCL or 0.1M NaOH .

The Tolerance of three tested strains to simulate gastric (SGJ) and small intestinal juices (SIJ) was determined as follows: mixing 0.2 ml of each washed cell suspensions (A1-ml aliquot of each culture was centrifuged at 5000x g for 5 min and washed three times in phosphate- buffered saline, pH 7.0) with 1.0 ml of gastric (pH 2.0) or intestinal juices (pH 8.0). After brief vortexing the mixtures were incubated at 37ºC. When assaying gastric transit tolerance aliquots of 0.1ml were removed after 60,120 and 180 min for determination of total viable count. When assaying for small intestinal transit tolerance, the sampling times were 60,240 and 360 min. the experiment was repeated twice.

8. Preparation of Acidophilus- Bifidus -Yoghurt: the fresh standardized buffalo's milk (3.0% fat) was heated to 90± 1.0C for 15 min., rapidly cooled to 42 C, inoculated with mixture of yoghurt culture, ***L. acidophilus*** and ***B.bifidum*** (1:2:1.5) at ratio of active starter culture (6.0%) as described by Sezgin et al (1996). Inoculated milk was equally distributed into plastic cups, incubated at 42 C until complete coagulation and then ket in the refrigerator at 6 ±2.0°C.

9. Feeding experiment: the Japanese quail chicks were randomly and equally divided into four groups, three birds each. The electrical batteries were used in brooding of birds and equipped with water. The temperature degree and humidity percentages were daily measured and recorded approximately 17 ±2.0C and 40±3.0% as averages at the experimental period. Before feeding the birds were acclimatized on basal diet for one week. At the end of adaptation period (7 days), the birds were starved for 18 hours. The feeding was by injection in the mouth daily 25g for each bird and the, water were offered ad libitum for birds during the experimental period.

The first group was fed on 15g basal diet plus 10g acidophilus-Bifidus-Yoghurt (ABY), after two hours of feeding, the chicks were slaughtered , defeathered , evacualted and taking the intestine for ***L. acidophilus*** and ***B.bifidum*** enumeration.

The remaining three groups were feeding for 7 days, faeces from birds were collected daily in sterile Petri dishes and analysed for ***E.coli*** counts at the end of feeding period, thus , the second group was given 25g basal diet and served as control I, while the third group was received 15g basal diet plus 10g traditional yoghurt (1:1 ***S. thermophilus*** EMCC 11044 and ***L. delbrueckii subsp bulgaricus*** EMCC 11102) and served as control II. The fourth group was offered 15g basal diet + 10g ABY.

10. Microbiological analysis: For selective enumeration of ***L. acidophilus*** MRS- salicin medium was used. While, modified MRS (m-MRS) medium consisted of MRS medium supplemented with 0.5 g/L. L-cystein HCL and 3.0g/L. Lithium chloride, was used for ***B.bifidum*** enumeration. The plates were incubated anaerobically at 37 C for 48h. For feces analysis, 1.0g portion of wet feces taken from the interior of the stool samples of birds was added to 99 ml dilution blank containing 1.0% peptone. For ***E.Coli*** count, violet red bile agar (VRBA) suggested by Misra and Kuila (1994b) was adopted.

Results And Discussion

1- In vitro studies on some probiotic criteria:

The majority of probiotic bacteria belong to two bacterial genera i.e ***Lactobacillus*** and ***Bifidobacterium***. To be considered as probiotic , these bacteria should become a part of the normal microbial flora in the intestine, survive the gastrointestinal passage and be able adhee and colonize the intestinal tact (Havenarr et al., 1992). The gastrointestinal tract of healthy human is a harsh environment because it contains gastric juices, digestive enzymes and bile acids.Thes conditions impose a significant threat to probiotic strains.

Therefore, a stringent selection criteria for identification of probiotic strains is required in order to achieve consistent and positive

probiotic effect. Essentially, these criteria suggested that the selected strains must be able to tolerance low pH, high bile concentrations, survive gastric and intestinal juices.

1.1. Acid tolerance:

Bacteria used as probiotic adjuncts are commonly delivered in a food system and therefor being their journey to the lower intestinal tract via the mouth. In this respect, Berradanet al ., (1991) reported the time from entrance to release from the stomach to be 90 min. Although, cellular stress begins in the stomach, which has pH as low as 1.5 (Lankaputhra and shah,1995) in most in vitro assays pH 2.0 and 3.0 has been preferred (Siskovic et al., 1997 and Garriga et al., 1998).

Therefore, three tested ***L.acidophluus, L. johnsinii*** and ***B. bifidum*** strains were examined for their tolerance to low pH (2.0 and3.0) in MRS broth data obtained were summarized in Table 1 and graphically presented in Figare 1.

It could be noticed from the obtained results that pH 2.0 seemed to be more damaging to the test strains. In this connection. Maffei and Nobrega (1975) stated that the bactericidal effect of acid is evident at pH values below 2.5.

Also, it might be gathered that ***L.johnsinii*** ATCC 33200 was the most acid tolerance and possessed the highest value for viable cell count, actually 7.61 log cfu/ml after 180 min. of incubation at pH 2.0. Moreover, it was of interest to notice that after 180 min. of exposure to low pH, more than 97% and 99% of the tested ***L.johnsonii*** cells remained viable at pH 2.0 and 3.0, respectively (Fig.1).

In contrast, ***L.acidophilus*** ATCC 4356 was the most acid sensitive and strongly affected at pH 2.0, where the strain showed great reduce in viable count, actually 2.95 log cycle and exhibited the lowest population, peing 5.00 log cfu/ml, after 180 min of incubation. Furthermore. Tested ***L.acidophlus*** strain ranked the highest growth inhibition %, being 37.11 followed by ***B.bifidume*** ATCC 2203, actually 13.41 % after exposure to pH 2.0 for 180 min.

Continuously, from the foregoing results, it could be stated the ***B.bifidum*** ATCC 2203, strain survived better than ***L. acidophilus*** ATCC 4356 culture. The same finding was previously by Vinderola et al., (2000).

1.2. Bile tolerance:

Once the bacteria reach the intestinal tract, bile entering the duodenal section of small intestine has been found to reduce survival of bacteria. Bile salts are surface- active chemical produced in the liver from

The catabolism of cholesterol (Brandt and Bernstein, 1972). Therefore, probiotics must have an ability to tolerance bile (Kimoto et al., 2000).

Although, the bile concentration of the human gastrointestinal tract varies, the mean intestinal bile concentration is believed to be 0.3 % w/v. (Suskovic et al., 1997 and Garriga et al., 1998).

Therefore, this part of study was conducted on in vitro experiment to screen the three tested strains for their ability to tolerate bile concentration of 0.3 , 0.5 and 1.0 % (w/v)to mimic approximate levels in the intestinal tract, results obtained plotted in Table 2 and Figure2.

As shown from data obtained, ***L.acidophilus*** ATCC 4356 was selected as the highest bile tolerance strain and possessed the least growth inhibition % varied from 0.39 % to 6.98 % when incubation time extended from 60 to 180 min at 0.3% (w/v) bile concentration. In this respect. Oh et al., (2000) stated that ***L.acidophilus*** was capable of surviving in the presence of bile due to its ability to deconjugate bile acids.

Continuously, ***L.johnsonii*** ATTCC 33200 showed relatively high tolerance at 0.5 and 1.0 % (w/v) bile concentration and able to maintain 86.7 % and 84.2 % viability after 180 min. of incubation at 0.5 and 1.0 % (w/v) bile, respectively. In general, our finding confirms the results previously reported by Oh et al., (2000) and Mathara et al., (2008).

In contrast, ***B.bifidum*** ATCC 2203 was the most sensitive strain at different tested bile salts concentrations and exhibited great reduction in its viable count after 180 min. of incubation, being 1.95, 2.07 and 2.81 log cycles at 0.3,0.5 and 1.0 % (w/v) bile concentrations, respectively.

1.3.Tolerance to simulated gastric juice.

About 2.5L of gastric juice is secreted each day having a pH of approximately 2.0 and a salt content of not less than 0.5% (w/v) (Hill, 1990). Thus, the effect of simulated gastric juice on the viability of the three tested bacteria was assessed. Results obtained for tolerant gastric juice are shown in Table (3) and Figure (3).

From these results, it could be noticed that gastric juice exerted a strain exhibited a slight influence on the growth of tested cultures. In general, ***B.bifidum*** strain exhibited more gastric juice resistance than ***L. acidophilus*** culture.

Moreover, it might be gathered from data obtained that the most tolerance strain to gastric juice was ***L.johnsonii*** ATT 33200 fowled by ***B.bifidum*** ATCC 2203 , they gave survival % of 100 % and 98.45 % respectively, after 60 min. of exposure, which is the time required for stomach content to empty nearly completely. On contrary, he most

sensitive culture to gastric juce was ***L. acidophilus*** ATCC 4356, which possessed the highest growth inhibition %, actually 4.38 %, under the same previous conditions. This finding confirms the results of Mathara et al., (2008) they stated that tolerance to gastric transit was observed among strains showed much higher acid tolerance.

From the foregoing results, it could be mentioned that, although a slight reduction in the viable cell numbers for all tested strains was detected after exposure to gastric juice, the three tested culture exhibited acceptable levels of survivability under these conditions since at least 5.5 x 106 cfu/ml (in case of ***L acidophilus*** ATCC 4356) survived after 180 min of exposure. In this regard, Guerra et al., (2005) considered ***Enterococcus faecium*** CECT 410 strain intrinsically tolerant to gastric juice, since at least 2x106 cfu/ml survived after 180 min. of exposure. Finally, our present results revealed that all tested cultures were considered intrinsically tolerant to gastric juice and could be successfully transit the stomach and reaching the intestinal tract and functioning effectively there.

1.4. Tolerance to simulated intestinal juice:

About 0.7 l. of pancreatic juice is secreted into the proximal intestine each day having a pH of about 8.0 and a salt content of not less than 0.5% w/v. (Keele and Neil, 1965). This secretion present a pH and enzymatic barrier to the survival of ingested microorganisms during digestion. Therefore, the effect of simulated intestine juice on the viability of tested cultures were carried out, and results obtained illustrated in Table 4.

As shown from data obtained, ***L. johnsonii*** ATCC 33200 retained viability during growth in simulated small intestine juice and is considered intrinsically tolerant to intestinal juice. Contrarily, ***L. acidophilus*** ATCC4356 showed growth inhibition % of 1.75 % after incubation for 360 min. in simulated intestinal juice. In this respect, Kim et al. (2008) reported that the viability of ***L. acidophilus*** ATCC 43121 decreased by 12.16% after 24h of exposure to artificial intestinal juice.

Also, it is noteworthy from the same table that ***L. johnsonii*** ATCC 33200 was markedly better with regard to intestinal juice tolerance, followed by ***B. bifidum*** ATCC2203. From these results, it could be noticed that counts of ***L. johnsonii*** ATCC 33200 increased by 0, 0.73 and 2.19% after 60,240 and 360 min of exposure, respectively. While, ***L. acidophilus*** ATCC 4356 exhibited 0.03, 0.11 and 0.13 log cycle decreases in viability under the same previous conditions.

In general, from the previous results, it could be concluded that all tested cultures exhibited acceptable levels of survivability ranged from 98.25% to 102.19% after 360min. of exposure to intestinal juice.

This statement meant that these cultures were considered tolerant to intestinal juice and could be successfully functioning effectively in the intestinal tract.

2. In vivo feeding experiment:

In order to estimate the amount of ingested bacteria surviving transit through the gastrointestinal tract of Japanese quail chicks., feeding experimental was carried out. ***L. acidophilus*** ATCC 4356 and ***B. bifidum*** ATCC 2203 strains were incorporated in the manufacture of yoghurt, resulted in new cultured milk designated acidophilus-bifidus – yoghurt, which ingested in the tested chicks.

From results presented from Table 4, it could be gathered that after two hours of feeding ***L. acidophilus*** ATCC 4356 count recovered from the small intestine showed noticeable decreased, actually 1.23 log cycle, where its count reduced from 4.91 log cfu/g to 3.68 log cfu/g. While, ***B. bifidum*** ATCC 2203 population showed less decreased, actually 1.09 log cycle, in which the viable ingested count reduced from 5.83 log cfu/g to 4.74 log cfu/g when recovered after 2 h. from the small intestine. In this connection, Abou Dawood (2002) reported that ***B. bifidum*** count decreased by 2 log cycle when recovered from the small intestine after 2h of feeding Kareish cheese. However, our finding suggest that the tested ***L. acidophilus*** ATCC 4356 was more sensitive to the harsh environment of the gastrointestinal tract as compared with ***B. bifidum*** ATCC 2203 culture.

Moreover, results obtained revealed that the survival% of ***L. acidophilus*** and ***B. bifidum*** strains during transit through gastrointestinal tract were 74.95% and 81.30% respectively. However, Abou Dawood (2002) gave close figure for survival % of ***B. bifidum***, being 71.43% In contrast, Bquhnik et al (1992) gave a lower value for survival rate of bifidobacteria through gastrointestinal tract of adults, being about 30%. In addition, Marteau et al (1993) reported that the maximal survival rate of lactobacilli during transit through gastrointestinal tract, varied between 2 and 5%.

Our previous results strongly the important of in vitro selection criterion for probiotic microorganisms. Also, it was evident from the obtained data that ***L. acidophilus*** ATCC 4356 and ***B. bifidum*** ATCC 2203were successfully transit through gastrointestinal tract and they have ability to reach , survive and persist in the environmental in which they were tended to act also, their populations attained about 107 cfu/g. in order to explain this statement Charteris et al (1998) stated that milk proteins may function as both buffering agent and inhibitors of digestive protease activity in vivo, therapy protecting ingested bacterial strains during gastrointestinal transit.

The intestinal micro floracomprise a complex ecosystem of a large variety of bacteria. These complex flora can produce negative and positive effects, and altering the intestinal microbiota in a beneficial way can improve the health of a host. Probiotics have been considered potentially useful in this respect. When consumed the transiting Probiotics in the gastrointestinal tract are capable of delivering enzymes and other substances into the intestine which possibly help to control intestinal flora (Ayebo et al.,1980).

3. Antagonistic effect of probiotics against *E.coli*

The present study explores the potential of ***L. acidophilus*** ATCC 4356 and ***B. bifidum*** ATCC 2203 in inhibiting E. coil strains. The two selected strains were administered to japanese quail chicks to evaluate the immediate effect in controlling the intestinal microflora of the host birds. However, the counts of ***E.coli*** in the feces samples are graphically plotted in Figure 5. As shown from this result, in the first experimental the administration of basal diet for 5 days led to increase in the count of ***E.coli*** in feces by about 3.87 log cycles as compared with the initial count in basal diet being, 3.88 log cfu/g. in the second trail, when birds fed on traditional yoghurt for 5 days , the results obtained showed noticeable effect, where the count of ***E.coli*** reduced by 1.85 log cycles, decline in ***E.coli*** counts in the fecec of birds may be attributed to antagonistic effect of yogurt cultures against coliforms (Aslim et al., 2000).

Subsequently , the highest reduction in ***E.coli*** counts in feces samples, being 35.1% after5 days of proving acidophilus-bifidouls-yoghurt, where the counts decreased by about 5.03 log cycles. This finding may be ascribed to antagonistic behavior of ***B. bifidum*** (Misra and Kuila, 1994b) and ***L. acidophilus*** (Misra et al ., 1997).

Table (1) Effect of low pH values on viability of tested strains.

Tested strains	pH	Incubation time (min)						
		Zero	60		120		180	
		Log cfu/ml	Log cfu/ml	G inh (%)	Log cfu/ml	G inh (%)	Log cfu/ml	G inh (%)
L.acidophilus ATCC 4356	2	7.95	7.26	8.68	6.64	16.48	5.00	37.11
	3	7.48	7.00	6.42	6.89	7.89	6.70	10.40
L.johnsonii ATCC33200	2	7.79	7.76	0.39	7.70	1.16	7.61	2.31
	3	7.90	7.90	0.00	7.89	0.13	7.88	0.21
B.bifidum ATCC2203	2	7.83	7.74	1.15	7.45	4.85	6.78	13.40
	3	7.54	7.52	0.27	7.52	0.27	7.48	0.80

cfu/ml = Colony forming unit / ml

G inh = Growth inhibition precent

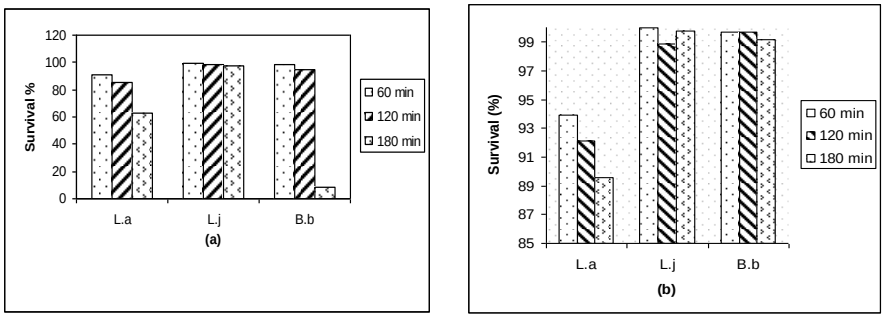
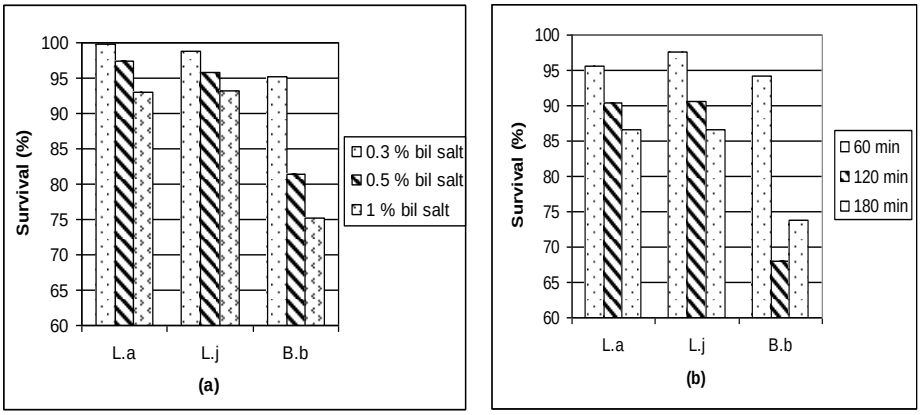


Fig. (1) Survival (%) of tested strain at pH 2 (a) and pH3 (b)
La= *L.acidophilus* Lj= *L.johnsonii* Bb= *B.bifidum*

Table (2) Effect of different bile salt concentration on viability of tested strains.

Tested strains	Bile Salt Con	Incubation time (min) Zero Log cfu/ml	60 Log cfu/ml	60 G inh (%)	120 Log cfu/ml	120 G inh (%)	180 Log cfu/ml	180 G inh (%)
L.acidophilus ATCC 4356	0.3	7.74	7.71	0.39	7.54	2.58	7.20	6.98
	0.5	7.68	7.34	4.43	6.94	9.64	6.65	13.41
	1.0	7.87	7.11	9.66	6.55	16.77	5.85	25.67
L.johnsonii ATCC33200	0.3	7.08	7.00	1.13	6.78	4.24	6.60	6.78
	0.5	6.98	6.81	2.44	6.33	9.31	6.05	13.32
	1.0	7.04	6.87	2.41	6.18	12.22	5.93	15.77
B.bifidum ATCC2203	0.3	7.86	7.48	4.83	6.40	18.58	5.91	24.81
	0.5	7.88	7.43	5.71	6.15	21.95	5.81	26.27
	1.0	7.85	7.08	9.81	5.81	25.99	5.04	35.80

Cfu/ml = Colony forming unit / ml G inh = Growth inhibition percent



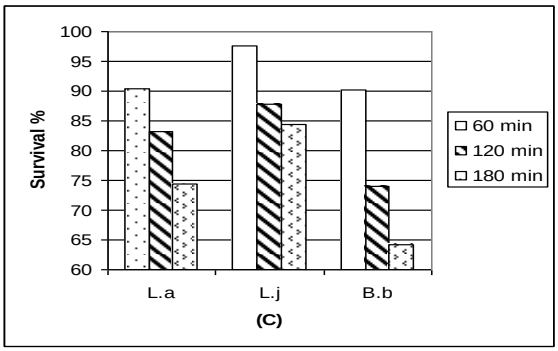


Fig. (2) Survival (%) of tested cells at 0.3 % (a) , 0.5 (b) and 1 % bile salt concentration
La= *L.acidophilus* Lj= *L.johnsonii* Bb= *B.bifidum*

Table (3) Effect of simulated gastric juice on viability of tested strains.

Tested strains	Incubation time (min)						
	Zero	60		120		180	
	Log cfu/ml	Log cfu/ml	G inh (%)	Log cfu/ml	G inh (%)	Log cfu/ml	G inh (%)
L.acidophilus ATCC 4356	7.30	6.98	4.38	6.90	5.48	6.74	7.67
L.johnsonii ATCC33200	6.98	6.98	0.00	6.95	0.43	6.90	1.15
B.bifidum ATCC2203	7.74	7.62	1.55	7.45	3.75	7.20	6.98

cfu/ml = Colony forming unit / ml G inh = Growth inhibition present

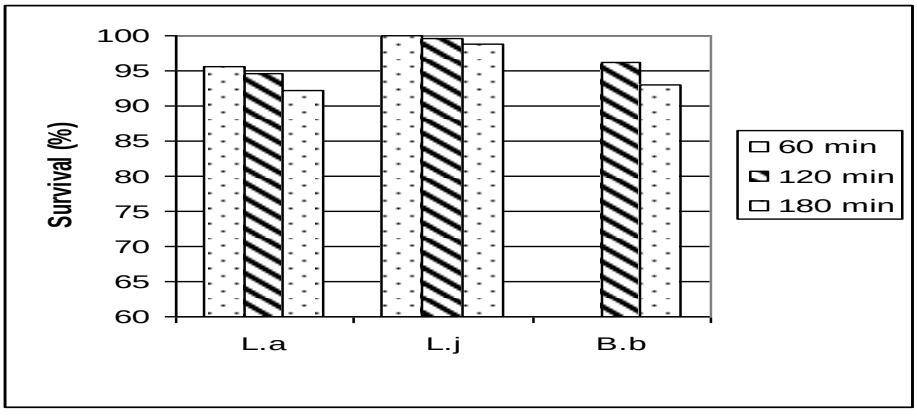


Fig. 3) Survival of simulated gastric juice on viability of tested strains.
La= *L.acidophilus* Lj= *L.johnsonii* Bb= *B.bifidum*

Table (4) Effect of simulated intestinal juice on viability of tested strains.

Tested strains	Incubation time (min)						
	Zero	60		240		360	
	Log cfu/ml	Log cfu/ml	G inh (%)	Log cfu/ml	G inh (%)	Log cfu/ml	G inh (%)
L.acidophilus ATCC 4356	7.41	7.38	(-) 0.40	7.30	(-) 1.48	7.28	(-) 1.75
L.johnsonii ATCC33200	6.85	6.85	0.00	6.90	(+) 100.73	7.00	(+) 102.19
B.bifidum ATCC2203	7.30	7.34	(+) 100.55	7.23	(-) 0.96	7.30	0.00

Cfu/ml = Colony forming unit / ml G inh = Growth inhibition percent

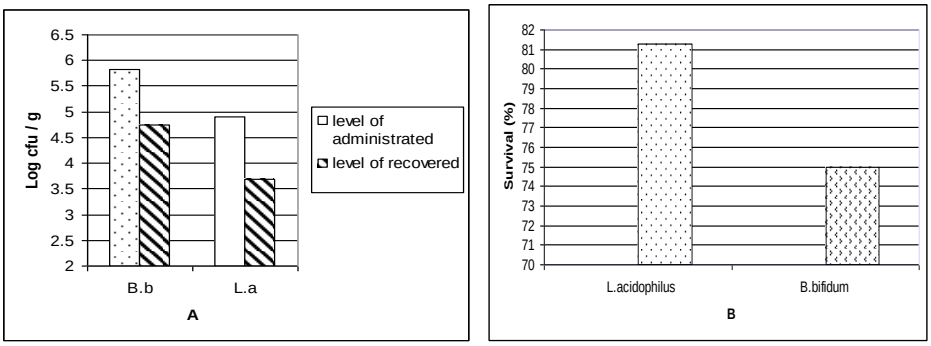


Fig. 4 Level of administrated of *L.acidophilus* and *B. bifidum* to quail chicks in ABY (A). Survival % of *L.acidophilus* and *B.bifidum* during transit through gastrointestinal tract (B)

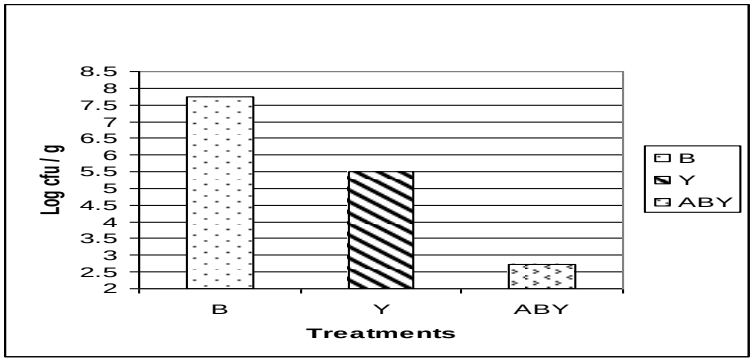


Fig .5 Viable plate count of *E.coli* in the feces of Japanese quail chicks.
B = Basal Diet **Y=** Traditional Yoghurt **ABY =** AcidophilusBifidouls- Yoghurt

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دراسات معملية وتطبيقية على بعض مدعمات الحياة فى القناة الهضمية

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الملخص:

فى محاولة لتقييم تاثير ظروف القناة الهضمية على بعض السلالات المحتمله كمدعمات حياة، استخدمت ثلاث سلالات بكتيريه و هى *L. johnsonii, L. acidophilus, B. bifidum* وتم اختبارها معمليا لمدى قدرتها على النمو على درجة حموضة منخفضة pH 2 ، 3 بالاضافة الى القدرة على النمو فى تركيزات مختلفة من املاح الصفراء (0.3 ، 0.5 ، 1 %) و كذا قدرتها على تحمل كل من العصارة المعديه والمعويه. كما اجريت تجربه تطبيقيه يتغذيه السمان اليابانى على احد منتجات الالبان المتخمرة (اسيدوفيلس – بفيدس - يوجورت) والذى استخدم فى انتاجه بالاضافة الى بادىء اليوجورت المعتاد كل من السلالتين *L. acidophilus, B. bifidum* و بعد ساعتين من التغذية تم ذبح الطيور واجراء عد لكل من البكتيريا *L. acidophilus, B. bifidum* فى الامعاء الدقيقة . كما تم دراسة التاثير المضاد Antagonistic effect على البكتريا *L. acidophilus, B. bifidum* على بكتيريا *E.coli* . اوضحت النتائج المعملية المتحصل عليها انا سلالة (*L. johnsonii*) كانت الاقدر على مقاومة كل من الحموضة المنخفضة ، النمو فى العصارة المعدية و المعوية ياليها فى ذلك سلالة *B. bifidum* . فى تجربة املاح الصفراء اضهرت سلالة *L. acidophilus* مقدرة اكبر على تحمل تركيز 0,3% ياليها سلالة *L. johnsonii* بينما على تركيزات الاعلى 0.5 ، 1 % كانت سلالة *L. johnsonii* الاقدر على تحمل تلك الظروف .

فى تجربة التغذية اوضحت النتائج ان سلالة *L. acidophilus* انخفضت اعدادها المعزولة من الامعاء الدقيقة بعد ساعتين من التغذية الى 3.68 خلية لكل جرام بينما انخفضت اعداد بكتريا *B. bifidum* بصورة اقل الى 4.74 خلية لكل جرام وهذا يعد مؤشر جيد على قدرة السلالتين على تحمل ظروف القناة الهضمية الصعبة والمرور خلالها .

بينما فى تجربة التاثير المضاد لبكتريا مدعمات الحياة على بكتريا *E.coli* اضهرت نتائج عد خلايا بكتيريا *E.coli* فى عينات اخراج الطائر (البراز) ارتفاع اعداد بكتريا *E.coli* لأعلى معدلاتها عند التغذية العليقة الغذائية الاساسية Basal diet لمدة 5 ايام حيث بلغت 7.75 خلية لكل جرام وتناقصت تلك الاعداد الى 5.90 خلية لكل جرام بعد التغذية لمدة 5 ايام على اليوحورت العادى يعزى ذلك الى تاثير سلالات بادىء اليوحورت على بكتريا *E.coli* حيث كان الانخفاض فى اعدادها اكثر وضوحا على منتج (اسيدوفيلس – بفيدس - يوجورت) حيث انخفض عدد بكتريا *E.coli* الى 2.72 خلية لكل جرام و هذا يعزى الى قدرة خلايا *L. acidophilus, B. bifidum* على انتاج مواد مضادة لنمو بكتريا *E.coli* مثل الاحماض العضوية) حمض اللأكتيك ، حمض الخليك) والكحول الأثيلى .