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Effect of Various Microorganisms Culture Feeding Against Salmonella Infection in Broiler Chicks

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ABSTRACT

Effect of various microorganisms on the performance of broilers and control of Salmonella infection in broiler chicks was studied. *Lactobacillus acidophilus* and *Aspergillus oryzae* were isolated from the intestinal contents of healthy birds while *Streptococcus thermophilus* was obtained from the yogurt. Stock cultures were maintained with viable count of $\geq 10^8$ CFU in separate pure cultures. These (single / and in combinations) cultures were administered to different experimental groups for two weeks at the dose rate of $\geq 10^8$ microorganisms per litre of drinking water. Feed intake, water intake and weight gain of the birds were recorded per week. Various experimental groups were also monitored for immune response against Newcastle disease and infectious bursal disease by measuring geometric mean titres upto 45 days of age. At the age of three weeks, five birds from each group were challenged with *Salmonella gallinarum* at the dose rate of $1x10^6$ microorganisms per litre of drinking water. Clinical signs, morbidity and mortality were recorded after challenge. There was more feed intake, water intake, weight gain and antibody titres in the group administered combined beneficial microorganisms. After challenge, no mortality was observed in this group indicating the effective role of microorganisms culture in the prevention of Salmonella infection in broiler chicks.

Key Words: Effective microorganisms; Broiler chicks; Performance; Immune response; *Salmonella gallinarum*

INTRODUCTION

Poultry may serve as viable and quick source to meet the food shortage of ever increasing human population. However, poultry industry is confronted due to prevalence of various infectious diseases which are still havoc for the newly established Pakistan poultry industry. Among these, salmonellosis is highly infectious and most important zoonotic disease. It is vertically transmitted disease and there is a decreased production and low weight gain in the infected flocks. Mortality in susceptible birds may reach upto 90%.

For the control of these infectious diseases, the use of antibiotics in poultry industry is increasing day by day. This lavish use of antibiotics is causing not only serious problems (Bacterial resistance, Dysbiosis) in poultry but also responsible for harmful residual effects in meat and eggs thus leading to public health hazards. Effective microorganisms (probiotics) may be suitable replacements of antibiotics. According to Fuller (1989), probiotics are live microbial feed supplements that beneficially affect the host by improving its intestinal microbial balance. These microbial species are obtained from the soured and fermented milk, yogurt, cheese and intestinal contents of poultry gut (Fuller, 1992; Medina *et al*., 2001). The probiotics act through competitive exclusion, sticking to specific sites located in the intestinal epithelium thus decreasing colonization by pathogenic microorganisms (Fox, 1988; Jin *et al*., 1997). Besides their use for controlling pathogenic bacteria, probiotics have also been used to improve the growth of poultry (Stavric & Kornegay, 1995). *Salmonella enteritidis* infection in commercial ducklings has been controlled by oral chicken egg-derived antibodies alone or in combination with probiotics (Fulton *et al*., 2002).

The present study was planned to isolate and characterize non-pathogenic microorganisms from indigenous poultry as well as from yogurt and to study the effect of feeding these microorganisms on the performance, immune response and prevention of Salmonella infection in chicken broilers.

MATERIALS AND METHODS

Isolation and identification of microorganisms. Intestinal contents were collected from different parts of the gut of local healthy chickens. These contents were inoculated on Rogosa agar and potato dextrose agar for the isolation of *Lactobacillus acidophilus* and *Aspergillus oryzae,* respectively. For the isolation of *Streptococcus thermophilus*, yogurt sample were inoculated on yeast lactose agar and incubate at 37°C for 48 h (Cappucino & Sherman, 1996; Harrigan & McCance, 1976). Isolated microorganisms were identified by their cultural, morphological and biochemical characteristics (Buxton & Fraser, 1977).

Pathogenicity test. Four purified cultures of bacteria and fungi were separately given to various groups of healthy chicks (1 week) through drinking water with final concentration of $\geq 10^8$ CFU/100 mL continuously and the

chicks were kept under strict observations for two weeks. No bird revealed any clinical signs and there was no mortality.

Counting of microorganisms. The microorganisms were counted by plate count method (Salle, 1979). After counting, dilution of pure culture and mixed cultures were prepared in sterilized phosphate buffer solution at the rate of $\geq 10^8$ CFU/ mL for feeding to the experimental birds.

Experimental trials. One hundred and sixty, day-old broiler chicks (Hubbard) were procured from the hatchery, weighed and randomly divided into eight groups (A, B, C, D, E, F, G and H) of 20 chicks each. The chicks were maintained in the experimental animal unit of the department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan. Both feed and drinking water were offered *ad libitum*. Groups A, B and C were given *Lact. acidophilus*, *Strept. thermophilus* and *Asp. oryzae,* respectively, daily for 2 weeks @ $\geq 10^8$ CFU/L of drinking water. Groups D, E and F were given mixed cultures of *Lact. acidophilus* and *Strept. thermophilus*, *Lact. acidophilus* and *Asp. oryzae,* and *Strept. thermophilus* and *Asp. oryzae,* respectively. Group G was given mixed culture of *L. acidophilus*, *S. thermophilus* and *Asp. oryzae* while group H was maintained on untreated control diet.

Challenge trial. For challenge trial, 10 birds from each experimental group were given *Salmonella gallinarum* suspension @ $1x10^6$ microorganisms / drinking water at the age of three weeks for three consecutive days.

RESULTS

Various microorganisms including *Lact. acidophilus, Strept. thermophilus* and *Asp. oryzae* were isolated from the native intestinal contents of healthy birds as well as from fresh yogurt samples. These were identified by studying their cultural, morphological and biochemical characteristics (Buxton & Fraser, 1977). All the cultures were found apathogenic in chicks.

Feed intake and FCR. Maximum feed intake and minimum feed conversion ratio (FCR) was recorded in group G, which was fed with combination of all three microorganisms while minimum feed intake and maximum feed conversion ratio (FCR) was recorded in group in group H that was control group. The results of feed intake, weight gain and feed conversion ration are presented in Table I.

Immune response against Newcastle Disease. The geometric mean titres (GMT) against Newcastle disease (ND) at day 15, 30 and 45 (Haemagglutination & Haemagglutination Inhibition tests) are presented in Table II. The birds of group G had the maximum antibody titre against ND.

Immune response against infectious bursal disease. The geometric mean titre against infectious bursal disease (IBD) at day 15, 30 and 45 were recorded through indirect haemagglutination test and are presented in Table III. The group G had the maximum antibody titre against IBD and the minimum being in-group H.

Table I. Average feed intake, weight gain and FCR in Broiler chicks at the end of 6 weeks in different treated and control groups

Groups	Feed Intake (g)	Weight (g)	FCR
A	3672	1666± 95.71 bcd	2.20
B	3633	1612± 84.43 cde	2.25
C	3682	1586± 76.62 de	2.33
D	3657	1738± 98.18 ab	2.10
E	3663	1720± 92.38 b	2.13
F	3660	1680± 85.37 bc	2.18
G	3685	1802 ± 79.69a	2.02
H (control)	3670	1548 ± 82.30e	2.38

Table II. Serum antibody titre of broiler chicks against Newcastle disease using HI test in treated and control groups

Days	Groups							
	A	B	C	D	E	F	G	H
15	42	37	32	37	32	32	42	37
30	84	74	84	84	64	84	147	48
45	74	64	48	37	42	56	145	32

Table III. Serum antibody titre of broiler chicks against Infectious bursal disease using IHA test in treated and control groups

Days	Groups							
	A	B	C	D	E	E	G	H
15	42	32	56	48	42	42	84	37
30	128	64	64	84	64	56	169	48
45	97	56	56	48	42	37	168	42

Groups A to H: A= Fed with *Lactobacillus acidophilus*, B= Fed with *Streptococcus thermophilus,* C= Fed with *Aspergillus oryzae,* D= Fed with *L. acidophilus* and *S. thermophilus,* E= *S. thermophilus* and *A. oryzae*, F= Fed with *S. thermophilus* and *A. oryzae*, G= Fed with combination of all three microorganisms, and H= Control group without feeding of microorganisms

Challenge exposure trials. Post-challenge clinical signs (morbidity/mortality) recorded in percentage in experimental groups at different days. Maximum mortality was recorded in-group H, which was 10%, 11%, 25% and 33% at day 3, 6, 8 and 9 respectively. Minimum mortality recorded in-group F was 10% at day 5 while no mortality was recorded in-group G that was given combination of all the microorganisms.

DISCUSSION

The present investigations were undertaken to investigate the effects of various beneficial microorganisms on the performance as well as their role for the prevention of *Salmonella gallinarum* in broiler chicks. The microorganisms which were used in the trial may

collectively be called as "probiotics". Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). The parameters included in this study were feed intake, weight gain, feed conversion ratio (FCR), humoral immune response against Newcastle disease (ND), Infectious bursal disease (IBD) and prevention of *Salmonella gallinarum* colonization post probiotics administration.

Various microbes used in this trial included *Lact. acidophilus, Strep. thermophilus and Asp. Oryzae.* These microorganisms were administered singly or in various combinations. The data collected revealed that Group G which was given combination of all microorganisms had higher weight gain, better FCR, higher antibody titres and no post challenge mortality as compared with other groups. The findings of the present study are supported by Tortuero *et al.* (1973) and Dilworth and Day (1978) who reported that supplemented Lactobacillus culture in broiler diet resulted in significant improvement in weight gain. Similar results are also obtained by ZuAnon *et al.* (1998). They fed probiotics to broiler chicks and observed increased feed intake, weight gain and feed to gain ratio as compared to those fed control diet.

Findings of Mohan *et al.* (1996) also supported the results of this study. They fed broiler chicks with probiotics containing *Lactobacillus casei, Lact. acidophilus, Bifidobacteria bifidum, Aspergillus oryzae* and Torulopsis and reported positive effect on bogy weight. Similar results are also reported by Zulkiffi *et al.* (2000) who found that broilers offered feed supplemented with Lactobacillus culture had increased weight gain, better feed efficiency and higher antibody titre against Newcastle disease. Nurmi and Rantala (1973) reported that the introduction of gut microflora from adult healthy birds into newly hatched chicks resulted in resistance to infection when subsequently challenged with food poisoning Salmonella and *E. coli*. The inhibition is still known as Nurmi's concept of competitive exclusion.

CONCLUSIONS

On the basis of the results of this study it may be inferred that the mixture of non-pathogenic cultures may be declared as probiotics which have positive effects on weight gain, feed efficiency, FCR, immune response against ND and IBD and prevention of Salmonella infection in broiler chicks. There is a gap for further studies to compare with antibiotics and growth promoters. Probiotics are a prospective alternative to the antibiotic growth promoters (AGPs) that are still used in poultry and animal rations but have shown to lead to the development of antibiotic resistant bacterial strains, which may impose a serious health hazard for animals as well as for humans. More investigations are necessary for the evaluation of the probiotics to be used as therapeutic agents for the treatment of different diseases.

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