FIELD EVALUATION OF SOME USEFUL MICROBES AS GROWTH PROMOTERS IN BROILERS

THESIS

BY

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Submitted to

CHAUDHARY SARWAN KUMAR
HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA

IN

Partial fulfilment of the requirements for the degree

OF

MASTER OF VETERINARY SCIENCE
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Dedicated
to
my parents

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CERTIFICATE I

This is to certify that the thesis entitled **"Field evaluation of some useful microbes as growth promoters in broiler"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Science** in the subject of **Animal Nutrition** of Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, is a bonafide research work carried out by **Ms. Tamanna Gupta** daughter of Sh. K.K. Gupta under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation has been fully acknowledged.

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Place: Palampur
Dated: 31.7.04

CERTIFICATE II

This is to certify that the thesis entitled **"Field evaluation of some useful microbes as growth promoters in broilers."** Submitted by **Tamanna Gupta** daughter of Sh. K.K. Gupta to the Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Sciences** in the subject of **Animal Nutrition** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
AIA	Acid insoluble ash
AOAC	Association of Official Analytical Chemist
Av.	Average
B.	Bacillus
BIS	Bureau of Indian Standards
Ca	Calcium
CF	Crude fibre
COVAS	College of Veterinary and Animal Sciences
CP	Crude protein
CSKHPKV	Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya
DCP	Digestible Crude Protein
DFM's	Direct Fed Microbials
DM	Dry matter
DORB	De-oiled rice bran
DS	Double strength
E. coli	*Escherichia coli*
EE	Ether extract
et al.	*Et alli* (and others)
etc.	Et cetera (and the rest)
FCR	Feed conversion ratio
Fig.	Figure
g	Gram
GIT	Gastro intestinal tract
GIW	Gain in weight
GNC	Groundnut cake
h	Hours
H.P	Himachal Pradesh
H_2SO_4	Sulphuric acid
i.e.	*Id est* (That is)
I.U.	International Units
KCal	Kilo calories
Kg	Kilograms
L.	Lactobacillus
LSDM	Lactobacillus-Streptococcus differential medium
ME	Metabolizable energy
mg	Milligram
Min.	Minimum
N	Nitrogen
NaCl	Sodium Chloride
NDF	Neutral detergent fibre
NFE	Nitrogen free extracts
NH_3	Ammonia
No.	Number
NPN	Non-Protein Nitrogen
P	Phosphorus
$P<0.01$	Significant at 1% level

P<0.05	Significant at 5% level
P<0.0001	Significant at 1% level
pH	Hydrogen ion concentration
Rs.	Rupees
S.	*Saccharomyces*
Spp.	Species
SSA	Streptococcus selection agar
T	Treatment
TA	Total ash
TTC	Tri phenyl tetrazolium chloride
Vit.	Vitamin
Viz	Videlecet (Namely)
Vs	Versus
w/v	Weight by volume
Wt.	Weight
YFB	Yeast Fermentation Broth

INTRODUCTION

CHAPTER 1

INTRODUCTION

The major aim of any enterprise is to produce maximum with minimum input and poultry industry is no exception. Feed alone accounts to 70% of total poultry production. The constant increase in cost of poultry feed ingredients and compounded feeds is going to narrow the economic returns of the poultry farmers. Therefore, balanced and efficient feeding is the most important requisite to a superior germplasm for economic poultry production. The growth promoters like synthetic hormones and antibiotics have been extensively used for enhancing poultry production but due to their residual effects in finished products, they lead to various health hazards to consumers. Thus the best alternative appears to be the use of probiotics ie. direct fed microbials (DFM's). The probiotics not only enhance productivity but also provide hazard free animal products.

The concept of microbial manipulation in gastrointestinal tract was first appreciated by Metchnikoff (1907) who viewed the consumption of yoghurt by Bulgarians peasants as conferring a long span of life. The term 'probiotic' was first coined by Parker (1974) who described this as "microorganism or substance, which contribute to the intestinal microbial balance." In 1989, Fuller defined the probiotic as "a live microbial fed supplement, which beneficially affects the host animals by improving its intestinal microbial balance." The term 'probiotic' is derived from a greek word 'Probios' meaning for life." Probiotics have been described as opposite of antibiotics (Stephens & Bekanyl, 1990). While antibiotics destroy life, probiotics build up or promote life.

At present, probiotics are classified as GRAS (Generally recognized as safe) ingredient by the US Food and Drug Administration. Now it is generally accepted that

certain viable microbial cultures beneficially affect the productive performance of poultry. The function of a probiotic is to improve the growth and development of the normal, desirable microbial population in the gut, allowing them to maintain domination over the undesirable organisms. A probiotic can be a live (viable) culture of microbial species, a dead (non viable) product of microbial fermentation or an extract of plant origin.

The most important feature of a well functioning gastrointestinal tract is microbial balance, which means a beneficial ratio between lactic acid producing bacteria and other microbes. The birds are submitted to several stress factors, such as transport from the incubator to commercial farms, overpopulation aviaries, vaccination and temperature changes. This tends to induce a misbalance in the intestinal microflora and damages to the bird's corporal defence mechanism (Jin *etal.*, 1997) causing low productive performance, intestinal infections, as intestine rotting with formation and liberation of toxins, jeopardizing of the growth, reduction of the meat's quality and reduction of the reproductive efficiency; possibility that opportunistic bacteria because pathogenic; emergence of infectious diarrhoea and anemia (Fox, 1988).

The use of probiotics as daily supplement has become a popular routine in commercial poultry industry. Beneficial bacteria also help to fight off the pathogenic bacteria that pass through the system, before it has a chance to take hold. The intestinal tract contain receptor sites, when probiotic enter the digestive system, they occupy these sites, restricting the attachment of harmful bacteria i.e. their adhesion and colonization (Salminen *et al.*, 1996). Probiotics keep the gut at a desirable pH thus allowing the growth of beneficial bacteria, which produce a natural antibiotic like

substance called bacteriocins (Suskovic *et al.*, 1997), which helps to eliminate unwanted coliforms.

Some strains of microbes have been reported to shows their beneficial effects in monogastrics depending upon their source, type of feed given to stock and their strain. Probiotic preparation may consist any number upto eight strains. The attraction of multiple strain preparation is that they are active against a wider range of conditions. The species, which have been most commonly in use in the probiotic preparation, are *Lactobacillus bulgaricus, L. acidophilus, L. casei, L. salivarius, L. helveticus, L. lactis, L. plantarum, L. fecalis, Lactococcus thermophilus, Lactococcus uberis, Enterococcus faecium, Saccharomyces cerevisiae* and *Torulopsis sphaerica* etc. Lactococci and *Lactobacilli* are the most commonly used groups of bacteria in the production of probiotic. Besides yeast and unicellular fungi are also known for their fermentative ability. Yeast produces enzymes such as amylases, proteases, lipases, cellulases as well as B complex vitamins in the medium in which they grow.

The benefits of feeding probiotics to poultry are that they stimulate immune system (Sanders, 1984), improve utilization of proteins, intestinal tract health (Siitonen et al., 1990), increases feed conversion ratio, strengthens beneficial microbial populations and suppress harmful bacterial growth in the digestive system, reduces colon and bladder cancer in monogastrics and ruminants (Rafter, 1995), counter acts ill effect of antibiotic treatment by sustaining the population of beneficial bacteria, increases egg size and help in nutrient synthesis and increases its bioavailability (Sahani and Chandan, 1979).

Though much research work has been done on probiotic and its utility at university level but information regarding its use in commercial broiler production at

field level is still far from being complete. Thus the present study was conducted to ascertain its effectiveness under practical feeding conditions.

Keeping in view the above information, the present investigation was undertaken with the following objectives:

1. To test the efficacy/validity of *Lactobacillus acidophilus* (bottle gourd), *Lactococcus uberis* (bitter gourd) and *Saccharomyces cerevisiae* (bitter gourd) at three different locations (farmers' level) on the biological performance of commercial broilers from 1-day to six weeks of age.
2. To work out the cost benefit ratio of broiler production.

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

The literature has been reviewed under the following heads:

2.1 Chemical composition

2.2 Probiotics in the diets of broilers

2.1 Chemical composition

2.1.1 Chemical composition of feed ingredients

2.1.2 Proximate composition

Bhatt (1993) reported the CP, CF, EE and ash per cent as 9.00, 2.50, 3.20 and 1.9 for maize; 13.10, 3.20, 2.30 and 2.90 for wheat; 39.00, 8.50, 8.10 and 6.20 for groundnut (expeller); 34.20, 20.00, 2.10 and 10.20 for sunflower cake; 47.50, 8.90, 1.20 and 6.90 for soya flakes and 48.00, 0.00, 5.80 and 18.00 for fish meal. Katoch (1996) reported the CP, CF, EE and ash per cent as 9.00, 3.10, 3.00 and 5.30 for maize; 12.10, 1.70, 3.00 and 3.20 for wheat; 40.50, 9.00, 2.10 and 6.90 for groundnut (expeller); 29.60, 8.20, 9.00 and 9.00 for sunflower cake; 42.10, 4.00, 1.00 and 7.00 for soya flakes and 45.50, 2.00, 4.50 and 6.00 for fish meal. Similarly, Gupta (2003) reported the CP, CF, EE and ash per cent as 9.00, 3.11, 3.00 and 5.31 for maize; 12.50, 1.60, 3.00 and 3.20 for wheat; 40.24, 8.90, 2.12 and 6.88 for groundnut (extraction); 32.22, 8.21, 9.04 and 9.03 for sunflower cake (extraction); 42.63, 3.91, 1.00 and 7.04 for soya flakes and 51.38, 2.01, 4.55 and 6.07 for fish meal (jawla) and 16.89,14.03, 1.15 and 13.60 for de-oiled rice bran.

2.1.3 Cell wall components of feed ingredients

Bhatt (1993) reported the NDF, ADF, hemi-cellulose and cellulose per cent as 19.25, 16.25, 3.00 and 3.80 for maize; 18.00, 12.40, 3.60 and 4.00 for wheat; 19.50, 11.50, 8.00 and 6.50 for groundnut cake (expeller); 38.30, 28.50, 9.80 and 19.70 for sunflower cake (deoiled) and 25.20, 13.10, 12.10 and 8.50 for Soya flakes. Katoch (1996) reported the NDF, ADF, hemi cellulose and cellulose per cent as 19.10, 16.10, 3.10 and 3.75 for maize; 15.90, 12.20, 3.70 and 4.05 for wheat; 19.30, 11.60, 7.70 and 6.60 for groundnut cake (expeller); 38.10, 28.40, 9.70 and 19.80 for sunflower cake (deoiled) and 25.50, 13.00, 12.50 and 6.50 for soya flakes. Similarly, Gupta (2003) reported the NDF, ADF, hemicellulose and cellulose per cent as 19.37, 14.55, 4.82 and 3.72 for maize; 15.99, 10.79, 5.20 and 4.09 for wheat; 19.30, 10.59, 8.71 and 6.67 for groundnut cake (extraction); 38.11, 11.10, 27.01 and 6.48 for sunflower cake (deoiled) and 25.49, 16.89, 8.60 and 6.48 for soya flakes.

2.2 Probiotics in the diet of broilers

2.2.1 Concept of probiotic

Probiotics are viable bacterial cell preparation or food containing viable bacterial cultures or components of bacterial cells that have beneficial effect on the health of the host (Delley *et al.*, 1990, Hertel et al., 1991, Ehrmann *et al.*, 1992 & 1994, Charteris *et al.*, 1997).

In the modern intensive poultry production, newly- hatched chicks have little chance to contact with their mother, thereby the growth and colonization of normal micro flora in the intestine is slow (Fuller, 1989). This situation makes the chicks likely to be affected by pathogenic bacteria due to sterile condition of intestine, then subsequently causing food-borne diseases to human beings (Pivnick and Nurmi, 1982).

Probiotic, which is a live microbial feed supplement and beneficially affects the host animal by improving its intestinal microbial balance, has been used as the alternative tool for helping newly hatched chicks to colonize normal micro flora as conventionally hatched chicks do (Fuller, 1989). There are two types of microflora, which colonize the gastrointestinal tract in animals viz; beneficial microbes that colonize gut surfaces in a symbiotic relationship with the host and undesirable that are potentially pathogenic. Under normal conditions, the beneficial organisms predominate, which are essential to normal physiological functions supplying nutrients to the host, aiding in digestion of dietary nutrients and competing with potential pathogens. Besides the therapeutic use for controlling pathogenic bacteria in poultry, probiotics also improves the performance of poultry. They also prevent contamination of carcasses by intestinal pathogens during processing and promote higher growth rate and feed conversion efficiency in growing chickens (Hose & Sozzi, 1991; Juven *et al*, 1991). When the adverse conditions prevails in the internal environment, the resistance of animals get lowered due to stress and thus normal micro-flora of GIT gets affected and pathogenic microbes proliferate which leads to various problems like reduced feed intake, lowered production, reduced performance, diarrhoea and gastroenteritis.

According to Fuller (1989), Probiotics are the food supplements based on live microorganism that affects beneficially the host animal, promoting balance intestinal microflora. Public disapproval and banning of antibiotics and growth hormones as feed additives in certain parts of the world has renewed the use of probiotics in chicken feeding (Hose and Sozzi, 1991).

The concept of probiotic was originated by Metchinikoff in 1907. The word "probiotic was first used by Parker (1974) to describe microorganism and substances

which contribute to intestinal microbial balance. Soagard and Suhr-Jenssen (1990) described probiotics as live microbial cultures that are administered to animals with the primary aim of preventing infectious diseases by strengthening the barrier functions of the gut microflora or by non-specific enhancement of the immune system. Gournier *et al* (1994) redefined probiotic as live microbial preparations used as nutritional additives and with beneficial action on the host by promoting digestion and intestinal balance. Lactic acid bacteria have anticarcinogenic activity as reported. Supplementation of lactic acid bacteria may reduce the risk of colon cancer in both animals and herd-man (Friend & Shahani, 1984). Miller *et al.*,(1985)suggested that Lactobacilli could be important in development of immunity in young animals, particularly during weaning when protection must be acquired against antigens likely to cause inflammatory reactions. The intensity of production system in the poultry industry produces a great stress on the birds. Beneficial effects with the use of probiotic have been obtained on productive parameters in broilers (Rincon *et al.*, 2000). Most of the probiotics now available in the market contain lactobacilli, lactococci and yeast cultures alone or in combination with each other. Although the production of probiotic would permanently colonize the gut and would require a limited administration. The permanent establishment of probiotic strains would require their displacement and would be difficult to induce. In the young ones, micro flora is in a more unstable condition thus administration of probiotics immediately after birth to them is most necessary to compete with acquired flora.

2.2.2 Bacterial Probiotics

The survival of beneficial bacteria in the gut depends on their colonization characteristics due to which they resist the antibacterial factors operating in the gut. Stable flora of intestine helps to resist the infections of gut. This phenomenon has

been described by various workers under different names as bacterial antagonism (Freter, 1965), bacterial interference (Dubos, 1963), barrier effect (Ducluzeau *et al.*, 1970), colonization resistance (Vander Waaij *et al.*, 1977). Among all these terms competitive exclusion is most commonly used. The competitive exclusion concept was first applied by Nurmi in Finland in 1973 and became known as " Nurmi concept". This can be defined as "early establishment of complete intestinal microflora to prevent colonization by enteropathogens". Nurmi and Rantals (1973) reported the protective effect of intestinal flora in hatched chicks, which were inoculated with adult chicken excreta by restricting the caecum colonization by *Salmonella infants* (Reynaldo and Hoyos 1992). Schneitze (1992) described the phenomenon of probiotics as " Early establishment of complete intestinal micro flora to prevent colonization by entero- pathogens".

The species of bacteria that are used in probiotic preparations are *Lactobacillus bulgaricus, L. acidophilus, L. casei, L. helveticus, L. lactis, L. salivarius, L. plantarum, L. faecalis, Streptococcus thermophilus, S. faecium, Enterococcus faecium, Enterobacteria faecalis and Bifidobacterium species.* Lactobacilli and Streptococci are most commonly used group in the production of probiotic. Lactic acid bacteria are commonly used in most probiotic preparation due to historical belief that they are the desirable members of the intestinal microflora and thus generally regarded as the safe (Tannock, 1997). However not all the lactic acid bacteria have the probiotic function and the primary characteristic required for the candidate probiotic are the ability to survive in the acidic stomach and to establish in the GIT of host (Conway *et al.*, 1987, Lee & Salminen, 1995). The specific role of lactic acid bacteria as the probiotic has been extensively discussed by Juven *et al* (1991). Lactic acid bacteria produce many kind of metabolites which might affect the

other microbes in the gut. The lactic acid produced by homolactic and heterolactic strains reduce the pH of the luminal contents, which is most obvious in the stomach of neonatal piglets (Cranwell *et al.*, 1976). Moreover, acetic acid and hydrogen peroxide produced by lactic acid bacteria are inhibitory against coliforms, salmonella and the clostridia in vitro (Nousiainen, 1993). Lactobacillus group in general and *L. sporogenes* in particular are largely used as probiotic and are claimed to improve the performance of layers and broilers by suppressing the harmful effect of *E. coli* in the digestive tract (Mohan Kumar & Christopher, 1988 and Mudalgi *et al.*, 1993). Lyons and Jacques (1987) reported that acidification of feed and/ or water complemented the role of probiotic bacteria.

2.2.3 Characteristics of an ideal probiotic

1. Should be a strain, which is capable of exerting a beneficial effect on the host animal e.g. increased growth or resistance to disease.
2. Should be non-pathogenic and non-toxic to animals and human.
3. Should be present as viable cells, preferably in large numbers although the minimum effective dose is not fully defined.
4. Should be able to withstand processing and storage.
5. High tolerance to bile and gastric acid pH.
6. Adhere to epithelium or mucus.
7. Persist in intestinal tract.
8. Produce inhibitory compounds.
9. Modulate immune response.
10. Alter microbial activity.

2.2.4 Mode of action of probiotics

The beneficial effects of probiotic may be mediated by direct antagonistic effects against specific group of organisms, resulting in a decrease in number or by an effect on their metabolism or by synthesis of some essential nutrients or by stimulation of immunity.

1. Suppression of viable count

   a) By production of antibacterial compounds.

      i) The antibacterial substances like lactocidin, organic acids, and bacteriocins.

      ii) By production of hydrogen peroxide, organic acids.

   b) By competitive exclusion

      i) Competition for adhesion sites.

      ii) Competition for nutrients. Pathogenic and non pathogenic bacteria usually compete for food. Non pathogenic bacteria usually have high competitive power, colonizing the intestine better (Fox, 1988).

2. Alteration of microbial metabolism

   a) By increased digestive enzyme activity e.g. α amylase, β galactosidase etc which aid in digestion of various carbohydrate, fat and protein and absorption of nutrients.

   b) By decreased bacterial enzyme activity e.g. of β glucoronidase, nitroreductase and azoreductase which are produced by some pathogenic bacteria.

   c) By decreasing ammonia production.

3. Stimulation of immune system, making birds less vulnerable to disease.

   a) By increasing antibody levels.

b) By increasing macrophage activity.

4. Decreasing gut pH by production of lactic acid
5. Offering digestible proteins, vitamins, enzymes and other cofactors.
6. Absorption of toxins.
7. Increasing the area of absorption of small intestine.

2.2.5 Yeast culture or Yeast probiotics:

The role of yeast is not exactly clear as yet but it is understood that they are normal inhabitants, in small numbers, of the rumen and intestines of herbivores. Yeast is concerned with sugar fermentation and cellulose digestion. Under certain conditions they may suppress the growth of pathogens, synthesize B-complex vitamins and also may help in stabilizing normal micro flora of GIT. Most common genera of yeast are namely; *Candida, Trichosporon, Rhodtoirula and Saccharomyces* have been reported in the GIT of ruminants.

Yeasts are unicellular fungi with strong fermentative ability. *Saccharomyces cerevisiae* is most commonly used yeast in poultry industry. It is one of the highly resistant bugs. It neither gets interfered nor interferes with the activity of antibiotics. This yeast multiplies by phenomenon of budding and has oval and rounded morphology. When the new bud arises from the parent cell, it grows and thus separates finally to become a new yeast cell. They have the ability to utilize the wide range of sugars including fructose, sucrose, glucose and maltose when used as additive in animal feed. Yeast cultures are dehydrated product of yeasts and media on which they are grown. Yeast culture which is used as feed additive is unique in that the, culture process, leads to production of enzymes in the residual growth medium. Due to their ability to absorb bitter compounds, yeasts have bitter taste. Baker's yeast and Torula yeast are the most commonly used additives in animal feeds.

2.2.6 Mechanism of action of yeast in monogastrics

pH is the most important prerequisite factor for yeast growth and pH of 4.5 is supposed to be the optimum pH for yeast growth. But in case of poultry birds' pH of digestive tract is highly acidic and is far below this range, as a result of this hyper acidic environment, yeast produces chemical compounds such as amino acids, nucleic acids, some B-complex vitamins and some of the lytic enzymes. In the presence of hyper acidic pH, yeast becomes unable to adjust to such adverse environment. As a result of this, cellular metabolic machinery reacts and leads to over production of these metabolites. When the lyses occur, there is release of these compounds in excess, which increases the micronutrients to stimulate the digestive process. Under optimal environmental conditions, these compounds gets incorporated into cellular compounds and leads to stimulation of autolysis as a result of which there is release of cell compounds like amino acids, fatty acids and sugars in to the intestinal environment. Yeast has absorbent properties towards wide variety of compounds and act as a nutrient source for intestinal flora (Rose, 1987).

2.2.7 Mechanism of action of *Saccharomyces cerevisiae*

Saccharomyces cerevisiae releases mannoligosacchride (MOS) through its cell wall. Mannoligosacchride with mannan sugar can preferentially bind to bacterials lectins before they get attached to surface carbohydrates of gut epithelia and literally carry them out of the gut. Hence, pathogens pass through the gut without getting colonized. It is a classic competitive exclusion. Several pathogens *E. coli* and salmonella possess lectins, which are specific to mannose and hence get bound by MOS. Apart from that it produces certain cofactors, which are beneficial to birds. In addition, they are also proved to bind mycotoxins present in feed.

2.2.8 Probiotics in the diets of broilers:

2.2.8.1 Effect on growth rate and gain in weight:

Various studies have proved that there is marked increase in body weight when broilers are fed with probiotics of various types at different levels. Probiotic also improve general health of chicks (Chapman and Lyons, 1989; Buche *et al.*, 1992; Holoubek, 1993; Sharma and Katoch, 1996; Ghadban 1998). Tortuero (1973) cited by Jernigan *et al.* (1985), compared the use of probiotic *Lactobacillus acidophilus* in the drinking water and / or antibiotic bacitracin Zn in the ration. The author concluded that poultry fed with probiotic and antibiotic gave better weight gain and feed conversion ratio. Krecov and Pujic (1975) fed two groups of 600 broilers LBA (*Streptococcus faecium*) @ 200g/400kg feed and observed that with and without LBA, total weight gain was 1570 and 1545g. Melluzzi *et al.* (1986) studied the effect of lactic acid bacteria and bifid bacteria in broiler chicks and observed that birds fed with 2 per cent of lactic acid bacteria culture gave significantly ($P \leq 0.05$) higher body weight than that of control given reconstituted sterile milk. Mohan and Christopher (1988) concluded that probiotic (*Lactobacillus sporogenes*) increased weight gain in broilers. Kim *et al.* (1990) observed increased weight gain of chickens offered maize diet supplemented with probiotics. Guillot and Yvore (1990) reported that the probiotic Bacillus species significantly ($P \leq 0.05$) promoted the growth of chicken. Shin (1991) reported that addition of yeast culture to the broiler diet at 0.1, 0.2 and 0.3 per cent, respectively improved broiler growth rate. It appeared that feeding live yeast culture had a stimulatory effect on the lactic acid bacteria of small intestine, resulting in suppression in coliform bacterial population.

Moses (1992) reported that when probiotics and antibiotics combination was used, the body weights at seventh week were 1.6 kg against 1.2 kg in control. Cho *et*

al. (1992) reported the growth promoting ability of *Lactobacillus casei* with antibiotics and observed that 3.4 to 6.0 per cent increase in body weight gain with *Lactobacillus casei.* Prasad and Sen (1993) reported that broilers receiving probiotic Biospur containing *Lactobacillus sporogenes* grew faster than control upto six weeks of age. Mudalgi *et al.* (1993) observed that birds offered probiotic cultures (*L. acidophilus* and *L. bulgaricus*) appeared to gain numerically higher weights than those fed the control diets irrespective of protein level. Chicks fed *L. bulgaricus* with low and high protein diet gained 5.7 and 6.5 per cent, more weight, respectively over the control birds. Stanley *et al.* (1993) observed that when broiler chicks were fed for 4 weeks from day-old with diet containing *Saccharomyces cerevisiae* at 0.00, 0.05 or 0.1 per cent of feed and aflatoxin at 0 or 5 ppm (mg/kg feed). Chicks receiving aflatoxin contaminated feed had suppressed body weight (456g), which significantly improved (516g) with inclusion of 0.1 per cent *Saccharomyces cerevisiae.* Bhatt (1993) reported significantly ($P \leq 0.05$) higher live weight gain in broiler stock supplemented with *Streptococcus lactis* and *Saccharomyces cerevisiae.* Manicham *et al.* (1994) recorded a highly significant ($P \leq 0.01$) difference in weight gain between control and treatment groups when Lactobacillus based probiotic was given at the rate of 1g per liter of drinking water for a period of 0-6 weeks. Sapcota *et al.* (1994) found that the supplementation of probiotic LSP (*Lactobacillus sporogenes* powder) @ of 150 million spores/kg feeds, leads to maximum weight gain followed by group fed standard recommended dose of 100 million spores/kg of feed. Singh *et al.* (1994) fed three microbial cultures viz., *L. casei, L. acidophilus and L. bulgaricus* and found that at 6 weeks of age the body weights were 1182, 1205 and 1186 g, respectively. Maruta (1993) observed an increase of musculature and decrease of abdominal fat, mainly in

males and reduction in poultry characteristic smell when fed diets with probiotic *Bacillus subtilis*.

Taklimi *et al.* (1994) reported that the feeding of diets supplemented with live yeast (*Saccharomyces cerevisiae*) to broilers improves the growth performance. Chiang and Hsiela (1995) observed better weight gain ($P<0.01$) in birds fed with probiotic supplemented diets (mixture of Lactobacillus, Bacillus and Streptococcus) as compared to the birds fed probiotic unsupplemented diets. They also conclude that excreta ($P\leq0.05$) and litter ($p\leq0.01$) ammonia levels were reduced by dietary probiotic supplementation. Ignacio and Sefton (1995) observed that at the end of the 42-day trial period, average weights were 1492g and 1725g for control birds and birds fed 1g of a commercial yeast culture, respectively. Hamid *et al.* (1996) reported that feeding of broiler chicks with diets supplemented with *L. acidophilus* cultures showed higher gain in weight (945.5 g) as against control (773.5g). Wohkle *et al.* (1996) studied the effect of *Bacillus* natoo in different dosages (0, 50, 75 & 100 $X10^9$ spores/ration t) on broiler performance and concluded that male chicken presented an improvement in weight gain and feed conversion. Katoch *et al.* (1996) supplemented different probiotics in broiler diets and observed improvement in body weight gains in the range of (1063.47g to 1213.28g) against (961.85g) in control. Verma *et al.* (1996) observed that *S. lactis* (S_1) and *Saccharomyces cerevisiae* (Y_3) microbial cultures when added in diets of growing broiler chicks, improved growth rates. Jin *et al.* (1996) reported that the weight gain in chickens given feeds incorporated with *Bacillus subtilis* and *Lactobacilli* was significantly ($P< 0.05$) higher than those of the control. Kaistha *et al.* (1996) compared the effect of supplementation of *L. acidophilus*, *S. uberis* and *S. cerevisiae*, standard strains, *S. cerevisiae* (Y3), *L. bulgaricus* (L4) and *S. lactis* (S1) and also with the control group of broilers upto six

weeks of age. They observed that although there were no differences in live weight gain during starter phase, however, improvements were observed ($P<0.05$) during the finisher phase. Where as Katoch *et al.* (1996) reported that the growth performance of broilers was significantly ($P\leq0.05$) better in treatments fed Y_3 strain of *S. cerevisiae* during 1-6 weeks of age. Verma *et al.* (1996) observed that *S. lactis* (S_1) and *Saccharomyces cerevisiae* (Y_3) microbial cultures when added in diets of growing broiler chicks, improved growth rates. Joy and Samuel (1998) reported that supplementing diets of broilers with *Lactobacillus sporogenes* @ of 100 million spores orally per chick from 1 to 42 days leads to increased ($P<0.01$) weight gain.

Pedron *et al.* (1997) reported that when *Pediococcus acidilactici* fed to the broilers raised for eight weeks, the growth of the treatment groups was higher than that in control. Sarkar *et al.* (1997) observed that when different cultures of yeast and antibiotics were used in broiler ration, the weight gain at 6 weeks was ranging between 1677.27 to 1733.35 against 1644.91 g in control. Wolke *et al.* (1997) carried out an experiment where broilers were fed without or with the probiotic *B. natto,* weight gains were better ($P\leq0.05$) in male chickens fed with probiotic and no significant differences were observed in female chickens. Samanta and Biswas (1997) reported that the dietary supplementation of *Lactococcus spp.* improved body weight of broilers along with reduction in mortality without affecting the feed intake and feed conversion ratio. Senani *et al.* (1997) reported that the dietary supplementation of different levels of *lactobacilli* in the broilers resulted in better microbial colonization in the gut region along with decrease in invasion of the pathogenic organisms. Bhatt *et al.* (1997) reported the effect of dietary supplementation of different strains of *Streptococcus lactis* on the biological performance of commercial broilers (Starbro broilers). S_1 strain procured from CFTRI, Mysore, was the most effective because it

showed significantly higher broiler live-weight gains coupled with efficient feed efficiency ratio and minimum chick mortality during both the starter and finisher phase as compared to other strains - HPKV, Palampur and control group. Katoch *et al.* (1998) reported that dietary supplementation of the selected strains of *Lactobacillus bulgaricus* (L_4), *Streptococcus lactis* (S_1) and *Saccharomyces cerevisiae* (Y_3) alone and in different combinations on 'IBL-80' broilers gave significantly ($P \leq 0.05$) higher growth rates and feed efficiency ratio coupled with zero mortality as compared to control during the starter phase. On the basis of overall picture with respect to growth rate, feed efficiency ratio, mortality per cent, the combination of three microbes as mentioned above and L_4 strain of *L. bulgaricus* was the most promising probiotics.

Zaho Yanbing and Zaho (1999) reported that when Lactobacillus was fed to 60 chickens, their average daily weight gain increased by 24.4 per cent as compared to control. Georgieva *et al.* (2000) found significantly highest body weight in broiler chicks coupled with better FCR on feeding probiotic Lacto Sac @ 1kg/ton of feed as compared to other group containing antibiotic and negative control. Katoch *et al.* (2000) studied the dietary performance of combination of *Lactobacillus acidophilus*, *Streptococcus faecalis and Saccharomyces carlsbergensis* isolated from Leopard-excreta and the combination of their respective standard counterparts ie. *L. bulgaricus (L4)*, *Streptococcus lactis* (S_1) and *Saccharomyces cerevisiae* (Y_3) on commercial broilers up to six weeks of age. During the starter, grower and overall phases, both the microbial combinations gave significantly ($P \leq 0.05$) higher gain in weight only in "Vancob strain" of broilers as compared to control group. Shome (2000) reported that when mixture of *Lactobacillus acidophilus and L. salivarius* was fed to broiler, the average live weight of chicken was higher during starter phase in experimental group

as compared to control. Banday and Risam (2001) coducted an experiment to determine the efficiency of probiotic (Biospur) at 3 different levels-25g/100kg, 50g/100kg and 75g probiotic/100kg. It was observed that chicks fed with probiotic grew faster than control and the highest live weight gain was obtained in group fed @ 75g probiotic/100kg. Kumar *et al.* (2002) observed that supplementation of EY Micromix @ 30g and 40g/q of feed showed significantly higher gain in weight at 1 and 5 per cent level of significance, respectively at marketable age. Upendra and Yathiraj (2002) observed that supplementation of 'Lactosac' (a combination of *S. cerevisiae*, *L. acidophilus* and *S. faecium*) @ 250g per ton of feed resulted in numerical increase in body weight gain (1.7% mean body weight) as compared to control. Kumar *et al.* (2003) studied the effect of supplementing *L. acidophilus*, Mannon oligosacchride and native gut culture in chickens which were experimentally infected with *Salmonella gallinarum* and reported singnificantly ($P<0.05$) increase in weight gain and increase in organ versus body weight percentage of lymphoid organs in probiotic supplemented group as compared to control indicating immuno stimulatory action of probiotic with consequent disease resistance. Upendra and Yathiraj (2003) evaluated the effect of *S. cerevisiae*, *L. acidophilus* and MOS in four groups of broiler chicks (1-control, 2- *S. cerevisiae*^{1026} ($36X10^7$/g), 3- *L. acidophilus* ($30X\ 10^4$), 4- MOS respectively) @ of 1g/kg of feed. The results revealed that mean body weight of broiler chicks on day 14, 28 and 48 days were significantly $P\leq0.05$ higher in group 2, group 3 and group 4 as compared to group 1 i.e. control. Gupta (2003) supplemented different strains of Lactococci isolated from excreta of Sambhar, Himalayan Black bear and Monal and their standard counterpart- *L. lactis* (CFTRI, Mysore) and Bacitracin. He found that differences in gain in weight were significantly ($P\leq0.05$) different in all the treatment groups as compared to unsupplemented control

and also concluded that groups fed with Bacitracin and Lactococcus species isolated from Monal showed highest per cent increase in gain in weight i.e. 6.80 and 5.44 per cent over control.

Some researchers have also reported non-significant differences in live weight among the groups fed high quality rations or with probiotics (Adler and DaMassa, 1980; Watkins and Kvatzer, 1984 & 1988; Maiolino *et al.*, 1992; Sarkar *et al.*, 1997).

Mandal *et al.* (1994) investigated that when 10 g of live yeast culture of *S.cerevisiae* was used in feed, the weight gain was 1653.27 Vs 1705.67 g in control. Yadav *et al.* (1994) observed that baker's yeast does not play any significant role in broilers when used as a probiotic. Samanta and Biswas (1995) reported that probiotic (Lactobacillus species) as well as lactic acid feeding did not produce significant results on the growth performance of broiler chicks. Wambeke *et al.* (1995) observed that when broilers were given a conventional diet without and with paciflor, body weight was not affected by treatment at 3 weeks and 6 weeks of age by paciflor supplement. Kahraman *et al.* (1996) fed broiler chickens diets supplemented with sodium bicarbonate or probiotic Fastrack R in different combinations. They observed that addition of Fastrack R alone or with sodium bicarbonate to the diet did not influence the growth performance of broiler chickens. Senani *et al.* (1997) reported that addition of *L. casei* to broiler diet lead to non significant improvement in growth rate, FCR and survivability. Miazzo and Kraft (1998) reported that when diet of broilers added with brewer's yeast (*S. cerevisiae)* at 0.5 and 1.5 per cent from 29th to 53rd day and 0.3 and 0.6 per cent yeast from 18th to 35 th day and 0.3 and 0.5 per cent yeast from 18th to 50th day results improvement in weight gain. Saha *et al.* (1999) observed non significant effect of baker's yeast supplementation on growth, nutrient utilization and carcass quality of broilers. Erdogan-Z (1999) studied the effects of two

different probiotics (Thepax R and Fastrack R) and zinc bacitracin on body weight gain of broiler chickens. No significant improvement was recorded. Panda *et al.* (2001) studied the effect of dietary supplementation of probiotics on growth and gut microflora of broilers. No significant effect on body weight gain was reported however a significant decrease in *E. coli* count was reported.

2.2.8.2 Feed consumption and feed efficiency:

Mohan and Christropher (1988) reported that feed efficiency in broiler was improved with probiotic *Lactobacillus sporogenes*. Mohan (1991) reported that though there was a marginal increase in cumulative feed intake, varying from 40-69 g for the 5 weeks period, it resulted in better utilization of feed and thus improved the feed efficiency by 1.85 and 1.84 in 75 and 100 mg probiotic supplemented group, respectively, as compared to 1.94 in control birds. Cho *et al.* (1992) reported that the supplementation of *L. casei* improved feed conversion ratio by 0.3 to 3.1 per cent when compared with control and groups supplemented with antibiotics or other probiotics. Moses (1992) reported that suppementation of probiotic product Biospur in diet of broiler resulted in improved FER (2.18 Vs 2.9) at 17^{th} week of age.

Manickam *et al.* (1994) found that the feed conversion efficiency for the probiotic supplemented group and control group was 2.55 ± 0.01 and 2.36 ± 0.01, respectively, which was highly significant ($p<0.05$). Bhatt *et al.* (1995) used different strains of *S. cerevisiae* and recorded feed consumption in range of 2522.1 to 2717.5 Vs 2555.2 g in control. Chiang and Hsiegh (1995) observed that when broiler chickens fed diets supplemented with six probiotic levels upto 6 weeks of age, Gain: Feed ratio was improved with reduction in ammonia production in litter. Hamid *et al.* (1996) observed improved feed gain ratios of birds fed basal diet plus biomass of *L. acidophilus* culture on whey and zinc bacitracin from 2.32 to 2.01. Lin and Quarles

(1996) reported that when broilers were fed ration containing Primalac at the different rate in feed during different stages and observed improvement in feed efficiency from 2.193 to 2.083 in control and treated groups.

Singh and Sharma (1996) investigated the effect of feeding of *L. sporogenes* added at 0.00, 0.02, 0.03 and 0.04 per cent level in broilers. The probiotic combination did not affect the feed consumption, however, at 0.02 per cent level Lactobacillus, improved the feed efficiency, significantly. Verma *et al.* (1996) recorded improvement in feed efficiency, when *L. lactis* (S_1) and *S.* cerevisiae (Y_3) microbial culture supplemented diets were fed to the broiler chicks. Pedron *et al.* (1997) reported that when *Pediococcus acidilactis* MA 18-5M fed to the broilers raised for 8 weeks at high and low levels, they observed that feed efficiency was improved from 2.064 to 1.938 at high level and 1.969 at low level. Takahashi *et al.* (1997) found that when chicken raised in dirty conditions were fed with *Bacillus cereus* improvement in feed conversion efficiency was noticed, however, no improvement in live weight gain or feed intake was noticed. Sarkar *et al.* (1997) reported that when different cultures of yeast and antibiotics were used, the FCR was in the range of 1.89-2.02 against 2.13 in control. Georgieva *et al.* (1998) observed that when broilers were fed with probiotic "Lactosac" (1g/kg), the feed/gain ratio (FCR) was improved by 8.2 per cent. Gohain and Sapcota (1998) reported improvement in the FCR of broiler chicks fed *L. acidophilus* and *S. faecium* microbial supplemented diets. They reported better FCR (2.25) in probiotic treated group as against 2.44 in control. Rajmane and Sonawane (1998) reported that when broilers were treated with probiotic through water @ 20 g/1000 chicks to group B for first five days and from sixth day onwards @ 50 g/tonne of feed till the end of 42nd day of age, the better performance in terms of FCR from 2.37 as compared to control (2.04) was recorded.

Mahajan (1999) studied the effect of probiotic (Lactosac) feeding during summer and winter on the growth performance and carcass quality of broiler. Feed consumption and feed conversion ratio on cumulative basis were significantly ($P<0.05$) higher in probiotic fed broiler during winter and summer, and winter only, respectively. Kumar *et al.* (2002) supplemented broiler diet with EY Micromix @ 30 and 40 g/q and found that FCR at 30 g/q dose level was 2.54 and 2.58 at 40 g/q dose level in comparison to control group.

Upendra and Yathiraj (2002) observed that supplementation of Lactosac @ 250g/t of feed resulted in an environment of FCR to the tune of 10.86 per cent as compared to flocks not supplemented with Lactosac. Statistically significant difference ($P\leq0.05$) was noticed in mean FCR when Lactosac supplemented and non-supplemented flocks were compared. Orilic *et al.* (2002) with his experiment showed that probiotic have beneficial effects on feed conversion and also reduced salmonella in GIT, thus reducing the risk of contamination of poultry meat and eggs. Gupta (2003) supplemented different strains of Lactococci and Bacitracin. He observed that all the test diets showed ($P\leq0.05$) lower FCR than control. Test diet T-6 Bacitracin showed highest cost: benefit ratio of 17.87per cent followed by test diet T-4 (Lactococci isolated from excreta of Monal) 16.98 per cent and control group showed the lowest cost: benefit ratio of 6.22 per cent.

2.2.8.3 Livability:

Hussein and EI- Ashry (1991) found that a Lactobacillus concentrate, 0.5g/kg starter mixture, given to broiler chickens decreased the incidence of diarrhoea and mortality. Talukdar (1992) reported a non-significant difference between different probiotic treatments; however, pure Lactobacilli culture fed groups showed marginally lower mortality. Adsul (1993) recorded total cumulative mortality of 3.05, 4.14, 3.04 and

4.19 per cent in birds fed Lactobacilli culture; enzyme feed supplement, Lactobacilli culture plus enzyme supplement and control, respectively. He concluded that the groups received Lactobacilli culture showed lesser mortality. Lee *et al.* (1994) noticed that viability of groups given antibiotics and probiotics was higher than that of the control diet, which was statistically non-significant. Bhatt *et al.* (1995) reported that chick receiving L_4 strain of *L. bulgaricus* showed lesser mortality when compared with chickens fed control and other strains of *L. bulgaricus*, which was statistically non-significant. Samanta and Biswas (1995) reported that the dietary supplementation of *Streptococcus spp.* improved body weight of broilers along with reduction in mortality without affecting the feed intake and feed conversion ratio. Hamid *et al.* (1996) have observed favorable results due to addition of probiotic in to the diet of broilers on livability. Kaistha *et al.* (1996) also recorded lesser mortality in broiler chicks fed diets supplemented with *L. acidophilus, Str. uberis and S. cerevisiae.*

Miljkovic *et al.* (1998) fed diets to the broilers supplemented with probiotic Acid-Pak-4-way at 0.5% and recorded reduction in percentage mortality. Rajmane and Sonawane (1998) also reported reduction in chick mortality from 7 to 2 per cent on the oral administration of probiotic through drinking water @ 20 g/1000 chicks. Mahajan *et al.* (1999) reported lower cumulative mortality in Lact Sacc fed broilers in both season when compared to control. Shome *et al.* (2000) reported zero mortality in broiler fed with *L. acidophilus* and *L. salivarius* and 12.7 per cent mortality in unsupplemented control, mostly due to bacterial enteropathogens.

Upendra and Yathiraj (2002) recorded significant ($P \leq 0.05$) reduction (54.25%) in chick mortality when chicks were fed diets supplemented with Lacto sacc.

2.2.9 Probiotics in the diets of layers:

Probiotic also have proved to be beneficial in case of layers. Bacteria like Lactobacilli, Streptococci and yeasts like *Saccharomyces cerevisiae* are the most commonly used probiotic in layers.

2.2.9.1 Effect of probiotic on hen-day egg production:

Goodling *et al.* (1987) reported that the addition of liquid, non viable lactobacillus fermentation product to the feed of cage or floor housed laying hens for 48 weeks did not improve hen day egg production. Hsu *et al.* (1987) found no significant difference in the rate of egg production when a *Lactobacillus bulgaricus* factor was added to the diet of a single comb White Leg horn laying hens fed on diets of equal energy and nitrogen value and containing maize or barley as a carbohydrate source. Mohan and Christopher (1988) concluded that *Lactobacillus sporogenes* increased egg yield in hens. Chapman (1988) reported that probiotics in poultry feeds reduce symptoms of stress, acts as natural growth promoters and improve production and general health. Gippert (1992) fed fowls with Lacto-sac and Yea-sac containing diets (1kg/1000kg feed) and found that egg production was 0.4 and 1.1 per cent, respectively. Jadhav *et al.* (1992) fed 120 White Leg horn pullets on isoenergetic and isonitrogenous mash diets containing dried *Lactobacillus sporogenes* @ 0, 20, 40 and 60g/100g feed and found that egg production was 77.14, 74.65, 78.41 and 78.31 per cent, respectively. Nahashon *et al.* (1994) carried out two experiments with White Leghorn laying pullets by feeding them with lactobacillus supplement at the rates of 1100 and 2200 mg/kg had significantly ($P \leq 0.05$) better hen day production. They concluded that Lacto-Sac at 1100 mg/kg in diets of layers stimulated appetite and improved egg production. Nahashon *et al.* (1994) conducted experiments on single comb White Leghorn layers and concluded that Lactobacillus supplementation of

maize- soybean meal significantly improved (P<0.05) hen day-egg production. Okumura *et al.* (1994) found no significant differences in egg production in 84 weeks old laying hens fed with diets supplemented with gluco-ligo-saccharide and biobacteria (*Streptococcus faecalis, Clostridium butyricum, Bacillus mesentericus).* Katoch *et al.* (1994) reported significantly (P<0.05) higher per cent hen egg production in layer stock fed diets supplemented with *Lactobacillus bulgaricus.* Panda *et al.* (2000) recorded improvement in egg production in White Leghorn layers (48 to 64 weeks of age) in the decline phase of production with feeding of diets supplemented with probiotic @ 100 mg/kg diet. Yalcin *et al.* (2002) carried out a study to determine the effects of the usage of enzyme, probiotic or antibiotic alone or in combinations in the rations on egg production of laying hens and recorded improvement in egg production with the supplementation of enzyme, antibiotic, enzyme+ antibiotic, antibiotic+ probiotic and enzyme+ probiotic to the rations.

2.2.9.2 Effect of probiotic on egg weight and egg size:

Hsu *et al.* (1987) reported that feeding of pantethine (*Lactobacillus bulgaricus* factor) to single comb White Leg horn layers had non significant effect on egg weight. Jadhav *et al.* (1992) reported that *Lactobacillus sporogenes* in diet of White Leg horn pullets had no effect on the mean egg weight. Gerend i and Gippert (1992) fed fowls Lacto sac and Yea sac (1 kg/1000kg feed) in diet and found that egg weight was increased by 0.2 g and 0.5 g in the treatment groups. Lactobacillus supplementation in diet of single comb White Leg horn layers improved significantly (p< 0.05) egg size and egg weight (Nahason *et al.* 1994) They further reported that addition of fat to lacto containing diets resulted in better egg weight and egg size. Katoch *et al.* (1994) reported that layer stock supplemented with Lactobacillus and combination of *Streptococcus lactis* + *L. bulgaricus* + *S. cerevisiae* showed higher

egg mass and average egg weight. Yalcin *et al.* (2002) also recorded an increase in egg weight and egg size with the usage of enzyme, probiotic or antibiotic alone or in combinations in the rations of laying.

2.2.9.3 Effect of probiotic on feed intake of layers:

Jadhav *et al.* (1992) reported no effect on feed intake/egg weight with supplementation of *L. sporogenes*. Nahason *et al.* (1994) reported that layers given lactobacillus 1100 mg/kg had better daily feed intake.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The research work was carried out in the Department of Animal Nutrition College of Veterinary and Animal Sciences, CSKHPKV, Palampur in collaboration with farmers group viz at three different villages namely Saner, Quor and Kutkhudiyal of district Kangra. In order to achieve the objectives of the research programme, the feeding experiments were conducted on day old broiler chicks of "Vancob strain". The information regarding collection and chemical analysis of feed samples, feed formulations, selection, distribution and maintenance of poultry stock at three different sites, observations recorded there on are presented in the following pages.

3.1 Collection and chemical composition of feed ingredients

Samples of feed ingredients like maize, wheat, groundnut exraction, soyaflakes, sunflower extraction, fishmeal and de-oiled rice bran were procured from feed store of the department. These were analysed for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract(EE), ash, acid insoluble ash(silica), Nitrogen free extract(NFE), Calcium(Ca), Phosphorus(P) and Metabolisable energy(ME).

3.1.1 Proximate composition

Standard methods as reported in AOAC (1985) were followed for determination of proximate composition of the various feed ingredients.

3.1.2 Cell and cell wall composition

Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) were estimated as per methods suggested by Goering and Van Soest (1970). Hemi cellulose was computed as the difference between NDF and ADF. Lignin was estimated on ADF residue by treating it with 72 per cent sulphuric acid for three hours at 20 to 30 ^{0}C.

3.1.3 Minerals

Calcium was estimated by standard method of AOAC (1985), while phosphorus was estimated by the method proposed by Parks and Dunn (1963).

3.1.4 Metabolizable energy (ME)

The ME contents of test diets used under different treatments were calculated as per the equation proposed by Lodhi et al. (1976), which is

ME (kcal/kg)= 32.95(%CP+%EE x 2.25+%NFE)-29.20

3.2 Statistical analysis

The data was analyzed by using unpaired t test (Statistical package, Graph Pad Instat tm. of Russel 1990).

3.3 Isolation, purification, multiplication standardization and preservation of different microbes.

For assessing the performance on broilers, the following isolated and characterized strains were used viz;

Isolated microbes	**Source**
Lactobacillus acidophilus	Bottle gourd
Lactococcus uberis	Bitter gourd
Saccharomyces cerevisiae	Bitter gourd

The techniques were standardised for the growth and preservation of different microbial strains as detailed below;-

3.3.1 Isolation and characterization of microbes from bitter gourd

2-3 g of finally chopped overripe bitter gourd was taken and incubated in yeast fermentation broth (YFB) at 30 °C overnight. Next day the precipitates that were deposited, taken on a glass slide and then warmed gently on a gas flame for fixation. After fixation, staining was done with gram crystal violet stain for 5 min. The slides

were observed under compound microscope under oil immersion lens. Mixed colonies consisting of cocci, darkly stained yeast with buds and contaminants were seen. The procedure was repeated 3 to 4 times till approximately 90 per cent contaminants were removed. Thereafter, the colonies were grown on differential medium namely Streptococcus selection Agar (SSA), Yeast fermentation broth with antibiotic.

For isolation of *Lactococcus uberis* the colonies were grown in SSA, which had the following composition:-

Ingredients	**g/l**
Tryptone	15.00
Soya peptone	5.00
Sodium chloride	4.00
L cystine	0.2
Sodium sulphite	0.70
Dextrose	5.50
Sodiumazide	0.20
Crystal violet	0.002
Agar	15.0

The 46.6 g of SSA was suspended in 1liter-distilled water. It was then heated to dissolve the contents completely. The pH was adjusted to 7. It was then sterilized by autoclaving at 15psi for 15min. The colonies that grew on SSA were streaked repeatedly 2-3 times on sterilized petri-plates containing medium till a pure colony of *Lactococcus uberis* was obtained.

For isolation of yeast, the colonies were grown in YFB (Hi media) mixed with antibiotic Oxytetracycline (10 mg/ml of medium). The colonies were streaked twice

to thrice on the petri-plates till pure colonies, free of any contamination were obtained.

3.3.2 Isolation of *Lactobacillus acidophilus* from bottle gourd

2-3 g of finally chopped overripe bottle gourd was taken and incubated in yeast fermentation broth (YFB) at 30 °C overnight. Next day the deposited precipitates were taken on a glass slide and warmed gently on a gas flame for fixation. After fixation, staining was done with gram crystal violet stain for 5 min. The slides were observed under compound microscope under oil immersion lens. Mixed colonies consisting of cocci, darkly stained yeast with buds and contaminants were seen. The procedure was repeated 3 to 4 times till approximately 90 per cent contaminants were removed. Thereafter, the colonies were grown on differential medium namely Lactobacillus selection broth. Lactobacillus selection broth was then heated to dissolve the medium completely. Thereafter the medium was autoclaved at 12 lbs pressure for 15 min. The pH was adjusted to 5.4±0.2 at 25°C. The colonies that grew on Lactobacillus selection broth were streaked repeatedly 2-3 times on sterilized petri-plates containing medium till a pure colony of Lactobacillus was obtained. The compositon of the Lactococcus Selection broth is as under:

Ingredients	**g/l**
Casein enzyme hydrolysate	10.00
Sodium acetate	25.00
Glucose	20.00
Monopotassium hydrogen phosphate	6.00
Yeast extract	5.00
Ammonium citrate	2.00
Polysorbate 80	1.00
Magnesium sulphate	0.575
Manganese sulphate	0.034

The pure cultures of *Lactobacillus acidophilus* and *Lactococcus uberis* were grown in Elliker broth (Hi media). The composition of which is given as under:

Ingredients	**g/l**
Tryptone	20.00
Yeast extract	5.00
Gelatin	2.50
Dextrose	5.00
Lactose	5.00
Saccharose	5.00
Sodium chloride	4.00
Sodium acetate	1.50
Ascorbic acid	0.5

Twenty grams of Elliker broth was dissolved in 800 ml of distilled water and was warmed to dissolve the ingredients completely and the then final volume was made to one litre with distilled water. The pH of the medium was adjusted to 5.4. Aliquots of 100 ml of the medium solution after dispersion in 250ml Erlenmeyer flasks sterilized by autoclaving at 15 psi for 15 minutes and were incubated at 30 ^{0}C to check the sterilization of medium.

One per cent inoculum each of *Lactobacillus acidophilus* and *Lactococcus uberis* was added to the medium and incubated at 30 ^{0}C for the growth of microbes for a period of 24 hours.

Saccharomyces cerevisiae was grown in yeast fermentation broth (Hi media), which had the following composition:

Ingredients	**g/l**
Peptone	10.00
Sodium chloride	5.00
Beef extract	3.00
Bromo cresol purple	0.04

18 g of above medium was dissolved in 980ml of distilled water. Glucose was added at the rate of 1 per cent. pH of medium was adjusted to 5.8. The final volume was made to one litre by adding distilled water. Medium was sterilized by autoclaving at 15 lb pressure for 15 min and incubated at 30 ^{0}C to test the complete sterilization of medium. 1 per cent inoculum of pure culture of *Saccharomyces cerevisiae* was added to medium by taking sterilized aliquots of 100 ml of the medium in the Erlenmeyer flasks and incubated at 30 ^{0}C. Growth of yeast was observed after 24 hours.

The pure colonies of microbes so obtained were characterized by following biochemical and microscopic methods as proposed by Cowan *et al.* (1974) and Hucker and Conn (1923). The isolation and characterization of the microbes was carried out in the Department of Animal Nutrition, in collaboration with the Department of Veterinary Microbiology.

The microbes thus characterized were:

1 *Lactobacillus acidophilus*

2 *Lactococcus uberis*

3 *Saccharomyces cerevisiae*

3.4 Experimental Plan

Test diet	Microbial treatment	Sources of microbes	Microbial concentration/kg of feed
T1	Control (with culture medium)	-	-
T2	*Lactobacillus acidophilus*	Bottle gourd	6.8×10^8
T3	*Lactococcus uberis*	Bitter gourd	6.8×10^8
T4	*Saccharomyces cerevisiae*	Bitter gourd	5.7×10^7

With the objective of testing the efficacy of isolated microbial strains in broilers, following series of feeding experiments were conducted at three different sites of Kangra district.

Experiment -1 Validation of *Lactobacillus acidophilus*

Site	Subdivision	Control	Treatment	Microbial concentration per kg of feed (cells)	Source
Kutkhudiyal	Jawali	51 birds ration with culture medium only.	51 birds *Lactobacillus acidophilus*	$6.8 X10^8$	Bottle gourd

Experiment -2 Validation of *Lactococcus uberis*

Site	Subdivision	Control	Treatment	Microbial concentration per kg of feed (cells)	Source
Quor	Baijnath	51 birds ration with culture medium only.	51 birds *Lactococcus uberis*	$6.8 X 10^8$	Bitter gourd

Experiment -3 Validation of *Saccharomyces cerevisiae*

Site	Subdivision	Control	Treatment	Microbial concentration per kg of feed (cells)	Source
Saner	Kangra	51 birds ration with culture medium only.	51 birds *Saccharomyces cerevisiae*	$5.7 X 10^7$	Bitter gourd

In all the three experimental trials day-old broiler chicks (N =102) of "Vancob Strain" were procured from a local hatchery.

3.5 Method of supplementation

The probiotics were supplemented through mixing the culture containing specific probiotics in the broiler mash (Table 1). For bacterial cultures, 1 ml of microbial culture ($6.8X10^8$ cells/ml) was diluted with 200 ml of sterile normal saline and was manually mixed with 1kg of feed. Similarly for yeast culture, 1ml of microbial culture ($5.7 X 10^7$ cells/ml) was diluted with 200 ml of sterile normal saline and was manually mixed with 1kg of feed. The same way the mixing of each culture was carried out with the required quantity of broiler starter and finisher mash for each site.

3.6 Management of broiler chicks

Selection of site and adoption of farmers group was done from the beneficiaries selected under DBT project "Development of poultry farmers as self sustaining unit for promotion of rural women entrepreneurship." Beneficiaries were imparted practical training at the University and were also provided with various inputs along with broiler starter and finisher ration for the establishment of farms at their doorsteps. At each site 102, day old broiler chicks of "Vancob Strain" were given. The chicks were wing banded, weighed and grouped to two groups (one control and other experimental) on equal weight basis containing 51 broiler chicks in each group and each group is further subdivided into three subgroups containing 17 chicks in each subgroup. The chicks were reared on deep litter system. The chicks were reared under the brooders during the first week and thereafter shifted in the poultry pens, created from the locally available material. During starter and finisher phase, the broiler chicks were fed standard starter and finisher mashes (Table 1) supplemented with specific microbial culture in experimental groups. Free access to clean drinking water was made from first day of the life of chicks. The feed was also

made available on ad- lib basis during the entire experimental period. The following parameters were recorded during the entire course of feeding trial.

Gain in body weight

Feed intake

Feed conversion ratio

Mortality percentage

The overall data was statistically analyzed by the standard method of Snedecor and Cochran (1968).

Table 1. Per cent composition of broiler mash

Ingredients	Starter mash (Parts/100kg)	Finisher mash (Parts/100kg)
Maize	25.00	54.705
Wheat	24.855	-
Groundnut extraction	12.00	10.00
Sunflower extraction	06.00	04.00
Mustard oil cake	04.00	04.00
Soya flakes	16.00	15.00
Fish meal	05.00	5.00
Lime powder	01.00	01.00
Di Calcium Phosphate (DCP)	01.00	01.00
Molasses	05.00	01.00
Choline chloride	-	100.00
Premix	+	+

Premix was prepared by mixing the following in 200 g maize flour:

1) Ventri mix DS 20g
(Vit.A, 82,500 i.u, B2, 50mg, D3, 12,000 i.u.
Vit.k, 10mg/g)
2) Ventri bee plus 25g
(Vit. B1, 25mg, B6, 35mg, B12, 250 ug, E, 225mg
Panto- thenate, 225mg, Niacinamide, 300mg
Folic acid, 20 mg/5g)
5) Trace minerals 100g

Ferrous oxide, 2g,	Dicalcium phosphate, 54g,
Copper sulphate, 2g,	Manganese sulphate, 3g,
Zinc oxide, 2.5g,	Magnesium sulphate, 25g
Ferrors sulphate, 10g,	Potassium iodide, 2.5 g
Zinc sulphate, 0.6g	

RESULTS

CHAPTER 4

RESULTS

4.1 Proximate composition

In the formulation of poultry diets, cereals, oil seed cakes and their byproducts play an important role, as they are rich sources of energy as well as proteins. The various feed ingredients, which were used for formulating different test diets under different feeding experiments, were analyzed for the proximate composition and the data have been presented in table 2.

On perusal of data in this table indicated that dry matter value of maize, wheat groundnut extraction, sunflower extraction, soya flakes, fishmeal and de-oiled rice bran were 87.70, 88.00, 91.46, 91.50, 85.20, 86.40 and 90.28 per cent, respectively. The crude protein values for all these ingredients were 9.00, 12.50, 40.20, 32.23, 42.50, 51.38 and 16.87 per cent, respectively. The ether extracts values were 3.01, 3.00, 2.12, 9.04, 1.01, 4.55 and 1.15 per cent, respectively. The crude fibre values were 1.59, 1.60, 8.90, 8.22, 3.90, 2.01 and 14.04 per cent, respectively. The ash contents of all these ingredients were 5.31, 3.20, 6.88, 9.04, 7.04, 6.06 and 13.60 per cent, respectively. The NFE contents of all these ingredients were 79.60, 79.00, 41.85, 41.50, 45.44, 35.95 and 54.33 per cent, respectively. The calcium content of these ingredients were 0.03, 0.09, 0.24, 0.80, 0.20, 6.71 and 0.20 per cent, respectively, whereas phosphorus content of all these ingredients were 0.24, 0.66, 0.54, 0.30, 0.39, 3.47 and 1.18 per cent, respectively.

4.2 Cell and cell wall components

The various ingredients used for formulating different test diets under different microbial treatments were also analyzed for the cell and cell wall composition and the results are presented in table-3

Table 2. Chemical composition of feed ingredients (per cent on dry matter basis)

Feed ingredients	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	NFE (%)	Ca (%)	P (%)
Maize	87.70	9.00	3.01	1.59	5.31	79.60	0.03	0.24
Wheat	88.00	12.50	3.00	1.60	3.20	79.00	0.09	0.66
Groundnut extraction	91.46	40.20	2.12	8.90	6.88	41.85	0.24	0.54
Sunflower extraction	91.50	32.23	9.04	8.22	9.04	41.50	0.80	0.30
Soya flakes	85.20	42.50	1.01	3.90	7.04	45.44	0.20	0.39
Fish meal	86.40	51.38	4.55	2.01	6.06	35.95	6.71	3.47
DORB	90.28	16.87	1.15	14.04	13.60	54.33	0.20	1.18

Each value is a mean of duplicate determinations.

The study indicated that the cell soluble contents for maize, wheat, groundnut extraction, sunflower extraction, soya flakes and de-oiled rice bran were 80.56, 84.02, 80.70, 61.85, 74.51, and 34.02 per cent, respectively. The NDF values for all these ingredients were 19.73, 15.90, 19.31, 38.20, 25.50, and 66.00 per cent, respectively. Similarly ADF values were found to be 4.83, 5.20, 8.71, 27.01, 8.61 and 34.97 per cent, respectively in all the ingredients. The hemi cellulose values for all these ingredients were 14.49, 10.79, 10.59, 11.10, 16.88 and 31.00 per cent, respectively. The values for cellulose were 3.82, 4.06, 6.67, 19.82, 6.48 & 26.72 per cent, respectively. The values for lignin in all these ingredients were found to be 1.10, 1.11, 2.02, 7.20, 2.12 and 8.25 per cent, respectively.

Table 3. Cell and cell wall components of feed ingredients (per cent on dry matter basis)

Feed ingredients	Cell soluble (%)	NDF (%)	ADF (%)	Hemi cellulose (%)	Cellulose (%)	Lignin (%)
Maize	80.56	19.73	4.83	14.49	3.82	1.10
Wheat	84.02	15.90	5.20	10.79	4.06	1.11
Groundnut extraction	80.70	19.31	8.71	10.59	6.67	2.02
Sunflower extraction	61.85	38.20	27.01	11.10	19.82	7.20
Soya flakes	74.51	25.50	8.61	16.88	6.48	2.12
DORB	34.02	66.00	34.97	31.00	26.72	8.25

Each value is a mean of duplicate determination.

4.3 Chemical composition of experimental diets

Data regarding the proximate composition, cell and cell wall composition of the broiler starter and finisher mashes have been given in table 4. The study of data indicated that DM, CP, CF, EE, total ash, NFE, acid insoluble ash (silica), Ca, P, NDF, ADF, cellulose, lignin and hemi cellulose content of broiler starter mash were 89.38, 23.06, 3.48, 2.50, 5.84, 65.15, 2.58, 1.48, 0.77, 18.24, 7.40, 5.74, 1.64 and 10.85 per cent, respectively. The broiler finisher mash was found to contain DM, CP, CF, EE, total ash, NFE, acid insoluble ash (silica), Ca, P, NDF, ADF, cellulose, lignin and hemicellulose as 88.42, 20.05, 3.06, 2.57, 5.38, 68.94, 2.13, 2.37, 1.16, 17.85, 6.38, 4.84, 1.54 and 11.47 per cent, respectively. The calculated values for metabolisable energy (ME) were 2825.00 and 2956.56 Kcal/kg in case of starter and finisher mash, respectively. All the nutrients were as per the specifications given by BIS (1992).

Table 4. Chemical composition of experimental diets (per cent on dry matter basis)

Nutrient	Broiler starter mash	Broiler finisher mash
Dry matter (DM)	89.38	88.42
Crude protein (CP)	23.06	20.05
Crude fiber (CF)	3.48	3.06
Ether extract (EE)	2.50	2.57
Total Ash	5.84	5.38
Nitrogen free extract (NFE)	65.15	68.94
Acid insoluble ash	2.58	2.13
Calcium	1.48	2.37
Phosphorus	0.77	1.16
Neutral detergent fiber (NDF)	18.24	17.85
Acid detergent fiber (ADF)	7.40	6.38
Cellulose	5.74	4.84
Lignin	1.64	1.54
Hemi cellulose	10.85	11.47
ME (kcal/kg)	2825.00	2956.56

The total expenditure for the establishment of each commercial broiler unit of 100 chicks for one to six weeks was Rs. 4600 (Table 5)

Table 5: Total expenditure incurred for establishment of broiler unit (100 chicks) at farmers site

Sr. No.	Items	Amount (Rs.)
1.	Chicks	1175.00
2.	Maize	11.00
3.	Feed (starter)	1000.00
4.	Feed (finisher)	1250.00
5.	Saw dust	60.00
6.	Bulbs	24.00
7.	Wire	15.00
8.	Phenyl	50.00
9.	Vimeral	112.00
10.	Cadiplex	11.40
11.	Feeder	240.00
12.	Waterer	70.00
13.	Steel Parat	44.00
14.	Thermometer	55.00
15.	Electricity charges	101.00
16.	Labour charges	360.00
		4600.00

The total expenditure for the establishment of each commercial broiler unit of 100 chicks for one to six weeks was Rs. 4600.

4.4 Experiment 1:

Effect of dietary supplementation of *Lactobacillus acidophilus* isolated from bottle gourd on the biological performance of commercial broilers from day 1 to six weeks of age.

4.4.1 Effect on biological performance during starter phase (1-4 weeks of age):

The data regarding average body weight, average gain in live weight and feed conversion ratio (FCR) during the starter phase has been presented in table-6. The average live weight gains were 825.12±15.63 g and 990.20±15.68 g for control and treatment respectively. The average live weight gain in microbial supplemented group

was significantly P (<0.0001) better than control. The FCR for control and treatment group calculated were 1.22±0.01 and 0.99±0.03 respectively. There was significant (P<0.0001) improvement in FCR in *Lactobacillus acidophilus* treated group as compared to control. The % increase in gain in weight of treatment over control was 20.00.

4.4.2 Finisher phase (5-6 weeks of age):

The data regarding gain in weight, feed intake and feed conversion ratio have been presented in table 6.

Table 6. Biological performance of "Vancob strain" of broiler chicks fed test diets supplemented with *Lactobacillus acidophilus* (bottle gourd) from one to six weeks of age.

Particulars	Control	Test diet
Initial Av weight (g)	55.2±1.12	55.2±1.13
Performance at 1-4 weeks		
Av weight at 4 week (g)	878.32±15.63	1042.20±7.26
Gain in weight (g)	825.12±15.63[a]	990.20±15.68[b]
Feed intake	1014±30.90	986±32.09
FCR	1.22±0.01[b]	0.99±0.03[a]
Performance at 5-6 weeks		
Av weight at 6 week (g)	1909.02±12.57	2048.43±10.17
Gain in weight (g)	997.76±24.18	1014.84±14.62
Feed intake (g)	1286.32±25.60[a]	1217.2±26.93[b]
FCR	1.28±0.05[b]	1.19±0.01[a]
Overall performance in 1-6 weeks		
Gain in weight (g)	1853.82±6.66[a]	1993.23±14.35[b]
Feed intake (g)	2300.32±25.53	2203.22±24.46
FCR	1.29±0.02[b]	1.10±0.10[a]
Benefit per broiler (Rs)	56.90	68.82
Cost benefit ratio (Rs)	1:2.24	1:2.49
Mortality (%)	2.00	-

*The values with different superscripts in a row are significantly (P<0.0001) different from each other.

The average live weight gain of chicks fed test diet was 1014.84±14.62 g against 997.76±24.18 g in control group. The gain in weight in treatment group is numerically higher than control group but statistically this difference was non

significant. The FCR of treatment group was 1.19±0.01 which was significantly (P<0.0001) better than control group (1.28± 0.05). The per cent increase in growth rate of treatment group over control group was 1.71 per cent.

4.4.3 Overall biological performance (1-6 weeks of age):

Overall biological performance has been given in table 6. The gain in live weight was 1993.3±14.35 g and 1853.82±6.66 g in treatment and control, respectively. The difference in gain in weight was significantly (P<0.0001) higher in treatment group as compared to control (fig.1) The per cent increase in gain in live weight as compared to control was 7.52 per cent. FCR of treatment group and control group were 1.10±0.10 and 1.29±0.02, respectively (fig. 2). The FCR of treatment group was significantly (P<0.0001) better than control group. The benefit per broiler for treatment group was Rs 68.82 against Rs 56.90 in control group. The cost benefit ratio for treatment and control were 1:2.24 and 1:2.49, respectively. Zero mortality was recorded in treatment group with 2% mortality in control group.

4.5 Experiment 2:

Effect of dietary supplementation of *Lactococcus uberis* isolated from bitter gourd on the biological performance of commercial broilers from day one to six weeks of age.

4.5.1. Effect on biological performance during starter phase (1-4 weeks of age):

The data regarding average body weight, average gain in live weight and feed conversion ratio (FCR) during the starter phase has been presented in table 7. The average live weight gains were 621.70±5.93 g and 716.80±5.93 g for control and treatment group, respectively. The average live weight gain in microbial supplemented group was significantly (P<0.0001) better than control group. The FCR for control and treatment group were 1.40±0.01 and 1.36±0.02, respectively. There

Fig. 1 Gain in weight of broilers

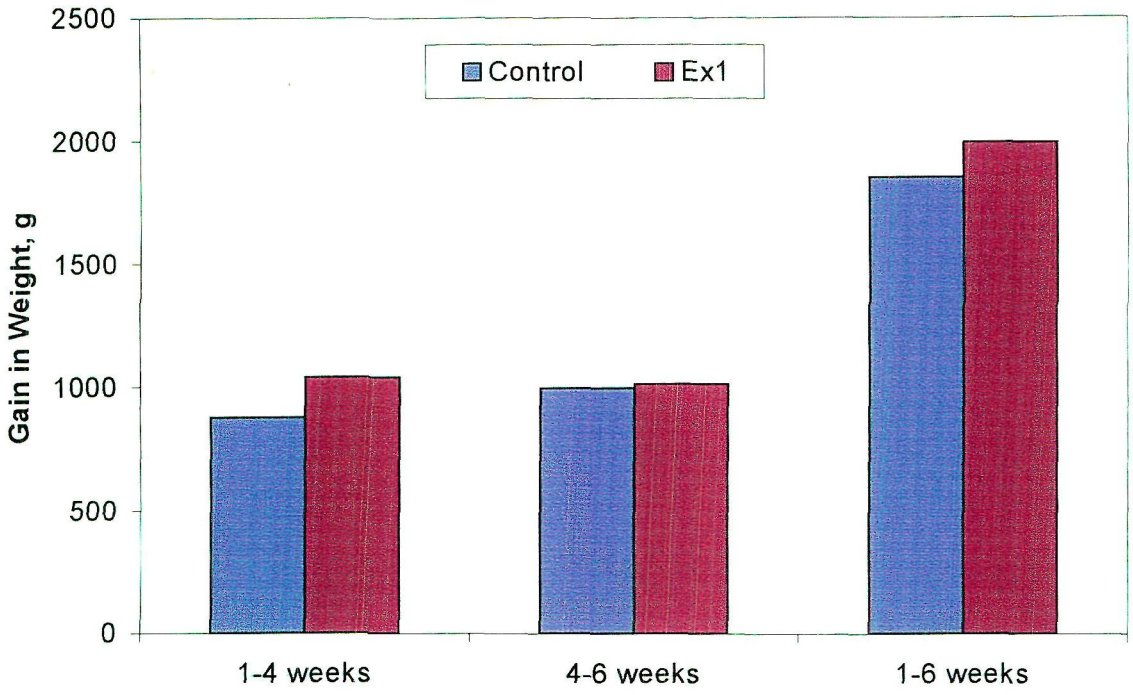
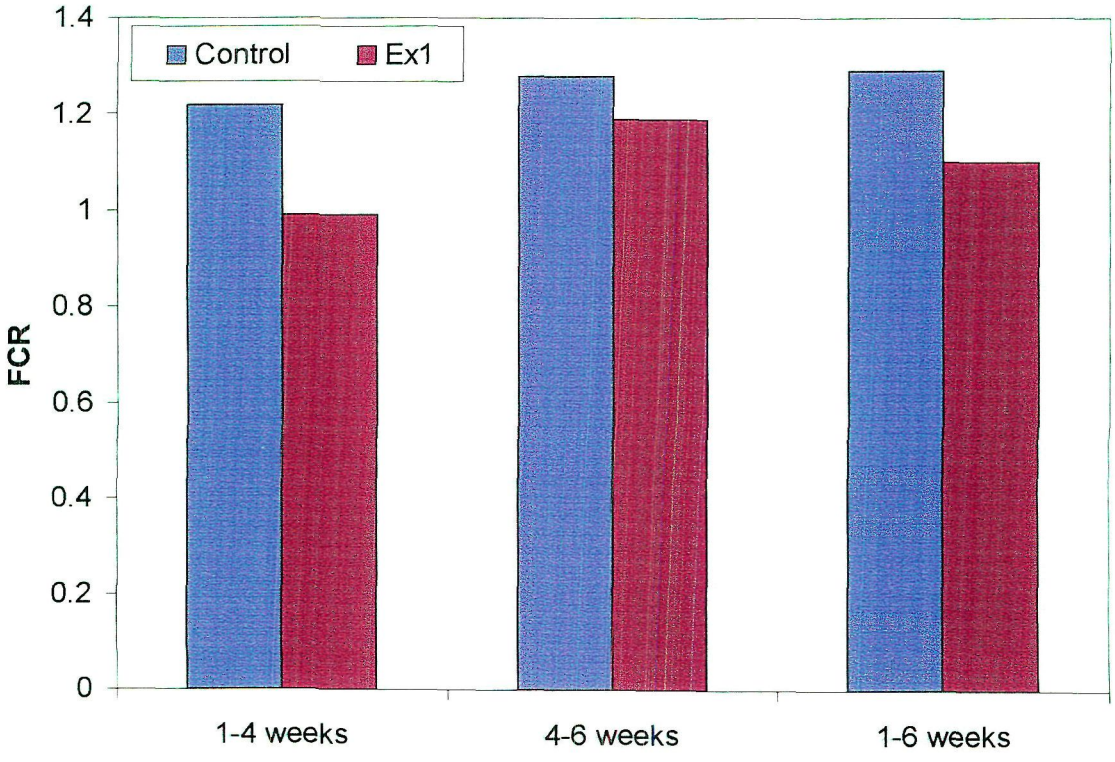


Fig. 2. Feed conversion ratios (FCR) of broilers



was significant (P<0.0001) improvement in FCR in *Lactococcus uberis* treated group as compared to control group. The per cent increase in gain in weight of treatment group over control group was 15.29 per cent.

4.5.2 Finisher phase (5-6 weeks of age):

The data regarding gain in weight, feed intake and feed conversion ratio have been presented in table 7. The average live weight gain of chicks fed test diet was 1444.66±11.60g against 1334.6±12.02g in control. The gain in weight in treatment group was numerically higher than control group but statistically this difference was non significant. The FCR in treatment group was 1.85± 0.05 which was significantly (P<0.0001) better than control group (1.96±0.04). The per cent increase in growth rate of treatment group over control group was 2.40 per cent.

Table 7. Biological performance of "Vancob strain" of broiler chicks fed test diets supplemented with *Lactococcus uberis* (bitter gourd) from one to six weeks of age.

Particulars	Control	Test diet
Initial Av weight (g)	55.5±1.13	55.6±2.17
Performance at 1-4 weeks		
Av weight at 4 week (g)	676.28±8.48	770.3±6.92
Gain in weight (g)	621.70±5.93^{a}	716.804±5.93^{b}
Feed intake	1010±30.04	980±39.29
FCR	1.40±0.01^{b}	1.36±0.02^{a}
Performance at 5-6 weeks		
Av weight at 6 week (g)	1334.6±12.02^{a}	1444.66±11.60^{b}
Gain in weight (g)	656.22±11.31	672.06±10.45
Feed intake (g)	1290.32±30.90	1223.22±32.09
FCR	1.96±0.04^{b}	1.85±0.05^{a}
Overall performance in 1-6 weeks		
Gain in weight (g)	1277.83±9.94^{a}	1389.06±5.54^{b}
Feed intake (g)	2300.32±25.92^{a}	2203.22±25.61^{b}
FCR	1.99±0.04	1.63±0.08
Benefit per broiler (Rs.)	20.14	32.13
Cost benefit ratio (Rs.)	1:1.44	1:1.70
Mortality (%)	2.00	1.00

* The values with different superscripts in a row are significantly (P<0.0001) different from each other.

4.5.3 Overall biological performance (1-6 weeks of age):

Overall biological performance has been given in table 7. The gain in live weight was 1277.83±9.94 g and 1389.06±5.54 g in control and treatment, respectively. The difference in gain in weight was significantly (P<0.0001) higher in treatment group as compared to control group (fig. 3). The per cent increase in gain in live weight as compared to control was 8.70 per cent. FCR of treatment group and control group were 1.63±0.08 and 1.99±0.04, respectively (fig. 4). The FCR of treatment group was significantly (P<0.0001) better than control group. The difference in FCR of treatment and control was significant (P<0.0001). The benefit per broiler for treatment group was Rs. 32.13 against Rs. 20.14 in control. The cost benefit ratio for treatment and control were 1:1.70 and 1:1.44, respectively. Two per cent mortality was recorded in treatment group and one per cent mortality in control group.

4.6 Experiment 3:

Effect of dietary supplementation of *Saccharomyces cerevisiae* isolated from bitter gourd on the biological performance of commercial broilers from day one to six weeks of age.

4.6.1 Effect on biological performance during starter phase (1-4 weeks of age):

The data regarding average body weight, average gain in live weight and feed conversion ratio (FCR) during the starter phase has been presented in table 8. The average live weight gains were 256.84±7.09 g and 363.69±7.26 g for control group and treatment group, respectively. The average live weight gain in microbial supplemented group was significantly (P<0.0001) better than control group. The FCR for control and treatment group calculated were 4.2±0.035 and 3.16±0.02 respectively. There was significant (P<0.0001) improvement in FCR in

Fig. 3. Gain in weight of broilers

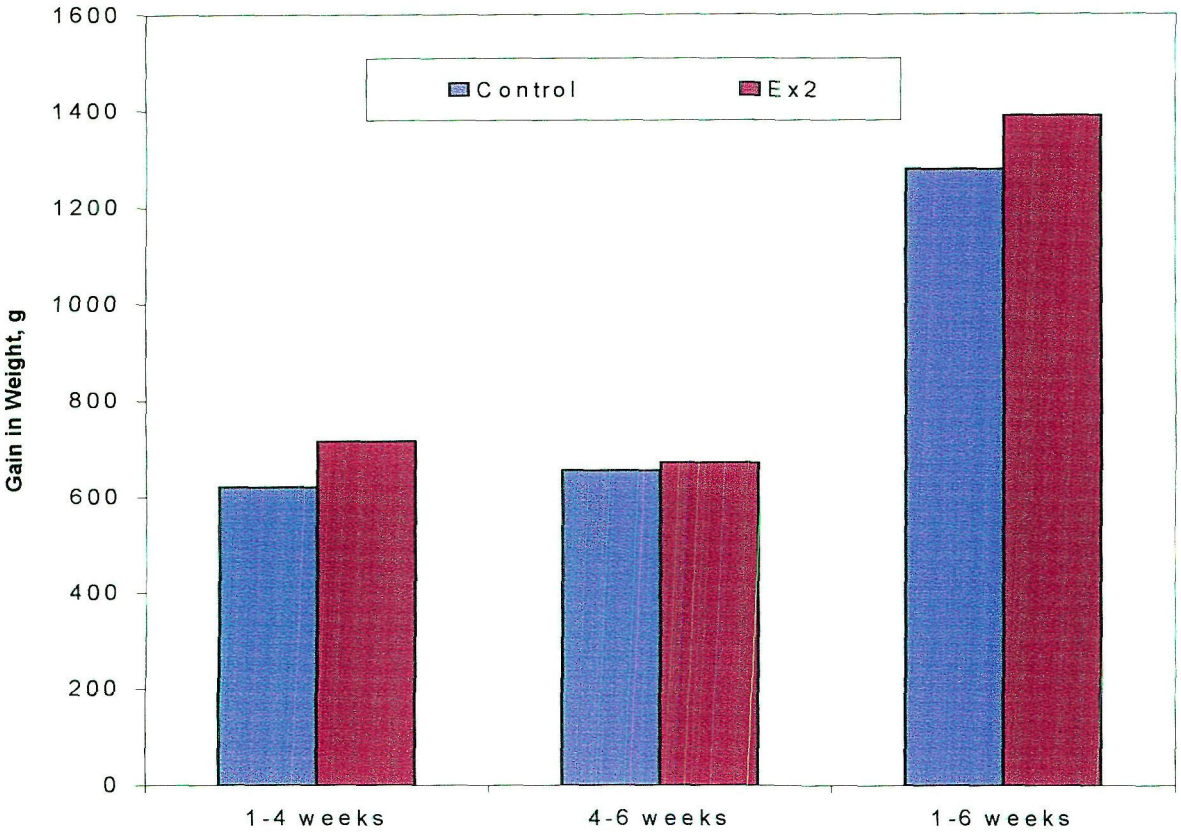
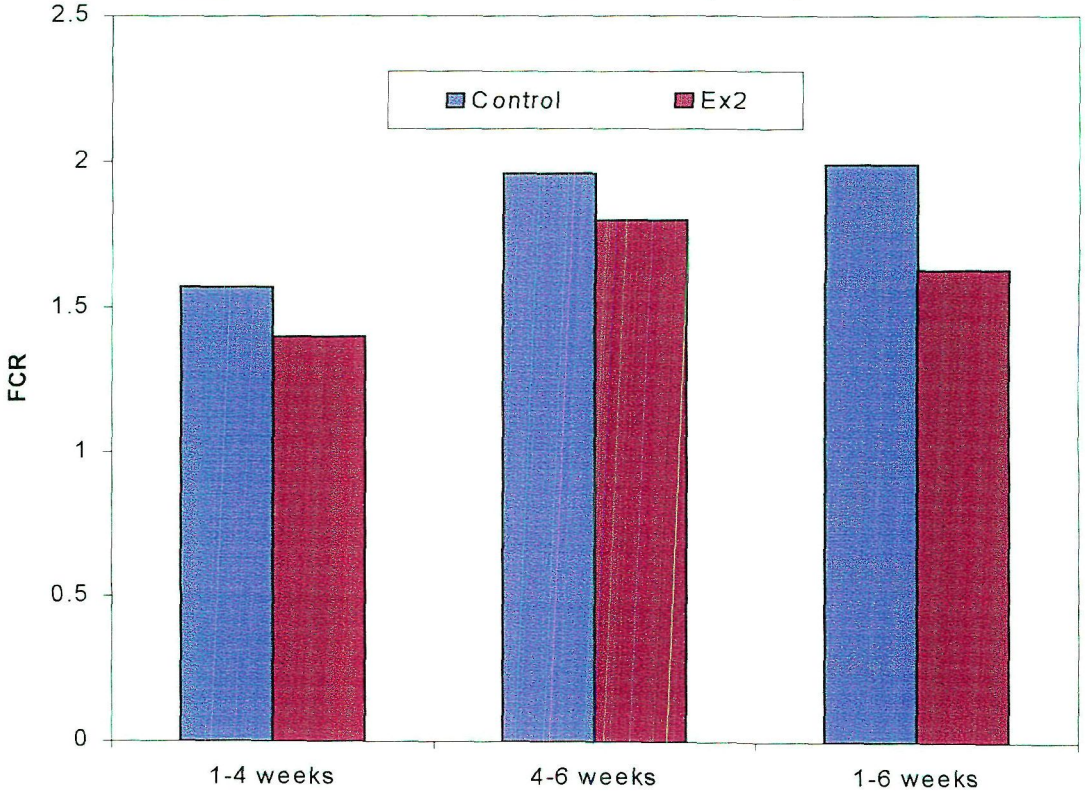


Fig. 4. Feed conversion ratios (FCR) of broilers



Saccharomyces cerevisiae treated group as compared to control group. The per cent increase in gain in weight of treatment group over control group was 50 per cent.

4.6.2 Finisher phase (4-6 weeks of age):

The data regarding gain in weight, feed intake and feed conversion ratio have been presented in table 8. The average live weight gain of chicks fed test diet was 1164.45±10.93 g against 847.32±12.53 g in control. The gain in weight in treatment group was statistically significant as compared to control. The FCR of test group was 1.4± 0.02 which was significantly (P<0.0001) better than control group (2.3±0.06). The per cent increase in growth rate of treatment group over control group was.37.60 per cent.

Table 8. Biological performance of "Vancob strain" of broiler chicks fed test diets supplemented with *Saccharomyces cerevisiae* (bitter gourd) from one to six weeks of age.

Particulars	Control	Test diet
Initial Av weight (g)	52.4±1.12	52.3±1.11
Performance at 1-4 weeks		
Av weight at 4 week (g)	256.84±7.09[a]	363.69±7.26[b]
Gain in weight (g)	206.42±5.05[a]	311.39±9.45[b]
Feed intake	1015±33.86[a]	985±35.87[b]
FCR	4.2±0.035[b]	3.16±0.02[a]
Performance at 5-6 weeks		
Av weight at 6 week (g)	847.32±12.53[a]	1164.45±10.93[b]
Gain in weight (g)	585.2±7.45[a]	805.28±10.97[b]
Feed intake (g)	1358±28.82[a]	1135.76±25.60[b]
FCR	2.3±0.06[b]	1.4±0.02[a]
Overall performance in 1-6 weeks		
Gain in weight (g)	794.92±13.07[a]	1111.7±10.98[b]
Feed intake (g)	2373.0±25.33[a]	2120.76±24.76[b]
FCR	3.32±0.08[b]	2.02±0.09[b]
Benefit per broiler (Rs)	4.24	16.24
Cost benefit ratio (Rs)	1:0.95	1:1.35
Mortality (%)	2.00	-

*The values with different superscripts in a row are significantly (P<0.0001) different from each other.

4.6.3 Overall biological performance (1-6 weeks of age):

Overall biological performance has been given in table 8. The gain in live weight was 794.92±13.07 g and 1111.7±10.98 g in control and treatment, respectively. The difference in gain in weight was significantly ($P<0.0001$) higher in treatment group as compared to control group (fig. 5). The per cent increase in gain in live weight of treatment group as compared to control was 39.85 per cent. FCR of treatment and control group were 2.02±0.09 and 3.32±0.08, respectively (fig. 6). The difference in FCR of treatment and control was significant ($P<0.0001$). The benefit per broiler for treatment group was Rs. 16.24 against Rs. 4.24 in control. The cost benefit ratio for treatment and control were 1:1.35 and 1:0.95, respectively. No mortality was recorded in treatment group however 2 per cent mortality was there in the control group.

4.7 Comparison of field results with laboratory results:

Kaistha *et al.* (1996) conducted a trial on commercial broilers to check the efficacy of *Lactobacillus acidophilus*, *Lactococcus uberis*, and *Saccharomyces cerevisiae* isolated from bottle gourd, bitter gourd and bitter gourd at the university level.

With supplementation of *Lactobacillus acidophilus* in diet of broilers, it was observed that during starter phase, GIW and FCR were statistically non significant as compared to control. However in the present investigation using the same probiotic isolated from same source, significantly ($P<0.0001$) higher gain in weight and improvement in FCR in supplemented group was recorded as compared to the control. During finisher phase significantly ($P<0.05$) better GIW and FCR in treatment group as compared to unsupplemented control were recorded at the university level. However, at the field level non-significant difference in GIW of treatment group as

Fig. 5. Gain in weight of broilers

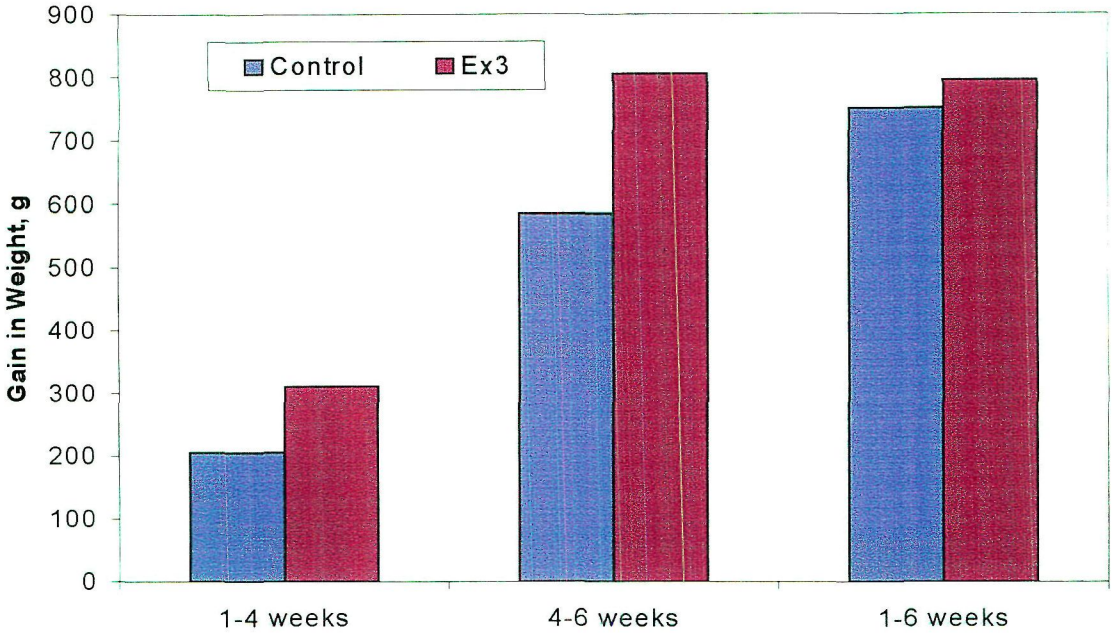
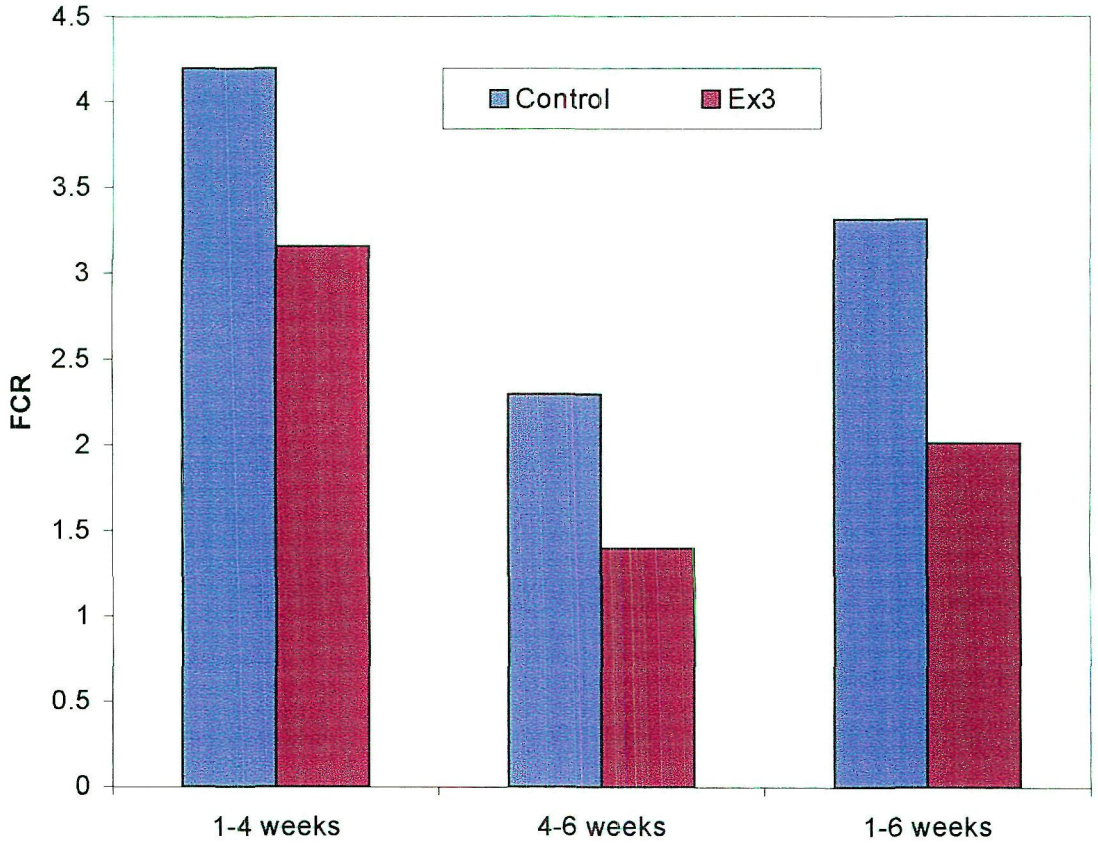


Fig. 6. Feed conversion ratios (FCR) of broilers



gave significantly (P<0.0001) higher gain in weight coupled with improvement in FCR in supplemented group as compared to the control group. During the finisher phase significantly (P<0.05) better GIW and FCR in treatment group as compared to unsupplemented control was reported by Kaistha, *et al.* 1996. Similarly in field trial significantly higher (P<0.0001) GIW and FCR in treatment group were also recorded with the same microbial isolate On comparing overall biological performance during 1-6 weeks of age, we found significantly higher GIW and improvement in FCR in the findings of both laboratory and field trials. Thus, from above comparison, it is clear that the *Saccharomyces cerevisiae* isolated from bitter gourd showed equally promising results at field level also.

4.8 Comparison of three different microbes:

These three useful microbes viz *Lactobacillus acidophilus, Lactococcus uberis* and *Saccharomyces cerevisiae* were tested at three different sites with different managemental practices and variable climatic conditions. The comparison has been made on the basis that same strain of chicks, along with similar type of starter and finisher mash were offered to the chicks. Birds at three different sites were reared on deep litter system under the brooders. The per cent increase in gain in weight during 1-6 weeks of age of "Vancob strain" broilers, fed diets supplemented with *Lactobacillus acidophilus* (bottle gourd), *Lactococcus uberis* (bitter gourd) and *Saccharomyces cerevisiae* (bitter gourd) were 39.85, 8.7 and 7.52 per cent, respectively.

Thus, it can be concluded that on the overall *Saccharomyces cerevisiae* (bitter gourd) performed better followed by *Lactococcus uberis* (bitter gourd) and *Lactobacillus acidophilus* (bottle gourd).

DISCUSSION

CHAPTER 5

DISCUSSION

The effects of inclusion of microbial cultures of *Lactobacillus acidophilus*, *Lactococcus uberis, Saccharomyces cerevisiae* isolated from bottle gourd, bitter gourd and bitter gourd respectively, were studied on the biological performance of commercial broiler chicks of "Vancob-strain" from day one to six weeks of age at three different sites of Kangra district.

5.1 Chemical composition of feed ingredients:

The various feed ingredients, which were used for formulating different experimental diets under different feeding experiments, were analysed for their proximate composition and the data is presented in table 2.

The perusal of data indicated that the dry matter value of maize, wheat, groundnut extraction, sunflower extraction, soya flakes, fishmeal and de-oiled rice bran were 87.70, 88.00, 91.46, 91.50, 85.20, 86.40 and 90.28 per cent, respectively, where as the corresponding DM values of these ingredients reported by Katoch (1996) were 87.80, 88.50, 91.00, 91.40, 86.10, 86.20 and 90.00 per cent, respectively. The DM values of different ingredients were comparable to those reported by Katoch (1996) except in soya-flakes, which were on higher side. The slight difference might be due to handling procedures and analytical techniques. Gupta (2003) recorded the DM values for maize, wheat, groundnut extraction, sunflower extraction, soya flakes, fishmeal and de-oiled rice bran as 87.75, 88.32, 91.57, 91.50, 85.20, 86.45 and 90.28 per cent, respectively.

The crude protein values for all these ingredients were 9.00, 12.50, 40.20, 32.23, 42.50, 51.38 and 16.87 per cent, respectively, where as the corresponding crude protein values of these ingredients reported by Katoch (1996) were 9.00, 12.10,

40.50, 29.60, 42.10, 45.50 and 16.90 per cent, respectively. The CP values of different ingredients were comparable to those reported by Katoch (1996) except in sunflower extraction and fishmeal, which was on higher side. This difference in CP values of sunflower extraction might be due to difference in processing method and in case fishmeal was due to difference in quality of ingredients available. Gupta (2003) reported that the CP values for maize, wheat, groundnut extraction, sunflower extraction, soya flakes, fishmeal and de-oiled rice bran as 9.00, 12.50, 40.24, 32.22, 42.63, 51.38 and 16.89 per cent, respectively. The ether extract values of maize, wheat, groundnut extraction, sunflower extraction, soya flakes, fishmeal and de-oiled rice bran were 3.01, 3.00, 2.12, 9.04, 1.01, 4.50 and1.01per cent, respectively, where as the corresponding ether extract values of these ingredients reported by Katoch (1996) were 3.00, 3.00, 2.10, 9.04, 1.00, 4.50 and 1.00 per cent, respectively and are matching with each other. Gupta (2003) recorded the ether extract values for all these ingredients as 3.00, 3.00, 2.12, 9.04, 1.00, 4.55 and 1.15 per cent, respectively.

The crude fibre values for maize, wheat, groundnut extraction, sunflower extraction, soya flakes, fishmeal and de-oiled rice bran were 1.59, 1.60, 8.90, 8.22, 3.90, 2.01 and14.04 per cent, respectively, where as the corresponding crude fibre values of these ingredients reported by Katoch (1996) were 3.10, 1.70, 9.00, 8.20, 4.20 and 14.00 per cent, respectively and are almost matching except for slight difference in values of wheat, groundnut extraction and soya- flakes which were on higher side and might be due to variation in their quality. Gupta (2003) recorded the CF values for all these ingredients as 3.11, 1.60, 8.90, 8.21, 3.91, 2.01 and 14.03 per cent, respectively.

The ash content of all these ingredients were 5.31, 3.20, 6.88, 9.04, 7.04, 6.06 and 13.66 per cent, respectively, where as the corresponding ash content of these

ingredients reported by Katoch (1996) were 5.30, 3.20, 6.90, 9.00, 7.00, 6.00 and 13.50 per cent, respectively. Gupta (2003) recorded the ash content of these ingredients as 5.31, 3.20, 6.88, 9.03, 7.04, 6.07 and 13.60 per cent, respectively.

The NFE contents of all these ingredients were 79.60, 79.00, 41.85, 41.50, 45.44, 35.95 and 54.33 per cent, respectively, where as the corresponding NFE values of these ingredients reported by Katoch (1996) were 79.60, 80.00, 41.50, 44.20, 45.90, 42.00 and 54.60 per cent, respectively. Gupta (2003) recorded the NFE content of these ingredients as 79.61, 79.80, 41.86, 41.50, 45.42, 35.99 and 54.33 per cent, respectively.

The calcium content of these ingredients was 0.03, 0.09, 0.24, 0.80, 0.20, 6.71 and 0.20 per cent, respectively where as the corresponding calcium contents of these ingredients reported by Katoch (1996) were 0.03, 0.08, 0.25, 0.80, 0.20, 6.70 and 0.20 per cent, respectively. Gupta (2003) recorded the calcium content of these ingredients as 0.03, 0.09, 0.25 0.80, 0.20, 6.71 and 0.20 per cent, respectively.

The phosphorus content of all these ingredients were 0.24, 0.66, 0.54, 0.30, 0.39, 3.47 and 1.18 per cent, respectively, where as the corresponding P value of these ingredients reported by Katoch (1996) were 0.25, 0.65, 0.55, 0.30, 0.30, 3.50 and 1.20 per cent, respectively. Gupta (2003) recorded the phosphorus content of these ingredients as 0.24, 0.66, 0.54 0.30, 0.29 3.47 and 1.19 per cent, respectively.

5.2 Cell and cell wall components:

The various feed ingredients used for formulating different test diets under different microbial treatments were also analysed for the cell and cell wall components and the results are presented in table 3.

The perusal of data indicated that the cell soluble values for maize, wheat, groundnut extraction, sunflower extraction, soya flakes and de-oiled rice bran were

80.56, 84.02, 80.70, 61.85, 74.51 and 34.02 per cent, respectively, where as cell soluble values for these ingredients reported by Katoch (1996) were 80.90, 84.40, 80.70, 61.90, 74.50 and 35.00 per cent, respectively. Gupta (2003) recorded these values as 80.63, 84.01, 80.70, 61.89, 74.51 and 34.00 per cent, respectively.

The NDF values for all these ingredients were 19.73, 15.90, 19.31, 38.20, 25.50 and 66.00 per cent, respectively where as NDF values for these ingredients reported by Katoch (1996) were 19.10, 15.90, 19.30, 38.10, 25.50 and 65.00 per cent, respectively. Gupta (2003) recorded the NDF values for these ingredients as19.37, 15.99, 38.11, 25.49 and 66.00 per cent, respectively.

The ADF values for all these ingredients were found to be 4.83, 5.20, 8.71, 27.01, 8.61and 34.97 per cent, respectively, whereas the corresponding ADF values for these ingredients reported by Katoch (1996) were 3.10, 3.70, 11.60, 28.40, 13.00 and 35.00 per cent, respectively. Gupta (2003) recorded the ADF values for these ingredients as 4.82, 5.20, 8.71, 27.01, 8.60 and 34.97 per cent, respectively.

The hemi cellulose values were found to be 14.49, 10.79, 10.59, 11.10, 16.88 and 31.00 per cent, respectively where as hemi cellulose values for these ingredients reported by Katoch (1996) were 16.10, 12.20, 7.70, 9.70,12.50 and 30.00 per cent, respectively. The values for cellulose were 3.82, 4.06, 6.67, 19.82, 6.48 and 26.72 per cent, respectively. Gupta (2003) recorded the hemi cellulose values for these ingredients as 14.55, 10.79, 10.59, 11.10, 16.89 and 31.03 per cent, respectively.

The values for cellulose were reported to be 3.82, 4.06, 6.67, 19.82, 6.48 and 26.75 per cent, respectively; where as cellulose values for these ingredients reported by Katoch (1996) were 3.75, 4.05, 6.60, 19.80, 6.50 and 25.92 per cent, respectively. Gupta (2003) recorded the cellulose values for these ingredients as 3.72, 4.09, 6.67, 19.81, 6.48 and 26.72 per cent, respectively.

The values for lignin in all these ingredients were found to be 1.10, 1.11, 2.02, 7.20, 2.13 and 8.28 per cent, respectively where as lignin values for these ingredients reported by Katoch (1996) were 1.10, 1.10, 2.00, 7.20, 2.10 and 8.10 per cent, respectively. Gupta (2003) recorded the values for lignin as 1.10, 1.11, 2.04, 7.20, 2.12 and 8.25 per cent, respectively.

5.3 Chemical composition of test diet:

Data regarding the proximate composition, cell and cell wall components of broiler starter and finisher mash are given in table 4.

The perusal of data revealed that the broiler starter mash was found to contain DM, CP, CF, EE, total ash, NFE, acid insoluble ash (silica), Ca, P, NDF, ADF, cellulose, lignin and hemi cellulose as 89.38, 23.06, 3.48, 2.50, 5.84, 65.15, 2.58, 1.48, 0.77, 18.24 7.40, 5.74, 1.64 and 10.85 per cent, respectively, where as values for these nutrients in broiler starter mash reported by Katoch (1996) were 89.00, 23.01, 3.48, 2.50, 5.80, 65.21, 2.50, 1.46, 0.72, 18.24, 7.38, 5.03, 1.62 and 10.86 per cent, respectively. Gupta (2003) recorded these values as 89.36, 23.05, 3.48, 2.50, 5.82, 65.15, 2.57, 1.46, 0.77, 18.25, 7.40, 5.76, 1.64 and 10.85 per cent, respectively. The corresponding constituents in the broiler finisher mash were 88.42, 20.05, 3.06, 2.57, 5.38, 68.88, 2.13, 2.37, 1.16, 17.85, 6.38, 4.84, 1.54 and 11.47 per cent, respectively, whereas values for these nutrients in finisher mash reported by Katoch (1996) were 88.50, 20.02, 3.08, 2.67, 5.30, 68.93, 2.12, 2.35, 1.16, 17.82, 6.37, 4.78, 1.52 and 11.45 per cent, respectively.

The calculated values for Metabolisable Energy (ME) were 2825.00 and 2956.56 Kcal/Kg in case of starter and finisher mash respectively, whereas value for ME in starter and finisher mashes reported by Katoch (1996) were 2826.00 and 2956.50 (Kcal/Kg), respectively. Gupta (2003) recorded the ME values for starter and

finisher mash as 2827.00 and 2956.56 (Kcal/Kg), respectively. All the nutrients were as per the specifications given by BIS (1992).

5.4 Effect on biological performance of broilers with supplementation of their diet with *Lactobacillus acidophilus*, *Lactococcus uberis*, *Saccharomyces cerevisiae* in experiment 1, experiment 2 and experiment 3, respectively.

5.4.1 Effect on biological performance of broiler, during starter phase (1-4 weeks of age):

The data regarding the average body weight, gain in weight and feed conversion ratio during the starter and finisher phase have been presented in tables 6, 7, 8 for experiments 1, 2 and 3 respectively. The overall observation of result indicated a better response with respect to higher gain in weight and better FCR in chicks fed diets supplemented with *Lactobacillus acidophilus* in experiment 1, *Lactococcus uberis* in experiment 2 and *Saccharomyces cerevisiae* in experiment 3 as compared to unsupplemented control. These results of present investigation are in accordance with the findings of Katoch (1996) who reported that the broilers fed on microbial cultures, showed higher gain in weight and better FCR.

5.4.2 Effect on biological performance of broiler, during finisher phase (5-6 weeks of age)

During finisher phase also the GIW and FCR values were significantly ($P<0.0001$) better in treatment group as compared to the control group in all the three experiments. These results of present investigation are in accordance with the findings of Kaistha (1996) who reported that the broilers fed on microbial cultures viz. *Lactobacillus acidophilus, Lactococcus uberis and Saccharomyces cerevisiae* showed significantly ($P<0.05$) higher GIW and improvement in FCR as compared to non-supplemented control. Similar findings had also been reported by Katoch (1996).

5.4.3 Effect on overall biological performance of broiler (1-6 weeks of age):

The overall perusal of data revealed that the GIW were significantly ($P<0.0001$) higher in treatment group as compared to non-supplemented control.

These results of present investigation are in accordance with the findings of Kaistha (1996) who reported that the broilers fed on microbial cultures viz. *Lactobacillus acidophilus, Lactococcus uberis and Saccharomyces cerevisiae* showed significantly ($P<0.05$) higher GIW as compared to non- supplemented control. The higher body weight gains observed during the present study are also in agreement with the findings of Mandal, *et al.* (1994), Samanta and Biswas (1995), Bhatt (1997), Katoch, *et al.* (2000), Upendra and Yathiraj (2003), Sapcota (1998) and Gupta (2003). These authors ascribed the beneficial effects of probiotics, might be due to reduction of pathogenic microflora in the gut. In addition to that reduction in pH by lactic acid forming microflora, alteration of metabolism and production of cofactors, which might have helped in digestion and absorption of nutrients.

Feed conversion ratio (FCR) in terms of feed consumption g/ g gain is important tool to measure the efficiency of feed utilization. FCR values of treatments group are significantly ($P<0.0001$) higher as compared to control. The better FCR in present study might be due to the use of probiotic and better managemental conditions maintained during investigation. These results of present investigation are in accordance with the findings of Kaistha (1996) who reported that the broilers fed on microbial cultures viz. *Lactobacillus acidophilus, Lactococcus uberis and Saccharomyces cerevisiae* showed significantly better ($P<0.05$) FCR as compared to non- supplemented control. The improved FCR observed during the present study are in agreement with the findings of Cho, *et al.* (1992), Kaistha, et al (1996), Georgieva, et al (1998), Gohain and Sapcota (1998), Katoch (1996), Gupta (2003).

The drastic reduction in mortality in these three treatment groups as compared to control group was also observed. This might be due to competitive exclusion of pathogens, enhanced growth of favourable microbes in GIT, increased immune

response and decreased incidence of disease. These results of lower mortality or no mortality in present study was in accordance with findings of several researchers, Hussein and El-Ashry (1991), Samanta and Biswas (1995), Kaistha, *et al.* (1996) and Rajmane and Sonawane (1998).

The cost benefit ratio in treatments groups was also improved due to higher growth rate in broilers, improvement in feed conversion ratio along with decrease in chick mortality. Similar trend of cost benefit ratio was observed by Gupta (2003).

SUMMARY

CHAPTER 6

SUMMARY

In broiler production all endeavours are towards increasing growth rate and obtain maximum feed efficiency. As the feed costs accounts for 70 to 80% of total cost in poultry farming and the rocketing price only serve to increase the vulnerability of the poultry farmers, many of who may not be able to survive the unequal battle against inflation. It is, therefore, absolutely necessary to reduce the cost of poultry production by enhancing the availability of nutrients and by using suitable biotechnological tools.

Public disapproval and banning of antibiotics and growth hormones as feed additive in certain parts of world has renewed the use of probiotic in chicken feeding. Probiotic, not only promote higher growth rate and feed conversion efficiency in growing chickens but also prevent carcass contamination by intestinal pathogens during processing. Improvement in poultry production has to be oriented with the primary aim of ensuring the healthy environment and the safety of the consumers.

In the present investigation an attempt has been made to test the efficacy of *Lactobacillus acidophilus, Lactococcus uberis, Saccharomyces cerevisiae* isolated from bottle gourd, bitter gourd and bitter gourd, respectively on the biological performance of commercial broilers from day one to six weeks of age under field conditions. Keeping in view the present investigation was undertaken with the following objectives:

1. To test the efficacy/validity of *Lactobacillus acidophilus* (bottle gourd), *Lactococcus uberis* (bitter gourd) and *Saccharomyces cerevisiae* (bitter gourd) at three different locations (farmers' level) on the biological performance of commercial broilers from 1-day to six weeks of age.

2. To work out the cost benefit ratio of broiler production.

On analysing the chemical composition of broiler diets, the broiler starter mash wash found to contain DM, CP, CF, EE, total ash, NFE, AIA(silica), Ca, P, NDF, ADF, cellulose, lignin and hemi cellulose as 89.38, 23.06, 3.48, 2.50, 5.84, 65.15, 2.58, 1.48, 0.77, 18.24, 7.40, 5.74, 1.64 and 10.85 per cent, respectively. The broiler finisher mash was found to contain DM, CP, CF, EE, total ash, NFE, AIA (silica), Ca, P, NDF, ADF, cellulose, lignin and hemi cellulose as 88.42, 20.05, 3.06, 2.57, 5.38, 68.88, 2.13, 2.37, 1.16, 17.85, 6.38, 4.84, 1.54 and 11.47 per cent, respectively. The calculated values for Metabolisable Energy (ME) were 2825.00 and 2956.56 Kcal/Kg in case of starter and finisher mash, respectively.

The experiment 1 was conducted to study the effect of dietary supplementation of *Lactobacillus acidophilus*-bottle gourd on the biological performance of commercial broilers. *Lactobacillus acidophilus* was supplemented in standard concentration of 6.8 X 10^8 cells /kg of feed. Broilers were fed standard starter diet up to first four weeks and finisher diet during the last two weeks. During starter phase, the difference in live weight gain, feed intake and feed conversion ratio for treatment and control was statistically ($P<0.0001$) significant.

Similarly during the finisher phase FCR was significantly higher in treatment group as compared to control. The gain in weight of treatment group was numerically higher than control group but not statistically significant during finisher phase.

Overall broiler growth performance from 1-6 weeks of age also showed significantly ($P<0.0001$) higher gain in weight and FCR of treatment group as compared to control. The per cent increase in gain in live weight of treatment group as compared to the control was 7.52 per cent. The cost benefit ratio for treatment was 1:2.49 against 1:2.24 of control.

The experiment 2 was conducted to study the effect of dietary supplementation of *Lactococcus uberis* - bitter gourd on the biological performance of commercial broilers. *Lactococcus uberis* was supplemented in standard concentration of 6.8 X 10^8 cells /kg of feed. Broilers were fed standard starter diet up to first four weeks and finisher diet during the last two weeks. During starter phase, the difference in live weight gain, feed intake and feed conversion ratio for treatment and control was statistically ($P<0.0001$) significant.

During finisher phase the GIW of treatment group was significantly ($P<0.0001$) higher than control group. But FCR of the treatment group was numerically higher than control but statistically insignificant during finisher phase.

The GIW and FCR were significantly ($P<0.0001$) higher for the treatment group as compared to control during one to six weeks of age. The per cent increase in gain in live weight of treatment group over the control was 8.70 per cent. The cost benefit ratio for treatment was 1:1.70 against 1:1.44 of control.

The experiment 3 was conducted to study the effect of dietary supplementation of *Saccharomyces cerevisiae* – bitter gourd on the biological performance of commercial broilers. *Saccharomyces cerevisiae* was supplemented in standard concentration of 5.7X 10^7 cells /kg of feed. Broilers were fed standard starter diet up to first four weeks and finisher diet during the last two weeks. During starter phase, the difference in live weight gain, feed intake and feed conversion ratio for treatment and control groups was statistically ($P<0.0001$) significant.

FCR and GIW showed the same trend during finisher phase as in starter phase. During 1-6 weeks of growth GIW and FCR of treatment group were significantly ($P<0.0001$) higher than control. The per cent increase in gain in live weight of

treatment group over the control was 39.85 per cent. The cost benefit ratio for treatment was 1:1.35 against 1:0.95 of control.

On the overall at both the laboratory and field levels, the same trend of growth rates and FCR in broilers fed diets supplemented with the similar microbes was observed.

Hence, the overall study revealed that all these three useful microbes i.e. *Lactobacillus acidophilus*, *Lactococcus uberis* and *Saccharomyces cerevisiae* isolated from vegetable sources could be used to improve the broiler production at field levels that will in turn result in more profitable broiler farming.

Comparison of three strains- *Lactobacillus acidophilus* – bottle gourd *Lactococcus uberis* – bitter gourd and *Saccharomyces cerevisiae* – bitter gourd, was done on the basis of per cent increase in gain in weight along with better FCR, decreased mortality and improved cost benefit ratio during the period of 1-6 weeks of commercial broiler rearing. *Saccharomyces cerevisiae* supplemented group performed well followed by *Lactococcus uberis* and *Lactobacillus acidophilus* and therefore, can be ranked in the following order:

Rank 1- Saccharomyces *cerevisiae*

Rank 2- *Lactococcus uberis*

Rank 3- *Lactobacillus acidophilus*

The salient findings of the present study are:

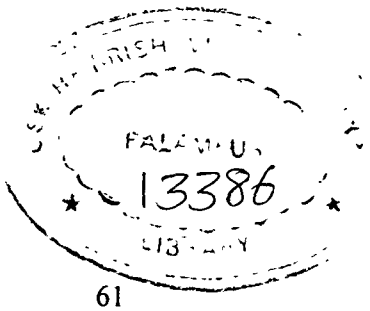
1. All the three microbes (*Lactobacillus acidophilus*, *Lactococcus uberis*, and *Saccharomyces cerevisiae*) gave higher gain in weight as compared to the control.
2. FCR was also improved in all the treatments as compared to control.
3. Mortality in supplemented groups was reduced to great extent. No mortality observed in *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* supplemented groups however mortality was reduced by 50 per cent with *Lactococcus uberis* as compared to control.
4. Highest per cent increase in GIW was showed by *Saccharomyces cerevisiae* followed by *Lactococcus uberis* and *Lactobacillus acidophilus.*
5. Cost benefit ratio was also improved in all three supplemented groups as compared to the control
6. On the basis of overall biological performance these three microbes can be ranked as

Rank 1- Saccharomyces *cerevisiae*

Rank 2- *Lactococcus uberis*

Rank 3- *Lactobacillus acidophilus*

Thus overall results of the present investigation revealed that Probiotics are the most suitable means, to achieve economic growth performance, higher gain in live weights, coupled with better FCR and for good quality of chicken, safe for human consumption.



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