PERFORMANCE OF EARLY WEANED CROSSBRED (LANDRACE X DESI) PIGLETS FED ON LIVE *SACCHAROMYCES CEREVISIAE*

THESIS

Submitted in partial fulfilment of the requirements for the degree

of

Master of Veterinary Science

in

ANIMAL NUTRITION

By

Dr. Sachin Kumar

Roll No. 4763

To

DEEMED UNIVERSITY

INDIAN VETERINARY RESEARCH INSTITUTE

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Dated: 30 **July, 2011**

Certificate

Certified that the research work embodied in this thesis entitled **"Performance of early weaned crossbred (Landrace x Desi) piglets fed on live *Saccharomyces cerevisiae*"** *submitted by* **Dr. Sachin Kumar, Roll No. 4763,** *for the award of* **Master of Veterinary Science** *degree in* **Animal Nutrition** *at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.*

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Dated : 25. 8. 2011

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Date: **(Sachin Kumar)**

Place: Izatnagar

Abbreviations

ADF	:	Acid detergent fibre
ADG	:	Average daily gain
ALT	:	Alanine aminotranferease
ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemists
AST	:	Aspartate aminotransferase
BCP	:	Bromo- cresol purple
BCG	:	Bromo-cresol green
BP	:	Boiling point
C	:	Crypt depth
Ca	:	Calcium
CB	:	Crossbred
CF	:	Crude fibre
CFU	:	Colony forming unit
CMI	:	Cell mediated immunity
CP	:	Crude protein
d	:	Day
DAM	:	Diacetyl monoxime
DCP	:	Digestible crude protein
DDM	:	Digestible dry matter
dl	:	Decilitre
DM	:	Dry matter
DMB	:	Dry matter basis
DMI	:	Dry matter intake
DOM	:	Digestible organic matter
DOMI	:	Digestible organic matter intake
DTH	:	Delayed type hypersensitivity
EE	:	Ether extract
FAO	:	Food and Agriculture Organization
FCR	:	Feed conversion ratio
g	:	Gram(s)

G.I.T.	:	Gastro-intestinal tract
GOD	:	Glucose oxidase
h	:	Hour(s)
H_2SO_4	:	Sulphuric acid
HCl	:	Hydrochloric acid
IVRI	:	Indian Veterinary Research Institute
i/m	:	Intramuscular
kg	:	Kilogram (s)
$kgW^{0.75}$	:	Metabolic body size
L	:	Litre (s)
M	:	Molar
mA	:	Milliangstrom (s)
mg	:	Milligram(s)
min	:	Minute(s)
ml	:	Millilitre(s)
mmol	:	Millimolecular weight
N	:	Nitrogen
NaOH	:	Sodium hydroxide
NDF	:	Neutral detergent fibre
NRC	:	National Research Council
NFE	:	Nitrogen free extract
nm	:	Nano meter(s)
NS	:	Non significant
OD	:	Optical density
OM	:	Organic matter
OMD	:	Organic matter digestibility
OMI	:	Organic matter intake
P	:	Phosphorus
PBS	:	Phosphate buffer saline
PHA	:	Phytohaemagglutinin
POD	:	Per oxidase
PW	:	Post weaning
rpm	:	Revolutions per minute

S	:	Standard
SEM	:	Standard error of mean
SRBC	:	Sheep red blood cell
T	:	Test
V	:	Villus height
TA	:	Total ash
TDN	:	Total digestible nutrients
YC	:	Yeast culture
YEPG	:	Yeast extract peptone glucose
%	:	Per cent
µmol	:	Micromolecular weight
µm	:	Micro meter
^{0}C	:	Degree celcius

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Introduction

Agriculture and allied sectors is the mainstay of the Indian economy as these sectors are the main contributors of the gross domestic production. Livestock sector in India is closely linked with agriculture and plays an important complimentary role in the rural economy. It provides high quality human food (milk, meat and eggs), wool, fibre and manure, in addition to gainful employment and supplementary income to the majority of rural population. The contribution of livestock sector is about 25% of the value of India's total agriculture output. Presently India is naturally gifted with the largest livestock population in the world, which is more than 484.9 million with 178 million cattle, 98.7 million buffaloes, 125.45 million goats, 64.6 million sheep and 14 million pigs (FAO, 2007). Among the different livestock pigs are believed to be the most prolific. Porcine production has a great potential to contribute to high economic gain. Pigs have high fecundity, high feed conversion efficiency, early maturing, short generation interval and relatively small space requirement. Pork is the most popular meat consumed in the world today. The growth in meat consumption in the developing world is far greater than that of the developed countries. Total pig meat production in 2007 was 98.8 million tonnes which is 36% of total world meat production (FAO, 2007).

In the pig farming, weaning is an important managemental practice. Weaning is a stressful experience for young piglets because of adjustment from a liquid to a dry diet. The standard age of weaning in case of piglets is 42-56 days. This has started to decrease and now, weaning at an early age (21-35 days) is a normal commercial practice. Early weaning increases the potential for annual production of piglets per sow and profit of farmer. At the time of weaning, young piglets are subjected to several stressors such as nutritional, environmental, social and microbial imbalance (Fraser *et al.,* 1998; Nabuurs, 1998). As a result, low feed intake, impaired intestinal morphology and function (Pluske *et al.,* 1995), a high incidence of diarrhoea and growth depression are commonly seen immediately after weaning (Pluske *et al.,* 1997). So weaning is regarded as the most critical period in a pig's life. It is well established that this process is multifactorial, and that post weaning (PW) anorexia and undernutrition are major aetiological factors (Pluske *et al.,* 1997 and Lallès *et al.,* 2004). Subtherapeutic use of antibiotics has been widely applied to nursery pigs to solve post weaning problems (Barton, 2000). However, the use of in-feed antibiotics is banned by the European Union from 2006 onwards because there is an increasing evidence of microorganism becoming resistant to antibiotics in both animal and human (Cohen, 1992; Newman, 2002). Thus, public concern over the use of antibiotics urged scientists to find alternatives (to the antibiotics) (Cassel, 1995; Mathew *et al.,* 1998; Bach Knudsen, 2001 and Smith *et al*., 2002). Growing public disquiet over the use of antibiotics as feed additives has encouraged recent commercial interest in prebiotics, probiotic and synbiotic as an alternate therapy against harmful pathogens in the gastrointestinal (G.I.) tract and to ameliorate the weaning stress.

Prebiotics are defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth

and activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Probiotics are preparations of non-pathogenic microorganisms, prepared for animal and human use, that have beneficial effects on the digestive ecosystem and confer resistance to infection (Fuller, 1992). A concept of synbiotic, a combination of prebiotcs and probiotics components has been proposed to characterize health enhancing foods and supplements used as functional food ingredients in humans (Gibson and Roberfroid, 1995). Nemcova *et al* (1999) observed that feeding a diet with synbiotics to young pigs increased *Lactobacillus* and *Bifidobacterium* compared to prebiotics and probiotics alone.

Probiotics have been reported to increase feed intake, growth (Lessard and Brisson, 1987), immune responses (Isolauri *et al,* 1995), the numbers of *Lactobacilli* and decrease the numbers of *E. coli* (Xuan *et al.*, 2001; Deprez *et al.,* 1986). However, experiments have failed to show consistent and beneficial responses (NRC, 1998). The effect of yeast culture (*Saccharomyces cerevisiae*) supplementation on the growth performance of piglets has been well documented (Veum *et al.*, 1988; Jurgens *et al.,* 1997; Mathew *et al.*, 1998; Bontempo *et al.*, 2006 and Shen *et al.,* 2009) and has generated considerable scientific interest over the last two decades. It is clear from many previous research efforts that yeast culture (YC) supplements can beneficially modify microbial activities, fermentative and digestive functions in the GIT of piglets. It is recognized that microbial fermentation within the G.I. tract is very important for the pig (Williams *et al.,* 2001). These microorganisms are supposed to influence the microbial activity in the G.I. tract of the animals leading to prevention of infections and increases growth performance, nutrient utilization and immune status. Supplementation of live yeast or yeast culture has been reported to improve growth performance and average daily gain (ADG)

in weanling pigs (Veum *et al.,* 1988; Jurgens *et al.,* 1997; Maloney *et al.,* 1998a**;** Mathew *et al.,* 1998; Bontempo *et al.,* 2006 and Shen *et al.,* 2009). However, results are variable, as some workers have reported no benefit of yeast supplementation (Jurgens, 1995 and Kornegay *et al.,* 1995). Van Heugten *et al.* (2003) reported that live yeast supplementation had positive effects on nursery piglets performance, but only when diets contained growth promoting antimicrobial substances. Shen *et al.* (2009) observed that YC supplementation had positive effect on immune response in nursery pigs. Supplementation of probiotics *(Bacillus cereus var. toyoi)* affected the intestinal immune system of the piglets at the time of weaning (Scharek *et al.,* 2007). However information regarding effect of live *Saccharomyces cerevisiae* feeding on the crossbred (Landrace X Desi) early weaned piglets is lacking.

Keeping above in view, the present study has been proposed to assess the effect of feeding live *Saccharomyces cerevisiae* on crossbred (Landrace X Desi) early weaned piglets with following objectives:

- **To study the effect of live *Saccharomyces cerevisiae* feeding on growth performance and nutrient utilization in early weaned piglets.**
- **To study the effect of live *Saccharomyces cerevisiae* feeding on metabolic profile and immune response in early weaned piglet.**

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Review of Literature

2.1 PIGLETS AND WEANING

The weaning transition is a complex period during which the piglets have to cope with abrupt separation from their mother, mixing with other litters in a usually new environment and switch from highly digestible (liquid) milk to a less digestible more complex solid feed, hence weaning is a stressful experience for the piglets. Weaning at an early age (21–35 days) is a normal commercial practice, which increases general stress in the weaned piglets. The aim of early weaning is to optimize reproductive efficiency of the sow. Early weaning of piglets is often associated with gastrointestinal disturbances and severe growth check. This practice is accompanied by many challenges such as low and variable feed intake, diarrohea, body weight loss, damage to intestinal function and health (Pluske *et al.*, 1997). This process is multifactorial and post weaning (PW) anorexia and undernutrition are major aetiological factors (Pluske *et al.*, 1997 and Lallès *et al.*, 2004). Moreover, weaning is associated with villous atrophy in the small intestine. This may impair digestion and absorption of the gut. However, high standards of management and efficient feeding can dramatically reduce post weaning losses and improve growth rates by moderating the stress of weaning. Antibiotics have been applied to nursery pigs at sub-therapeutic levels

to solve post weaning problems (Barton, 2000). But worldwide concern about development of antimicrobial resistance to these antibiotics urged scientists to find alternatives to the use of antibiotics (Cassel, 1995; Mathew *et al.,* 1998; Bach Knudsen, 2001 and Smith *et al.,* 2002) that could enhance the natural defence mechanisms of animals and reduce the massive use of antibiotics. One way is to use specific feed additives or dietary raw materials to favourably affect animal performance and welfare, particularly through the modulation of the gut microbiota, which plays a critical role in maintaining host health (Tuohy *et al.,* 2005). A balanced gut microbiota constitutes an efficient barrier against pathogen colonization, produces metabolic substrates (eg. vitamins and short chain fatty acids) and stimulates the immune system in a non inflammatory manner. In this context feeding of probiotics, prebiotics and synbiotics could be possible solutions. The main effects of these feed additives are the improved resistance to pathogenic bacteria colonization and enhanced host mucosal immunity; thus resulting in a reduced pathogen load, an improved health status of the animals (Choct, 2009 and Williams *et al.,* 2001) and a reduced risk of food borne pathogens in feeds.

2.2 POSTWEANING GROWTH CHECK

Pigs weaned at 2 (Smith and Lucas, 1957), 7 (Hartman *et al,* 1961), 10 (Lucas *et al.,* 1959) or 14 days (Ewan, 1970) of age gain slower than nursing pigs. The poor performance of early weaned pigs may be related to inadequate digestive development resulting in poor utilization of the nutrients in the starter diet.

Early weaning increases stress on the immature piglet, which is accompanied by many challenges such as low and variable feed intake, diarrohea, body weight loss, damage to intestinal function and health (Pluske *et al.,* 1997). Weaning usually causes a reduction

in villous height and in brush border enzyme activity for the first few days after weaning (Pluske *et al,* 1995). The reduction in villous height is associated with a low feed intake. Thus, low feed intake that is usually seen immediately after weaning causes a serious problem, and this leads to reduction in growth rate which is called “postweaning growth check" (Dunshea *et al.,* 2002 and Le Dividich and Seve, 2000). The postweaning check which occurs in pigs is greater and lasts longer in early weaned pigs (Dunshea *et al.*, 2002 and Power *et al.*, 1996).

Geary and Brooks (1998) observed that dry matter intake in the first week after weaning significantly affects the 28 day postweaning weight of pig. Feed intake during one week after weaning was only 60-70% of that consumed prior to weaning (Le Dividich and Seve, 2000). This reduction in feed intake is mainly due to shift in diet from liquid to solid. Hence, the low feed intake after weaning may be avoided partly by feeding a liquid diet instead of dry diet (Odle and Harrell, 1998). However, the above mentioned methods do not have similar effects of growth promoting antibiotics. It is assumed that prebiotics and probiotics could be potential alternative to growth promoting antibiotics. Because prebiotics, which are non-digestible carbohydrates may serve to provide nutrient source for bacteria and also influence the gastrointestinal ecosystem (Buddington, 2001) so beneficial lactic acid bacteria may improve gut health and feed intake. The improved gut health by feeding pre and probiotics may have potential to improve growth of young pigs (Abe *et al.*, 1995)

2.3 POSTWEANING STRESSORS

Weaning of piglets is accompanied by multi-faceted stressors to young ones such as nutritional, psychological, environmental, microbiological and immunological stresses. If some of the postweaning stresses can be overcome, then the postweaning growth

check would probably be less severe. This would allow animals to reach their potential weight at weaning. Thus, it is important to understand postweaning stressors for successful adaptation of weanling pigs.

2.3.1 Nutritional stress

Postweaning nutritional stress may occur as the source of nutrients changes from a liquid to a dry diet. The rapid change of pancreatic enzyme production occurring at weaning (Friend *et al.*, 1970 and Shields *et al.*, 1980) indicates that the digestive system is ill-prepared to digest typical starter diets. This may cause alterations of the gastrointestinal tract of weanling pig. As a result, piglets often do not eat during the first few days after weaning. This is associated with an adverse change in gut histology such as villous atrophy, increasing in crypt depth, reduction in digestive and absorptive functions of nutrient leading to postweaning malabsorption syndrome (Pluske *et al.*, 1997). Thus, a continuous supply of nutrients to the gut after weaning may prevent the detrimental changes of gut morphology and function of newly weaned pig (Pluske *et al.*, 1996 and Bruininx, 2002).

2.3.2 Environmental and psychological stress

Weaning of piglets imposes the environmental stress and psychological stress. Boe (1993) observed that post-weaning environment has a major influence on the frequency of abnormal behaviours in weaned piglets. When piglets are weaned they are separated from their mother, this causes psychological stress to pigs (Funderburke, 1985). Piglets are commonly mixed with other litters after weaning. This may also cause psychological stress and depress immunological function (Blecha *et al.*, 1983) and health status of the animal.

2.4 PROBIOTICS

The term "Probiotics" was first coined by Parker (1974) who described this as "microorganism or substance, which contributes to the intestinal microbial balance". The term probiotic means "for life" and has a contrast with the term antibiotic which means "against life". At present, probiotics are classified by the US Food and Drug Administration as generally recognised as safe (GRAS) ingredients. Probiotics are preparations of non pathogenic microorganisms, prepared for animal and human use, that have beneficial effects on the digestive ecosystem and confer resistance to infection (Fuller, 1992). Many definitions have been proposed for the term "probiotic". The more widely accepted one is "live microorganisms, when they were administered in adequate amounts, confer a health benefits on the host" (FAO/WHO, 2002). This definition implies that a health effect must be demonstrated for the probiotic. The beneficial modes of action include: regulation of intestinal microbial homeostasis, stabilization of the gastrointestinal barrier function (Salminen *et al.*, 1996), expression of bacteriocins (Mazmanian *et al.*, 2008), enzymatic activity inducing absorption and nutrition (Hooper *et al.*, 2002 and Timmerman *et al.*, 2005), immunomodulatory effects (Salzman *et al.*, 2003), inhibition of procarcinogenic enzymes and interference with the ability of pathogens to colonize and infect the mucosa (Gill, 2003).

2.4.1 Microorganism commonly used as probiotics

- *Lactobacillus- acidophilus, brevis, fermentum, gallinarum, plantarum, casei, gasseri, johnnsonii, salivaris*
- *Saccharomyces- cerevisiae, boulardii*
- *Aspergillus- oryzae*
- *Bacillus- cereus, coagulans, licheniformis, subtilis*
- *Enterococcus- faecium*

2.4.2 Mode of action of probiotics

The normal gut microflora have a supportive role in disease protection and digestion of food. However, stressful conditions such as introduction of weaning result in change of diet and increase in gut pH, which favour growth of pathogenic organisms. The probiotics bring about its beneficial effects through one of the following mechanisms.

2.4.2.1 Neutralization of toxin

Probiotics neutralize the various enterotoxins produced by various pathogenic microorganisms in gastrointestinal tract. The protective effect of *Saccharomyces cerevisiae* is due to the reduction in the available amounts of the toxins secreted by pathogens and by competition for its adhesion sites in the presence of the yeast. Generally, toxins bind to specific receptors on intestinal epithelial cells and induce changes resulting in loss of water and electrolytes. Inhibition of the toxins production or of the effects of the toxins has been well described with *Clostridium difficile* (Corthier *et al.*, 1986), *Vibrio choleris* (Vidon *et al.*, 1986). Castagliulo *et al.* (1996) indicated that certain strains of *Saccharomyces cerevisiae* can excrete a serine protease that can hydrolyze toxin A coming from 50 *Clostridium difficile*, which is resistant to trypsin, and inhibits binding of this toxin to its brush border glycoprotein receptor.

2.4.2.2 Antibacterial activity

Probiotic microorganisms after establishment in the gut may produce substances such as lactoferrin, lysozyme, hydrogen peroxide as well as several other organic acids with bactericidal or bacteriostatic properties (bacteriocins). These substances have a detrimental effect on harmful bacteria, which is primarily due to a lowering of the gut pH. A decrease in pH may partially offset the low secretion of hydrochloric acid in the stomach of weaned piglets.

2.4.2.3 Competition for adhesion sites

The competition for space to adhere between indigenous bacteria and exogenous pathogens results in the competitive exclusion of exogenous pathogens from the intestinal lumen. The concept of competitive exclusion indicates that cultures of selected, beneficial microorganisms, supplemented to the feed, compete with potentially harmful bacteria in terms of adhesion sites and organic substrates (mainly carbon and energy sources). Probiotics may colonise and multiply in the gut, thereby blocking receptor sites and preventing the attachment of other bacteria including harmful species such as enteropathogenic *E. coli* or *Salmonella.* Probiotics have the potential to decrease the risk of infections and intestinal disorders.

It is generally accepted that the adhesion of bacteria to epithelial cells is an early stage in bacterial infection of mucous membranes. Bacteria possess binding molecules on their surfaces that are capable of interacting stereospecifically with host cell membranes in a manner analogous to antigens-antibodies interaction. Evidence has been established that certain strains of *E. coli* or *Salmonella* possess a fimbrial adhesin, which binds to mannose residues on epithelial cell membranes (Ofek *et al.*, 1977). Such bacteria, or their isolated fimbriae (Korhonen, 1979) also agglutinate yeast containing mannan in the outer layer of their cell wall. This agglutination is inhibited by solutions of D-mannose (Ofek *et al.*, 1977).

Binding of pathogens to yeast cell wall induces a protective effect since the complex *Saccharomyces cerevisiae*-pathogen is then rapidly eliminated from the digestive tract (Gedek, 1989). Competition between yeast and pathogens for binding to intestinal cells could help to explain the beneficial action of yeast, since adhesion is crucial

to the expression of the cytopathogenic effect. Frequency of *Salmonella typhimurium* colonisation was significantly reduced in broilers, due to both mannose (Oyofo *et al.*, 1989), and yeast treatment (Line *et al.*, 1998), although *Campylobacter* colonisation was not affected by yeast supplementation.

2.4.2.4 Stimulation of immunity

Probiotics stimulate the immune system by increased production of antibodies. Probiotic increases macrophage activity shown by the enhanced ability to phagocytose microorganisms. The action of yeast cell wall material on the complement system has been known for a long time (Pillemer *et al.*, 1954). Generally these properties are related to the presence in the inner part of yeast cell wall of glucans, that are constituted of main chains of beta-(1-3)-linked D-Glucose molecules to which are attached linear side chains of beta-(1-6) linked residues. These macromolecules have an ability to stimulate certain aspects of the immune system in mammals especially inflammatory response and reticuloendothelial system (RES).

2.4.2.5 Prevention of amine synthesis

Amines produced from the decarboxylation of amino acids by coliform bacteria, are toxic which may irrigate the gut and lead to diarrhoea. Probiotic prevents the proliferation of coliform bacteria thus amine production is also prevented.

2.5 RESPONSES OBTAINED FROM ADDITION OF LIVE MICRO-ORGANISMS

2.5.1 Growth performance and nutrient utilization

The most important feature during post-natal life of piglets is growth. The growth performance of pig is economically very important. Growth is generally measured as an increase in body weight. Growth

rate of the pig is one of the key indicators affecting the profitability of pig meat production. Improvement in growth rate and feed to gain ratio results in improved profitability due to greater output and reduction in overhead costs (Campbell, 1997). An increased growth rate due to high dry matter intake and better feed conversion efficiency has been reported by feeding probiotics. However, the dietary composition and environmental conditions influence the growth response of animals to yeast supplementation. Positive effects were observed by Maxwell *et al.* (1983) and Hong *et al.* (2002) as a result of supplementation with *Lactobacillus* based probiotics on nutrient digestibility. However, other reports show no effect on digestibility of Dry Matter (DM), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), amino acid when pigs were fed probiotics containing *Lactobacillus* or *Bacillus* cultures (Hale *et al.,* 1979 and Kornegay and Risley, 1996).

Mahan *et al.* (1998) and Quiniou *et al.* (2002) observed that the age and weight at weaning are closely related to postweaning growth. Many studies have demonstrated that weaning weight influences postweaning growth performance and also influences performance during the subsequent grower and finisher phases (Le Dividich, 1999). An increase in pig weight at weaning with one kg will result in a pig which reaches slaughter weight at least 10 days faster (Cole and Close, 2001). It is also accepted that average daily gain during the first week postweaning has a major impact on subsequent growth performance (Tokach *et al.,* 1992).

Muirhead and Alexander (1997) have reported that the use of growth promoting antibiotics in pigs results in 4 to 6.5% increase of their growth and feed efficiency. Now a days prebiotic, probiotic and /or synbiotic are using as a substitute of antibiotics. Prebiotics (non-

digestible oligosaccharides such as FOS) may have potential to increase weight gain of young pigs. Abe *et al.* (1995) and Russell *et al.* (1996) have reported that feeding probiotics (beneficial bacteria) to piglets or calves improves weight gain and feed efficiency. In addition, Nemcova *et al.* (1999) have found that synbiotics, combining FOS and *Lactobacillus paracasei*, increase beneficial bacteria and decrease harmful bacteria in weaned pigs.

Jurgens *et al.* (1997) studied the effect of dietary active dry yeast supplementation on postweaning pig performance. They observed that dry yeast supplementation had little difference in feed intake but significant improvement in average daily gain ($P<0.05$) and feed efficiency ($P<0.05$) by pigs whose dams, as well as themselves, received supplemental active dry yeast. Mathew *et al.* (1998) studied the effect of *S. cerevisiae* on performance of weaning piglets. They reported that pig fed with the yeast diets had shown better total intake as well as overall gains when compared with pig fed with the control diet. Bontempo *et al.* (2006) studied the effect of yeast supplementation (2g/kg of diet providing 2×10^6 cfu /g of feed) on piglet growth. They observed that control piglets were heavier ($P<0.05$) than treated piglets at weaning but the later ones were significantly heavier at 30 days post weaning ($P<0.01$). They also observed that piglets fed yeast had a significantly greater ADG (474±0.01g) from weaning throughout 30 days post weaning than non-supplemented group (432± 0.01g). Van Heugten *et al.* (2003) evaluated the effects of live yeast supplementation on nursery pig performance, nutrient digestibility and faecal microflora and also determined whether live yeast could replace antibiotic and growth promoting concentration of Zn and Cu in nursery pigs. They observed that live yeast supplementation had a positive effect on nursery pig performance when diets contained growth promoting antimicrobials.

Table 2.1 : Influence of probiotic strains on performance of pigs

Animal	Probiotic	Effect	Reference
Weaning	*Saccharomyces cerevisiae*	Little difference in feed intake but significant improvement in average daily gain and feed efficiency	Jurgens *et al.* (1997)
Weaning	*Saccharomyces cerevisiae*	better total intake as well as overall gains	Mathew *et al.* (1998)
Weaning	*Saccharomyces cerevisiae*	Increase in body weight, greater ADG	Bontempo *et al*. (2006)
Nursery	*Saccharomyces cerevisiae*	Positive effect on nursery pig performance when diets contained growth promoting antimicrobials.	Van Heugten *et al.* (2003)
Weanling	*Saccharomyces cerevisiae*	No benefit of yeast supplementation	Kornegay *et al*. (1995)
Weanling	*Lactobacillus sp.*	Feed conversion ratio (↑), Nitrogen and fibre digestibility (↑)	Hale and Newton, (1979)
Weanling	*L. bulgaricus, L. casei, S. thermophilus*	Feed intake (↑), Growth (↑), FCR (No effect)	Lessard and Brisson, (1987)
Weanling	*L. acidophilus S. faecium*	Growth and FCR (↑), Growth (↓).	Pollmann *et al.* (1980a)
Weanling	*L. acidophilus*	Some depressed growth rate	Harper *et al.* (1983)
Weanling	*Lactobacillus sp*	Growth (↑)	Ogle and Inborr, (1987)
Weanling	*L. reuteri*	Decreased growth rate, lower FCR	Ratcliffe *et al.* (1986)
Weanling	*Bacillus licheniformis*	Weight gain (↑),	Collinder *et al.* (2000)
Weanling	*Saccharomyces cerevisiae*	No benefit of yeast supplementation	Jurgens (1995)
Weanling	*B. licheniformis, Bacillus toyoi*	Growth (↑), Growth (↑)	Kyriakis *et al.* (1999)
Weanling	*Bacillus*	Growth (↑)	Collinder *et al.* (2000)
Growing Finishing	*Lactobacillus sp*	No effect	Hale and Newton, (1979)
Growing Finishing	*L. acidophilus S. faecium*	No effect	Pollmann *et al.* (1980a)
Weanling	*Bacillus toyoi*	Growth and feed efficiency (↑), Diarrhoea, mortality (↓)	Kyriakis *et al.* (1999)
Weanling	*L. acidophilus and Streptococcus faecium or Bacillus toyoi*	Growth and feed efficiency (↑), Feed intake (No effect), Nitrogen retention and Biological Value (↑)	Fialho *et al.* (1998)

* (↑) and (↓) are either significantly increased or decreased.

However, results are variable, as some workers have reported no benefit of yeast supplementation (Jurgens, 1995 and Kornegay *et al*., 1995).

2.6 IMMUNE RESPONSE

An immune system is a system of biological structures and processes within an organism that protects against disease by identifying and killing pathogens and tumour cells. It detects a wide variety of agents, from viruses to parasitic worms and needs to distinguish them from the organism's own healthy cells and tissues in order to function properly. The immune system is separated into two branches: humoral immunity, for which the protective function of immunization could be found in the humor (cell-free body fluid or serum) and cellular immunity, for which the protective function of immunization was associated with cells.

The humoral immune response (HIR) is the aspect of immunity that is mediated by secreted antibodies (as opposed to cell mediated immunity, which involves T lymphocytes) produced in the cells of the B lymphocyte lineage (B cell). B Cells (with co stimulation) transform into plasma cells which secrete antibodies.

Cell mediated immunity is an immune response that does not involve antibodies or complement but rather involves the activation of macrophages, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes and the release of various cytokines in response to an antigen.

The pig placenta does not transport maternal immunoglobulin and therefore newborn piglets acquire maternal immunoglobulin from colostrums during the first 24 h to 48 h of life. At birth the mucosal immune system and more especially the T-cell component of the

intestinal mucosa of the newborn piglet is poorly developed. During the first few weeks of life, it undergoes a rapid period of expansion and specialization. It is clear then that mucosal T-cells must not only be in surveillance and the expression of active responses to potential pathogens, but also in the regulation and maintenance of mucosal tolerance. Weaning is a period during which there is a great change in the magnitude and diversity of exposure to environmental antigens derived from food and potentially pathogenic organisms. In recent years, scientific knowledge in the field of microbiology has expanded and it has been suggested that the gastrointestinal microflora has a role in maintaining animal health. The immunomodulatory effects of probiotics are related to important parts of their beneficial effects. Initially, ingested probiotic bacteria interact with gut epithelial cells. In studies using cell lines, such as Caco-2 or HT-29, probiotic *Lactobacillus* stimulated the production of pro and anti-inflammatory cytokines by these cell lines in a strain dependent manner (Delcenserie *et al.*, 2008). Because intestinal epithelial cells regulate the intestinal immune response (Lu and Walker, 2001 and Hase and Ohno, 2006), probiotic *Lactobacillus* may modulate the intestinal immune response through the stimulation of certain cytokine secretion by epithelial cells (Lu and Walker 2001 and Delcenserie *et al.*, 2008). Shen *et al.* (2009) observed that IFN-γ, which can activate phagocytosis by macrophages was increased in gut mucosa by YC supplementation. It was also shown that CD4+ lymphocyte numbers increased after weaning in the case of control group, whereas the number of CD4+ lymphocytes in YC group did not increase by 14 days post weaning. Scharek *et al.* (2007) observed that supplementation of probiotics (*Bacillus cereus var. toyoi*) was shown to affect the intestinal immune system of the piglets at the time of weaning and shortly thereafter such that the intestinal epithelium

CD8/CD3 double positive cell populations were enhanced in the probiotics group. Dietary supplementation with phosphorylated mannans derived from yeast *(S. cerevisiae)* cell wall improves growth response and modulates immune function of weaning pigs (Davis *et al.*, 2004).

Probiotics are able to prevent intestinal diseases through both humoral and cell mediated immune modulation (Erickson and Hubbard, 2000). Perdigon *et al.* (1999) reported that probiotics may lead to an increased IgA production and stimulation of macrophage. Moreover, several studies have reported that probiotics are able to regulate both anti- and pro-inflammatory cytokine production (Matsuzaki and Chin, 2000 and Chiang *et al.*, 2000). However, the results are quite conflicting and the mechanisms of action of probiotics on cytokine expression are not understood it. In pigs, weaning is associated with a transient inflammatory response in the gut, as indicated by the up regulation of several inflammatory cytokines, such as IL-1β, tumor necrosis factor TNF-α and IL-6 (Pollmann *et al.*, 1980b), which might be implicated in the development of post-weaning diarrhoea. Some studies reported that treatment of piglets with *B. lactis* increased blood leukocyte phagocytic and T-lymphocyte proliferative responses (Shu *et al.*, 2001).

2.7 EFFECT ON MICROFLORA

Weaning of pigs is associated with the change of diet from sow's milk to a solid weaner diet and other postweaning stressors. Microflorae in the digestive system of pigs play a very important role in the defense mechanisms of the body. One of the important abilities of stable microflora in gastrointestinal tract is colonization résistance. The major intestinal florae of pig are *Lactobacilli, Saccharomyces, Bifidobacteria, Streptococci, Bacteriodes, Clostridium perfringes* and *E.*

Table 2.2. Influence of probiotic strains on the microflora in pigs

Animal	Probiotic	Effect	Reference
Nursery Pigs	*Saccharomyces cerevisiae*	*E. coli* (↓).	Shen *et al.* (2009)
Weanling pigs	*Saccharomyces cerevisiae*	Total faecal bacteria (↓)	Van Heugten *et al.* (2003)
Weanling pigs	*Saccharomyces cerevisiae*	Yeast colony-forming units in the feces (↑), Total number of fecal coliforms (↓)	Maloney *et al*. (1998)
Weanling pigs	*Saccharomyces cerevisiae*	No influence on intestinal microflora	Mathew *et al*. (1998)
Suckling piglet	*B. thermophilum* and *B. pseudolongum*	Reinforced the normal intestinal flora and alleviated clinical symptoms of scouring	Kimura *et al.* (1983)
Piglet	*Strectococci, Ent. Faecum cernelle 68*	Faecal *E. coli* and haemolytic *E. coli* (↓).	Deprez *et al.* (1986)
Weanling	*Ent. faecalis*	Faecal *E. coli* (↓).	Danek, (1986)
Piglet	*L. acidophilus*	Lactobacillus and *E. coli* in stomach (↑), but no influence in other digestive tract	Pollman *et al.* (1980b)
Weanling	*Lactobacillus*	Scouring (↓)	Hale and Newton (1979)
Weanling	*Bacillus cereus, Lactobacillus spp. Streptococccus*	No influence on mortality, clinical symptoms and fecal hemolytic *E. coli*	Cupere *et al.* (1992)
Suckling piglet	*Lactobacillus*	Faecal lactobacillus (↑)	Jonsson (1986)
Weanling	*Bifidobacterium globosum A*	No consistent effect on scour scores, faecal or gastrointestinal pH and cell-mediated immune response	Apgar *et al.* (1993)
Weanling	*Bacillus subtilis*	Streptococci and bifidobacteria (↑), Bacteroides (↓)	Ozawa *et al.* (1983)
Suckling piglet	*Lactobacillus*	Coliforms (↓), lactobacillus (No effect)	Newman (1990)
Suckling Weanling	*Bifidus bifidum ID*	Less incidence of diseases	Ervolder *et al.* (1985)

* (↓) and (↑) are either significantly increased or decreased.

coli, they change with age. It has been suggested that it may take 4 to 6 weeks to establish a stable flora (Mul and Perry, 1994). When piglets are weaned, the intestinal microflora of piglets are altered (Jensen, 1998). It has been well recognized that *E. coli* populations, especially haemolytic *E. coli,* are markedly increased in the anterior small intestine after weaning, and enteropathogenic *E. coli* is the major infection agent for post-weaning diarrhoea. Withdrawal of sow's milk that contains natural immunoglobulin stops preventing the proliferation of haemolytic *E. coli* (Deprez *et al.,* 1986). The dietary and environmental change after weaning may also be associated with change in intestinal microflora (Jensen, 1998 and Conway, 1994).

Yeast supplements may have the ability to stimulate digestion and aid in maintaining microbial equilibrium in the gut of young pigs. In addition, enzymes, vitamins, and other nutrients or growth factors contained in yeast have been proposed to produce beneficial production responses in pigs (Kornegay *et al.,* 1995). Anderson *et al.* (1999) observed that microbial composition in the gastrointestinal tract can be affected by dietary differences. Changes in the microflora, due either to yeast cell wall components (mannans) or to a direct effect of live yeast, could reduce pathogenic bacteria and toxic metabolites and subsequently improve animal health and growth performance (Anderson *et al.,* 1999). Several studies have demonstrated that YC or yeast cell wall components can affect the composition of intestinal microflora (Firon *et al.,* 1987; Naughton *et al.,* 2001 and White *et al.,* 2002). Dietary supplementation of YC and Antibiotic growth promotive (AGP) did not affect microbial populations tested however, only the number of *E. coli* in the cecum of YC and AGP group was decreased compared with the control group (Shen *et al.,* 2009). Mathew *et al.* (1998) reported that YC did not change

microflora in the stomach, duodenum, ileum, caecum, or colon. Van der Peet- Schwering *et al.* (2007) also reported that YC did not have a clear effect on microbial composition in the ileum.

2.8 SMALL INTESTINE MORPHOLOGY

At weaning there are a number of well documented changes in the histology and morphology of the small intestine. The gastrointestinal tract is the main digestive and absorptive organ in animal. The small intestine has a huge absorptive surface. The gastrointestinal tract permits the uptake of dietary substances into systemic circulation and it also excludes pathogenic compounds simultaneously (Gaskins, 1997). The presence of villi, microvilli and Kerckring's folds in the small intestine results in a much larger surface area than that of a cylindrical tube (Caspary, 1992). There is a reduction in villous height (villous atrophy) and an increase in crypt depth (crypt hyperplasia) at weaning (Kelly *et al.*, 1991 and Pluske *et al.*, 1996). Pluske *et al.* (1997) observed that villous atrophy was associated with either an increased rate of cell loss from the villous apex or a reduced rate of cell renewal. These changes are clear at 5 days postweaning and continued in the first to second week after weaning (Kelly *et al.*, 1991). The villous height was reduced to 50-75% of pre-weaning values (Kelly *et al.*, 1991). Other report demonstrated that higher feed intake immediately after weaning reduced the histological changes of small intestinal morphology (Pluske *et al.*, 1996). Thus, it is important to increase feed intake immediately after weaning.

Shen *et al.* (2009) found that jejunal villus height and villus height to crypt depth ratio of pigs fed YC were greater than controls. Bontepmo *et al.* (2006) observed that villus height and crypt depth were greater and villus : crypt ratio was smaller in treated piglets than

controls. Villus height and crypt depth are indirect indications of the maturity and functional capacity of enterocytes, and more long the villi and crypts are, a greater number of enterocytes are present (Hampson, 1986). Baum *et al.* (2002) also found that villus length was greater in the small intestine of piglets fed yeast than controls.

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Materials and Methods

The present study was carried out to investigate the effect of live *Saccharomyces cerevisiae* feeding on growth performance, nutrient utilization and immune response in crossbred (Landrace X Desi) early weaned piglets. The experimental materials employed and methods adopted during the course of study are described briefly as under.

3.1 EXPERIMENTAL ANIMALS AND GROUPING

A total of 48 (24 Male (M) and 24 Female (F)) crossbred (Landrace X Desi) piglets weaned at 28 and 42 days were selected for the present study. The piglets were assigned to four different treatments each containing 4 replicates with equal male female ratio of three animals in each as detailed below:

3.1.1 T1- 12 (6 M and 6 F) piglets weaned at 28 days of age without live *S. cerevisiae*

3.1.2 T2-12 (6 M and 6 F) piglets weaned at 28 days of age with live *S. cerevisiae*

3.1.3 T3- 12 (6 M and 6 F) piglets weaned at 42 days of age without live *S. cerevisiae*

3.1.4 T4-12 (6 M and 6 F) piglets weaned at 42 days of age with live *S. cerevisiae*

3.2 HOUSING AND MANAGEMENT

The piglets were kept on cement floored pens provided with separate feeders and waterers and a run behind. The piglets were reared under standard hygienic and uniform managemental conditions throughout the experimental period. Clean potable water was made available throughout the day.

3.3 YEAST CULTURE

The yeast (*Saccharomyces cerevisiae)* procured from NDRI, Karnal, was maintained by repeated sub culturing on agar slants. The composition of yeast extract peptone glucose (YEPG) medium is shown in Table 3.1.

Table 3.1. Composition of medium (YEPG Agar)

Ingredients	Composition/1000ml
Yeast extract	3 g
Peptone	5 g
Glucose	10 g
Agar	15 g
Distilled water	1 lit.

3.3.1 Preparation of yeast culture for feeding

From the agar slant of yeast a loopful of yeast culture was transferred aseptically to 100 ml of sterilized broth (Table 2). The broth was incubated for 24 hours at 39°C. Crushed maize (1000g) mixed with 1000 ml of tap water was inoculated with the 100 ml of 24 hours old yeast culture (10% of the total feed) and incubated for 24 hours at 39°C. The fermented material was fed to the animals and the same fermented material was used as inoculum (20% of concentrate mixture) for preparation of next day's fermented feed. After 15 days, fresh culture was taken as described above and used consecutively for next 15 days.

Table 3.2. Composition of medium for growing *Saccharomyces cerevisiae*

Ingredients	Composition/1000ml
Yeast extract	3 g
Peptone	5 g
Glucose	10 g
Distilled water	Up to 1000 ml

3.3.2 Colony forming units (cfu) of yeast

Medium for yeast counting (Table 3.2) and physiological normal saline (0.85% sodium chloride in distilled water) was prepared. Ninety ml saline in 250 ml Erlenmeyer flasks and 9 ml in each test tube was taken. Medium, saline tubes and flasks were autoclaved at 121ºC for 20 minutes. Saline tubes and Petri dishes were labelled and two petri dishes for each dilution were numbered. Ten g of fermented feed was mixed with 90 ml of autoclaved physiological saline. The content was mixed slowly for 4-5 minutes and the supernatant was used for the counting. The sample prepared (10 g of sample in 90 ml of saline) was having ten times dilution and thus it was marked as 10^1. 1 ml of 10^1 diluted sample was added to a next normal saline tube (9 ml of saline) to get a 10^2 dilution and contents was mixed on vortex test tube mixer. Serially the dilutions were done to get the required dilutions. From the last four dilutions, 1 ml inoculum in two petri dishes for each dilution was taken. The medium was heated on boiling water bath to melt it and then was cooled to 45ºC and there after antibiotic (chloramphenicol 12 µg/ml) was added after autoclaving as they are heat labile and get inactivated during autoclaving. Fifteen ml medium was poured into each of the dishes and incubated at 37ºC in an inverted position in incubator. After 24-48 h of incubation petri dishes were observed for yeast colonies. Number of colonies was counted and the average of duplicate plates was taken. The average number of colonies was multiplied by the dilution factor to get the number of colony forming units (cfu) per g of sample.

3.4 FEEDS AND FEEDING

Basal diet was formulated (Table 3.3) with maize, soya bean meal, fish meal, wheat bran, salt, mineral and vitamin supplements as per NRC (1998). Crushed maize was fermented with *S. cerevisiae* (2-3x10^6 cfu/g feed) and fed at the rate of 200g (as fed basis) per piglet. Piglets were fed as follows:

3.4.1 T1- Basal diet without live *Saccharomyces cerevisiae*

3.4.2 T2-Basal diet with live *Saccharomyces cerevisiae*

3.4.3 T3- Basal diet without live *Saccharomyces cerevisiae*

3.4.4 T4- Basal diet with live *Saccharomyces cerevisiae*

Table: 3.3. Ingredient composition of ration for piglets

Ingredients (Parts/100 Kg)	Body weights (kg) 5-10	10-20	20-50	50-80
Crushed maize	46	54	62	71
Deoiled soya bean meal	30	22	15	10
Wheat bran	16	16	15	13
Fish meal	06	06	06	04
Mineral mixture	1.5	1.5	1.5	1.5
Common salt	0.5	0.5	0.5	0.5
Calculated CP (%)	23.7	20.9	18.3	15.6
Calculated DE (kcal/kg)	3400.1	3399.45	3399.37	3390

3.5 GROWTH AND PERFORMANCE

Weighed quantities of feed were offered once daily so as to provide *ad libitum* feeding. The left over residues were weighted after 24 hours of offering and record was maintained daily. Body weight of animals in each group was recorded at fortnightly intervals in the morning before feeding.

3.6 METABOLISM TRIAL

In order to assess the digestibility of nutrients, nitrogen balance and plane of nutrition a metabolism trial of 6 days duration was

Fig. 3.1 : Piglets in metallic metabolic cages during metabolism trial

Fig. 3.2 : Feeding of piglets during metabolism trial

conducted on experimental piglets after 90 days from the initiation of the experiment. The piglets were adapted for a period of 4 days duration in the metallic metabolic cages in order to attain uniform feed intake and faeces and urine output. The environmental temperature during the metabolism trials was 25 to 37°C with relative humidity of 65 to 80%. The animals were placed in specially designed metabolic cages with facility for separate collection of faeces and urine. To ensure clean separation of faeces and urine the screens were cleaned daily and also the angle and position of the collector was suitably adjusted. During the metabolism trial the feeding schedule of the animals remained same as during feeding period and they were fed their respective diet at 9.00 AM daily. Body weight of the animals was recorded before and after the metabolism trial.

3.6.1 Collection, sampling and preservation of feed, faeces and urine samples

A representative sample of the ration (100g) offered and residues left was collected daily to estimate the dry matter intake and a pooled sample was taken for estimation of proximate principles.

At the time of metabolism trial, faeces from the individual animal was collected manually as soon as the animal defecated. The total quantity of faeces voided during the preceding 24 hours period was pooled and weighed daily at 9:00 am. The faeces collected was thoroughly mixed in a clean plastic tub and representative sample of about 250g was taken for dry matter and nitrogen estimation for each animal separately.

Faecal samples ($1/50^{th}$) for dry matter were kept in hot air oven overnight at 100 ± 1°C. Dried samples were pooled for 6 days for each animal. For the estimation of faecal nitrogen excretion, an aliquot ($1/100^{th}$) of faeces was preserved with dilute (1:4) sulphuric acid and stored in a preweighed stopper plastic jar daily.

Urine excreted in 24 hours was collected in a tub kept under the metabolic cage and the volume was measured daily at about 9:00 A.M. For the estimation of urinary nitrogen excretion a suitable aliquot ($1/500^{th}$) in duplicate of daily urine voided was taken in the Kjeldahl flask containing 40 ml of commercial sulphuric acid. Another fraction of the urine sample was preserved in plastic bottles and kept in deep freeze for calcium and phosphorus estimation.

3.7 PROXIMATE COMPOSITION

The methods as described in AOAC (1995) were followed for estimation of proximate composition. Details of estimation of individual components are as follows.

3.7.1 Dry matter (DM)

Samples of feed and faeces were taken in pre-weighed moisture cups and dried in a hot air oven at 100±1°C for 12 h until constant dry weight is achieved. Dried samples were cooled in desiccator and weighed. The loss in weight reflected the moisture content of the sample. DM (%) was calculated using the following formula:

$$\text{DM (\%)} = \frac{\text{Wt. of dried sample}}{\text{Wt. of fresh sample}} \times 100$$

3.7.2 CRUDE PROTEIN (CP)

Nitrogen content of sample was determined by the standard Kjeldahl method. One gram of feed/faeces (10g) sample was digested on heater after adding 50 ml commercial grade sulphuric acid and 2.5 g digestion mixture (copper sulfate: sodium sulfate in 1: 9 ratio) until became clear. The flask was allowed to cool and about 50 ml of water poured slowly along the neck of the flask. The content was shaken and transferred completely into 250 ml volumetric flask by

giving repeated washings with distilled water and the final volume was made up to 250 ml. For urine samples, 30-40 ml of urine sample was digested with 30 ml of concentrated sulphuric acid and 2.5-3 g of digestion mixture and volume was made to 250 ml. Ten ml aliquot of digested feed, faeces or urine samples were distilled in a Micro Kjeldahl assembly by adding 10 ml of 40% sodium hydroxide solution. Gaseous ammonia thus released was trapped in 10 ml boric acid containing Toshiro's indicator (boric acid 20 g, 1 % solution of methyl red, 12ml; 1 % solution of bromocresol green, 6ml; dehydrated alcohol, 200ml and distilled water 782 ml). The nitrogen trapped in boric acid was estimated by titrating it against N/7 sulphuric acid. The normality of acid was checked by titrating against sodium carbonate using methyl orange as indicator. The value of blank was subtracted from sample's readings. The crude protein content of samples was determined by the formula:

$$\textbf{Crude protein (\%)} = 100 \times Y \times (B-B_1) \times 0.002 \times 6.25 / X \times W$$

Where,

Y = volume (ml) made out of digested sample
X = volume (ml) of aliquot taken for distillation
B = volume (ml) of N/7 H_2SO_4 consumed for titration of samples.
B_1 = volume (ml) of N/7 H_2SO_4 consumed for titration of blank distillate
W = weight (g) of oven dried sample taken for digestion
0.002=1 ml of N/7 H_2SO_4 is equal to 0.002 g N
6.25 = Factor for converting nitrogen into crude protein of sample

3.7.3 Ether extract (EE)

A weighed quantity (about 2-3 g) of ground sample was taken in a thimble, plugged with cotton and extracted for 8-10 h with petroleum ether (boiling point: 60-80°C) in Soxhlet apparatus. The extracted oil in the oil flask was dried to constant weight at 100±1°C. The ether extract was estimated as the difference in the weight of oil flask after and before extraction and was expressed as percent on DM basis as follows:

Ether Extract (%) = 100 x Weight of the ether extract / Weight of sample on DM basis

3.7.4 Total ash (TA)

Approximately 3-4 g of feed sample (exactly weighed) was taken in a preweighed silica crucible and charred on heater to make smoke free. The crucible along with the sample was ignited at 550±5°C for 3h in Muffle furnace. The furnace was allowed to cool down and the crucibles containing ash was taken out and kept in desiccator to make it moisture free and then weighed. The difference in the weights of empty silica crucible and with ash gave the amount of total ash (TA). The TA on DM basis was calculated from the following formula:

Total Ash (%) = 100 x (Weight of ash / Weight of sample on DM basis)

3.7.5 Organic Matter (OM)

Percent Organic matter in the samples was arrived by deducting total ash (%) from hundred.

OM (%) = 100 – Total ash (%)

3.7.6 CALCIUM AND PHOSPHORUS ESTIMATION

Preparation of mineral extract

The total ash samples were transferred into a 250 ml beaker using concentrate hydrochloric acid and distilled water and digested on a hot plate for 30 minutes and then cooled. The digested samples were then filtered through Whatman filter paper No.1 in a 250 ml volumetric flask followed by several washings with hot distilled water (till it was free from acid) and volume was made upto the mark.

3.7.6.1 Calcium estimation

Reagents required:

a) Dilute hydrochloric acid (1:4)

b) Alcoholic methyl red indicator (0.1%)

c) Dilute ammonia solution

d) Dilute sulphuric acid solution

e) Standard N/10 potassium permanganate($KMNO_4$) solution

f) Saturated ammonium oxalate solution.

Procedure

Calcium in feed, faeces and urine samples was estimated from the mineral extract following procedure of Talapatra *et al.* (1940). A 25 ml aliquot of mineral extract was transferred into a 250 ml beaker and to it 10 ml of 1:2 hydrochloric acid and 10 ml of saturated ammonium oxalate solution were added with constant stirring. Then two drops of methyl red indicator were added and then1:4 liquor ammonia was added to it till the colour changed to yellow with constant stirring and pH was adjusted by addition of hydrochloric acid (1:2) so as to get faint pink colour. Beaker contents were heated on a hot plate for few minutes and kept overnight for proper precipitation of calcium as calcium oxalate. Next day, beaker contents were filtered through Whatman filter paper no. 40 with minimum five washings using hot distilled water. After that, filter paper containing whitish calcium oxalate precipitate was transferred back to same beaker and dissolved by adding dilute sulphuric acid, followed by heating and titration against standard N/10 $KMNO_4$ solution. Calcium (%) in the sample was calculated as follows:

$$\text{Ca (\%)} = \frac{\text{N/10 KMNO}_4\text{ used (ml) x 0.002 x dilution factor}}{\text{Wt. of sample (DM basis)}} \text{ x 100}$$

3.7.6.2 Estimation of phosphorus

The phosphorus content of feed, faeces and urine was estimated from the mineral extract as per AOAC (2000) using UV visible spectrophotometer (UV-1101, Precision Biotek Instruments Pvt Ltd, New Delhi) as described below:

Reagents required

a) Molybdo-vandate reagent

40g ammonium molybdate tetrahydrate was dissolved in 400 ml hot distilled water and cooled. In another flask, 2g of ammonium metavandate was dissolved in 250 ml hot distilled water and cooled and to it 250 ml of 70% perchloric acid ($HClO_4$) was added. Molybdate solution was gradually added to vandate solution and volume was made upto 2 liters mark with distilled water.

b) Phosphorus standard solution

1) Stock solution (2 mg P/ml) - 0.8788g potassium dihydrogen phosphate (KH_2PO_4) was dissolved in distilled water and volume was made up to 100 ml.

2) Working solution (0.1mg P/ml) – 5 ml stock solution was diluted to 100 ml.

Preparation of standard curve

Different volumes of working standard solution were taken in the test tubes so as to have 0, 0.02, 0.04, 0.06, 0.08 and 0.1mg P and volume was made to 6 ml by adding distilled water. Then, 4 ml of molybdo-vandate reagent was added to all the test tubes and mixed. After 10 minutes optical density (OD) was taken at 400 nm in a spectrophotometer and regression equation was developed.

Estimation of phosphorus

Suitable aliquot of mineral extract (about 0.2 ml) were taken in test tubes and to this distilled water was added to make the total volume to 6 ml. Then 4 ml of molybdo-vandate reagent was added in each tube, mixed and OD was taken at 400 nm after 10 minutes. The amount of phosphorus present in the samples was determined by using regression equation.

3.7.7 Van Soest method of analysis

Samples of feeds and faeces were analyzed for neutral detergent fibre and acid detergent fibre as per the method given by Van Soest *et al.* (1991).

3.7.7.1 Neutral detergent fibre (NDF)

Reagents required

a) Neutral detergent solution (NDS):
Chemicals required for NDS:

Sodium lauryl sulphate	30.0g
Disodium ethylene diamine tetracetate dehydrate (EDTA)	18.61g
Sodium borate decahydrate	6.81g
2-Ethoxy ethanol (ethylene glycol)	10.0 ml
Disodium hydrogen phosphate (anhydrous)	4.56g

b) Decahydro-naphthalene (decaline) – (AR grade)

c) Acetone – (AR grade)

Neutral detergent solution (NDS) preparation

EDTA and sodium borate decahydrate were taken in a large beaker having small quantity of distilled water and dissolved by heating and shaking. After that, sodium lauryl sulphate and 2-ethoxy-ethanol were added to it. In another beaker, disodium hydrogen phosphate (Na_2HPO_4) was dissolved in small quantity of distilled water by heating. Both the solutions were mixed together and volume was made upto 1 litre and checked for pH (6.9 - 7.1).

Procedure

A weighed quantity (About 0.5 g) of ground feed/faeces sample was refluxed in a spoutless beaker. To it 100 ml of neutral detergent solution and 2 ml decaline were added and refluxed for one hour after the initiation of boiling. Contents were filtered through pre-

weighed sintered glass crucible (Grade-1) and washed repeatedly with hot water followed by 2 washings with acetone. The sintered crucible containing residue was then kept in hot air oven (100±1°C) for overnight drying and weighed after cooling it in a desiccator. The difference in the weight of crucible plus residue and that of empty crucible was recorded as NDF and expressed on DM basis.

$$\text{NDF (\%)} = \frac{\text{Weight of crucible +NDF-Weight of crucible}}{\text{Weight of sample (DM basis)}} \times 100$$

3.5.6.2 Acid detergent fibre (ADF)

Reagents required

a) Cetyl trimethyl ammonium bromide (CTAB)

b) Acetone – (AR grade)

c) Sulphuric acid (AR grade) – 72% (w/v)
Acid detergent solution (ADS) preparation:
ADS was prepared by dissolving 20g CTAB in 1N H_2SO_4.

Procedure

A weighed quantity of dried ground feed/faeces sample was taken in a spoutless beaker and to it 100 ml of acid detergent solution was added. Beaker contents were refluxed for 1 h after onset of boiling. The contents of the beaker were then filtered through a pre-weighed sintered glass crucible and washed several times with hot water followed by two washings with acetone. The crucible was dried in a hot air oven at 100±1^{0}C overnight and weighed after cooling it in a desiccator to know the amount of ADF (%) on DM basis.

$$\text{ADF (\%)} = \frac{\text{Weight of ADF}}{\text{Weight of sample (DM basis)}} \times 100$$

3.7.8 Digestibility (apparent)

The apparent digestibility of DM, OM, CP, EE, NDF and ADF was calculated from the difference of the quantity of these substances

consumed and excreted by the individual animal. It was calculated by the following formula:

Digestibility (%) = (Intake- Outgo in faeces) x 100 / Intake

3.8 FAECAL CHARACTERISTICS

Faecal characteristics were determined to know the effect of *Saccharomyces cerevisiae* feeding on faecal count.

3.8 1 Physical examination

Faecal consistency was divided into three categories: firm, semi-soft and soft.

3.8 2 Yeast count

Ten g of faecal sample mixed with 90 ml of autoclaved physiological saline and the content was mixed slowly for 4-5 minutes and the supernatant was used for the counting. The yeast counting was done as in section 3.3.2.

3.8 3 Coli form count

Coliform bacteria count in the fecal sample was done by most probable number technique.

Medium for coliform counting (Table 3.4 and 3.5) and physiological normal saline (0.85% sodium chloride in distilled water) were prepared. Seven ml of BCP MacConkey was dispensed in the test tubes and 9 ml saline in the test tube. Medium and saline tubes were autoclaved at 121ºC for 20 minutes. Serial dilutions of the fecal sample were made to get the required dilutions. One ml each of diluted samples was inoculated in triplicate tubes of MacConkey broth. All tubes were incubated at 37^0 C for 24 h and observed for acid and gas production (acid production is evident by the change in colour to yellow). The tubes were marked positive showing change in

colour. The results of the tubes were tabulated. Three consecutive dilutions were selected, containing both negative and positive tubes. The numbers of coliform bacteria were calculated from the standard table of Most Probable Number.

Table 3.4 Composition of bromo-cresol purple (BCP) MacConkey broth:

Ingredients	Composition/1000ml
Peptone	20 g
Lactose	10 g
Bile salts	5 g
Sodium chloride	5 g
BCP indicator	2 ml

Final volume was prepared 1000 ml and pH was adjusted to 7±0.1.

Table 3.5. Composition of BCP indicator

Ingredients	Composition/1000ml
Bromo-cresol purple	1.6 g
95% ethanol	50 ml
Distilled water	50 ml

BCP was dissolved in ethanol, water was added and filterd.

3.9 IMMUNOLOGICAL STUDIES

Immunological studies were performed during last month of the experiment alongwith different blood parameters.

3.9.1 Humoral immune response

Humoral response was studied by microhemagglutination assay as described by Wagmann and Smithies (1966) against injecting 1ml of a suspension of 20% sheep red blood cells (SRBC) in phosphate buffer saline solution (PBS) i/m in the ham region of pig. Blood samples were taken by venipuncture immediately before injection on day 0

and after injection on day 7, 14, 21 and 28. Pigs were again challenged with SRBC on day 28 to investigate the secondary immune response on day 35. Blood was centrifuged, serum was collected and stored at -20°C until it was analysed.

3.9.1.1 Preparation of suspension of 20% SRBC

About 20-40 IU of heparin (anticoagulant) was taken in sterile syringe and blood was collected by venipuncture from jugular vein of healthy sheep. Blood was immediately centrifuged at 2000-2500 rpm for about 15-20 min after transferring into centrifuge tube. The supernatant was carefully removed without disturbing the sediment of SRBC. The washing of SRBC was done by suspending them to the original volume with sterile PBS solution, mixed gently, centrifuged at 2000-2500 rpm for about 15-20 min and supernatant was discarded. This process was repeated about 2-3 times to finally get the volume of packed SRBC which was further diluted with requisite sterile PBS solution to make 20% suspension of SRBC.

Antibody titer against SRBC was measured by microtiter hemagglutination assay (Wagmann and Smithies, 1966). Two-fold serial dilution of heat inactivated serum (30 min at 56°C) was made in PBS and a 2% SRBC suspension was added. This two-fold serial dilution was made by keeping 50µl PBS to all well and then adding 50µl heat inactivated serum to first well, mixed gently and transferred 50µl to next well. This process is repeated till the last well for finally getting the two-fold serial dilution of serum. Lastly 50µl of 2% SRBC suspension was added and mixed. Plates were incubated for 30 min at 37°C. Titres were expressed as $\log_2$ of the reciprocal of the highest dilution showing agglutination of SRBC.

3.9.2 Cell mediated immune (CMI) response

CMI response was studied by PHA skin test (Blecha *et al.* 1983) as modified by Kornegay *et al.* (1989) to investigate *in vivo* cellular

immunity. Piglets were injected subcutaneously with 0.1 ml of PHA (1.5 mg/ml) on the left inside flank, approximately 5 cm posterior to the last nipple and 5 cm from the midline. A PBS solution was injected similarly on the right side of the pig and served as a control. Skin thickness was measured using callipers on 0, 6, 12, 24, 36 h after injection. Finally data were expressed as skin thickness of PHA-injected site minus skin thickness of PBS-injected site in centimeters.

3.10 BLOOD PARAMETERS

Blood collected at 0 day and 120 day of experimental feeding was centrifuged, serum was collected and stored at -20°C until it was analysed. Finally after thawing, serum was analysed for various biochemical and enzymatic profiles, as per the methods described below.

3.10.1 Total protein (TP)

Total protein in serum was estimated by the Biuret modified end point method (Gornell *et al.*, 1949). Peptide bonds of protein react with cupric ions in alkaline solutions to form a colored chelate, the absorbance of which is measured at 578 nm. The Biuret's reagent contains sodium-potassium tartarate to complex cupric ions and maintains their solubility at alkaline pH. The absorbance data are proportional to protein concentration and expressed as g/dl.

3.10.2 Albumin and globulin

Albumin in serum was estimated by bromocresol green (BCG) dye binding method of Gustaffson (1978). At pH 3.68, albumin acts as cation and binds to the anionic dye BCG, forming a green complex, the absorbance of which is measured at 630 nm. Globulin was determined as the difference between total protein and albumin concentration in the serum.

3.10.3 Urea

Serum urea was estimated by improved diacetyl monoxime (DAM) method of Rahmatullah and Boyde (1980). Urea reacts with DAM in an acidic medium to produce a pink coloured complex. Use of thiosemicarbazide and a cadmium salt intensifies the colour so obtained. The absorbance of the colour complex at 520 nm was proportional to the urea concentration in the sample. The values were multiplied with 0.467 to get the blood urea nitrogen (BUN) content (mg/dl).

3.10.4 Aspartate amino transferase (AST/SGOT)

The AST activity in serum was determined as per Reitman and Frankel (1957). The AST catalyzes transfer of amino group from L-aspartate to á-ketoglutarate with formation of oxaloacetate and glutamate. The oxaloacetate so formed is allowed to react with 2,4 dinitrophenyl hydrazine to form 2,4 dinitrophenyl hydrazone derivative which is brown coloured in alkaline medium. The absorbance of this hydrazone derivative is measured on spectrophotometer at 505 nm and correlated to AST activity by plotting a calibration curve using oxaloacetate standard.

3.10.5 Alanine amino transferase (ALT/SGPT)

The ALT activity in serum was determined as per Reitman and Frankel (1957). ALT catalyzes transfer of amino group from L-alanine to á-ketoglutarate with formation of pyruvate and glutamate. The pyruvate so formed is allowed to react with 2, 4 dinitrophenyl hydrazine to form 2, 4 dinitrophenyl hydrazone derivative which is brown coloured in alkaline medium. The absorbance of this hydrazone derivative is measured on spectrophotometer at 505 nm and correlated to ALT activity by plotting a calibration curve using pyruvate standard.

3.10.6 Serum glucose

Serum glucose was estimated by the glucose oxidase (GOD) and peroxidase method (POD) as described by Henry (1963). Glucose was oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalyzed reaction, the oxygen liberated was accepted by the chromogen system to give a red coloured quinoneamine compound. The red colour so developed was measured at 505 nm and was directly proportional to glucose concentration (mg/dl blood serum).

3.10.7 Total Cholesterol

Cholesterol in serum samples was determined by the method of Wybenga *et al.* (1970). Cholesterol present in serum reacted with hot solution of ferric perchlorate, ethyl acetate and sulphuric acid and gave a lavender coloured complex, absorbance of which was measured at 560 nm.

3.10.8 Triglycerides

The triglycerides content of the serum samples was estimated adopting the enzymatic (glycerol-3-phosphate oxidase; GPO) method as per McGowan (1983). Triglycerides were hydrolysed by lipoprotein lipase (LPL) to produce glycerol and free fatty acid. In presence of glycerol kinase, adenosine triphosphate phosphorylates glycerol to produce dihydroxy acetone phosphate and hydrogenperoxide. In the presence of peroxidase, hydrogen peroxide coupled with 4-aminoantipyrine and 4-chlorophenol to produce red quinoneimine dye. Absorbance of colored dye was measured at 505 nm and is proportional to trigycerides concentration in the sample. Glycerol free fatty acid was calculated by subtracting 10 from triglyceride level. Thus, glycerol free fatty acid was estimated as non esterified fatty acid (NEFA).

3.11 SMALL INTESTINE MORPHOLOGY

At day 120 post-weaning, two female and two male piglets per group (total number of animals per group = 12) were slaughtered and the systemic necropsy was conducted. The entire intestinal tracts were removed and jejunum was collected from each animal and promptly fixed in 10% neutral buffered formalin. The specimens were then dehydrated in graded alcohols, cleared with xylene and embedded in paraffin & serial microtome sections (6 µm thick) were stained with hematoxylin and eosin and examined to assess micro anatomical structure and number of villi in 100µm distance, villus height, crypt depth, width of villi (in middle), intervillous distance, number of goblet cells and infiltration in lamina propria were determined at 10× magnification using light microscope and villus height and crypt depth ratio was calculated.

3.12 STATISTICAL ANALYSIS

The experimental data generated were analyzed using statistical package SPSS 16.0 / SAS 12.0 adopting standard statistical procedures (Snedecor and Cochran, 1994).

❑❑❑

Results

The results observed in present study to assess the effect of live *Saccharomyces cerevisiae* feeding on growth performance, nutrient utilization and immune response in crossbred (Landrace X Desi) early weaned piglets have been presented in this chapter.

4.1 CHEMICAL COMPOSITION OF FEEDS

The chemical composition of the feed ingredients used in ration formulation for experimental feeding is presented in the Table 4.1. Crushed maize, deoiled soyabean meal (DSBM), wheat bran (WB) and fish meal (FM) were the major feed ingredients in ration for different treatments whose chemical composition were almost similar to the standard values. The values of different proximate principles and the fibre fractions in diet offered to the piglets are presented in Table 4.2.

Table 4.1 Chemical composition (% on DM basis) of feed ingredients used in dietary formulation

Attributes	Maize	SBM	WB	FM
DM	90.49	90.45	88.89	90.45
OM	98.24	93.25	95.14	57.98
CP	9.80	45.10	14.90	55.30
EE	2.45	2.01	2.44	6.31
CF	1.70	7.48	9.44	7.41
NFE	82.51	32.74	66.10	0.56
Ash	1.76	6.75	4.86	42.02

Table 4.2 Chemical composition (% on DM basis) of diet offered to piglets during metabolic trial

Attributes	Concentrate mixture
Organic matter	93.03
Crude protein	18.22
Ether extract	2.52
Neutral detergent fibre	22.50
Acid detergent fibre	7.32
Hemicellulose	15.18
Calcium	0.67
Phosphorous	0.82

4.2 INTAKE AND DIGESTIBILITY OF NUTRIENTS

Experimental piglets remained in good health throughout the experiment without any clinical symptoms. The intake, digested and digestibility coefficients of dry mater, organic matter, crude protein, ether extract, neutral detergent fibre, acid detergent fibre, hemicellulose and total carbohydrates are summarized in Table 4.3.

The total DM intake (g/d) was 1431.7±86.16, 1574.9±36.66, 2264.9±156.49, 2272.6±85.96 in groups T1 (weaned at 28 days of age without live *S. cerevisiae*), T2 (weaned at 28 days of age with live *S. cerevisiae*), T3 (weaned at 42 days of age without live *S. cerevisiae*) and T4 (weaned at 42 days of age with live *S. cerevisiae*), respectively. The dry matter intake and digested was significantly higher in 42 days weaned piglets (T3 and T4) as compared to 28 days weaned piglets (T1 and T2) irrespective of treatments, However, there was no significant difference between 28 days weaned groups (T1 and T2) and between 42 days weaned groups (T3 and T4). The mean values of digested DM (g/d) differed significantly ($P<0.05$), with values 1211.3±79.22, 1353.8±31.69, 1890.4±124.93 and 1941.2±70.62 in T1, T2, T3 and T4, respectively. However, there was no significant

difference between T1 and T2 and between T3 and T4. The mean DM digestibility (%) was statistically similar, with values 84.5±0.79, 86.0± 0.63, 83.6±0.92 and 85.5±0.82 in T1, T2, T3 and T4, respectively.

The organic matter intake, digested and digestibility followed the similar trend as in case of DM. Intake of OM (g/d) was 1331.9±80.16, 1465.1±34.10, 2106.9±145.57 and 2114.1±79.97 in T1, T2, T3 and T4, respectively. The respective values of OM digested (g/ d) were 1154.9±74.23, 1287.7±29.97, 1806.7±121.02 and 1849.2±66.55 in T1, T2, T3 and T4. The mean OM digestibility (%) was statistically comparable with values of 86.6±0.67, 87.9± 0.55, 85.8±0.72 and 87.5±0.70 in T1, T2, T3 and T4, respectively.

The crude protein intake and digested also followed the similar trend. The respective values of intake of CP (g/d) were 257.7±15.50, 283.5±6.60, 407.7±28.16 and 409.1±15.17 in T1, T2, T3 and T4. The respective values of CP digested (g/d) were 202.3±14.48, 232.4±5.56, 340.1±24.88 and 351.8±15.08 in T1, T2, T3 and T4. The mean values of digestibility (%) of CP differed significantly ($P<0.05$) with values 78.3±1.22, 82.0±0.50, 83.3±0.65 and 85.9±0.48 in T1, T2, T3 and T4, respectively. The values in T1 was significantly ($P<0.05$) lower as compared to T2, T3 and T4. The value in group T2 was significantly ($P<0.05$) lower as compared to T4 and significantly ($P<0.05$) higher as compared to T1, but comparable with T3. However, T3 and T4 were comparable.

The ether extract intake followed the similar trend as in case of DM, OM, and CP intake. The respective values of intake of EE (g/d) were 36.1±2.17, 39.7±0.92, 57.1±3.94 and 57.3±2.16 in T1, T2, T3 and T4. The values of digested EE (g/d) were 24.9±2.16, 30.2±0.74, 36.3±2.67 and 44.5±1.99 in T1, T2, T3 and T4, respectively and showed significant difference ($P<0.05$) among groups. The value in T1 was

significantly (P<0.05) lower as compared to T3 and T4 but comparable with T2. The value in T2 was significantly (P<0.05) lower as compared to T4, but comparable with T1 and T3. The value in T3 was significantly (P<0.05) lower as compared to T4 and significantly (P<0.05) higher as compared to T1 but comparable with T2. However, values in T2 and T3 were comparable. The digestibility (%) of EE was significantly (P<0.05) lower in T3 (64.4±4.04) as compared to T2 (76.0±1.50) and T4 (77.6±1.90) but comparable with T1 (68.5±2.34). However, there was no significant difference in T1, T2 and T4.

The neutral detergent fibre intake also followed the similar trend. The NDF intake (g/d) was 322.1±19.39, 354.4±8.25, 509.6±35.21 and 511.3±19.34 in T1, T2, T3 and T4, respectively. There was significant difference (P<0.05) in NDF intake among four groups. However, the values were statistically similar between 28 days weaned groups (T1 and T2) and between 42 days weaned groups (T3 and T4). The values of digested NDF (g/d) were 188.5±16.70, 229.6±8.52, 289.7±21.21 and 327.4±14.27 in T1, T2, T3 and T4, respectively and showed significant (P<0.05) difference. The value in T1 was significantly (P<0.05) lower as compared to T3 and T4 but comparable with T2. The value in T2 was significantly (P<0.05) lower as compared to T4, but comparable with T1 and T3. The value in T3 was significantly (P<0.05) higher as compared to T1 but comparable with T2 and T4. The digestibility (%) of NDF was significantly (P<0.05) lower in T3 (56.9±2.21) as compared to T2 (64.8±1.94) and T4 (64.2±2.24) but comparable with T1 (58.2±2.34). However, there was no significant difference in T1, T2 and T4.

The intake of acid detergent fibre also followed the similar trend as in NDF. The mean ADF intake (g/d) was 322.1±19.39, 354.4±8.25, 509.6±35.21 and 511.3±19.34 in T1, T2, T3 and T4,

respectively. There was significant (P<0.05) difference in ADF intake among 4 groups. However, the values between T1 and T2 and T3 and T4 were statistically similar. The values of digested ADF (g/d) were 37.2±6.10, 50.4±1.48, 56.4±7.27 and 72.1±6.76 in T1, T2, T3 and T4, respectively and showed significant (P<0.05) difference. The value in T1 was significantly (P<0.05) lower as compared to T4 but comparable with T2 and T3. The value in T4 was significantly (P<0.05) higher as compared to T1 but comparable with T2 and T3. The respective mean values of ADF digestibility (%) 35.0±4.58, 43.9±1.96, 34.1±3.74 and 43.8±4.34 in T1, T2, T3 and T4 showed non-significant difference among 4 groups.

The hemicellulose intake, digested and digestibility also followed the similar trend as in case of DM or OM. The mean values of hemicellulose intake (g/d) were 217.3±13.08, 239.1±5.56, 343.3±23.76 and 345.0±13.05 in T1, T2, T3 and T4, respectively. The respective values of hemicellulose digested (g/d) were 151.4±11.88, 179.2±8.14, 233.3±15.48 and 255.3±12.22 in T1, T2, T3 and T4. The respective mean values of hemicellulose digestibility (%) were statistically similar, with values 69.3±1.64, 74.8±2.56, 68.0±1.51 and 74.0±1.83 in T1, T2, T3 and T4.

The total carbohydrates intake, digested and digestibility also followed the same trend as in other nutrients. Total carbohydrates intake (g/d) was 1038.4±62.49, 1142.3±26.58, 1642.7±113.50 and 1648.3±62.35 in T1, T2, T3 and T4, respectively. The respective values of total carbohydrates digested (g/d) were 928.1±57.98, 1025.8±23.92, 1430.9±94.68 and 1453.4±50.13 in T1, T2, T3 and T4. The respective mean values of total carbohydrates digestibility (%) 89.3±0.76, 89.7±0.58, 87.2±0.76 and 88.3±0.88 in T1, T2, T3 and T4 showed non-significant difference.

4.3 PLANE OF NUTRITION

The plane of nutrition of piglets during experimental period is presented in Table 4.4. The mean body weights of piglets 45.8±1.49, 47.9±2.10, 50.5±2.19 and 58.1±1.85 kg in T1, T2, T3 and T4, respectively were significantly ($P<0.05$) different. The mean values of T1 and T2 were statistically similar to each other, however, they were significantly ($P<0.05$) lower as compared to T4 and comparable with T3. The mean value of T3 was comparable with T1, T2 and T4. The mean metabolic body weights (kg $W^{0.75}$) of piglets 17.6±0.43, 18.2±0.60, 18.9±0.61 and 21.0±0.50 kg in respective T1, T2, T3 and T4, were significantly ($P<0.05$) different and followed the similar trend as mean body weight.

DMI when expressed as g/kg $W^{0.75}$/d; the values were 81.7±5.14, 86.9±2.44, 120.3±8.92 and 108.0±2.92 in respective groups. The value of T1 was comparable to T2 but significantly ($P<0.05$) lower than T3 and T4. The value of T2 was comparable to T1 and T4 but significantly ($P<0.05$) lower than T3, however, the values of T3 and T4 were similar.

The respective values of CP intake (g/d) were 257.7±15.50, 283.5±6.60, 407.7±28.16 and 2114.1±79.97 in T1, T2, T3 and T4. When the intake of CP was expressed as g/kg $W^{0.75}$, the values were 14.7±0.93, 15.7±0.44, 21.7±1.60 and 19.4±0.53 in respective groups with no significant difference between T3 and T4, however, the value of T1 was significantly ($P<0.05$) lower as compared T3 and T4, but comparable with T2. The mean value of T2 was significantly ($P<0.05$) lower as compared to T3, but comparable with T1 and T4.

The respective DCP intake (g/d) was 202.3±14.48, 232.4±5.56, 340.1±24.88 and 351.8±15.08 in T1, T2, T3 and T4.

The mean value of T3 and T4 were significantly (P<0.01) higher as compared to T1 and T2, but their was no difference between similar age weaned group.

The TDN intake (g/d) was 1186.4±76.87, 1325.0±30.75, 1852.7±123.06 and 1905.2±68.86 in T1, T2, T3 and T4, respectively. There was significant (P<0.05) difference in TDN intake among the 4 groups. The mean values of 28 days weaned groups (T1 and T2) were significantly (P<0.05) lower as compared to 42 days weaned group (T3 and T4), however, the values between 28 days weaned groups (T1 and T2) and 42 days weaned groups (T3 and T4) were comparable. When expressed as g/kg $W^{0.75}$, the values were 67.6±4.41, 73.1±1.95, 98.5±7.51 and 90.6±2.60 in respective groups with no significant difference between T3 and T4, however, the value of T1 was significantly (P<0.05) lower as compared T3 and T4, but comparable with T2. The mean value of T2 was significantly (P<0.05) lower as compared to T3, but comparable with T1 and T4.

4.4 GROWTH PERFORMANCE, FEED INTAKE AND FEED CONVERSION RATIO (FCR)

4.4.1 Body weight changes

The detailed observations related to body weight (BW) changes are presented in Table 4.5. The weekly pattern of BW changes is depicted in Fig. 4.1. While comparing the data among the groups initial body weight of two weeks period has been excluded in group T1 and T2. So the initial body weights of the piglets in group T1, T2, T3 and T4 were 8.5±0.67, 8.8±0.57, 9.3±0.37 and 9.4±0.21 kg, respectively, which exhibited increase over the period of the study to reach the final BW of 52.0±3.66, 61.1±2.58, 65.8±2.70 and 73.9±2.83 kg, respectively at the end of 120 days of experimental feeding. It was thus evident that supplementation of yeast induced a significant

($P<0.01$) increase in body weights. The final BW mean value of T4 was significantly ($P<0.05$) higher as compared to early weaned group T1 and T2 but comparable with T3.

The net BW gain of the piglets in T1, T2, T3 and T4 was 43.4±3.26, 52.3±2.37, 56.5±2.51 and 64.4±2.71 kg, respectively. The respective values of average daily gain (ADG) of the piglets in T1, T2, T3 and T4 were 365.0±27.44, 439.4±19.92, 474.5±21.13 and 541.4±22.79g, respectively. The net BW gain (kg) and ADG (g) followed the similar trend as final body weights.

4.4.2 Voluntary Feed intake

The detailed observations related to weekly feed intake and daily feed intake (on DM basis) is presented in Table 4.6. While comparing the data among the groups feed intake of initial two weeks period has been excluded in T1 and T2. The total feed intake by the piglets in T1, T2, T3 and T4 was 405.1±16.08, 449.4±19.14, 477.6±24.15 and 527.7±20.80 kg, respectively. The total feed intake was comparable among groups T1, T2 and T3 and groups T2, T3 and T4. However, total feed intake was significantly higher ($P<0.01$) in group T4 as compared to T1.

The daily DM intake followed the similar trend as total DM intake. The respective mean values of daily feed intake by the piglets in T1, T2, T3 and T4 was 1.14±0.04, 1.26±0.05, 1.34±0.07 and 1.48±0.06 kg.

4.4.3 Feed conversion ratio (FCR)

The detailed observations related to weekly FCR are presented in Table 4.7. While comparing the data among four groups feed intake of initial two weeks period has been excluded in T1 and T2. The overall FCR values were 3.15±0.14, 2.92±0.05, 2.85±0.03 and 2.75±0.05 in T1, T2, T3 and T4 respectively. The value was significantly lower in T4 and higher in T1.

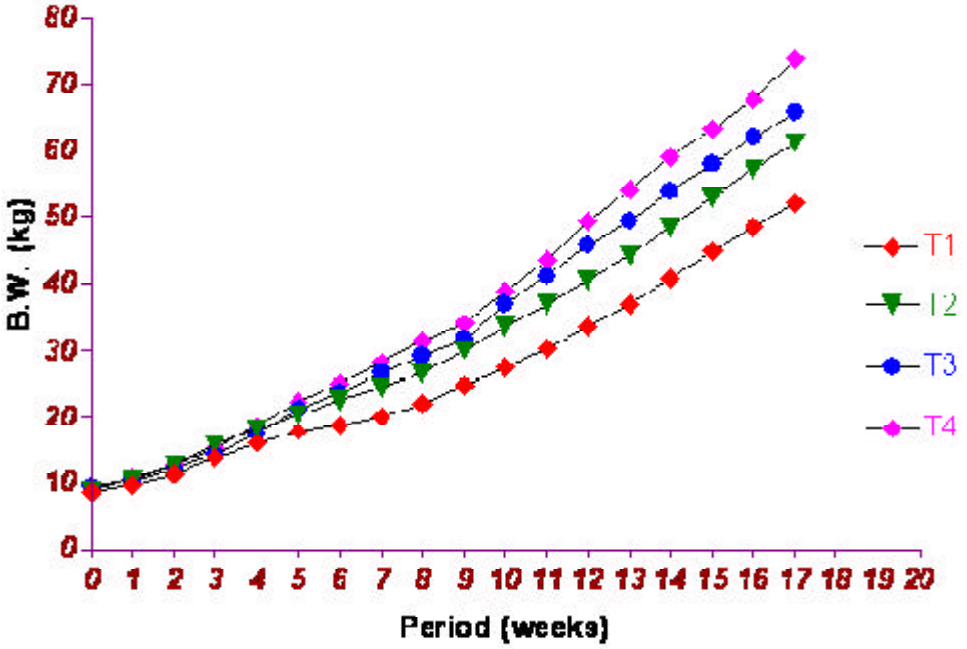

Fig. 4.1 : BW changes in piglets of different groups

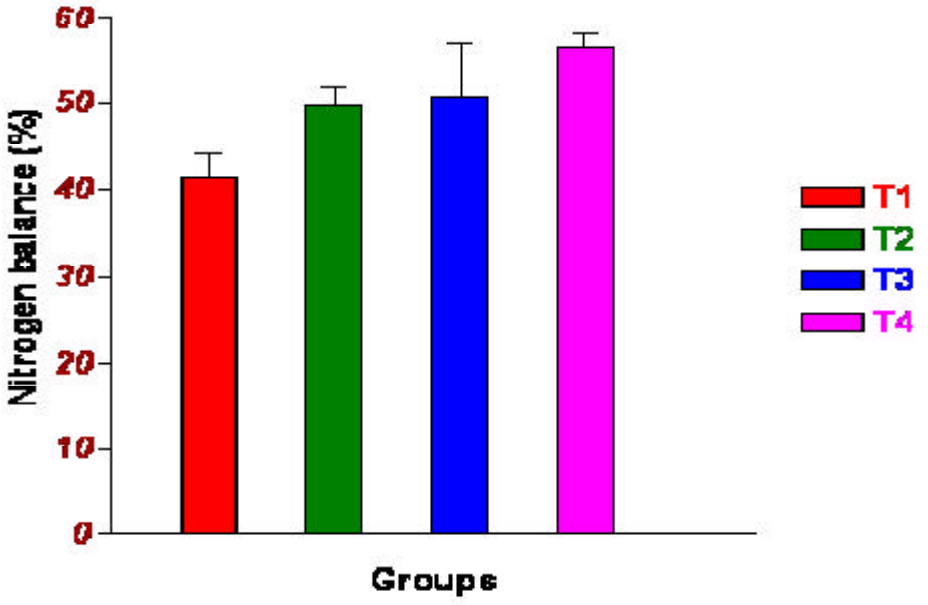

Fig. 4.2 : Nitrogen balance (%) in piglets of different groups

4.4.4 Economics of feeding

The mean values of feed cost and feed cost per unit gain was estimated and presented in Table 4.8. The feed cost (Rs) per kg gain in group T1, T2, T3 and T4 was 52.3±3.77, 47.4±0.88, 46.7±0.45 and 45.2±0.37, respectively. Though the values did not differ statistically, however, feed cost per unit gain reduced by 13.57% in T4 as compared to T1 and 9.36% in T2 as compared to T1. This reduction in feed cost for gain was primarily due to better FCR in yeast supplemented groups.

4.5 BALANCE OF NUTRIENTS

4.5.1 Nitrogen balance

Nitrogen balance of the piglets in respective groups is presented in Table 4.9. The Nitrogen balance of the piglets is depicted in Fig 4.2. The intake of nitrogen (g/d) was 41.5±2.50, 45.7±1.06, 65.7±4.54 and 65.9±2.49 in T1, T2, T3 and T4, respectively. There was significant difference in nitrogen intake among four groups. However, there was no significant difference between 28 days weaned group (T1 and T2) and 42 days weaned group (T3 and T4). The value in yeast supplemented group was statistically similar.

Nitrogen balance (g/d) in T1, T2, T3 and T4 was 17.5±2.28, 22.8±1.12, 34.4±5.32 and 37.4±2.47, respectively. The value in T4 was significantly ($P<0.01$) higher as compared to T1 and T2, comparable with T3. The nitrogen balance as percent of intake in T1 (41.4±2.84) was comparable with T2 (49.8±1.99) and T3 (50.7±6.24), however, the value was significantly lower as compared to T4 (56.5±1.76). The mean values of T2, T3 and T4 were statistically similar.

The faecal nitrogen (g/d) voided was significantly ($P<0.05$) higher in T3 (10.8±0.61) than T1 (8.9±0.41), T2 (8.2±0.29) and T4

(9.2±0.17). The urine nitrogen (g/d) excreted in T3 (20.4±2.08) was significantly (P<0.05) higher as compared to T1 (15.1±0.66) and T2 (14.7±0.73) while comparable to T4 (19.3±0.76). The mean values of T1, T2 and T4 were comparable and the values of T3 and T4 were comparable. Total nitrogen outgo (g/d) was significantly higher (P<0.01) in T3 (31.2±2.13) as compared to T1 (24.0±0.52) and T2 (22.9±0.96) while comparable to T4 (28.5±0.73).

4.5.2 Calcium balance

Calcium balance of the piglets in different groups is furnished in Table 4.10. The Ca balance of the piglets is depicted in Fig 4.3. The respective value of Ca intake (g/d) was 9.5±0.57, 10.5±0.24, 15.0±1.04 and 15.1±0.57 in T1, T2, T3 and T4. There was significant (P<0.01) difference in Ca intake among four groups. However, there was no significant difference between 28 days weaned groups (T1 and T2) and 42 days weaned groups (T3 and T4). The value in yeast supplemented group was numerically higher; however, they were statistically similar. The calcium (g/d) voided in faeces, urine (g/d) and the total loss did follow the similar trend as intake.

The respective values of calcium balance (g/d) in T1, T2, T3 and T4 were 4.2±0.23, 5.0±0.18, 7.2±0.52 and 7.9±0.31 respectively. The value in T4 was significantly higher (P<0.01) as compared to T1 and T2, comparable with T3. The Ca balance as percent of intake in T1 (43.7±0.69) was significantly lower as compared to T2 (47.7±0.79), T3 (47.8±1.05), and T4 (52.4±1.27). Groups T2 and T3 were statistically similar.

4.5.3 Phosphorus balance

Phosphorus balance of the piglets in different groups is presented in Table 4.11. The P balance of the piglets is depicted in

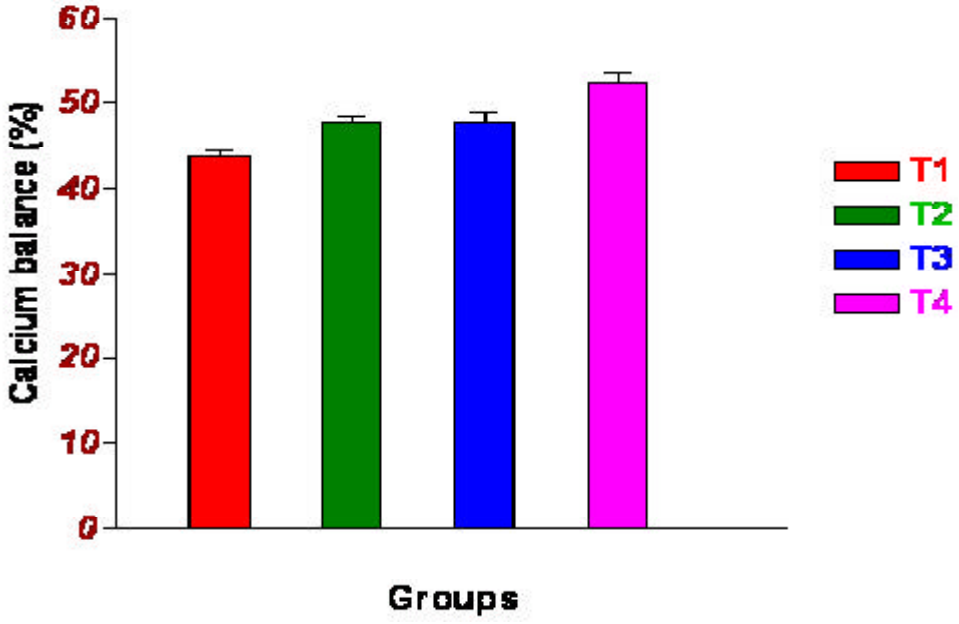

Fig. 4.3 : Calcium balance (%) in piglets of different groups

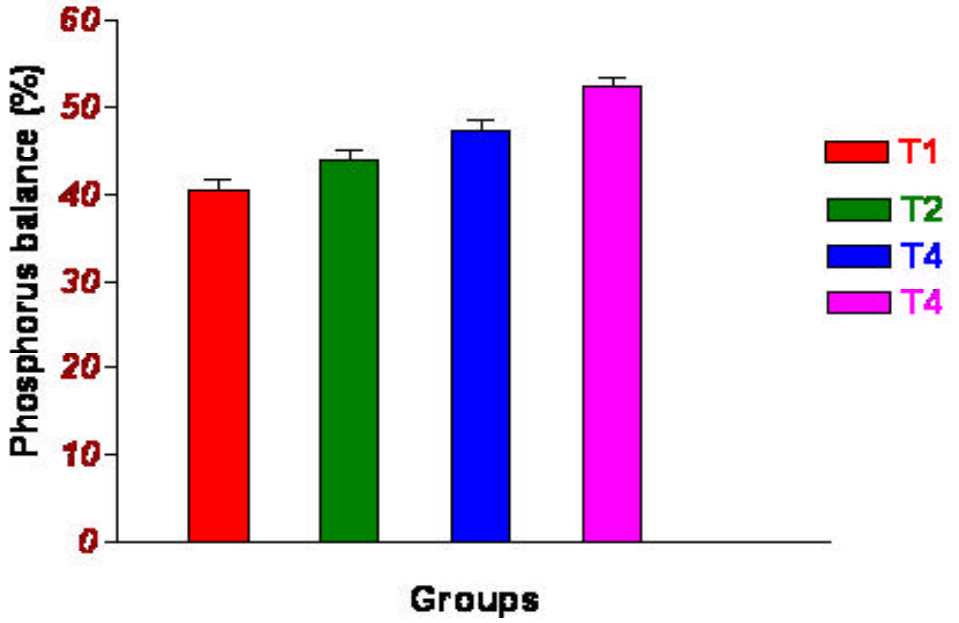

Fig. 4.4 : Phosphorus balance (%) in piglets of different groups

Fig 4.4. The phosphorus intake (g/d) was 11.7±0.71, 12.9±0.30, 18.5±1.28 and 18.6±0.70 in T1, T2, T3 and T4, respectively. There was significant difference ($P<0.01$) in phosphorus intake among four groups. However, there was no significant difference between groups T1 and T2 and between group T3 and T4. The value in yeast supplemented group was numerically higher; however, they were statistically similar. The phosphorus (g/d) voided in faeces, urine (g/d) and the total loss showed the similar trend as intake.

The respective values of phosphorus balance (g/d) in T1, T2, T3 and T4 were 4.7±0.35, 5.7±0.21, 8.8±0.78 and 9.8±0.49, respectively. The value in T3 and T4 was significantly higher ($P<0.01$) as compared to T1 and T2. The P balance as percent of intake in T1 (40.4±1.38) was significantly ($P<0.01$) lower as compared to T3 (47.2±1.24) and T4 (52.5±0.93) but the value was statistically similar to T2 (44.0±1.06).

4.6 FAECAL CHARACTERISTICS

4.6.1 Physical examination

Faecal consistency was recorded 3 times per week using a score of +, ++, +++, in which + was identified as firm faeces, ++ as semi-soft faeces, and +++ as soft faeces. The faecal consistency was judged per pen by recording the number of pigs within a category of faecal consistency scores. At last scores of group T1, T2, T3 and T4 were +++, ++, ++, + respectively.

4.6.2 Yeast and coliform count

The results of yeast and coliform count in faeces are presented in Table: 4.12. The pattern of faecal yeast count is depicted in Fig. 4.5. The mean values of yeast count ($\times 10^4$/g) differed significantly ($P<0.05$), with values 52.3±2.23, 87.5±3.13, 54.8±3.36 and 82.1±2.46 in T1, T2, T3 and T4, respectively. The values in T2 and T4 (live

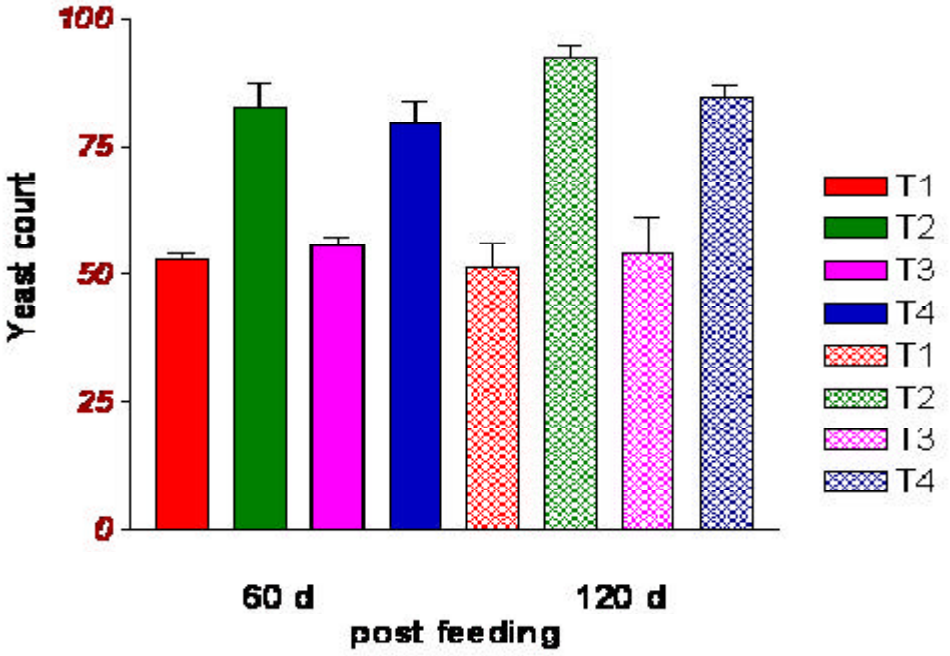

Fig. 4.5 : Faecal yeast count in piglets of different groups

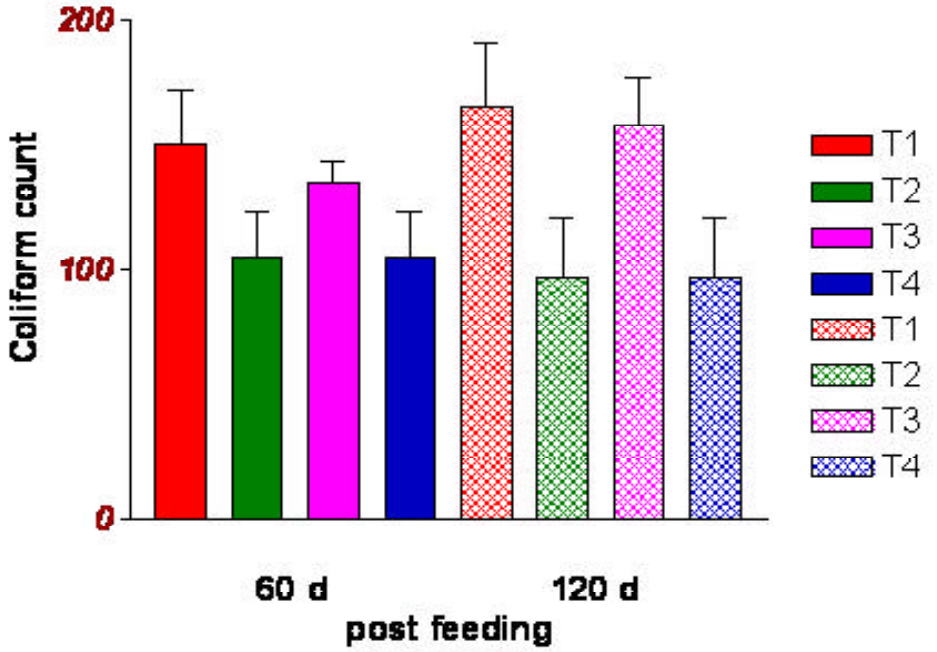

Fig. 4.6 : Faecal coliform count in piglets of different groups

Saccharomyces cerevisiae supplemented groups) were significantly higher as compared to T1 and T3. The mean value (70.6±5.10) at 120 days post-feeding was statistically similar with the mean value (67.7±3.80) at 60 days post-feeding but numerically higher.

The pattern of faecal coliform count is also depicted in Fig. 4.6. The respective mean values of coliform count ($\times 10^4$/g) differed significantly ($P<0.05$), with values 157.5±15.78, 101.0±13.96, 146.3±10.51 and 101.0±13.96 in T1, T2, T3 and T4. The respective values in T2 and T4 were significantly lower as compared to T1 and T3. The mean value (129.1±9.24) at 120 days post-feeding was statistically similar with the mean value (123.8±9.24) at 60 days post-feeding maintaining nearly at constant level.

4.7 IMMUNOLOGICAL STATUS

4.7.1 Humoral immune response

Humoral immunity of growing pigs in different group T1, T2, T3 and T4 was assessed by microhemagglutination (HA) assay as described by Wagmann and Smithies (1966) against sheep red blood cells (SRBC). It was evaluated at day 0, 14 and 28 against injecting SRBC (i/m) and found significantly ($P<0.01$) different among the different groups. Antibody response (SRBC response, HA units) to sheep red blood cells among different dietary groups are presented in Table 4.12. The pattern is depicted in Fig. 4.7.

The values in group T2 and T4 (live *Saccharomyces cerevisiae* supplemented groups) were significantly ($P<0.01$) higher as compared to T1 and T3. The mean value at 0 day against SRBC injection was significantly ($P<0.05$) lower as compared to 14 day and 28 day while the value at 14 day was numerically higher as compared to day 28 but statistically similar.

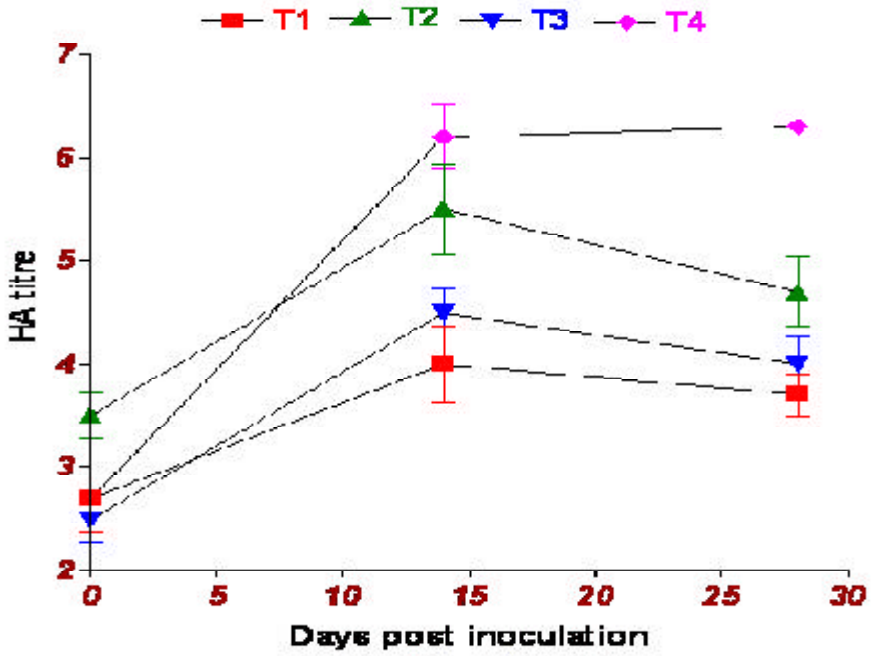

Fig. 4.7 : Antibody response (HA unit) against SRBC

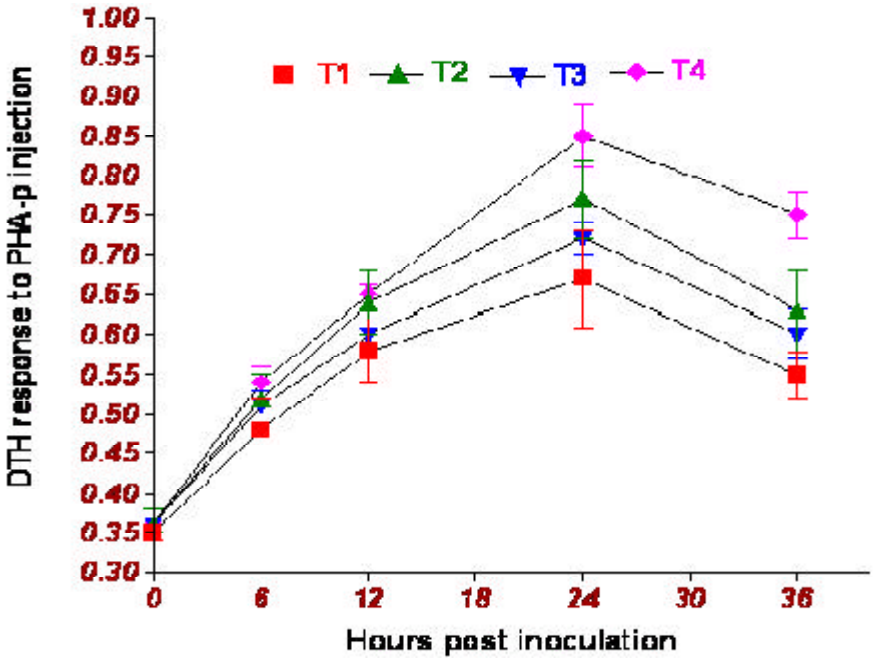

Fig. 4.8 : Skin thickness response to PHA-p in piglets of different groups

4.7.2 Cell mediated immune (CMI) response

The result pertaining to delayed type hypersensitivity (DTH) to ascertain cell mediated immune (CMI) response in PHA sensitized growing pigs on different dietary regimes at different hours of interval are presented in Table 4.13. The pattern is depicted in Fig. 4.8. The skin thickness measured at 0 h in T1, T2, T3 and T4 were 0.35±0.01, 0.36±0.02, 0.36±0.00, 0.36±0.00 cm, respectively, showing similar values ($P>0.05$). However, the dermal enduration showed a steady increase over the post-inoculation (PI) periods in all the four groups, with the values at 6, 12 and 24 h PI showing greater increase ($P<0.01$) in comparison to that of 0 h, before subsiding to basal levels at 36 h PI. When compared among the different groups, there was a significant ($P<0.05$) increase in skin thickness in group T4 as compared to T1 and T3 but the value was comparable to T2. The results revealed that CMI response of the group weaned at 28 days supplemented with *Saccharomyces cerevisiae* was comparable with 42 days weaned group (T3 and T4), however, significantly higher as compared to T1.

4.8 INTESTINAL MORPHOLOGY

The result pertaining to small intestinal morphology is shown in Table 4.14. Histometric analysis showed that average no. of villi in 100µm distance was significantly ($P<0.01$) lower in T1 (3.3±0.25) as compared to T2 (4.8±0.48) and T4 (4.8±0.25) and comparable with T3. There was no significant difference among T2, T3 and T4. The respective mean values of jejunal villus height (µm) were 285.4±13.11, 468.2±21.83, 397.4±35.38 and 478.3±18.77 in T1, T2, T3 and T4. The results indicated that the values of T2, T3 and T4 was significantly higher ($P<0.01$) as compared to T1, however, statistically similar to each other. Histological evaluation of jejunal villus width and crypt depth indicated no significant

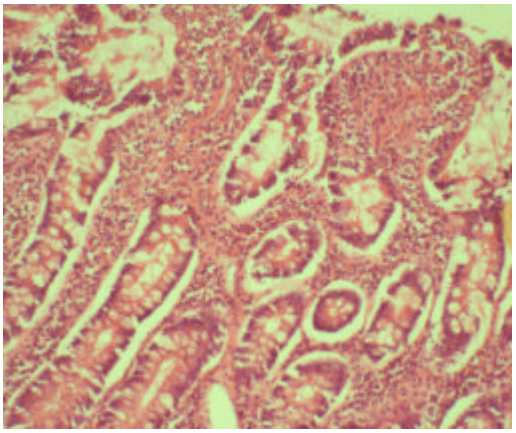

Fig. 4.9 : Showing height of villi in group T1 (H. & E. X 400).

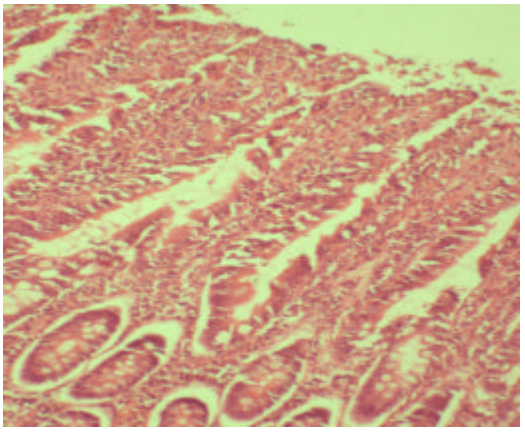

Fig. 4.10 : Showing height of villi in group T2 (H. & E. X 400).

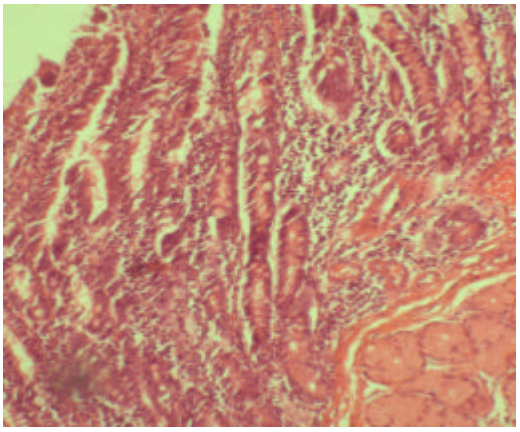

Fig. 4.11 : Showing height of villi in group T3 (H. & E. X 400).

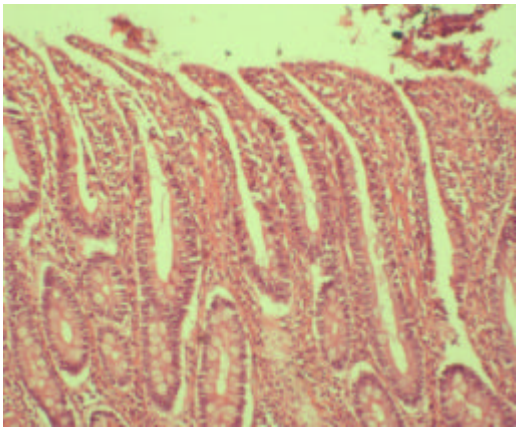

Fig. 4.12 : Showing height of villi in group T4 (H. & E. X 400).

effect of yeast supplementation. The mean values of villus width (µm) in T1, T2, T3 and T4 were 105.4±13.39, 96.4±17.44, 137.5±18.37 and 110.2±13.45, respectively while the respective mean values of crypt depth (µm) were 316.6±8.68, 299.2±17.83, 385.1±38.25 and 341.1±20.25 in T1, T2, T3 and T4, respectively.

Jejunal crypt width in T1 (55.0±3.01) was significantly higher ($P<0.05$) as compared to T2 (39.3±2.95) and T4 (38.9±2.83) but statistically similar with T3 (45.1±4.88). The value of T3 was comparable with T1, T2 and T4. The results of the present study revealed that early weaning induced an increase in crypt width in T1 which can be decreased by yeast supplementation in T2 and the value was comparable with T3 and T4, However, in 42 days weaning, yeast supplementation reduced the value numerically but not statistically.

The viilus height (V) to crypt depth (C) ratio in T1 (0.9±0.03) was significantly lower ($P<0.01$) as compared to T2 (1.6±0.14) and T4 (1.4±0.14) but statistically similar with T3 (1.0±0.01). The value of T2 was significantly higher as compared to T1 and T3 but comparable with T4. The value of T3 was significantly lower as compared to T2 but comparable with T1 and T4. The results of the present study revealed that early weaning induced a reduction in V:C ratio (T1) which can be enhanced by yeast supplementation.

Infiltration in lamina propria and no. of goblet cells were recorded using a score of +, ++, +++, in which + was identified as mild, ++ as moderate, and +++ as prominent level. The result of infiltration in lamina propria was +++, ++, ++ and + in T1, T2, T3 and T4, respectively. The no. of goblet cells did follow the same trend.

4.9 BLOOD BIOCHEMICAL CONSTITUENTS

The serum total protein, albumin, globulin, glucose, total cholesterol, triglycerides, serum urea, AST/SGOT and ALT/SGPT levels in different groups are presented in Table 4.15.

4.9.1 Serum proteins

The concentrations of serum total proteins, albumin and globulin were within the normal range (Kaneko *et al.*, 1997). There were no significant differences in total proteins (g/dl) among the group T1, T2, T3 and T4 and the values were 6.5±0.13, 6.8±0.15, 6.6±0.11 and 6.9± 0.10, respectively. The albumin (g/dl) also did not differ significantly and the values were 3.9±0.12, 3.8±0.08, 4.0±0.10 and 4.1±0.15, respectively. Similar trend was observed for globulin (g/dl) whose corresponding values were 2.6±0.12, 2.9±0.15, 2.7±0.10 and 2.8±0.20, respectively. The period-wise comparison of mean values of serum albumin and globulin were statistically similar, however, period-wise comparison of protein was significant $P<0.01$).

4.9.2 Serum glucose

The concentration of serum glucose was within the normal range (Kaneko *et al.*, 1997). The mean glucose (mg/dl) value of T4 (107.5±2.35) was statistically higher than T1 (98.8±1.72) and comparable with T2 (103.8±2.13) and T3 (103.1±2.09). However, upon period-wise comparison, the value did show significant ($P<0.01$) influence of time.

4.9.3 Total cholesterol and triglycerides

The mean values of cholesterol showed a significant ($P<0.01$) variation among the groups. The concentration of cholesterol was within the normal range (Kaneko *et al.*, 1997). The plasma total cholesterol level (mg/dl) was found to be lower in T2 (46.4±0.84)

and T4 (47.1±1.23) as compared to T1 (53.4±2.00) and T3 (53.8±1.62). The period-wise comparison of mean values were statistically similar.

There were no significant differences on triglycerides levels among the group T1, T2, T3 and T4 and the values were 31.4±1.47, 30.1±1.17, 31.6±1.02 and 29.9±1.24, respectively. However, the value in yeast supplemented group (T2 and T4) was numerically lower than non-supplemented group. The period-wise comparison of mean values were statistically similar

4.9.4 Serum urea

The value of urea was in accordance within the normal range (Kaneko *et. al.*, 1997). There were no significant differences on serum urea levels (mg/dl) among the groups T1, T2, T3 and T4 and the values were 22.5±0.37, 22.2±0.24, 22.7±0.31 and 22.1±0.19, respectively. The period-wise comparison of mean values were statistically similar

4.9.5 Serum ALT/SGPT and AST/SGOT

The mean values (IU/ L) of ALT were 41.9±2.20, 40.7±2.00, 41.5±1.97and 41.2± 2.15, respectively. Similarly, values of AST were 85.2±1.50, 85.3±2.57, 85.5±1.80 and 84.6±2.21 in groups T1, T2, T3 and T4, respectively. The values of both ALT and AST were within the normal range (Kaneko *et. al.*, 1997) and no significant difference was evident in growing pigs irrespective of dietary treatments in ALT and AST. The period-wise comparison of mean values of ALT and AST did not show significant difference.

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Table 4.3. Intake (g/d), digested (g/d) and digestibility (%) of nutrients in different groups

Attributes	T1	T2	T3	T4	SEM	P value
			Dry matter			
Intake	1431.7±86.16^{b}	1574.9±36.66^{b}	2264.9±156.49^{a}	2272.6±85.96^{a}	93.22	0.000
Digested	1211.3±79.22^{b}	1353.8±31.69^{b}	1890.4±124.93^{a}	1941.2±70.62^{a}	77.45	0.000
Digestibility	84.5±0.79	86.0±0.63	83.6±0.92	85.5±0.82	0.42	0.187
			Organic matter			
Intake	1331.9±80.16^{b}	1465.1±34.10^{b}	2106.9±145.57^{a}	2114.1±79.97^{a}	86.71	0.000
Digested	1154.9±74.23^{b}	1287.7±29.97^{b}	1806.7±121.02^{a}	1849.2±66.55^{a}	74.13	0.000
Digestibility	86.6±0.67	87.9±0.55	85.8±0.72	87.5±0.70	0.35	0.166
			Crude protein			
Intake	257.7±15.50^{b}	283.5±6.60^{b}	407.7±28.16^{a}	409.1±15.17^{a}	16.78	0.000
Digested	202.3±14.48^{b}	232.4±5.56^{b}	340.1±24.88^{a}	351.8±15.08^{a}	15.63	0.000
Digestibility	78.3±1.22^{c}	82.0±0.50^{b}	83.3±0.65^{ab}	85.9±0.48^{a}	0.68	0.000
			Ether extract			
Intake	36.1±2.17^{b}	39.7±0.92^{b}	57.1±3.94^{a}	57.3±2.16^{a}	2.35	0.000
Digested	24.9±2.16^{c}	30.2±0.74^{bc}	36.3±2.67^{b}	44.5±1.99^{a}	1.79	0.000
Digestibility	68.5±2.34^{ab}	76.0±1.50^{a}	64.4±4.04^{b}	77.6±1.90^{a}	1.67	0.005
			Neutral detergent fibre			
Intake	322.1±19.39^{b}	354.4±8.25^{b}	509.6±35.21^{a}	511.3±19.34^{a}	20.97	0.000
Digested	188.5±16.70^{c}	229.6±8.52^{bc}	289.7±21.21^{ab}	327.4±14.27^{a}	13.38	0.000
Digestibility	58.2±2.34^{ab}	64.8±1.94^{a}	56.9±2.21^{b}	64.2±2.24^{a}	1.25	0.038

Table 4.3. Contd...

Attributes	T1	T2	T3	T4	SEM	P value
			Acid detergent fibre			
Intake	104.8±6.31^{b}	115.3±2.68^{b}	165.8±11.45^{a}	166.4±6.29^{a}	6.82	0.000
Digested	37.2±6.10^{b}	50.4±1.48^{ab}	56.4±7.27^{ab}	72.1±6.76^{a}	3.79	0.004
Digestibility	35.0±4.58	43.9±1.96	34.1±3.74	43.8±4.34	2.02	0.143
			Hemicellulose			
Intake	217.3±13.08^{b}	239.1±5.56^{b}	343.8±23.76^{a}	345.0±13.05^{a}	14.15	0.000
Digested	151.4±11.88^{b}	179.2±8.14^{b}	233.3±15.48^{a}	255.3±12.22^{a}	10.35	0.000
Digestibility	69.3±1.64	74.8±2.56	68.0±1.51	74.0±1.83	1.09	0.051
			Total carbohydrates			
Intake	1038.4±62.49^{b}	1142.3±26.58^{b}	1642.7±113.50^{a}	1648.3±62.35^{a}	67.61	0.000
Digested	928.1±57.98^{b}	1025.8±23.92^{b}	1430.9±94.68^{a}	1453.4±50.13^{a}	57.00	0.000
Digestibility	89.3±0.76	89.7±0.58	87.2±0.76	88.3±0.88	0.41	0.109

^{abc}Means bearing different superscripts in a row differ significantly

Table 4.4. Plane of nutrition in different groups during metabolism trial

Attributes	T1	T2	T3	T4	SEM	P value
Body wt (kg)	45.8±1.49^{b}	47.9±2.10^{b}	50.5±2.19^{ab}	58.1±1.85^{a}	1.33	0.001
Body wt (kgW$^{.75}$)	17.6±0.43^{b}	18.2±0.60^{b}	18.9±0.61^{ab}	21.0±0.50^{a}	0.37	0.001
DMI (g/d)	1431.7±86.16^{b}	1574.9±36.66^{b}	2264.9±156.49^{a}	2272.6±85.96^{a}	93.22	0.000
DMI (g/kgW$^{0.75}$)	81.7±5.14^{c}	86.9±2.44^{bc}	120.3±8.92^{a}	108.0±2.92^{ab}	4.14	0.000
CPI (g/d)	257.7±15.50^{b}	283.5±6.60^{b}	407.7±28.16^{a}	409.1±15.17^{a}	16.78	0.000
CPI (g/kgW$^{0.75}$)	14.7±0.93^{c}	15.7±0.44^{bc}	21.7±1.60^{a}	19.4±0.53^{ab}	0.75	0.000
DCPI (g/d)	202.3±14.48^{b}	232.4±5.56^{b}	340.1±24.88^{a}	351.8±15.08^{a}	15.63	0.000
DCPI (g/kgW$^{0.75}$)	11.5±0.84^{b}	12.8±0.39^{b}	18.1±1.45^{a}	16.7±0.50^{a}	0.70	0.000
TDNI (g/d)	1186.4±76.87^{b}	1325.0±30.75^{b}	1852.7±123.06^{a}	1905.2±68.86^{a}	76.12	0.000
TDNI (g/kgW$^{0.75}$)	67.6±4.41^{c}	73.1±1.95^{bc}	98.5±7.51^{a}	90.6±2.60^{ab}	3.40	0.000
DE (Mcal/d)	5.23±0.34^{b}	5.84±0.14^{b}	8.17±0.54^{a}	8.40±0.30^{a}	0.34	0.000

^{abc}Means bearing different superscripts in a row differ significantly

Table 4.5. Body weight change (kg) and average daily gain (g/d) in piglets of different groups

Week	T1	T2	T3	T4	SEM	P value
1*	7.0±0.59	7.2±0.51	-	-	0.38	0.858
2*	7.8±0.63	8.0±0.55	-	-	0.41	0.820
0	8.5±0.67	8.8±0.57	9.3±0.37	9.4±0.21	0.24	0.495
1	9.6±0.79	10.4±0.72	10.2±0.45	10.8±0.29	0.30	0.596
2	11.3±0.88	12.7±0.78	12.1±0.44	12.6±0.31	0.32	0.409
3	13.7±1.05	15.6±0.90	14.4±0.45	15.1±0.36	0.38	0.288
4	15.9±1.06	18.2±0.94	17.3±0.57	18.7±0.52	0.42	0.083
5	17.7 ± 1.20^{b}	20.4 ± 1.08^{ab}	21.1 ± 0.78^{ab}	22.3 ± 0.61^{a}	0.52	0.010
6	18.7 ± 1.29^{b}	22.7 ± 1.26^{a}	23.7 ± 0.80^{a}	25.0 ± 0.58^{a}	0.61	0.001
7	20.0 ± 1.66^{b}	24.6 ± 1.47^{ab}	26.6 ± 0.95^{a}	28.2 ± 0.83^{a}	0.76	0.000
8	22.0 ± 1.90^{b}	26.8 ± 1.58^{ab}	29.2 ± 1.29^{a}	31.4 ± 1.09^{a}	0.89	0.000
9	24.7 ± 2.30^{b}	30.1 ± 1.89^{ab}	31.8 ± 1.46^{a}	34.0 ± 1.17^{a}	0.99	0.004
10	27.5 ± 2.59^{b}	33.7 ± 2.10^{ab}	36.8 ± 1.85^{a}	38.8 ± 1.32^{a}	1.16	0.001
11	30.3 ± 2.84^{b}	36.9 ± 2.15^{ab}	41.1 ± 2.10^{a}	43.4 ± 1.48^{a}	1.29	0.001
12	33.5 ± 2.91^{c}	40.5 ± 2.29^{bc}	45.7 ± 2.15^{ab}	49.2 ± 1.56^{a}	1.40	0.000
13	37.0 ± 3.00^{c}	44.2 ± 2.41^{bc}	49.3 ± 2.21^{ab}	54.0 ± 1.88^{a}	1.49	0.000
14	40.8 ± 3.17^{c}	48.5 ± 2.57^{bc}	53.6 ± 2.46^{ab}	58.8 ± 2.17^{a}	1.60	0.000
15	44.9 ± 3.41^{c}	52.9 ± 2.68^{bc}	57.8 ± 2.48^{ab}	63.3 ± 2.27^{a}	1.65	0.000
16	48.5 ± 3.54^{b}	56.9 ± 2.70^{ab}	61.9 ± 2.57^{a}	67.7 ± 2.59^{a}	1.73	0.000
17	52.0 ± 3.66^{c}	61.1 ± 2.58^{bc}	65.8 ± 2.70^{ab}	73.9 ± 2.83^{a}	1.85	0.000
Net gain	43.4 ± 3.26^{c}	52.3 ± 2.37^{bc}	56.5 ± 2.51^{ab}	64.4 ± 2.71^{a}	1.72	0.000
ADG (g/d)	365.0 ± 27.44^{c}	439.7 ± 19.92^{bc}	474.5 ± 21.13^{ab}	541.4 ± 22.79^{a}	14.49	0.000

While comparing the data among groups, initial body weight of 2 weeks (1 and 2*) period have been excluded

[abc]Means bearing different superscripts in a row differ significantly

Table 4.6. Weekly feed intake (kg) in piglets of different groups per replica (each group was having 12 piglets with 4 replicas in each)

Week	T1	T2	T3	T4	SEM	P value
1*	3.44±0.15	4.62±0.89	-	-	0.47	0.242
2*	5.02±1.06	7.79±0.67	-	-	0.78	0.070
1	7.53±0.67^{bc}	11.91±0.91^{a}	6.13±0.40^{c}	8.84±0.43^{b}	0.62	0.000
2	9.89±0.86^{b}	14.67±0.82^{a}	10.16±1.33^{b}	11.03±0.66^{ab}	0.65	0.014
3	13.43±1.23^{b}	17.39±0.42^{a}	13.10±1.00^{b}	14.48±0.68^{ab}	0.59	0.020
4	17.22±1.02	19.68±1.22	15.41±1.33	18.17±0.94	0.65	0.112
5	18.22±0.85	19.99±0.61	19.73±0.70	21.00±0.43	0.39	0.073
6	16.79±0.48^{b}	20.20±1.08^{b}	25.10±1.12^{a}	24.81±0.58^{a}	0.97	0.000
7	16.00±1.54^{c}	20.48±1.44^{bc}	25.79±1.19^{a}	25.32±0.64^{ab}	1.17	0.000
8	19.70±0.53^{b}	22.15±1.42^{ab}	24.03±0.65^{a}	25.81±0.66^{a}	0.71	0.002
9	21.14±1.39^{b}	22.42±1.60^{b}	26.39±1.75^{ab}	29.97±1.63^{a}	1.15	0.008
10	21.95±1.78^{c}	25.04±1.13^{bc}	30.58±2.37^{ab}	33.98±1.76^{a}	1.46	0.002
11	26.08±1.51^{b}	29.53±1.60^{ab}	31.29±2.84^{ab}	36.49±2.53^{a}	1.38	0.037
12	30.19±2.09	30.80±2.57	33.43±1.64	38.93±2.54	1.34	0.064
13	32.14±1.75^{b}	32.92±2.35^{b}	37.00±2.95^{ab}	43.61±2.11^{a}	1.57	0.017
14	33.13±1.28^{b}	34.39±2.18^{b}	37.96±2.13^{ab}	45.01±2.95^{a}	1.55	0.011
15	38.77±2.17	40.19±3.57	43.29±2.14	47.71±2.85	1.51	0.156
16	39.60±2.29	41.88±2.73	46.32±4.04	49.49±2.15	1.63	0.125
17	43.39±1.84	45.78±2.16	51.89±3.97	53.05±2.64	1.61	0.082
Total intake	405.1±16.08^{b}	449.4±19.14^{ab}	477.6±24.15^{ab}	527.7±20.80^{a}	14.63	0.008
Average Daily intake per piglet	1.14±0.04^{b}	1.26±0.05^{ab}	1.34±0.07^{ab}	1.48±0.06^{a}	0.04	0.008

While comparing the data among groups, initial feed intakes of 2 weeks (1 and 2*) period have been excluded

^{abc}Means bearing different superscripts in a row differ significantly

Table 4.7. Weekly FCR in piglets of different groups

Week	T1	T2	T3	T4	SEM	P value
1*	1.72±0.42	1.90±0.35	-	-	0.26	0.750
2*	2.54±0.61	3.55±0.34	-	-	0.37	0.197
1	2.37±0.28	2.52±0.26	2.46±0.33	2.24±0.07	0.12	0.877
2	2.11±0.37	2.16±0.18	1.76±0.20	2.02±0.07	0.11	0.625
3	1.86±0.15	1.95±0.11	1.98±0.16	1.95±0.27	0.08	0.967
4	2.67 ± 0.12^{a}	2.56 ± 0.22^{a}	1.80 ± 0.24^{b}	1.72 ± 0.09^{b}	0.14	0.004
5	3.49 ± 0.48^{a}	3.17 ± 0.43^{ab}	1.74 ± 0.11^{c}	1.98 ± 0.17^{bc}	0.25	0.007
6	4.49±0.51	3.16±0.49	3.33±0.47	3.33±0.61	0.27	0.299
7	3.14±0.30	3.84±0.59	3.08±0.37	2.66±0.17	0.21	0.248
8	3.73±0.70	3.56±0.35	3.40±0.49	2.87±0.37	0.24	0.651
9	3.12±0.70	2.28±0.10	3.65±0.43	3.94±0.60	0.28	0.164
10	2.71±0.26	2.35±0.12	2.08±0.16	2.37±0.17	0.10	0.173
11	3.10 ± 0.16^{a}	3.10 ± 0.14^{a}	2.46 ± 0.23^{b}	2.68 ± 0.12^{ab}	0.10	0.045
12	3.32 ± 0.47^{a}	2.83 ± 0.11^{ab}	2.45 ± 0.05^{ab}	2.23 ± 0.10^{b}	0.15	0.040
13	3.23±0.47	3.14±0.34	3.42±0.36	3.08±0.15	0.16	0.904
14	2.94±0.21	2.68±0.18	2.97±0.12	3.19±0.26	0.10	0.405
15	3.40±0.50	3.11±0.34	3.63±0.38	3.67±0.32	0.19	0.742
16	3.71±0.38	3.54±0.25	3.73±0.21	3.80±0.26	0.13	0.927
17	4.21 ± 0.34^{ab}	3.71 ± 0.42^{ab}	4.49 ± 0.41^{a}	2.94 ± 0.27^{b}	0.22	0.052
Overall	3.15 ± 0.14^{a}	2.92 ± 0.05^{ab}	2.85 ± 0.03^{ab}	2.75 ± 0.05^{b}	0.05	0.025

While comparing the data among groups, initial values of 2 weeks (1 and 2*) period have been excluded

[abc]Means bearing different superscripts in a row differ significantly

Table 4.8. Economics of feeding in different groups

Attributes	T1	T2	T3	T4	SEM	P value
Net gain (kg)	43.4±3.26^{c}	52.3±2.37^{bc}	56.5±2.51^{ab}	64.4±2.71^{a}	1.72	0.000
Feed cost of total weight gain (Rs.)	6708.9±266.3^{b}	7441.8±317.0^{ab}	7908.8±399.9^{ab}	8738.5±344.5^{a}	243.2	0.008
Feed cost per unit gain (Rs.)	52.3±3.77	47.4±0.88	46.7±0.45	45.2±0.34	1.11	0.112

^{ab}Means bearing different superscripts in a row differ significantly

Table 4.9. Intake and balance (g/d) of nitrogen in different groups

Attributes	T1	T2	T3	T4	SEM	P value
Intake	41.5±2.50^{b}	45.7±1.06^{b}	65.7±4.54^{a}	65.9±2.49^{a}	2.70	0.000
Voided in faeces	8.9±0.41^{b}	8.2±0.29^{b}	10.8±0.61^{a}	9.2±0.17^{b}	0.28	0.001
Voided in urine	15.1±0.66^{b}	14.7±0.73^{b}	20.4±2.08^{a}	19.3±0.76^{ab}	0.77	0.006
Total N voided	24.0±0.52^{bc}	22.9±0.96^{c}	31.2±2.13^{a}	28.5±0.73^{ab}	0.91	0.000
N balance	17.5±2.28^{c}	22.8±1.12^{bc}	34.4±5.32^{ab}	37.4±2.47^{a}	2.26	0.001
As % of intake	41.4±2.84^{b}	49.8±1.99^{ab}	50.7±6.24^{ab}	56.5±1.76^{a}	2.05	0.062

^{abc}Means bearing different superscripts in a row differ significantly

Table 4.10. Intake and balance (g/d) of calcium in different groups

Attributes	T1	T2	T3	T4	SEM	P value
Intake	9.5±0.57^{b}	10.5±0.24^{b}	15.0±1.04^{a}	15.1±0.57^{a}	0.62	0.000
Voided in faeces	5.4±0.36^{b}	5.5±0.11^{b}	7.8±0.56^{a}	7.2±0.37^{a}	0.29	0.000
Voided in urine	0.013±0.002^{b}	0.017±0.002^{ab}	0.018±0.002^{ab}	0.022±0.002^{a}	0.001	0.040
Total Ca voided	5.4±0.36^{b}	5.5±0.11^{b}	7.8±0.56^{a}	7.2±0.37^{a}	0.29	0.000
Ca balance	4.2±0.23^{b}	5.0±0.18^{b}	7.2±0.52^{a}	7.9±0.31^{a}	0.36	0.000
As % of intake	43.7±0.69^{c}	47.7±0.79^{b}	47.8±1.05^{b}	52.4±1.27^{a}	0.79	0.000

^{abc}Means bearing different superscripts in a row differ significantly

Table 4.11. Intake and balance (g/d) of phosphorus in different groups

Attributes	T1	T2	T3	T4	SEM	P value
Intake	11.7±0.71^{b}	12.9±0.30^{b}	18.5±1.28^{a}	18.6±0.70^{a}	0.76	0.000
Voided in faeces	6.2±0.37^{b}	6.3±0.17^{b}	8.6±0.50^{a}	7.8±0.27^{a}	0.27	0.000
Voided in urine	0.81±0.05^{b}	0.89±0.03^{b}	1.19±0.08^{a}	1.17±0.06^{a}	0.04	0.000
Total P voided	7.0±0.41^{b}	7.2±0.19^{b}	9.7±0.57^{a}	8.8±0.31^{a}	0.30	0.000
P balance	4.7±0.35^{b}	5.7±0.21^{b}	8.8±0.78^{a}	9.8±0.49^{a}	0.49	0.000
As % of intake	40.4±1.38^{c}	44.0±1.06^{bc}	47.2±1.24^{b}	52.5±0.93^{a}	1.07	0.000

^{abc}Means bearing different superscripts in a row differ significantly

Table 4.12. Yeast and coliform count in faeces of piglet in different groups

Attributes	Treatment	Period		Mean	P value		
		60 d	120 d		T	P	T×P
Yeast count	T1	53.0±1.08	51.5±4.66	52.3±2.23[b]	0.000	0.311	0.429
	T2	82.5±4.99	92.5±2.06	87.5±3.13[a]			
	T3	55.5±1.71	54.0±7.02	54.8±3.36[b]			
	T4	79.8±4.13	84.5±2.72	82.1±2.46[a]			
	Mean	67.7±3.80	70.6±5.10				
Coliform count	T1	150.0±21.21	165.0±25.98	157.5±15.78[a]	0.016	0.714	0.828
	T2	105.0±18.37	97.0±23.70	101.0±13.96[b]			
	T3	135.0±8.66	157.5±18.87	146.3±10.51[ab]			
	T4	105.0±18.37	97.0±23.70	101.0±13.96[b]			
	Mean	123.8±9.24	129.1±13.30				

[ab]Means bearing different superscripts in a column differ significantly

Table 4.13. Effect of dietary treatment on antibody response (SRBC response, HA units[x]) to sheep red blood cells (SRBC)

Treatment	Period			Mean	P value		
	0 d	14 d	28 d		T	P	T×P
T1	2.7±0.33	4.0±0.37	3.7±0.21	3.4±0.22[b]	0.000	0.000	0.001
T2	3.5±0.22	5.5±0.43	4.7±0.33	4.6±0.27[a]			
T3	2.5±0.22	4.5±0.22	4.0±0.26	3.7±0.24[b]			
T4	2.7±0.33	6.2±0.31	6.3±0.42	5.1±0.45[a]			
Mean	2.8±0.16[b]	5.0±0.24[a]	4.7±0.26[a]				

[x]Values expressed as log base 2 of the reciprocal of the highest serum dilution that caused agglutination of red blood cells.
[ab]Means bearing different superscripts in a row and column differ significantly

Table 4.14. Skin thickness response* to PHA injection on different treatment

Treatment	Period					Mean	P value		
	0 hr	6 hr	12 hr	24 hr	36 hr		T	P	T×P
T1	0.35±0.01	0.48±0.04	0.58±0.04	0.67±0.06	0.55±0.03	0.52±0.03^{c}	0.000	0.000	0.259
T2	0.36±0.02	0.52±0.03	0.64±0.04	0.77±0.05	0.63±0.05	0.58±0.03^{ab}			
T3	0.36±0.00	0.51±0.02	0.60±0.02	0.72±0.02	0.60±0.03	0.56±0.02^{b}			
T4	0.36±0.00	0.54±0.02	0.65±0.01	0.85±0.04	0.75±0.03	0.63±0.03^{a}			
Mean	0.36±0.00^{d}	0.51±0.01^{c}	0.62±0.02^{b}	0.75±0.02^{a}	0.63±0.02^{b}				

*Values are expressed as skin thickness of the Phytohemagglutinin centiimetres, 0, 6, 12, 24, 36 h after injection.
^{abc}Means bearing different superscripts in a row and column differ significantly

Table 4.15. Number of villi, villus height (V) (µm), villus width (µm), crypt depth (C) (µm), crypt width (µm), V:C ratio, infiltration, goblet cells

Attributes	T1	T2	T3	T4	SEM	P value
No of villi	3.3±0.25[b]	4.8±0.48[a]	3.5±0.29[ab]	4.8±0.25[a]	0.23	0.010
Villus height	285.4±13.11[b]	468.2±21.83[a]	397.4±35.38[a]	478.3±18.77[a]	22.53	0.000
Villus width	105.4±13.39	96.4±17.44	137.5±18.37	110.2±13.45	8.10	0.334
Crypt depth	316.6±8.68	299.2±17.83	385.1±38.25	341.1±20.25	13.51	0.114
Crypt width	55.0±3.01[a]	39.3±2.95[b]	45.1±4.88[ab]	38.9±2.83[b]	2.30	0.023
V:C ratio	0.9±0.03[c]	1.6±0.14[a]	1.0±0.01[bc]	1.4±0.14[ab]	0.08	0.001
Infiltration in lamina propria	+++	++	++	+		
Goblet cells	+++	++	++	+		

[abc]Means bearing different superscripts in a row differ significantly

Table 4.16. Effect of different treatments on blood biochemical

Attributes	Treatment	Period		Mean	P value		
		0 d	120 d		T	P	T×P
Total Protein (g/dl)	T1	6.4±0.20	6.6±0.15	6.5±0.13	0.180	0.004	0.381
	T2	6.5±0.20	7.1±0.19	6.8±0.15			
	T3	6.6±0.17	6.7±0.15	6.6±0.11			
	T4	6.6±0.12	7.1±0.11	6.9±0.10			
	Mean	6.5±0.08[b]	6.9±0.08[a]				
Albumin (g/dl)	T1	3.8±0.11	4.0±0.21	3.9±0.12	0.536	0.598	0.587
	T2	3.7±0.08	3.9±0.13	3.8±0.08			
	T3	3.9±0.15	4.0±0.13	4.0±0.10			
	T4	4.1±0.22	4.0±0.23	4.1±0.15			
	Mean	3.9±0.07	4.0±0.09				
Globulin (g/dl)	T1	2.6±0.18	2.7±0.18	2.6±0.12	0.381	0.052	0.403
	T2	2.7±0.26	3.2±0.12	2.9±0.15			
	T3	2.6±0.17	2.7±0.12	2.7±0.10			
	T4	2.5±0.28	3.1±0.25	2.8±0.20			
	Mean	2.6±0.11[b]	2.9±0.10[a]				
Glucose (mg/dl)	T1	95.9±2.83	101.7±1.52	98.8±1.72[b]	0.001	0.000	0.009
	T2	96.0±1.20	111.5±0.83	103.8±2.13[ab]			
	T3	99.7±3.24	106.5±2.19	103.1±2.09[ab]			
	T4	99.0±1.46	116.0±0.99	107.5±2.35[a]			
	Mean	97.6±1.16[b]	108.9±1.19[a]				
Cholesterol (mg/dl)	T1	54.6±2.87	52.3±2.91	53.4±2.00[a]	0.001	0.265	0.992
	T2	47.4±0.52	45.4±1.57	46.4±0.84[b]			
	T3	54.3±2.26	53.2±2.47	53.8±1.62[a]			

Table 4.16. Contd...

Attributes	Treatment	Period		Mean	P value		
		0 d	120 d		T	P	T×P
	T4	47.9±1.61	46.4±1.94	47.1±1.23[b]			
	Mean	51.0±1.14	49.3±1.25				
Triglycerides (mg/dl)	T1	28.9±1.64	34.0±2.16	31.4±1.47	0.621	0.007	0.623
	T2	29.3±1.56	30.9±1.81	30.1±1.17			
	T3	29.3±1.31	34.0±1.09	31.6±1.02			
	T4	28.9±1.64	30.9±1.91	29.9±1.24			
	Mean	29.1±0.74	32.4±0.90				
Serum urea (mg/dl)	T1	22.5±0.56	22.5±.52	22.5±0.37	0.528	0.730	0.996
	T2	22.2±0.35	22.2±0.34	22.2±0.24			
	T3	22.6±0.47	22.8±0.44	22.7±0.31			
	T4	22.1±0.26	22.2±0.31	22.1±0.19			
	Mean	22.3±0.20	22.4±0.20				
SGOT (IU/L)	T1	84.1±2.59	86.2±1.64	85.2±1.50	0.993	0.969	0.948
	T2	85.6±1.80	84.9±5.00	85.3±2.57			
	T3	85.4±1.69	85.6±3.33	85.5±1.80			
	T4	85.3±3.00	84.0±3.43	84.6±2.21			
	Mean	85.1±1.12	85.2±1.69				
SGPT (IU/L)	T1	40.0±3.85	43.8±2.21	41.9±2.20	0.982	0.172	0.984
	T2	39.7±3.42	41.8±2.28	40.7±2.00			
	T3	39.7±2.82	43.4±2.78	41.5±1.97			
	T4	40.2±3.28	42.2±2.95	41.2±2.15			
	Mean	39.9±1.60	42.8±1.23				

[abc]Means bearing different superscripts in a row differ significantly

Discussion

The results obtained in the present study to assess the effect of live *Saccharomyces cerevisiae* feeding on growth performance, nutrient utilization and immune response in crossbred (Landrace X Desi) early weaned piglets are discussed on the basis of sound scientific justifications in light of the available literature in this chapter.

5.1 NUTRIENT INTAKE AND DIGESTIBILITY

The beneficial effect of *S. cerevisiae* is attributed to the fact that it is a naturally rich source of proteins, minerals and B-complex vitamins (Davis, 1976). Yeast culture and its cell wall extract containing 1,3-1,6 D-glucan and mannanoligosaccharide, are the important natural growth promoters for modern livestock and poultry production. *S. cerevisiae* is considered as one of the most popular probiotics that, when administered through the digestive tract, have a positive impact on the hosts' health through its direct nutritional effect (Patterson *et al.*, 2003).

In the present study though there was no significant difference in intake of various nutrients (DM, OM, CP, EE, NDF, ADF, hemicellulose and total carbohydrates) between 28 days weaned groups (T1 and T2) and between 42 days weaned groups (T3 and T4), but numerically all the values were higher in the probiotic fed groups

(T2 and T4) as compared to their respective controls (T1 and T3). The data indicate that feeding of *S. cerevisiae* to the piglets had positive impact on nutrients intake. Similarly Crenshaw *et al.* (1986) observed that pigs weaned at 3 wk gained faster ($P<0.01$), consumed more feed ($P<0.01$) and utilized the feed more efficiently ($P<0.01$) than pigs weaned at 2 wk.

There was no difference in DM, OM, ADF, hemicellulose and total carbohydrates digestibilites among the four groups, whereas crude protein digestibility was significantly higher in *S. cerevisiae* fed groups (T2 and T4) as compared to their respective groups (T1 and T3) which were without any additive. The digestibilites of NDF and EE were significantly higher in 42 day weaned with *S. cerevisiae* fed group (T4) than 42 day weaned without *S. cerevisiae* fed group (T3), however, these values were also numerically higher, though not significant, in 28 day weaned with *S. cerevisiae* fed group (T2) than 28 day weaned without *S. cerevisiae* fed group (T1). Chen *et al.* (2005) using complex probiotic (*Lactobacillus acidophilus*, 1.0×10^7 CFU/g; *S. cerevisae*, 4.3×10^6 CFU/g; *Bacillus subtilis* 2.0×10^6 CFU/g) who observed that digestibility of DM and N was statistically similar in probiotic treated and control group. Spriet *et al.* (1987) who have used *Bacillus* products in pigs diet found similar results. Similar results were reported by Kornegay and Risley (1996) using *Bacillus* products for finishing pigs and Hale and Newton (1979) who used a *Lactobacillus* fermentation product in a corn-based diet. However, research of Maxwell *et al.* (1983) revealed improvements in DM and N digestibility when pigs fed diet with probiotics comprised of different bacteria strains. These discrepant findings may be due to differences in sanitary conditions of animals, composition of diet, quantity and type of probiotic added to diet and cfu content of diet. In the present study also, there is a positive influence of live *S. cerevisiae* feeding

on digestibilities of some nutrients. According to Scholten *et al.* (1999), feeding of live culture gives better response than lyophilized culture, because lyophilized culture takes time to start its biological expression in GI tract, whereas live culture starts working immediately as it enters the GI tract.

5.2 PLANE OF NUTRITION

In the present study the mean body weights and mean metabolic body weights (kg $W^{0.75}$) in groups T1 and T2 were statistically similar to each other, however, they were significantly lower ($P<0.05$) as compared to T4 and comparable with T3. The numerical value was higher in *S. cerevisiae* fed groups compared to their respective controls. This suggests that feeding of probiotics had positive effect on body weight gain. Similar improvements in piglet performance as a result of yeast supplementation have been reported by Maloney *et al.* (1998) and Mathew *et al.* (1998). DMI (g/kg $W^{0.75}$/d) and CP intake (g/kg $W^{0.75}$) in T1 and T2 groups were similar but the numerical value was higher in group T2 which was fed with probiotic. Similar trend was followed in 42 days weaned groups.

In the present study there was no significant difference in DCP intake between 28 days weaned groups and 42 days weaned groups, but the numerical value was higher in *S. cerevisiae* fed groups as compared to their respective controls. There was no significant difference in TDN intake (g/kg $W^{0.75}$) between 28 days weaned groups (T1 and T2) and 42 days weaned groups (T3 and T4). The TDN intake (g/kg $W^{0.75}$) in group T2 was significantly lower as compared to T3 but mean metabolic body weights (kg $W^{0.75}$) was comparable. It may be due to better utilization of feed. Mathew *et al.* (1998) also observed that overall gain tended to be higher for pigs fed diets containing yeast 1g/kg of diet.

5.2 GROWTH PERFORMANCE, FEED INTAKE AND FCR

The initial BW of the piglets in all the groups was statistically similar which exhibited increase in body weight during the period of 120 days of experimental feeding.

The final mean BW of 42 day weaned with probiotic fed group (T4) was significantly higher as compared to 28 day weaned groups (T1 and T2), but comparable with 42 day weaned without probiotic fed group (T3). The net BW gain and ADG followed the similar trend as final body weight. Similar improvements in piglet average daily gain as a result of yeast supplementation have also been reported by Jurgens *et al.* (1997), Maloney *et al.* (1998) and Mathew *et al.* (1998), while other studies found that yeast supplementation to weanling piglets had no effect on average daily gain (Kornegay *et al.*, 1995 and Jurgens *et al.*, 1997). These discrepant findings may be due to differences in sanitary conditions of animals, composition of diet, quantity, quality and type of yeast added to the diet. Van Heugten *et al*. (2003) found that live yeast supplementation had positive effects on nursery piglet performance, but only when diets contained growth-promoting anti-microbial substances.

The total feed intake and average daily intake were lowest in T1 and highest in T4 group, but the value in 28 day weaned with probiotic fed group (T2) and 42 day weaned without probiotic fed group (T3) was statistically similar to each other. The FCR value was significantly lowest in T4 and highest in T1 group, but the value in 28 day weaned with probiotic fed group (T2) and 42 day weaned without probiotic fed group (T3) was statistically similar to each other. Similar to the present study Bontempo *et al.* (2006) observed that piglet fed yeast (containing cfu 2×10^6/g) grew more efficiently as compared to those fed basal diet but the difference was not statistically significant. Overall gain tended to be higher for pigs fed diets containing yeast 1g/kg of diet (Mathew *et al.*, 1998).

5.4 BALANCE OF NUTRIENTS

5.4.1 Nitrogen balance

There was no significant difference between 28 days weaned groups (T1 and T2) and 42 days weaned groups (T3 and T4). The values in yeast supplemented groups were numerically higher; however, they were statistically similar. The nitrogen balance as percent of intake in group T1 was comparable with T2 and T3 groups, however, the value was significantly lower as compared to T4. The numerical value of T2 and T3 were similar to each other. The results revealed that feeding of live culture of *S. cerevisiae* modulate nitrogen metabolism in a positive manner. Similar to our finding, Kornegay and Risley (1996) observed that N retention was not affected by feeding Bacillus products. Contrary to our finding, Scheuermann (1993) reported enhanced N retention as a percentage of N intake primarily due to reduced urinary N excretion.

5.4.2 Calcium and Phosphorus balance

Intake of Ca and P followed the same trend as N. The Ca balance as percent of intake in group T1 was significantly lower as compared to T2, T3, and T4. Groups T2 and T3 were statistically similar. The P balance as percent of intake in group T1 was significantly lower as compared to T3 and T4 but the value was statistically similar to group T2.

5.5 FAECAL CHARACTERISTICS

Initially faecal consistency in 28 days weaned groups were soft (diarrhoeic) as compared to 42 days weaned groups. It may be due to early weaning which predisposes the weaned piglets to mal-absorption, possible dehydration and diarrhoea. Supplementation of *S. cerevisiae* improved the condition by increasing the villi surface

area which may enhance the absorption and reduced the incidence of dehydration and diarrhoea. The microbiota in the gut are unstable during the first week post-weaning and that it takes two to three weeks post-weaning for the gut microbes to fully develop their fermentative capacity and to reach a high level of stability (Jensen, 1998). Thus, supplementation of probiotics can reinforce the microflora composition during the post-weaning period.

The increased faecal yeast count in the groups T2 and T4 fed with live *S. cerevisiae* indicated an increased number of yeast in the gut. Similarly the decreased faecal coliform count in the groups T2 and T4 fed with live *S. cerevisiae* in the present study revealed a decreased number of coliform in the gut. Thus, it indicates that *S. cerevisiae* feeding improved the health by increasing yeast count and decreasing the number of harmful bacteria. The other studies have shown that *S. boulardii* can exert antagonistic effects against several bacterial pathogens, such as *Clostridium difficile* (Corthier *et al.*, 1986), *Salmonella typhimurium* (Rodrigues *et al.*, 1996), and *E. coli* (Czerucka and Rampal, 2002). These findings showed that the use of yeast can potentially alter gut microflora by selectively stimulating growth of beneficial bacteria and suppressing the growth of pathogenic bacteria. Giang *et al.* (2011) observed that the inclusion of the lactic acid bacteria (LAB) complex together with the mixture of *Bacillus* and *Saccharomyces* increased faecal LAB counts and decreased faecal *E. coli* counts in the grower pigs, but not in the finisher pigs. However, Mathew *et al.* (1998) reported no differences in the microflora in the stomach, duodenum, ileum, cecum, or colon of pigs supplemented with live yeast (1g/kg).

5.6 IMMUNOLOGICAL STATUS

The results of the present study suggest that the humoral immune response was not affected by early weaning as the antibody

titre was similar in group T1 and T3. The findings are in line with the observations of Crenshaw *et al.* (1986), who reported that total antibody titres against human red blood cells on 0, 4 and 16 d postweaning were not affected by age at weaning. The findings of our study showed that the antibody titre to sheep RBC increased in *S. cerevisiae* supplemented groups (T2 and T4) with regardless to weaning age. Probiotics are able to prevent intestinal diseases through both humoral and cell mediated immunomodulation (Erickson and Hubbard, 2000).

The intradermal response to phytohemagglutinin was reduced in pigs weaned at 28 days as compared to 42 days. Similar to the present findings, Blecha *et al.* (1983) reported that weaning pigs when younger than 5 wk old causes physiological changes which are detrimental to cellular immune reactivity. These changes could alter disease susceptibility in young pigs. In the present study the intradermal response to phytohemagglutinin was increased in T2 and T4 which were fed on live in *S. cerevisiae* culture irrespective of weaning age. Verduzco *et al.* (2009) also observed that dietary supplementation of 0.05% of yeast cell wall increased local mucosal IgA secretions, humoral and cell-mediated immune responses and reduced parasite excretion in faeces in chickens.

The finding was in accordance with the observation of Davis *et al.* (2004) who reported that dietary supplementation with phosphorylated mannans derived from yeast *(S. cerevisiae)* cell wall improved growth response and modulated immune function of weaning pigs. Scharek *et al.* (2007) observed that supplementation of probiotics (*Bacillus cereus var. toyoi*) affected the intestinal immune system of the piglets at the time of weaning and shortly thereafter such that the intestinal epithelium CD8/CD3 double positive cell populations were enhanced in the probiotic fed group. The observed trend of the

results suggested that effect of the weaning stressors can be compensated by feeding of yeast culture. The increased immune response might be due to interaction of probiotic with gut epithelial cells resulting in stimulation of the production of pro- and anti-inflammatory cytokines by these cell lines in a strain dependent manner (Delcenserie *et al.,* 2008), increased IgA production and stimulation of macrophage (Perdigon *et al.*, 1999), increased phagocytosis by macrophages in gut mucosa by YC supplementation (Shen *et al.*, 2009), increased blood leukocyte phagocytic and T-lymphocyte proliferative responses (Shu *et al.,* 2001).

5.7 SMALL INTESTINAL MORPHOLOGY

The results of the present study demonstrated that early weaning was characterized by a decrease in villus height. This may be due to postweaning low initial feed intakes and change in the diet. The similar finding was observed by Gall and Chung (1982) who have suggested that feed intake, rather than chronological age, per se, may have a more prominant influence on small intestinal maturation. Stragand and Hagemann, (1977) suggested that luminal distention may be involved as a factor in alteration of crypt cell mitotic rate. Kelly *et al.* (1991) and Pluske *et al.* (1996) reported that there was a reduction in villous height (villous atrophy) and an increase in crypt depth (crypt hyperplasia) at weaning. Similar findings were observed by Cera *et al.* (1988), McCracken *et al.* (1999), Tang *et al.* (1999) and Berkeveld *et al.* (2007) who have reported significant postweaning reduction in villus height. Villus atrophy is due to an increased rate of cell apoptosis from the villous apex or programmed death and decreased rate of renewal, which can be affected by cellular factors or endogenous stressors (Pluske et al., 1997 and Van der Peet-Schwering *et al.,* 2007). The crypt depth was more in early weaned group. The observed finding may be due to crypt hyperplasia at weaning.

An increase in villus height and villus:crypt ratio in the jejunum was observed in this study in pigs of groups T2 and T4 fed *S. cerevisiae* irrespective of weaning age when compared with pigs fed without *S. cerevisiae* culture (T1 and T2). These results agree with those from a study conducted by Shen *et al.* (2009) who observed that yeast culture supplementation improved the villus height and villus:crypt ratio in the jejunum. Gao *et al.* (2008) also observed that dietary supplementation of YC improved the villus:crypt ratio in the duodenum and jejunum in broiler chicken. However, Van der Peet-Schwering *et al*. (2007) reported that dietary supplementation of YC did not change villus height and crypt depth as well as villus:crypt ratio. Decreased number of pathogenic bacteria in the gut may improve proliferation of epithelial cells to build villus in the gut and thus enhanced intestinal morphology (Mourâo *et al.*, 2005). Baum *et al.* (2002) also found that villus length was greater in the small intestine of piglets fed yeast than controls. In poultry, Bradley *et al.* (1994) found no effect of yeast feeding on villus height while Zaouche *et al.* (2000) found differences in the intestinal mucosa in rats given *S. boulardii* orally. These contrasting results might be due to species-specific differences in response to *S. boulardii* supplementation.

The marked and abrupt morphological responses to weaning in the small intestine, characterized by the transformation from a dense fingerlike villi population to a smooth, compacted tongue-shaped luminal villi surface may indicate critical consequences for the young pig's digestive capacity and subsequent nutrients utilization during the starter phase. Development of intestinal morphology could reflect the health status of the GI tract of an animal. New epithelial cells are produced in the intestinal mucosal crypts and migrate along with the villi to the top (Schat and Myers, 1991). During the postweaning period the villus surface area of the small intestine is dramatically changed, at least for 7 to 14 d postweaning period. The shortened villi surface

area of the small intestine could predispose the weanling pig to mal-absorption, possible dehydration, diarrhea and enteric infection the conditions that are frequently encountered in group T1.

A deeper crypt in case of in *S. cerevisiae* supplemented groups (T2 and T4) may indicate faster tissue turnover to permit renewal of the villus, which suggests that the host's intestinal response mechanism is trying to compensate for normal sloughing or atrophy of villus due to inflammation from pathogens and their toxins. More energy would be required to support faster tissue turnover. Taller villi indicate more mature epithelia and enhanced absorptive function due to increased absorptive area of the villus. Greater villus height increases the activities of enzymes secreted from the tips of the villi (Hampson, 1986), resulting in improved digestibility.

Infiltration in lamina propria and goblet cell number were increased in early weaned piglets, however, supplementation of yeast can compensate the effect of weaning. In agreement with this study, Bradley *et al.* (1994) reported that goblet cell number and crypt depth in the ileal mucosa were reduced when the broiler diet was supplemented with *S. cerevisiae*. Zhang *et al.* (2005) reported greater villus height and improved performance in birds with supplementation of whole yeast or yeast cell wall. Cell wall components of YC (*b*-glucans and *á*-mannans) may provide a protective function to mucosa by preventing pathogens from binding to villi and allowing fewer antigens to be in contact with the villi. Zhang *et al.* (2005) reported the positive role of yeast cell wall in ileal mucosal development of broiler chicks.

5.8 BLOOD BIOCHEMICAL CONSTITUENTS

5.8.1 Serum proteins

The serum protein level indicates the balance between anabolism and catabolism protein in the body. The plasma protein

concentration at any given time is in turn a function of hormonal balance, nutritional status, water balance and other factors affecting the state of health. In the present study, serum total proteins, albumin and globulin remained within normal range and did not differ significantly ($P>0.05$) among different dietary treatments. The period-wise comparison of mean values of serum albumin and globulin were statistically similar, however, period-wise comparison of serum protein was significant ($P<0.01$). This may be due to improvement in animal appetite and feed utilization by the animals.

Our results are paralleled with that recorded by Bakr *et al.* (2009) who reported no significant difference in the levels of serum albumin and globulin in probiotic treated calves, however, they observed a significant increase in the blood serum levels of total proteins. The finding was also in harmony with that recorded by Sayed (2003) in probiotic treated kid. Chen *et al.* (2005) concluded that there was no effect of complex probiotic feeding on total protein and albumin.

5.8.2 Serum glucose

The glucose level is an indicator of the normal physiological condition and well being of animals. The results in the study clarified that the activities of glucose were significantly changed along the period of the experiments. The present finding is in line with the observation of Bakr *et al.* (2009). Serum glucose values increased both in T2 and T4 due to supplementation of in *S. cerevisiae* though the difference was not significant. The serum glucose level of T4 was significantly higher as compared to T1. Significant increase in serum glucose levels in probiotic treated buffalo calves was observed by Bakr *et al.* (2009). In contrary to the present results Antunovic *et al.* (2005) recorded low glucose levels in probiotic treated lambs.

5.8.3 Total cholesterol and triglycerides

The level of cholesterol was significantly decreased ($P<0.01$) in yeast fed piglets as compared to control. The value of triglycerides was also lower but non-significantly. The findings of the study were in agreement with Zacconi *et al.* (1992) and Taranto *et al.* (1998) who observed the low levels of cholesterol synthesis in animals treated with probiotics. Probiotics have the ability to conjugate with bile acids enzymatically increasing their rate of excretion and lead to the reduction of serum cholesterol.

5.9.4 Serum urea

Serum urea level in the present study was within the normal physiological range (Kaneko *et. al.*, 1997) and there was no effect of *S. cerevisiae* feeding. The obtained results were consistent with that reported by Sadeik and Boehm (2001) and Sayed (2003).

5.8.5 Serum ALT/SGPT and AST/SGOT

The values of both ALT and AST were within the normal physiological range (Kaneko *et. al.*, 1997) and no significant difference was evident in growing pigs irrespective of dietary treatments. The levels of ALT and AST were insignificantly changed along the period of the experiments. The findings were in agreement with that detected by Nahashon *et al.* (1992) and Sadeik and Boehm (2001), who reported that the activities of AST and ALT were normal and were similar in control and probiotic treated animals.

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Summary & Conclusion

Early weaning (21-35 days) is a normal commercial practice. At the time of weaning, young piglets are subjected to several stressors such as nutritional, environmental, social and microbial imbalance which result in to low feed intake, impaired intestinal morphology and function, a high incidence of diarrhoea and growth depression. Sub therapeutic use of antibiotics has been widely applied to nursery pigs to solve post-weaning problems. Growing public disquiet over the use of antibiotics as feed additives has encouraged recent commercial interest in prebiotics, probiotic and synbiotic as an alternate therapy against harmful pathogens in the gastrointestinal (G.I.) tract and to ameliorate the weaning stress. Keeping above in view, the present study was undertaken with objectives to study the effect of live *Saccharomyces cerevisiae* feeding on growth performance, nutrient utilization, metabolic profile, intestinal morphology and immune response in early weaned piglet

Forty eight crossbred (Landrace X Desi) piglets were assigned to four different treatments. In each group there were 12 piglets containing 4 replicates with equal male:female ratio of 3 animals in each replicate. The first group (T1) and the second group (T2) were weaned at 28 days of age while the third group (T3) and the fourth group (T4) were weaned at day 42 of age. T1 and T3 were fed basal

diet without live *S. cerevisiae,* however, T2 and T4 were supplemented with live *S. cerevisiae*. The experimental feeding trial was conducted for a period of 120 days. One metabolic trial of 6 days was conducted after 90 days of feeding. Faeces and urine were collected separately during the trial. The samples were collected, processed and stored as per the standard protocol. Feed and faecal samples collected during the trials were analyzed for proximate principles and fibre fractions. Blood was collected at the 0 day and at end of feeding trial. Serum were separated and kept in small plastic ependrops (2 ml) and stored at deep freezer (-20^0 C) for further analysis. Immune responses were discussed during last month of experiments. Basal diet was formulated with maize, soya bean meal, fish meal, wheat bran, salt, mineral and vitamin supplements as per NRC (1998). Crushed maize was fermented with *S. cerevisiae* ($2\text{-}3x10^6$ cfu/g feed) and fed at the rate of 200 g (as fed basis) per piglet.

In the present study though there was no significant difference in intake of various nutrients (DM, OM, CP, EE, NDF, ADF, hemicellulose and total carbohydrates) between 28 days weaned groups (T1 and T2) and between 42 days weaned groups (T3 and T4), but numerically all the values were higher in the probiotic fed groups (T2 and T4) as compared to their respective controls (T1 and T3). The data indicate that feeding of *S. cerevisiae* to the piglets had positive impact on nutrients intake. There was no difference in DM, OM, ADF, hemicellulose and total carbohydrates digestibilites among the four groups, whereas crude protein digestibility was significantly higher in *S. cerevisiae* fed groups (T2 and T4) as compared to their respective groups (T1 and T3) which were without any additive. The digestibilites of NDF and EE were significantly higher in 42 day weaned with probiotic fed group (T4) than 42 day weaned without probiotic fed group (T3), however, these values were also numerically

higher, though not significant in 28 day weaned with probiotic fed group (T2) than 28 day weaned without probiotic fed group (T1). The mean body weights and mean metabolic body weights (Kg $W^{0.75}$) in groups T1 and T2 were similar to each other, however, they were significantly ($P<0.05$) lower as compared to T4 and comparable with T3. The numerical value was higher in *S. cerevisiae* fed groups compare to their respective controls. This suggests that feeding of *S. cerevisiae* had positive effect on body weight gain. The DCP intake (g/d) of T3 and T4 was significantly higher ($P<0.01$) as compared to T1 and T2 and the numerical value was higher in *S. cerevisiae* supplemented groups irrespective of their respective controls. There was no significant difference in TDN intake (g/kg $W^{0.75}$) between 28 days weaned groups (T1 and T2) and between 42 days weaned groups (T3 and T4). The TDN intake (g/kg $W^{0.75}$) in group T2 was significantly lower as compared to T3 but mean metabolic body weights (kg $W^{0.75}$) was comparable. It may be due to better utilization of feed. Similarly Mathew *et al.* (1998) observed that overall gain tended to be higher for pigs fed diets containing yeast 1g/kg of diet. The initial BW of the piglets in all the groups was statistically similar which exhibited increase in body weight during the period of 120 days of experimental feeding. The final mean BW of 42 day weaned with probiotic fed group (T4) was significantly higher as compared to 28 days weaned group (T1 and T2), but comparable with 42 day weaned without probiotic fed group (T3). The net BW gain and ADG followed the similar trend as final body weight. The total feed intake and average daily intake was significantly lowest in T1 and highest in T4 group, but the value in 28 day weaned with *S. cerevisiae* fed group (T2) and 42 day weaned without *S. cerevisiae* fed group (T3) was similar to each other. The FCR value was significantly lowest in T4 and highest in T1 group, but the value in 28 day weaned with *S. cerevisiae* fed group (T2) and 42 day weaned without *S. cerevisiae* fed group (T3) was similar to each other.

The intake of nitrogen (g/d) was 41.5±2.50, 45.7±1.06, 65.7±4.54 and 65.9±2.49 in T1, T2, T3 and T4 respectively. There was significant ($P<0.01$) difference in nitrogen intake among four groups. However, there was no significant difference between group T1 and T2 and between group T3 and T4. The nitrogen balance as percent of intake in group T1 (41.4±2.84) was comparable with group T2 (49.8±1.99) and T3 (50.7±6.24), however, the value was significantly lower as compared to T4 (56.5±1.76), respectively. The mean values of group T2, T3 and T4 was statistically similar. The Ca balance as percent of intake in group T1 (43.7±0.69) was significantly lower as compared to T2 (47.7±0.79), T3 (47.8±1.05), and T4 (52.4±1.27). Groups T2 and T3 were statistically similar. The P intake (g/d) was 11.7±0.71, 12.9±0.30, 18.5±1.28 and 18.6±0.70 in T1, T2, T3 and T4 groups, respectively. There was significant difference ($P<0.01$) in P intake among four groups. However, there was no significant difference between group T1 and T2 and between group T3 and T4. The value in *S. cerevisiae* supplemented group was numerically higher; however, they were statistically similar. The P balance as percent of intake in group T1 (40.4±1.38) was significantly lower as compared to T3 (47.2±1.24) and T4 (52.5±0.93) but the value was statistically similar to group T2 (44.0±1.06).

The mean values of yeast count ($\times 10^4$/g) differed significantly ($P<0.05$), with values 52.3±2.23, 87.5±3.13, 54.8±3.36 and 82.1±2.46 in T1, T2, T3 and T4 groups, respectively. The values in T2 and T4 were significantly higher as compared to T1 and T3. The respective mean values of coliform count ($\times 10^4$/g) differed significantly ($P<0.05$), with values 157.5±15.78, 101.0±13.96, 146.3±10.51 and 101.0±13.96 in T1, T2, T3 and T4 groups, respectively. The respective values in T2 and T4 were significantly lower as compared to T1 and T3.

Humoral immunity of growing piglets was evaluated at day 0, 14 and 28 against injecting SRBC (i/m). Antibody response to SRBC in group T2 and T4 was significantly higher as compared to group T1 and T3. The DTH response to intra-dermal inoculation of PHA-p revealed that the skin thickness measured at 0 h in group T1, T2, T3 and T4 were 0.35±0.01, 0.36±0.02, 0.36±0.00, 0.36±0.00 cm, respectively, showing similar values (P>.05). However, the dermal enduration when compared among the different groups, there was a increase over the post-inoculation (PI) periods in all the four groups, with the values at 6, 12 and 24 h PI showing greater increase (P<0.01) in comparison to that of 0 h, before subsiding to basal levels at 36 h PI. When compared among the different groups, there was a significant (P<0.05) increase in skin thickness in group T4 as compared to group T1 and T3 but the value was comparable to T2. The results revealed that CMI response of the group weaned at 28 days supplemented with *Saccharomyces cerevisiae* was comparable with 42 days weaned group T3 and T4, however, significantly higher as compared to T1.

Average no. of villi in 100µm distance was significantly (P<0.01) lower in group T1 (3.3±0.25) as compared to T2 (4.8±0.48) and T4 (4.8±0.25) and comparable with group T3. The mean values of jejunal villus height (µm) were 285.4±13.11, 468.2±21.83, 397.4±35.38 and 478.3±18.77 in group T1, T2, T3 and T4, respectively. The values were significantly higher in *S. cerevisiae* fed group. The results of the present study showed that early weaning induced an increase in crypt width (T1) which can be decreased by yeast supplementation (T2) and the value was comparable with T3 and T4. The viilus height (V) to crypt depth (C) ratio in group T1 (0.9±0.03) was significantly lower (P<0.05) as compared to group T2 (1.6±0.14) and T4 (1.4±0.14) but statistically similar with group T3 (1.0±0.01). Early weaning induced a reduction in V:C ratio (T1) which can be enhanced by yeast supplementation.

The concentrations of blood biochemical constituents were within the normal range (Kaneko *et al.*, 1997). There were no significant differences in total proteins, albumin, globulin, serum urea, ALT and AST among the group T1, T2, T3 and T4. The period-wise comparison of mean values of serum albumin and globulin were statistically similar ($P>0.05$), however, period-wise comparison of protein was significant ($P<0.01$). The mean glucose (mg/dl) value of T4 (107.5±2.35) was significantly higher ($P<0.05$) than T1 (98.8±1.72) and comparable with T2 (103.8±2.13) and T3 (103.1±2.09). However, upon period-wise comparison, the value did show significant ($P<0.01$) influence of time. The plasma total cholesterol level (mg/dl) was found to be lower in T2 (46.4±0.84) and T4 (47.1±1.23) as compared to T1 (53.4±2.00) and T3 (53.8±1.62). The value of triglycerides in yeast supplemented groups (T2 and T4) was numerically lower than non-supplemented groups.

CONCLUSIONS

From the results of the present study it can be concluded that:

- Weaning of piglets is characterized by stress and reduced growth performance.
- Supplementation of live *Saccharomyces cerevisiae* was effective in improving the performance of early weaned piglets.
- *Saccharomyces cerevisiae* feeding improved the immune response and intestinal health of the piglets.

FURTHER STUDIES

- Feeding of diets containing higher concentration of Saccharomyces *cerevisiae* needs to be done.
- Combinations of various probiotics may also be tried. *(Lactobacillus+Saccharomyces*).

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Mini Abstract

In order to assess the effect of feeding live *Saccharomyces cerevisiae* on the performance of early weaned crossbred (Landrace X Desi) piglets, an experiment was conducted using forty eight piglets. They were assigned to four different groups following completely randomization design, twelve piglets in each group having 4 replicates with equal male female ratio of three animals in each replicate. T1 and T2 were weaned at 28 days of age while T3 and T4 were weaned at day 42 of age. Piglets were fed concentrate mixture as per NRC (1998) consisting of maize, soyabean, wheat bran and fish meal as a major ingredients. T1 and T3 were fed basal diet without live *S. cerevisiae*, however, T2 and T4 were supplemented with live *S. cerevisiae* (200 g/d/piglet containing 2-3x10^6 cfu/g). The intake of all the nutrients was higher ($P<0.05$) in T3 and T4 as compared to T1 and T2. There was no significant difference in intake of various nutrients (DM, OM, CP, EE, NDF, ADF, hemicellulose and total carbohydrates) between 28 days weaned groups (T1 and T2) and between 42 days weaned groups (T3 and T4). There was no difference in DM, OM, ADF, hemicellulose and total carbohydrates digestibilites among the four groups, except crude protein digestibility was significantly higher in S. cerevisiae fed groups (T2 and T4) as compared to their respective control (T1 and T3). The digestibilites of NDF and EE were significantly higher in 42 day weaned with probiotic fed group (T4) than 42 day weaned without probiotic fed group (T3). Total body weight gain and ADG was higher ($P<0.05$) in T4, lower in T1 and comparable between T2 and T3. Values of FCR were higher ($P<0.05$) in T1, lower in T4 and comparable between T2 and T3. Similarly the cost of per kg BW gain was same in T2 and T3 but was higher in T1 and lower in T4. Piglets of all the groups were in positive balance of N, Ca and P. Faecal yeast count was higher ($P<0.05$) in T2 and T4 as compare to non-supplemented group, however, Coliform count was lower in T2 and T4 as compare to non-supplemented group. The immune response (Humoral and CMI) was higher in *S. cerevisiae* supplemented group (T2 and T4) as compared to non-supplemented group (T1 and T3). The villus height was increased in T2 and T4 group but the value was comparable between T2 and T3. Villus and crypt ratio was increased in T2 and T4 group but the value was comparable between T2 and T3. Based on the results obtained it was concluded that the stress and constraints of the early weaning may be ameliorated by the supplementation of live *S. cerevisiae* and performance of piglets may be enhanced.

लघु सारांश

शीघ्र दुग्ध विमुक्त संकर (लैंडरेसX देसी) शूकर शावकों के निष्पादन पर जीवित सैकरोमाइसिस सेरेविसी खिलाने के प्रभाव का आकलन करने के लिये अड़तालीस संकर शूकर शावकों पर एक प्रयोग किया गया। उन शूकर शावकों को पूर्णतया यादृच्छिक अभिसंरचना के आधार पर चार वर्गों में इस प्रकार विभक्त किया गया कि प्रत्येक वर्ग में बारह शावकों में नर एवं मादा का अनुपात बराबर रहे एवं बनायी गयी चार प्रतिकृतियों में प्रत्येक में तीन–तीन शूकर शावक रखे जायें। परीक्षण वर्ग 1 एवं 2 को जन्म के 28वें दिन तथा तीन व चार को जन्म के 42वें दिन दुग्ध विमुक्त किया। सभी शूकर शावकों को एन.आर.सी. (1998) के आधार पर दाना मिश्रण खिलाया गया, जिसमें मक्का, सोयाबीन, खली, चोकर एवं मछली के चूरें को मुख्य अवयव के रूप में रखा गया। परीक्षण वर्ग 1 व 3 को सैकरोमाइसिस सेरेविसी रहित आहार तथा वर्ग 2 व 4 को सैकरोमाइसिस सेरेविसी युक्त (2-3x10^6 सी.एफ.यू प्रति ग्राम 200 ग्राम/शूकर/प्रतिदिन) आहार दिया गया। वर्ग 3 व 4 में वर्ग 1 एवं 2 की तुलना में पोषक तत्वों की आहार ग्राहयता सार्थक रूप में अथिक पायी गयी जबकि इन वर्गों में आपस में तत्वों की ग्राहयता समान पायी गयी। अपरिष्कृत प्रोटीन की पचनीयता को छोड़कर (जो वर्ग 1 व 3 के सापेक्ष वर्ग 2 व4 में अधिक पायी गयी) अन्य तत्वों की पचनीयता समान पायी गयी। एन. डी.एफ. एवं ईथर सार की पचनीयता 42वें दिन दुग्ध विमुक्त एवं सैकरोमाइसिस सेरेविसी खिलाये गये वर्ग के शावकों में नहीं खिलाये गये वर्ग से अधिक पायी गयी। कुल भार वृद्धि एवं वृद्धि दर वर्ग 4 में सार्थक रूप से अधिक, वर्ग 1 में सबसे कम तथा वर्ग 2 व 3 में आपस में समान पायी गयी। आहार परिवर्तन अनुपात वर्ग 1 में अधिक 4 में सबसे कम तथा 2 व 3 में समान पाया गया। इसी प्रकार प्रति किग्रा भार वृद्धि की कीमत वर्ग एक में सर्वाधिक चार में सबसे कम तथा वर्ग 2 व 3 में समान पायी गयी। सभी वर्ग में शूकर शावकों में नाइट्रोजन, कैल्सियम एवं फास्फोरस का धनातम संतुलन पाया गया। वर्ग 2 व 4 के शूकर शावकों के विस्ठा में यीस्ट की संख्या सैकरोमाइसिस सेरेविसी नहीं खिलाये गये शावकों के सापेक्ष अधिक पायी गयी जबकि इन वर्गो में कोलीफार्म की संख्या इसके विपरीत पायी गयी। प्रतिरोधक क्षमता (हयूमोरल एवं सी.एम.आई) सैकरोमाइसिस सेरेविसी खिलाये गये शावकों में (वर्ग 2 व 4) नहीं खिलाये गये शावकों (वर्ग 1 व 3) के सापेक्ष अधिक पायी गयी। विलए की ऊँचाई वर्ग 2 व 4 में अधिक पायी गयी परन्तु वर्ग 2 व 3 में समानता पायी गयी। इसी प्रकार विलए एवं क्रिप्ट का अनुपात वर्ग 2 व 4 में बढ़ा हुआ पाया गया जबकि वर्ग 2 व 3 में इसमें समानता पायी गयी। उपरोक्त प्रभावों को देखते हुये यह निष्कर्ष निकलता है कि सैकरोमाइसिस सेरेविसी के संभूरण में संकर शूकर शावकों में शीघ्र दुग्ध विमुक्त होने वाले तनाव व दवाब को कम किया जा सकता है।

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