V. DISCUSSION

The results of the experimental study on the effects of *Saccharomyces cerevisiae* (*Sac. cerevisiae*), *Lactobacillus sporogenes* (*L. sporogenes*), their combination and Provilacc® on growth performance, relative organ weights, activity of digestive enzymes, serum biochemical components and deposition of nutrients in muscular, skeletal and hepatic tissues in broiler chickens have been discussed in this chapter.

5.1. Performance

5.1.1. Body weight gain (g)

As indicated in Table 1 and Fig. 1, it is observed that the body weight of broiler chicks on day 21, 28, 35 and 42 were significantly higher in all the live probiotic culture supplemented groups compared to the control group. The Group II supplemented with *Sac. cerevisiae* showed significant (*P*<0.05) improvement of body weight on day 21, 28, 35 and 42. These observations are in conformity with Bonomi *et al.* (1977a), Krueger *et al.* (1990), McDaniel and Sefton (1991), Stanely *et al.* (1993), Bradley *et al.* (1994), Kotrbacek *et al.* (1994), Upendra (1999), Kompiang (2002) and Upendra and Yathiraj (2003). However, Yadav *et al.* (1994) and Sarkar *et al.* (1997) did not observe significant increase in body weight in *Sac. cerevisiae* supplemented broiler chicks. Improvement in body weight gain in broiler chickens following supplementation of *L. sporogenes* (Group II) observed in this study on

In the present study, the improved body weight was also observed in the group supplemented with both *Sac. cerevisiae* and *L. sporogenes* on day 21, 28, 35 and 42. Similar observations have been made earlier by Burkett et al. (1977), Katoch et al. (1996), Kahraman et al. (1997) and Reddy (2001). However, Megharaja et al. (1996) did not observe significantly improved weight gain in broiler chickens in a biological trial of six weeks duration. In the Group V also, where the supplementation was with Provilacc®, a commercial probiotic with a mixture of four types of probiotic species, the body weight gain was significantly improved. Such observations in improvements of body weight when a mixture of probiotics were administered have been reported by Mishra and Khan (1994) with Biovet®, Georgieva et al. (2000) with Lacto-Sacc® and Bhat et al. (2003).
Significant increase in body weight gain on supplementation of probiotics in different groups observed in the present study could be possibly due to alleviation of stress in birds by providing necessary vitamins, release of unidentified growth factors, secretion of digestive enzymes and release of high biological value protein (Stanely et al., 1993), improving protein digestibility (Bonomi et al., 1977a), changing intestinal microflora to favorable side and reducing the population of pathogenic enteric bacteria by competitive exclusion (Lloyd et al., 1977; Guo et al., 1991; Juven et al., 1991; Seema and Johri, 1992; Barrow, 1992; Vandevoorde et al., 1993; Jin et al., 1997; Ingledew, 1999 and Yi et al., 2001), counteracting aflatoxins by adsorption of aflatoxins by the mannan-oligosaccharides present in the yeast cell wall (Devegowda et al., 1994) alteration in ileal morphology for better absorption of nutrients (Bradley et al., 1994) improving the capabilities of immune response in the host (Perdigon et al., 1995 and Jin et al., 1997), better nutrient utilization (Peppler, 1982; Lyons, 1986), production of lactate from lactobacilli (Jernigan et al. 1985), source of nutrients (Rose, 1987; Rowland, 1992), secretion of digestive enzymes (Goodenough and Kleyn, 1976; Rowland, 1992; Vranesic, 1992; Jin et al., 1997 and Buhler et al., 1998) and synthesis of vitamins (Rowland, 1992).

In the present study also, the increase in body weight gain was due to better nutrient utilization by increased secretion of digestive enzymes, viz., alpha amylase (Table 13 and 14 and Fig. 13 and 14), trypsin (Table
15 and Fig. 15) and lipase (Table 16 and Fig. 16) and probably due to better synthesis of vitamins, release of unidentified growth factors, acting as source of nutrients, alteration in ileal morphology, counteraction of aflatoxins, improved immune response, competitive exclusion of pathogenic enteric bacteria and alleviation of stress.

5.1.2. Feed conversion ratio

In the present biological trial it can be observed from Fig. 2 that the feed conversion ratio in broiler chicks on day seven, 21 and 28 were significantly (P<0.05) better in all the probiotics supplemented groups compared to control group. Also on day 35 and 42, the FCR was numerically better in probiotics supplemented groups compared to control group. The positive observations on FCR in the present study for the group receiving *Sac. cerevisiae* were in agreement with Bonomi *et al.* (1977a), Bonomi *et al.* (1977b), Krueger *et al.* (1990), McDaniel and Sefton (1991) and Upendra (1999). Contrary to this, Yadav *et al.* (1994) and Sarkar *et al.* (1997) did not report significant variation in feed efficiency in broiler chickens upon supplementation of *Sac. cerevisiae*. The better FCR observed in *L. sporogenes* received groups were in conformity with Tortuero (1973), Hussein and Ashrey (1991), Takalikar *et al.* (1992), Nahashon *et al.* (1993), Manickam *et al.* (1994), Ramesh *et al.* (2000) and Khalid *et al.* (2002). The improved FCR observed in the Group IV that received both *Sac. cerevisiae* and *L. sporogenes* were in conformity with Burkett *et al.* (1977) and Katoch *et al.* (1996). The
improved feed conversion efficiency observed in Provilacc® supplemented group were in conformity with Bhat et al. (2003).

Improvements in feed conversion efficiency recorded on supplementation with Sac. cerevisiae, L. sporogenes, their combination and Provilacc® could be due to the same factors responsible for improvement in body weight gain in broiler chickens.

5.1.3. Mortality

No mortality occurred in the present study at any stage of the experiment. This observation was in conformity with the opinions of various workers that it could be due to reduced pathogenic bacterial load in the gut (Lloyd et al., 1977; Guo et al., 1991; Juven et al., 1991; Seema and Johri, 1992; Barrow, 1992; Vandevoorde et al., 1993; Jin et al., 1997; Ingledew, 1999 and Yi et al., 2001), improved immune status of birds (Perdigon et al., 1995 and Jin et al., 1997), counteracting aflatoxins (Devegowda et al., 1994) and removal of stressors (Jin et al., 1997).

5.2. Organ weights

The mean relative crop weight in the probiotic supplemented groups (Group II, III, IV and V) was significantly (P<0.05) lower compared to control group (Table 3 and Fig. 3) on day seven. In the available literature there is paucity of information either on the promotion or inhibition of the growth of crop in probiotic supplemented groups. Based on the results obtained in the present investigation, it is opined that the probiotics established in the crop at the early stage of broiler growth, i.e.
at day seven, perhaps reduced the growth of crop, since the established probiotics themselves take up the production of alpha amylase (Table 13 and Fig. 13). Whereas in the control group, since the amylolytic action is to be carried out by host enzymes only, there was regular development of the crop to secrete sufficient quantities of alpha amylase and hence the significantly (P<0.05) increased mean relative crop weight in the control group.

The mean proventriculus (Table 4 and Fig. 4), gizzard (Table 5 and Fig. 5.), liver (Table 8 and Fig. 8.), heart (Table 9 and Fig. 9.), kidney (Table 10 and Fig. 10.) weights were not significantly different (P<0.05) at various stages of observation in the groups in the present investigation. This was in conformity with the observations of the earlier workers, viz., dietary supplementation of probiotic in broiler chickens ration did not show any influence on the weight of internal organs (Mandal, 1994; Kaistha et al., 1996; Mohan et al., 1996) and probiotic had no influence on the weight of internal organs such as liver, heart and gizzard (Panda et al., 2000). The growth of these organs followed isometric growth with increased body weight in probiotic supplemented groups.

However, the mean relative spleen weight in Provilacc® treated group (Group V) was significantly (P<0.05) higher from Group IV on day 35 and Group II on day 42. Organ body weight ratio was taken as one of the criteria to assess immune status of the birds (Arun, 1992 and Devegowda et al. 1994). Also, there are reports indicating that the probiotic supplementation improved the immune response (Perdigon et
In the present study, the significantly (P<0.05) increased spleen weight in the Provilacc® treated group is attributed to the immunostimulatory status achieved in the spleen, a lymphoid organ.

The mean intestinal weight (Table 6 and Fig. 6) on day 42 in Provilacc® supplemented group was significantly (P<0.05) higher from Group I that did not receive any probiotic and the mean values in Group II, III, IV were numerically higher compared to control group and hence the better weight gain (Table 1 and Fig. 1) in probiotic supplemented groups. These findings were in agreement with the opinions that the ability of the alimentary tract to digest large amounts of food was presumably related to relative size of the digestive tract (Dror et al., 1977), allometric growth of small intestine (Nitsan et al., 1991a) and development of digestive tract was an important aspect of growth (Sell et al., 1991 and Uni et al., 1995).

The mean relative weight of pancreas (Table 7 and Fig. 7) on day 42, in Group IV, supplemented with the combination of Sac. cerevisiae and L. sporogenes, differed significantly (P<0.05) from mean value of Group I, II and III. Palo et al. (1995) recorded increased pancreas weight in ad libitum fed birds compared to feed restricted birds and this property was attributed to demand for more exocrine secretion from the pancreas. It was also reported that Sac. cerevisiae contained 40 per cent protein, 25 per cent polysaccharides and 15 per cent nucleic acids (Rose, 1987). In the present study significantly (P<0.05) increased weight of pancreas in Group IV that received combination of Sac. cerevisiae and L.
sporogenes and the numerically higher pancreas weight observed in Group II and Group V that also received only Sac. cerevisiae and the Provilacc® that contained Sac. cerevisiae along with other probiotics, respectively, have positively stimulated the pancreas to increase the exocrine secretion to supply digestive enzymes which can virtually digest rich content of proteins, carbohydrates and nucleic acids present in the Sac. cerevisiae, the yeast.

5.3. Length of small intestine

The length of small intestine (Table 12 and Fig. 12) was not significantly different (P>0.05) in different groups on various days of observation. However, in the probiotic fed groups the small intestine length was numerically higher on day 21, 28, 35 and 42. Parallelism was observed between the slight increase in the length of small intestine and growth promotion. The observation is in concurrence with the hypothesis that the development of the gastrointestinal tract is an important aspect of growth (Sell et al., 1991).

5.4. Digestive enzymes

Statistically significant (P<0.05) difference of crop alpha amylase between control and the groups that received probiotics on all the days of observation was found (Table 13 and Fig. 13.). Certain carbohydrates are degraded in the crop and the breakdown was attributed to enzymes of dietary origin or bacterial fermentation (Bolton, 1965 and Pritchard, 1972). alpha amylase was present in crop (Sturkie, 1976), alpha amylase of crop may originate from the salivary glands, ingested food, and bacteria in the crop or regurgitated duodenal contents and a significant amount of starch digestion occurs in the crop of the chickens as the result of bacterial action (Duke, 1996). A number of workers
(Rose, 1980; Goodenough and Kleyn, 1976; Rowland, 1992; Vranesic, 1992; Stanely et al., 1993; Wedberg, 1996; Jin et al., 1997; Anon., 2002; Upendra and Yathiraj, 2003; Schneitz et al., 1998; Baghel and Singh, 2004 and Palod and Singh, 2004) attributed the gain in body weight to the release of enzymes by the probiotics. The findings of statistically significant (P<0.05) levels of alpha amylase enzyme in the crop contents of the probiotics supplemented groups are in corroboration with these workers.

Significantly higher (P<0.05) levels of intestinal alpha amylase (Table 14 and Fig. 14), be of pancreatic origin or probiotic origin or both, was found in probiotic treated groups (Group II, III, IV and V) on day seven, 14, 21, 28, 35 and 42. Similar results were also found with respect to intestinal lipase levels (Table 16 and Fig. 16). The trypsin levels (Table 15 and Fig. 15) were significantly higher (P<0.05) on day 35 in all the probiotic treated groups whereas on day 42 it was only in Group II. These increased levels of digestive enzymes in the present study are in accordance with the findings of earlier workers. Rose (1980) reported secretion of digestive enzymes: nucleases, proteases, glucanases, mannases, lipases and amylases by yeasts. Various workers have opined about the enzyme secretion by probiotics; lactic acid bacteria secrete lactase (Goodenough and Kleyn, 1976), Probiotics stimulate host enzymes involved in the digestion of complex nutrients or they provide a probiotic source of digestive enzymes (Rowland, 1992), probiotics simulate metabolic process relating to feed digestion (Vranesic, 1992). Sac. cerevisiae served as source of enzymes (Stanely et al., 1993), liberation of enzymes by yeasts (Wedberg, 1996), probiotics alter metabolism by increasing digestive enzyme activity (Jin et al., 1997). L. sporogenes is considered as live enzyme factory (Anon., 2002), increase in body weight in broiler chickens due to release of enzymes by probiotics (Upendra and Yathiraj, 2003), improved degradation of β glucans and arabinoxylans due to increased enzymatic activity in Broilact® treated
group (Schneitz et al., 1998), probiotics establish an environment to increase digestibility of feeds (Baghel and Singh, 2004) and probiotics causes better digestibility (Palod and Singh, 2004).

Intestinal maltase (Table 17 and Fig. 17) and sucrase (Table 18 and Fig. 18) levels were not significantly different (P>0.05) between control and probiotic supplemented groups. On the activities of disaccharidases and other enzymes of the intestinal origin, the earlier workers indicated that the bacterial enzymes enriched the digestive capacity in the lower intestine (March, 1979), activity of brush border enzymes was an index of maturity and absorptive capacity of mucosal cells in chicken gut (Allen, 1987), little information is available on intestinal disaccharidases in avians (Sell et al., 1989), significant effect of probiotics was observed on the development of sucrase, lactase and tripeptidase activities before weaning in piglets (Collington et al., 1990). However, in the available literature, the specific reports on the role of probiotics on the activities of disaccharidases in intestines of broiler chickens are lacking. The probiotics are capable of secreting disaccharidases and this character is exploited to produce disaccharidases commercially (Ingledew, 1999). The levels of disaccharidases in the present biological study did not vary significantly (P>0.05) in the probiotic treated groups from the control and it is attributed to lower level of secretion of these enzymes.
5.5. Variable blood serum metabolites

The mean serum metabolites, viz., glucose, total protein, calcium, phosphorus, triglycerides, cholesterol, high density lipoproteins and low density lipoproteins were not statistically significant (P>0.05) between untreated Group I and probiotic supplemented groups (Group II, III, IV and V).

There is paucity of information on blood glucose levels in probiotic supplemented groups. In the present study there was no significant (P>0.05) difference between different groups in serum glucose levels (Table 19 and Fig 19) and it is possibly due to higher metabolic rate in the avian species that regulates the blood glucose within normal limits to maintain constant internal environment.

In the present investigation the levels of serum total protein was not significantly different (P>0.05). The values are presented in Table 20 and Fig 20. The results of the present study are in confirmation with the reported non – significant effect on serum total protein levels in Sac. cerevisiae supplemented group (Victor et al., 1993) and in probiotic supplemented groups (Mandal et al., 1994 and Gohain and Sapkota, 1998).

The serum calcium (Table 21 and Fig 21) and phosphorus (Table 22 and Fig. 22) levels did not differ significantly (P>0.05) in the present study. Nahashon et al. (1994b) reported higher phosphorus retention with lactobacillus supplementation along with 0.25 per cent available phosphorus which resulted in increased levels of serum phosphorus. The findings of the present
study are in agreement with Mandal et al. (1994) and Swain et al. (2001) who reported non significant variation in the levels of serum calcium and phosphorus in probiotic supplemented groups. However, on day 42, in the Group V that received Provilacc®, the numerically lower levels of triglycerides (Table 23 and Fig. 23) were found compared to control and other probiotic supplemented groups. Panda et al. (2001) reported non significant effect of probiotics on triglycerides. The results of the present study are in conformity with Panda et al. (2001).

Also, on different days of observation numerically lower levels of cholesterol (Table 24 and Fig. 24) were observed in all the probiotic supplemented groups. In probiotic treated groups the significantly lowered cholesterol levels were observed by Victor et al. (1993), Mohan et al. (1996) and Panda et al. (2001). Panda et al. (2001) explained that the cholesterol lowering effect of probiotics was due to conjugating effects of bile salts with probiotics in the intestine. The findings of the present study are in affirmation with Gohain and Sapkota (1998) who reported numerically reduced serum cholesterol in probiotic fed groups and in the present study it is concluded that probiotics reduce serum cholesterol numerically. The HDL (Table 25 and Fig. 25) and LDL (Table 26 Fig. 26) levels were not significantly differing (P>0.05) between control and probiotic supplemented groups. The findings of the present study are in conformity with Panda et al. (2001) who reported non significant effect of probiotics on HDL and LDL levels.
The serum VLDL levels (Table 27 and Fig. 27) are significantly (P < 0.05) differing in Provilacc® fed group on day 14 with Group I, III and IV and on day 35 with Group I and II. But, Panda et al. (2001) observed no probiotic effect on VLDL levels. The results of the present study with respect to Group II, III and IV are in conformity with Panda et al. (2001).

In the present study, the biochemical components like serum glucose, total protein, calcium, phosphorus, high density lipoproteins and low density lipoproteins that did not differ significantly (P>0.05) in various groups is attributed to maintenance of constant milieu interior in fast growing birds fed with probiotics.

Since the people are becoming much aware about the contents of lipid components in broiler chicken meat, the effect of probiotics in reducing one or the other lipid components in the broiler chicken meat either statistically significantly or numerically lower levels is desirable and requires numerous studies with reproducible results. This aspect will help in producing biotechnologically low lipid chicken meat or low cholesterol chicken meat similar to low cholesterol eggs or diet eggs.

5.6. Liver Glycogen
The liver glycogen values observed in the present study are presented in Table 28 and Fig. 28. In the probiotic supplemented groups, the liver glycogen levels were not affected significantly (P>0.05) compared to control group. However, there are no reports in this regard in the available literature that could be correlated.
5.7 **Proximate components in breast muscle.**

The moisture per cent (Table 29 and Fig. 29), crude protein per cent (table 30 and Fig. 30), ether extract per cent (Table 31 and Fig. 31) and total ash per cent (Table 32 and Fig. 32) in breast muscle sample did not differ significantly (P > 0.05) between different groups at various days of observation. However, on day 42, there was numerically slight increase in crude protein and decrease in ether extract levels of probiotic fed groups. This observation of slight increase of crude protein per cent is in agreement with Buche *et al.* (1992) who reported maximum retention of nitrogen in broilers supplemented with probiotics.

The non–significant (P>0.05) levels of moisture, ether extract and total ash were in accordance with Bhatti *et al.* (2003) who reported non significant levels of these components in breast muscle of Fayoumi and Rhode Island Red birds. However, the information on changing pattern of proximate components, if any, in probiotic fed broiler chicken is not available.

5.8 **DNA content in breast muscle**

The DNA content in breast muscle (Table 33 and Fig. 33) was not significantly different (P>0.05) between control and probiotic supplemented groups. The values in all the groups are in conformity with the values reported by Scanes *et al.* (1975). However, the specific information on DNA content in muscle samples is not available in probiotics treated broiler chickens. The DNA content did not reveal any
tracings between slow growing and fast growing birds such as control and probiotics treated groups, respectively.

### 5.9 Total ash from tibial bones

The tibial total ash (Table 34 and Fig. 34) was not significantly different (P>0.05) between control and probiotic supplemented groups. Better phosphorus utilization was indicated by tibial ash in yeast included birds by Guevera et al. (1978) and maximum mineral retention in probiotic supplemented birds by Buche et al. (1992). Leterrier and Nys (1992) opined that lower mineral density and higher porosity is associated with increased rate of growth.

The findings of the present study are in conformity with Leterrier and Nys (1992) and since the birds are at growing stage the significant difference was not observed between various groups. Endorsement is also made on the opinion that the research into skeletal biology of farm species is relatively neglected and needs better understandings of the possible manipulation of skeletal growth and development (Loveridge, 1999).

In the present investigation, the levels of liver glycogen, proximate components and DNA in muscle as well as total ash in bone did not differ significantly (P>0.05). This unchanging pattern of chemical composition in end organs of growth in probiotic fed birds signifies that, it is the change in mass and length of the tissues that takes place during physiological growth than the changes in chemical composition.
5.10 Ultrasonographic studies for bone density

The qualitative bone density based on ultrasonographic studies (Plate 1a, Plate 1b and Plate 1c) are also in conformity with Leterrier and Nys (1992) and the density was indirectly depending on porosity of bones such that at younger age the bones were more porous and as the age advanced the bones became less porous. In the present ultrasonographic study, it is concluded that more porous cartilaginous growth plate is an indication of less mineral deposition in the bones at younger age pointing towards continuation of growth of skeletal system. Less porous cartilaginous growth plate indicated more mineral accumulation at advanced age leading to endochondral ossification that tend to cease the further growth of the skeletal system.