ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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International Journal of Poultry Science 8 (8): 763-767, 2009
ISSN 1682-8356

Lactobacillus as a Probiotic Feed for Chickens

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Abstract: The present study was carried out to isolate and identify microorganisms as a probiotic feed for chicken. The strains were isolated from gut region of the chick and was inoculated in to the MRS Medium and incubated at 37°C for 48 h under anaerobiosis. The identity of culture was based on the characteristics of Lactobacilli as presented in the Bergey's Manual of Determinative Bacteriology using Gram Staining, Motility, Triple Sugar Iron and Fermentation of different carbon sources. Based on the criteria *Lactobacillus fermentum* tolerated on Inhibitory substances, Temperature and agitation were identified and tested for probiotic use for chickens. *Lactobacillus fermentum* shows antimicrobial activity and shows the similar effects to antibiotic in the feed. It could be a suitable strain as a probiotic feed for chickens.

Key words: *Lactobacilli*, probiotic activity, MRS medium, anaerobiosis

INTRODUCTION

The poultry Industry is now facing a ban for the use of antibiotic feed additives for disease prevention and growth enhancing supplements. Probiotics were used to overcome this problem. To maintain the intestinal microflora balance in animals it is important to prevent diseases by controlling the overgrowth of potentially pathogenic bacteria. The control of infections through a non antibiotic approach is urgently requested. The natural bacterial flora (e.g. probiotic bacteria) represents a promising alternative therapy. Probiotics were defined as "living microorganisms that upon ingestion in certain numbers exert health effects beyond inherent basic nutrition" (Guarner and Schaafsma, 1998). Probiotic supplementation of the intestinal microflora in poultry, especially with *Lactobacillus* species, showed beneficial effects on resistance to infectious agents such as *Escherichia coli* (Jin *et al*., 1998), *Salmonella* sp. (Pascual *et al*., 1999),*Campylobacter* sp. (Stern *et al*., 2001) and more recently, *Eimeria acervulina* (Dalloul *et al*., 2003). Proposed mechanisms of pathogen inhibition by the probiotic microorganisms include competition for nutrients, production of antimicrobial conditions and compounds (volatile fatty acids, low pH and bacteriocins), competition for binding sites on the intestinal epithelium and stimulation of the immune system (Rolfe, 2000). These are not mutually exclusive mechanisms and some microorganisms may effect the change due to a single mechanism whereas others may use several mechanisms. The role of probiotic microorganisms as a sound alternative to antibiotic growth promoters, which beneficially affect the host animal by improving its intestinal microbial balance" So far, a variety of microbial species have been used as probiotics in poultry (Ghadban, 2002; Patterson and Burkholder, 2003). Probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida* and *Saccharomyces* have a beneficial effect on broiler performance (Kalavathy *et al*., 2003; Kabir *et al*., 2004; Gil De Los Santos *et al*., 2005), modulation of intestinal microflora and pathogen inhibition and immunomodulation (Zulkifli *et al*., 2000 and Koenen *et al*., 2004). The beneficial effect of lactobacilli has been attributed to their ability to colonize human and animal gastrointestinal tracts. In this work, adhesion assays with three lactobacillus strains and intestinal fragments obtained from chickens were assessed. *Lactobacillus animalis* and *L. fermentum* were able to adhere to three kinds of epithelial cells (crop, small and large intestines) with predominance to small intestine. Among the strains considered, *L. fermentum* subsp. *cellobiosus* showed the lowest and *L. animalis* the highest adhesion ability. Scanning electron microphotographs showing *L. animalis* and *L. fermentum* adhering to intestinal cells were obtained (Carlos Gusils *et al*., 1999). The Spent Culture Supernatant (SCS) of these *L. fermentum* strains showed antagonistic effect against the indicator bacteria, such as *Escherichia coli*, *Salmonella* spp., *Shigella sonnei* and some enterotoxigenic *Staphylococcus aureus*. some *L. fermentum* strains isolated from poultry were found to have the probiotic properties required for use in animal feed supplement. This study suggested that poultry digestive tract may serve as potential source for the isolation of probiotic lactic acid bacteria (Wen-Hsin Lin *et al*., 2007). Probiotic displayed a greater efficacy as growth promoters for broilers. Furthermore, the dietary supplementations resulted in an increase the height and depth of intestinal mucosa of broilers. The increase in the height and depth ratio was associated with improvement of growth performance for both synbiotic and probiotic. This indicates that the symbiotic and probiotic can be used as a growth promoter in broiler diets and can improve the

gut health. These products show promising effects as alternatives for antibiotics as pressure to eliminate growth-promotant antibiotic use increases (Awad *et al*., 2009).

MATERIALS AND METHODS

After postmortem the sample was collected from chicken gut region and inoculated in sterilized MRS broth. Decimal Dilution of the collected samples were made and suspended in MRS Broth for enrichment and incubated at 37°C for 48 h under anaerobiosis. The study was carried out during July 2008 to February 2009.

Identification of bacteria: The selected strain was identified by using staining, motility, physiological characters and biochemical utilization. The strain was identified as *Lactobacillus fermentum*.

Effect of *Lactobacillus* against biotolerance

Tolerance to Inhibitory substances: MRS broth contains 0.3 or 10% bile, 0.3 or 0.4% phenol and 4 or 8% sodium chloride, which was inoculated with 1% of the test organisms. The pour plate method was used and the plates were incubated in a Gas Pak jar at 37°C for 72 h and then the colonies (cfu) were counted.

Effect of temperature: The *Lactobacillus* culture was inoculated at 1% in MRS broth medium and incubated at different temperatures such as 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55°C for 24 h and the growth was monitored by measuring the absorbance value of broth at 540 nm.

Effect of agitation: The effect of agitation was investigated on bacterial growth and the culture was inoculated at 1% in MRS broth and incubated at 37°C under agitation at 200 rpm and static conditions. Sample was taken aseptically at time zero and at 2 h intervals up to 8 h. Total populations were determined by pour plate method by incubating the plates at 37°C for 48 h anaerobically.

Antimicrobial activity: Sterile MRS broth (pH 6.0) was inoculated with 1% level of an actively growing culture of each isolate from chicken and incubated at 37°C for 24 h. The test materials (compounds produced by the microbial cultures having antimicrobial activity) were obtained and the fermented MRS broth was centrifuged (20,000 g for 15 min) to remove the microbial cells. The resulting liquid was dried under vaccum using 45°C water bath and a rotary evaporator which was re-suspended in one-fifth, the original volume of water and filtered through the sterile 0.45 mm membrane filters. Two control test materials were also prepared using uninoculated MRS medium. The medium in one tube was adjusted to 6.0 (the initial pH of the MRS broth) and the other to pH 4.0 using formic acid.

Test organisms: The antimicrobial activity was detected by the following organisms grown in nutrient broth at 37°C for 24 h were *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*.

Bioassays: Antimicrobial activity was quantified by a ditch assay using the test organisms. Actively growing culture of the test organisms were mixed at a 2.5% (2.5 x 10 cfu/ml) with melted nutrient agar poured in sterile Petri dishes and was allowed to solidify. A one-cm wide ditch was cut in the agar across the centre of the dish. The test material obtained from the isolated cultures was diluted in an equal volume of melted bacteriological agar and then 0.2 ml of the mixture was pipette out into the dish. When the mixture solidified, the plates were first incubated at 4°C for 60 min to allow the test material to diffuse in the agar and then incubated at 37°C for 18 h. After incubation, the diameter of the clear zone was measured in centimeters from the centre of the well.

Evaluation of probiotic activity in chickens: The experiment was carried out with chicks to evaluate the influence of *Lactobacillus fermentum*. 120 chickens were taken and was separated based upon sex (Male and Female) into two divisions containing each 60.

The 10^{th} week chickens were selected for the test (1670 and 1075 gms initial weights respectively). The isolated *Lactobacillus fermentum* (10^7 cfu/ml) were administered along with feed without any antibiotics in feed for 12 weeks. Feed has been administered daily twice in early morning and late evening without the interruption of hot temperature. The feed administered was in the form of pellet crumble feed. It was carried out up to 22^{nd} week. After completion of the administration of feed for 12 weeks, the weight of each chicken is been weighed and compared with the standard chart.

RESULTS

The microbial strain isolated from gut region of chicken was identified as *Lactobacillus fermentum*, which showed short single and paired square bacilli in MRS broth after 24 h of incubation at 37°C in anaerobiosis. The colonies in MRS agar were smooth and convex. *Lactobacillus fermentum* exhibited actively motile in SIM media. The strain produces the gas from glucose and H_2S Production in Triple Sugar Iron Media.

Lactobacillus fermentum strain tolerates 0.3 and 10% bile, 0.3 and 0.4 phenol and 4% but not 8% of Sodium Chloride. After incubation, substance inhibited the growth of *Lactobacillus fermentum* in media (Fig. 1 and Table 1). Biotolerance has been described as an important factor for the survival and the growth of *Lactobacillus fermentum* in the intestinal tract.

The effect of temperature tolerated between 30-45°C on the inoculated culture. So after 45°C the growth gradually starts to decline (Fig. 2 and Table 2).

Table 1: Effect of inhibitory substances

Substances used	Log value (cfu/ml)
MRS	0.08
B (0.3%)	0.072
B (10%)	0.051
P (0.3)	0.082
P (0.4%)	0.078
NaCl (4%)	0.025
NaCl (8%)	0.011

B-Bile, P-Phenol, NaCl-Sodium Chloride

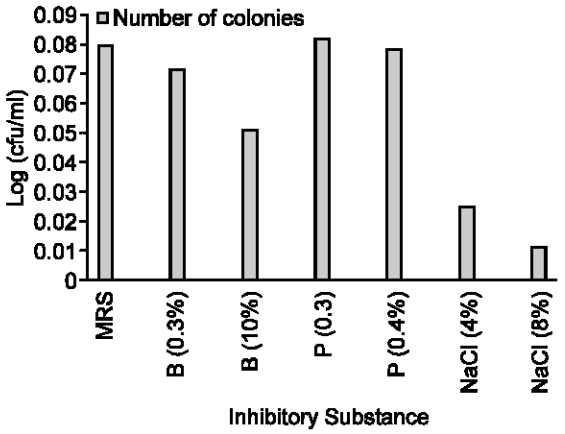

Fig. 1: Effect of Inhibitory substances on *Lactobacillus Fermentum*

The effects of agitation were taken aseptically at time Zero at 2 h interval, intervals up to 8 h. Total populations were determined by pour plate method and the plates were incubated at 37°C for 48 h anaerobically (Fig. 3 and Table 3).

Antimicrobial activity shows the size of inhibition zone obtained for *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus*. Inhibition zone were bigger or similar than control at pH 6.0. However when compared with the inhibition zone with the other control pH 4.0 strain was similar incase of *Escherichia coli* and *Staphylococcus aureus*. Thus, these effects were apparently due to a pH effect (Result of lactic acid production) and not to the production of any antimicrobial agent present in the materials tested (Table 4). The inhibition of microbial growth resulted from the presence of lactic acid produced or due to the production of other antimicrobial compounds showing inhibitory properties also noted the pH effect in fermentation analysis.

The isolate was evaluated for poultry feed supplement, the result shows that in comparison to the presence and effect of antibiotics *Lactobacillus fermentum* implantation resulted in a similar effect as that of antibiotic manifested by feed efficiency in growth of chick. The increase in weight and better feed efficiency has been determined (Fig. 4 and 5), (Table 5 and Table 6).

DISCUSSION

In the natural environment, the chicken's intestinal tract was colonized by a broad spectrum of microorganisms. However in commercial operation, high hygienic standards were maintained in the young chickens lack contact with the natural environment. So colonization of

Table 2: Effect of temperature

Temperature (°C)	Absorbance (540 nm)
0	0
5	0.029
10	0.037
15	0.039
20	0.044
25	0.049
30	0.068
35	0.075
40	0.087
45	0.095
50	0.071
55	0.062

Table 3: Effect of agitation (200 rpm)

Agitation Time (h)	Log value (cfu/ml)
2	7
4	8
6	8.6
8	8.9
10	9

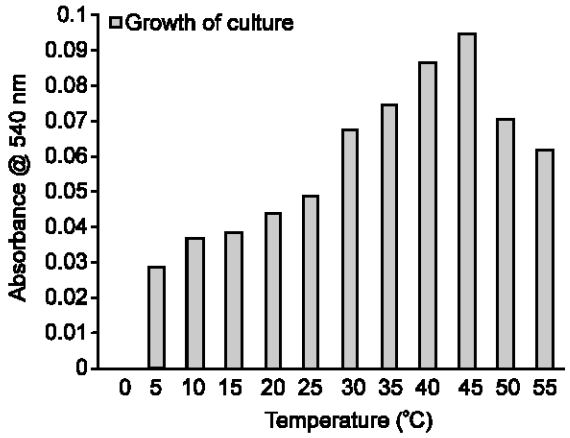

Fig. 2: Effect of temperature on *Lactobacillus fermentum*

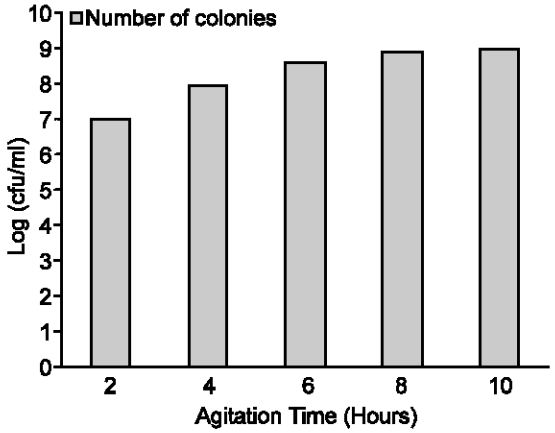

Fig. 3: Effect of Agitation (200 rpm) on *Lactobacillus fermentum*

Table 4: Antimicrobial activity of *Lactobacillus fermentum* towards various microorganisms

	Inhibition Zone (cm)	
Test Organism	pH 6	pH 4
Escherichia coli	1	2.5
Salmonella typhimurium	1	2.0
Staphylococcus aureus	1	1.5

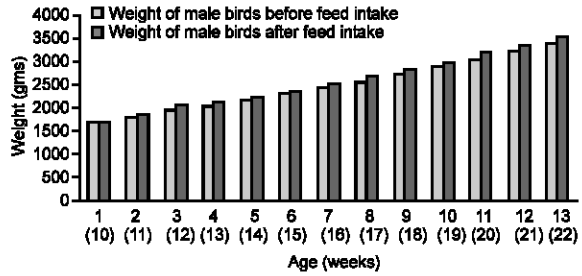

Fig. 4: Influence of *Lactobacillus fermentum* feed on Male birds

the intestinal tract was often a more prolonged process taking around 21 days to develop a balanced intestinal flora. After the first 21 days of life, other challenges such as stress, feed changes, antibiotic intervention and disease can also upset the intestinal flora and can lead to poor weight gain or considerable loss of stock.

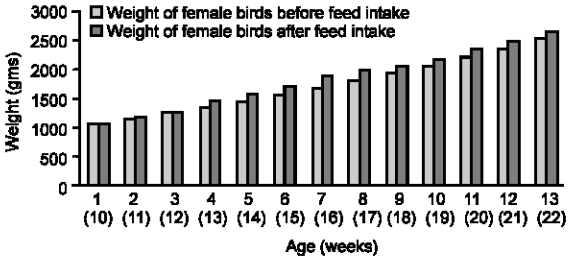

Fig. 5: Influence of *Lactobacillus fermentum* feed on Female birds

Conventionally intervention in animal production aiming to reduce disease causing pathogen include improved hygienic methods, vaccination and use of antimicrobial agent widely reduce the increase of disease in animal production. Alternative solution to the use of antibiotics have been sought and now available was a number of direct-fed microbial or probiotic products which help to

Table 5: Weight of Male birds before and after feeding of probiotics

		Male Birds Weight	
Age (in Weeks)	Amount of Feed Intake per week (gm)	Before Feed Intake (g)	After Feed Intake (g)
10	75	1670	1670
11	76	1800	1840
12	78	1920	2050
13	80	2040	2120
14	82	2160	2220
15	85	2290	2350
16	88	2420	2510
17	90	2560	2680
18	95	2710	2820
19	100	2870	2960
20	110	3040	3170
21	120	3210	3330
22	125	3370	3510

Table 6: Weight of female birds before and after feeding of probiotics

		Female Birds Weight	
Age (in Weeks)	Amount of Feed Intake per week (gm)	Before Feed Intake (g)	After Feed Intake (g)
10	61	1075	1075
11	63	1180	1200
12	64	1275	1310
13	66	1370	1490
14	67	1465	1600
15	69	1570	1720
16	76	1690	1900
17	82	1825	2000
18	88	1955	2080
19	94	2090	2190
20	102	2230	2380
21	109	2380	2520
22	118	2550	2670

maintain the balance of the intestinal microflora in the range of food animal species (Salminen *et al*., 1998).

If more were known about which specific organism of the flora were eliminated by antibiotics, appropriate probiotics should be administrated after antibiotic therapy to repair any damage to the flora by the antibiotics. Feed-type probiotic products have been demonstrated to help and maintain a positive balance of intestinal microflora resulting in the improvements in health and weight of the chickens throughout their short life span (Ouwehand *et al*., 2002).

Members of the genus *Lactobacillus* are particularly suited for development as probiotics, since they confer benefits to their host by improving properties of the indigenous flora. The Result of the experiment showed that substitution of antibiotic by probiotic did not affect the feed efficiency, thus paving way for substitution of antibiotics by probiotics. Since the antibiotic resistant of the pathogen now calls for antibiotic alternative (Sullivan *et al*., 1992 and Tortuero, 1973).

The strain of *Lactobacillus fermentum* showed similar effects to antibiotics in the feed. It could be a suitable strain for probiotic use for chicken because the administration of probiotic has little or no risk, their use may be worth while on a disease preventing strategy and to maintain good health.

ACKNOWLEDGEMENT

We thank the Department of Microbiology, Jamal Mohamed College (Autonomous), Tiruchirappalli-620 020, and Ramakrishna Poultry, Thalavapalayam, Karur for supporting and fulfilling all the needs to carry out this work.

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