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Original Research Article

Bioprocess optimization and evaluation of probiotics efficacy on growth performance of Broilers and Country Chicks

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A B S T R A C T

Poultry industry faces a serious threat by many infectious diseases caused by microbes and parasites that directly affect the productivity to a greater extent. Antibiotic usage to control these diseases disrupts the beneficial microbial communities in the intestine thus affecting the overall health and growth of the animal. Supplementation of probiotics in feeds imparts normalized intestinal microbiota composition, immunomodulation and metabolic effects in poultry. The probiotic effect of the microbes supresses the incidence of endogenous and exogenous pathogenic bacteria in the intestine. In the present study, the growth conditions of *Bacillus subtilis* (MTCC - 441), *Bacillus coagulans* (MTCC - 492), *Lactobacillus acidophilus* (NCDC - 417) and *Saccharomyces cerevisiae* (MTCC - 043) were optimized and these organisms were cultured in fermentor for higher cell count. Supplementation of these probiotics in the diet of broilers and country chicks reduces mortality and thereby increases the growth performance like feed intake, water intake, weight gain, feed conversion efficiency.

Keywords

Probiotics; broilers; country chicks; growth performances.

Introduction

Over the years the word probiotic has been used in several different ways. It was originally used to describe substances produced by one protozoan which stimulated by another (Lilly, D.M. *et al.*, 1965) but it was later used to describe animal feed supplements which had a beneficial effect on the host animal by affecting its gut flora (Parker, R.B.*et al.*, 1974). Fuller (Fuller, R.*et al.*, 1998) later gave a unique definition of probiotics as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. The US National Food Ingredient Association presented, probiotic (direct fed microbial) as a source of live naturally occurring microorganisms and this includes bacteria, fungi and yeast (Miles, R.D.*et al.*, 1991). According to the currently adopted definition by FAO/WHO, probiotics are: "live

microorganisms which when administered in adequate amounts confer a health benefit on the host" (Bellisle *et al.,* 1998). More precisely, probiotics are live microorganisms of non-pathogenic and nontoxic in nature, which when administered through the digestive route, are favourable to the host's health (Guillot, J.F.*et al.,* 1998).

During last two decades poultry industry has been one of the most growing sectors in the world, because the number of persons consuming chickens and the consuming amount is increasing in scale. It mainly helps to meet the protein requirement of human. Diseases through microorganisms such as viruses, bacteria, fungi, protozoa are the major barriers for the growth of the poultry industry (Lutful Kabir, 2009). An enteric disease such as coccidiosis is a realistic problem and one of the most important diseases of poultry worldwide. It is caused by a protozoan parasite known as *Eimeria* that invade the cells of the poultry intestine. Species of coccidia which commonly affect poultry are *Eimeria tenella, E. acervulina, E. necatrix, E. maxima and E. brunetti*.

The disease is characterised by enteritis, diarrhoea and mortality (Casterlow *et al.*, 2011). The bird develops reduced ability to absorb nutrients, which results in weight loss and eventually death. Sub clinically, it is manifested by poor performance, impaired feed conversion, poor flock uniformity and poor growth. The disease is considered as one of the most severe health and economic problems in poultry that causes an enormous loss to poultry producers worldwide.

Acquired immunity is important in protection against coccidiosis (Toms and Powrie, 2001). The effective use of anticoccidial feed additives over the past 50 years has played a major role in the growth of the poultry industry (Badran and Lukesova, 2006). This results in promoting host immunity and on the complex interactions between the gut microflora and immune system development (Kyungwoo Lee *et al.,* 2010).

Probiotics (in most cases, bacteria) are similar to beneficial microorganisms found in the human gut. They can be used as complementary and alternative medicine (CAM). Probiotics are available in foods and dietary supplements (for example, capsules, tablets and powders) and in some other forms as well. Examples of foods containing probiotics are yogurt, fermented and unfermented milk, and some juices and soy beverages. These are mostly *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus helveticus, Lactobacillus lactis, Lactobacillus salivarius, Lactobacillus plantarum, Enterococcu faecium, Enterococcus faecalis, Bifidobacterium spp. and Escherichia coli* are all intestinal strains (Fuller, R. 1998).

In probiotic foods and supplements, the bacteria may have been present originally or added during preparation (Salimen *et al*., 2005). The combine use of *Lactobacillus* and yeast cultures in the feed and water has been shown to be effective in reducing morbidity and mortality and improving growth performance and production (Choudhari, *et al*., 2008).

Dietary feed additives are products incorporated into animal feed to create favourable conditions in the animal's intestine for the digestion of feed. Growth promoters have been used extensively in

animal feeds and water all over world especially in the poultry (Charles and Duke, 1978). Probiotics reduce production of toxic components by bacteria and a chance in the morphology of the intestinal wall, thus preventing damage to the epithelial cells (Langhout, 2000).

From the safety point of view, the probiotic microorganisms should not be pathogenic, have no connection with diarrhoeagenic bacteria and no ability to transfer antibiotic resistance genes, as well as to maintain genetic stability. To be recognized as functional food components, they should demonstrate the following properties: acid- and bile-stability, resistance to digestive enzymes, adhesion to intestine surface, antagonistic activity against human pathogens, anti-carcinogenic and anti-mutagenic activity, cholesterol-lowering effects, stimulation of the immune system without inflammatory effects, enhancement of bowel motility, maintenance of mucosal integrity, improvement of bioavailability of food compounds and production of vitamins and enzymes (Ouwehand *et al*., 1999).

Materials and Methods

Microorganisms

Four microorganisms (*Bacillus subtilis* MTCC – 441, *Bacillus coagulans* MTCC – 492, *Lactobacillus acidophilus* NCDC – 417 and *Saccharomyces cerevisiae* MTCC - 043) were used in the present study.

Medium

The Nutrient Medium for bacteria and Yeast Medium for yeast were used to grow the organisms.

Nutrient medium	**(g/L)**	**Yeast medium**	**(g/L)**
Beef extract	1	Malt	3
Yeast extract	2	Yeast	3
Peptone	5	Peptone	5
Nacl	5	Glucose	10
pH – 7.0		pH – 7.0	

Optimization of Bioprocess Conditions

Optimizations of the cultures were carried for pH, temperature and incubation period.

Optimization of pH

For optimization of pH, the temperature and incubation period were kept constant with varied pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0). The OD values were taken using a UV-VIS spectrophotometer at 650 nm.

Optimization of Temperature

For optimization of temperature, the pH and incubation period were kept constant with varied temperatures. For bacterial cultures the temperatures kept were 28°C, 30°C, 34°C, 37°C and 40°C and for yeast the varied temperatures were 22°C, 25°C, 28°C, 32°C and 35°C. The OD values were taken using a UV-VIS spectrophotometer at 650 nm.

Optimization of Incubation Period

For optimization of incubation period, the temperature and pH were kept constant with varied incubation period. For bacterial cultures the incubation periods kept were 22hrs, 24hrs, 28hrs, 30hrs and 34hrs and for yeast culture the varied incubation period were 24hrs, 36hrs, 48hrs, 50hrs and 52hrs. The OD values were taken using a UV-VIS spectrophotometer at 650 nm.

Development of Probiotics using Labscale Fermenter

Lab scale cultivation of above cultures was done using Fermenter - Sterilizable Make: Bioengineering, Switzerland, Model: Insitu Bench top - type KLF 2000. Fermenter is filled with distilled water to the working volume (2.75 L) and sterilized at 121° C for 20 minutes. Acid and base reservoirs, oxygen supplying tubes ends were covered with cotton and sterilized using autoclave. Two litres of bacterial and yeast medium is made as per the given media composition and fed into the fermenter. The medium was sterilized in situ by autoclaving at 121° C for 20 minutes. The temperature of the fermenter is reduced using cooling water system. After the media attained the optimized temperature, 1.0 % of probiotic cultures were injected into the fermenter through sterilized needle & syringe aseptically. The fermentation process was carried out by the optimized growth conditions performed in shake flask method. Later the fermented broth was recovered by increasing the internal air pressure carefully without any contamination in a sterile container.

Formulation of Culture

The three bacterial cultures and yeast were formulated in the ratio of 1:1:1 by adding 1 g of preservatives such as Sodium benzoate and Sodium propionate each respectively for a litre

Tray Drying

After formulation, the broth were poured into the tray drier and dried at 40° C for 48 hours and collected in powder form.

Cell Count

Broth culture and dried probiotic powder was inoculated in the plates containing nutrient medium and yeast medium. After incubation, the microbial cells were counted using colony counter.

Probiotic Supplement Formulation

The air dried biomass of probiotics was powdered and supplemented at the concentrations of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 g/kg of meal for broilers and country chicks. The diets were formulated to be isonitrogenous, but differed in metabolizable energy content.

Animal Study

A total of 60 unsexed Vencob broiler chicks and 60 country chicks (ASEL) were randomly divided into six groups of ten chicks each for dietary treatments. Each group was placed and reared in a clean, disinfected pen measuring 2.65 x 2.55 m and a floor space of 0.225 m^2 per bird. The experiment comprises control birds of 10 numbers and treatment birds of 50 numbers in the both varieties.

Three days old chicks were used for the experiments in the both the varieties. The control and treatment birds were fed with diets of respective probiotic formulations for 45 days to broilers and 75 days to country chicks. The birds were left free to access for water and feed. Parameters such as food intake, water intake, body weight, feed conversion ratio and mortality were analysed at regular intervals.

Results and Discussion

Optimization of pH

All the four probiotic organisms exhibited maximum growth at pH 7.0.

Optimization of Temperature

The optimum temperature for *Bacillus subtilis, Bacillus coagulans, Lactobacillus acidophilus* and *Saccharomyces cerevisiae* were 30˚ C, 37˚ C, 35˚ C and 37˚ C respectively.

Optimization of Incubation Period

The optimum incubation Period for *Bacillus subtilis, Bacillus coagulans* and *Lactobacillus acidophilus* was 24 hours and for *Saccharomyces cerevisiae* it was 48 hours.

The probiotic cultures viz. *Bacillus subtilis, Bacillus coagulans, Lactobacillus acidophilus* and *Saccharomyces cerevisiae* were cultured individually in fermenter with the respective optimized growth conditions. The plate count method performed using pour plate technique to enumerate the cell counts in the broth showed 2.5 X 10^9 cells for three bacteria and 2 X 10^9 cells for yeast. The dried and powdered probiotic cultures showed 2.5 X 10^8 cells for three bacteria and 2 X 10^8 cells for yeast in plate count method.

Animal Study

In the present study it was observed that the probiotic dietary supplementation of 1.5 g/kg meal fed broilers showed the maximum feed consumption (4.54 kg), body weight (2.45 kg) and performance efficiency factor (132.22). Similarly the probiotic dietary supplementation of 1.5 g/kg meal fed country chicks showed the maximum feed consumption (4.00 kg), body weight (2.50 kg) and performance efficiency factor (156.25). The probiotic dietary supplementation at higher concentrations above 1.5 kg could not improve the growth performance. There were no morbidity or mortality observed in control and treatment birds of broilers and country chicks.

The effect of probiotics in broiler and country chicks on growth performance was evaluated in this study. The administration of probiotics in feed had beneficial effects of feed consumption, water intake, weight gain, feed conversion ratio and mortality rate (Zhou *et al.,* 2010). The previous studies showed that supplementation of *Lactobacillus* cultures to chickens, either as a single strain of *Lactobacillus acidophilus* or as a mixture of 12 *Lactobacillus* strains, increased significantly the body weight of broilers after 40 days of feeding (Jin *et al.,* 2000). Similar findings were obtained in the reports of Noh, 1997; Zulkifli *et al.,* 2000; Lan *et al.,* 2003; Timmerman *et al.,* 2006 which enhanced the growth performance of poultry.

It has been reported that inclusion of single cell protein in the broiler feed increased the feed consumption rate, body weight and performance efficiency factor (Chiou *et al.,* 2001).

Inclusion of probiotics in basal diet results in increasing the body weight, feed intake, feed conversion ratio, by varying the concentrations of probiotics. The body weight gain was 2.29 kg, feed consumption was 4.11 kg, feed conversion ratio of treatment birds was 1.81 (Mountzouris *et al.,* 2010).

Table.1 Effect of varying levels of probiotics on the growth performance of Broiler chicks on 49th day

Particulars	Level of probiotics (g/Kg)					
	0 (control)	**0.50**	**1.00**	**1.50**	**2.00**	**2.50**
Feed consumption (Kg)	4.48	4.51	4.52	4.54	4.52	4.50
Water intake (L)	10.40	11.20	11.40	11.60	11.80	12.00
Weight gain (Kg)	2.30	2.31	2.35	2.45	2.39	2.36
Feed conversion efficiency	1.948	1.952	1.923	1.853	1.891	1.907
Performance efficiency factor	118.07	118.34	122.20	132.22	126.39	123.75
Mortality (%)	Nil	Nil	Nil	Nil	Nil	Nil

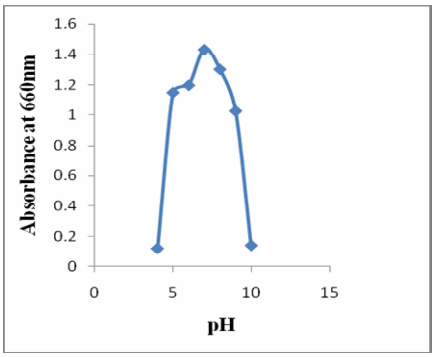
Values are the mean of 10 replicates

Table.2 Effect of varying levels of probiotics on the performance of Country chicks on 75th day

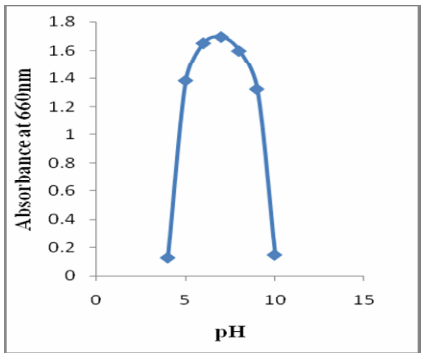
Particulars	Level of probiotics (g/Kg)					
	0 (control)	**0.50**	**1.00**	**1.50**	**2.00**	**2.50**
Feed consumption (Kg)	3.50	3.81	3.90	4.00	3.96	3.92
Water intake (L)	9.50	9.65	9.72	9.80	9.90	9.95
Weight gain (Kg)	2.30	2.35	2.40	2.50	2.38	2.33
Feed conversion efficiency	1.522	1.621	1.625	1.600	1.664	1.682
Performance efficiency factor	151.12	144.97	147.69	156.25	143.03	138.53
Mortality (%)	Nil	Nil	Nil	Nil	Nil	Nil

Values are the mean of 10 replicates

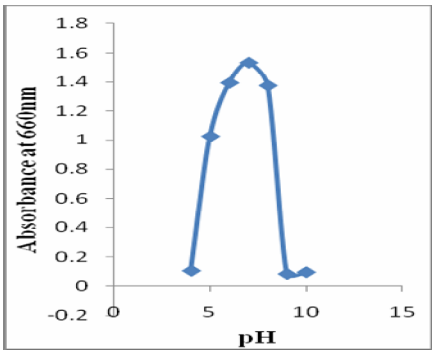
Figure.1 Optimization of pH



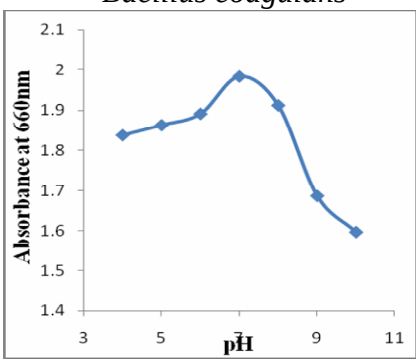
Bacillus subtilis



Bacillus coagulans

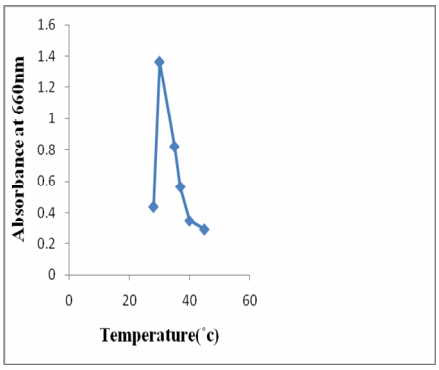


Lactobacillus acidophilus

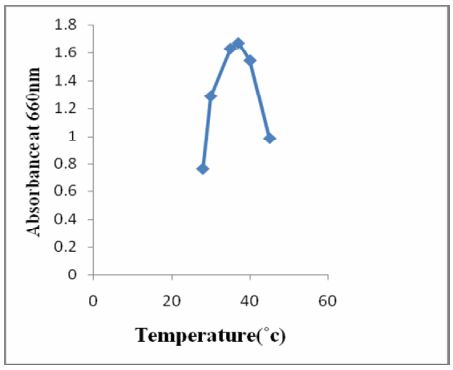


Saccharomyces cerevisiae

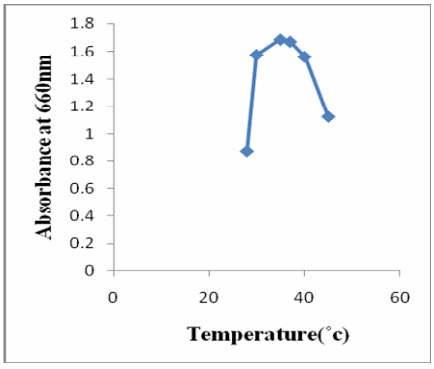
Figure.2 Optimization of Temperature



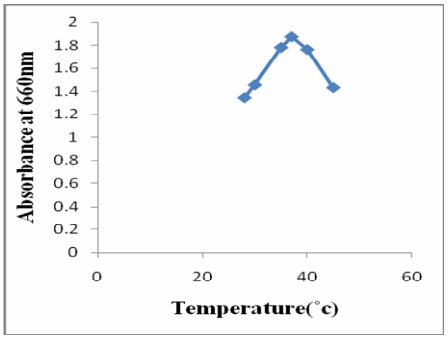
Bacillus subtilis



Bacillus coagulans

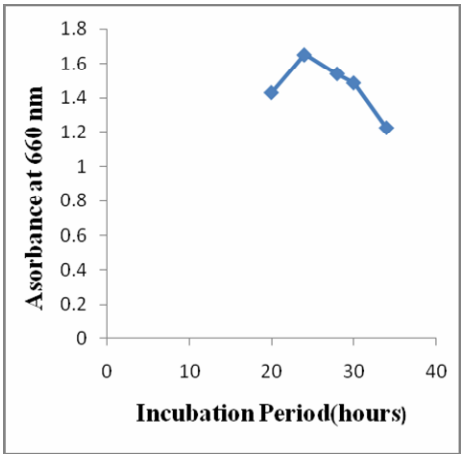


Lactobacillus acidophilus

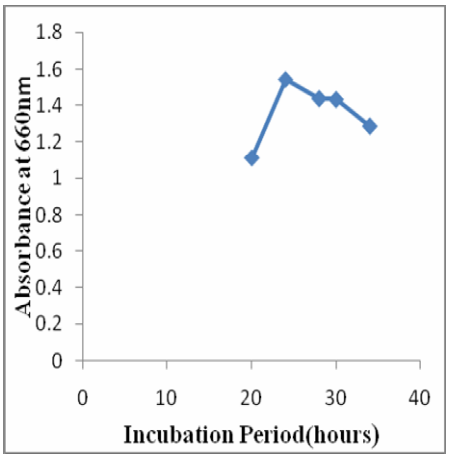


Saccharomyces cerevisiae

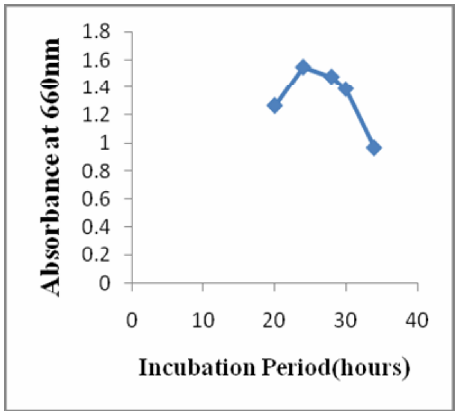
Figure.3 Optimization of Incubation Period



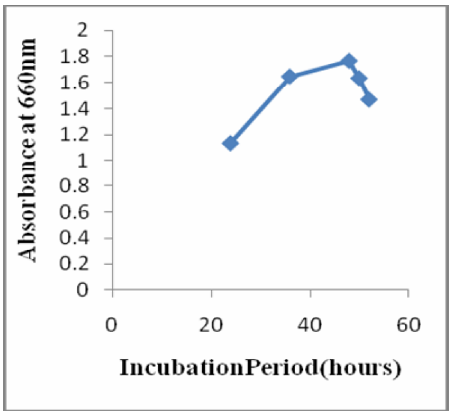
Bacillus subtilis



Bacillus coagulans



Lactobacillus acidophilus



Saccharomyces cerevisiae

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