CHAPTER V
DISCUSSION

The results of the biological trial conducted to evaluate the impact of enzyme addition to corn-soy based broiler diets have been discussed in this chapter.

5.1. Nonstarch polysaccharides composition of corn and soybean meal

Soybean meal (SBM) is the most commonly used vegetable protein source in the poultry diets. However, the inclusion of high concentrations of SBM as sole protein source in broiler diets can adversely affect performance due to presence of trypsin inhibitors, hemagglutinins, nonstarch polysaccharides (NSP) and phytate phosphorus (Irish and Balanave, 1993). The nutritive value of SBM also depends on the amount of indigestible carbohydrates in particularly, the amount of oligosaccharides and nonstarch polysaccharides (NSP).

SBM contained approximately six per cent oligosaccharides, six per cent soluble NSP and 18 to 21 per cent insoluble NSP (Knudsen, 1997). The values obtained in the present study are in accordance with the findings of Smith and Annison (1996), Kuo et al. (1998), Malathi and Devegowda (2001), and Ramesh and Devegowda (2004). Corn contained 9.8 per cent of total NSP and this observation was in accordance with the findings Nyman et al. (1984), Smits and Annison (1996) and Malathi and Devegowda (2001), whereas in contrast to this finding, Knudsen (1997) reported lower NSP content of six per cent with 0.5 per cent of soluble NSP in corn.

The results of the present study indicate that SBM too contain considerable/substantial quantity of oligosaccharides and NSP, which are not utilised by the poultry due to lack of endogenous enzymes. These data suggested that, there is a lot of scope to improve nutritive value of corn-soy based broiler diets with the use of exogenous enzymes.

5.2. Body weight
Addition of enzyme complex containing protease, amylase, xylanase, β-glucanase, pectinase, cellulase and phytase improved body weight gain of broiler chickens fed corn-soybean meal based diets.

The results are in accordance with Nagalakshmi and Devegowda (1992), Marsman et al. (1997), Zanella et al. (1999), Aravind, (1998), Channegowda et al. (2001), Ramesh and Devegowda (2004) who have showed that commercial carbohydrase and protease products improved weight gain (1.9-4.0%) in broilers fed maize-soya diet as a result of increased ileal digestibility of protein and NSP. These authors concluded that the added enzymes solubilised parts of the insoluble NSP which improved the overall NSP digestibility. Similarly Pack and Bedford (1997) recorded improved weight gain by four per cent and Kocher et al. (2003) reported that addition of enzyme complex containing β-glucanase, hemicellulase and pectinase resulted in a significant improvement in weight gain.

The improved body weight gain of broilers recorded in the present study with the addition of enzyme complex to corn-soy diets is also supported by the studies conducted by Schang et al. (1997), Roy et al. (2002), Rani et al. (2003) and Rao et al. (2003). Zanella et al. (1999) reduced the energy level by 104 Kcal, ME in the corn-soy based broiler diets and reported that supplementation of enzyme preparation containing amylase, protease and xylanazse produced similar results as compared to control diet. Based on these research findings, to extract the advantage of supplementation of enzyme complex, diet 3 was formulated to contain reduced energy (75 Kcal; ME, calcium (0.1%) and available phosphorus (0.1%) compared to control diet. However, the phytase activity maintained in the current study is 60 SPU/kg diet. Supplementation of enzyme complex (Allzyme SSF) to reformulated diet showed similar results as that of control diet and the results are in accordance with the findings of Denbow et al. (1995) and Zanella et al. (1999)

Several recent studies have demonstrated beneficial effects of combining phytase with glucanase in improving bird performance (Ravindran et al. 1999; Zyla et al. 1999;
Simbaya et al. (1996) reported that the use of phytase in combination with protease and carbohydrase in wheat-canola diets improved weight gain by 5.3 per cent.

The enzyme complex used in the present study is also a combination of phytase, protease and carbohydrase. This is also one of the possible reasons for the improved weight gain recorded in the present study. The improvement in body weight gain indicates that NSP degrading enzymes and phytase present in the enzyme complex were capable of releasing the cell trapped nutrients and facilitate the action of phytase resulting in an added increase in digestibility and absorption of nutrients.

5.3. Cumulative feed consumption

The addition of enzyme complex had no effect (P<0.05) on feed intake of broilers. The results are in accordance with Arun Babu and Devegowda (1997), Graham (1997), Pack and Bedford (1997) Kocher et al. (2002; 2003), Rani et al. (2003), Rivista (2004) and Ramesh and Devegowda (2004) However, the addition of enzyme complex to reformulated diet increased feed intake up to during second and third week of age. This is in accordance with findings of Deaton et al. (1979) and Musharaff (1991), who reported increased feed intake might be due to decreased energy levels of the diet. In the reformulated diet, the energy level was reduced by 75 Kcal ME. This may be the reason for increased feed intake.

5.4. Feed efficiency

The supplementation of enzyme complex significantly improved the feed conversion ratio (FCR) of broiler chicks by 3.39 per cent at sixth week and in reformulated diets with enzyme addition, the FCR was similar to that of control diet. The results of the present study are in agreement with Nagalakshmi and Devegowda (1992), Suresh and Devegowda (1996), Prakash and Devegowda (1996), Marsman et al. (1997), Pack and Bedford (1997), Zanella et al. (1999). The results are also concurrent to the findings of Heger et al. (1984), who reported that effect of enzyme addition is beneficial only during the later part (beyond 28 days). The beneficial effect of enzymes at later stage can be attributed to the sufficient concentration of substrate availability for enzymes.
in the finisher diets (Graham, 1996a) as concentration of substrate can vary the activity of enzymes. The improved FCR in the present study is attributed to better digestibility of neutral detergent fibre, starch (metabolisable energy), protein and minerals, due to breakdown of large mesh like NSPs into smaller polymers (Bedford, 1996, Rao and Devegowda, 1996 and Bhat, 1998) and thereby reducing entangling of nutrients and increasing the absorption (Anonymous., 1996) with the addition of NSP hydrolyzing enzymes. Similarly, the possible release of minerals and protein from the phytate bound minerals and improved protein utilization may be possibly the reasons for improved feed efficiency due to phytase enzyme.

5.6. Intestinal viscosity

Supplementation of enzyme complex to reformulated diet significantly reduced intestinal viscosity. The reduced ingredient level (corn and soy) in the reformulated diet to lower the energy level was also one of the reason to expect reduced intestinal viscosity compared to control diet. These findings are in accordance with Channegowda et al. (2001) and Ramesh and Devegowda (2004). Comparatively higher viscosity at fourth week in this study may be due to age factor as observed by Smits and Annison (1996). However, addition of enzyme complex to the control diet had no significant effect on intestinal viscosity. The results are in accordance with Marsman et al. (1997), Zanella et al. (1999), and Kocher et al. (2002), who reported that there was no change in intestinal viscosity of broiler chickens fed corn-soy diets with enzyme additions. In contrast to these findings, many authors had reported reduced intestinal viscosity with the enzyme addition leading to better performance of broilers in terms of weight gain and feed efficiency because of breakdown of NSPs (Choc et al., 1996; Crouch et al., 1997; Dussel et al., 1998; Steenfeldt et al. 1998) and also in layers (Shivaramu and Devegowda, 2004).

In general, enzyme addition reduced intestinal viscosity of broiler chickens in the present study. It is well established that soluble fraction of NSP when dissolved in water give viscous solution thereby increases the digesta viscosity and reduces nutrient digestion and absorption (Campbell et al., 1989; Annison, 1991; Bedford et al., 1991). The results of the current study indicated that the positive effects observed in terms of
performance parameters have largely been attributed to reduction in intestinal viscosity (Choct et al., 1996; Engberg et al., 2004; Murphy et al., 2004; Owens et al. 2004; Ramesh and Devegowda, 2004) and improved digestibility of NSP, crude protein and AME (Devegowda and Nagalakshmi, 1992; Marsmann et al., 1997; Pack and Bedford, 1997; Bhat, 1998; Rao and Devegowda, 1996; Zonella et al., 1999; Kocher et al., 2000).
5.7. Toe ash

Birds are lacking or limited in phytase, the enzyme necessary for breakdown of the phytase molecule and subsequent release of phytate bound phosphorus in plant feedstuffs. The addition of exogenous phytase enzyme offers promise in reducing phosphorus excretion by increasing the ability of chick to utilise a portion of the phytate phosphorus (Sebastian et al., 1998; Ravindran et al., 1999; Jayashree et al., 2001). Studies have demonstrated that dietary phosphorus levels in broilers can be reduced by supplementation with phytase (Broz et al., 1994). Toe ash is a sensitive measure of bone mineralisation and a direct correlation can be found between increased bone ash and higher amounts of calcium and nonphytate phosphorus in the diet and increased weight gain (Mitchell and Edward, 1996).

Toe ash content was increased with the addition of enzyme complex (200 PU/kg diet) in the present study. Similarly, other authors have also reported improvements in toe ash with the addition of phytase (Denbow et al., 1995; Ravindran et al., 1999; Jayashree et al., 2001; Hall et al., 2003; Silversides, 2004). However, these authors have added phytase at higher levels (>500 PU/kg) to the diets deficit in available phosphorus by more than 0.3 per cent.

An improvement in bone ash was also observed by Nelson et al. (1971), who found an increased tibia ash in chicks fed a phytase supplement. Increased bone ash suggests an improvement in bone mineralisation due to increased phosphorus and calcium utilisation, which was caused by the liberation of inorganic phosphorus and calcium from the phytate molecule by the phytase enzyme in the digestive tract.

Simons et al. (1990) improved the availability of both phosphorus and calcium with the use of dietary phytase. The effect of calcium on the phytase enzyme and competition between phosphorus and calcium for absorption has suggested a reduction in the level of dietary calcium below the normal requirements when phytase is added to the diet (Nelson et al., 1968b; Simons and Versteegh, 1990).
Schoner et al. (1991 and Vogt (1992) also found that growth and toe ash percentage of broilers were sensitive indicators for evaluation of phosphorus availability of birds. Several tibia, metatarsal and toe measurements, as well as body weight gain were examined in broilers fed deficient to adequate levels of phosphorus from seven phosphorus sources and a dicalcium phosphate dihydrate standard for three weeks (Ravindran et al., 1995). They found that body weight gain and toe ash percentage were equally or more sensitive for assessment of phosphorus availability than tibia ash percentage and that other measurements including tibia specific gravity, tibia shear force, toe shear force and metatarsal shear force were of limited value.
5.8. Gut bacterial load

The results from this study showed that addition of enzyme complex exerted a preferential stimulatory effect on a bacteria of the health promoting genus (*Lactobacillus*) while maintaining population of the unprofitable or potential pathogens (*Streptococcus, Coliforms And Total Count*) at relatively low levels both in ileal and caecal digesta. These findings are in accordance with the results of other studies (Apajapathi and Bedford, 1999; Engberg *et al*., 2004; Murphy *et al*., 2004). Tan and Hurby (2003) reported that the use of feed enzymes significantly reduced number of food poisoning bacteria like *Campylobacter* and *Clostridia* sp in the ceaca of broilers fed on corn-soy diet. They concluded that reduction in microbial populations with addition of enzymes appears to be linked to three key modes of actions.

1. A reduction in intestinal viscosity associated with increased feed passage rate, which means that less substrate available for bacteria.

2. Improved nutrient digestibility, resulting in fewer nutrients for the growth of bacteria.

3. An altered carbohydrate possible in the intestine – resulting in more of substrate preferred by beneficial bacteria eg., *Lactobacillus*.

Similarly, Choct *et al.* (1990) reported that addition of xylanase to wheat based diets reduced the microbial activity of ileal digesta as indicated by reduced concentration of volatile fatty acids. Sinale and Choct (2000) reported that addition of xylanase to a wheat-based diet reduced the number of undesirable organism such as *Clostridium perfringens* in caecal contents.

The soluble NSP are known to increase the residence time of digesta in the intestine (Gohl and Gohl, 1997) and thicken the unstirred water layer of mucosa by interacting with the mucopolysaccharides. This would decrease oxygen tension and favors the development of anaerobic microflora, which have negative effect on gut health, and performance of broilers (Choct *et al*., 1996; Wagner and Thomas, 1978). The degradation of arbinoxylans and pectic polysaccharides of soybean meal may result in a
reduction of intestinal viscosity. Nonstarch polysaccharide hydrolyzing enzymes are hypothesized to work on two steps, described as ileal and cecal phase (Bedford, 2000b). During ileal phase, 1) enzymes remove fermentable substrates or inhibit ileal fermentation by reducing bacterial numbers. During cecal phase, 2), degradation products of sugars (small oligomers and free sugars) are fermented by cecal bacteria, thus stimulating the production of volatile fatty acids and the growth of specific beneficial bacteria (Bedford, 2000b). The presence of viscous polysaccharides increased the intestinal microbial activity that are associated with the bile acid deconjugation, leading to an impaired lipid digestion and this has been suggested to be partly responsible for poor broiler performance.

In general, the result of the present study is clearly indicating that reduced intestinal viscosity with the addition of enzyme complex increased digesta passage rate and there being less substrate available for the bacterial community as indicated by Tan and Hurby (2003). The activity of the enzymes on the viscous polymers and cell wall carbohydrates produce sugars and oligomers, which are utilized preferentially by certain ileal and caecal bacterial species. Altered carbohydrate profile in the intestine resulting in more of the substrate preferred by beneficial bacteria (Bedford, 2000a).

5.9. Nutrient digestibility

The addition of enzyme complex resulted in significant improvement in the digestibility coefficient of crude protein. These findings are in accordance with Nagalakhmi and Devegowda, 1992; Graham. (1996); Swift et al. (1996); Pack and Bedford. (1997); Marsman et al. (1997); Bhat. (1998); Zanella et al. (1999); Kocher et al. (2002). Improvements in digestibility of crude protein have been reported in most studies. In general, the improvement in nitrogen digestibility with addition of enzymes in poultry fed corn-soy diet, ranged from 1.5 to 9.5%

5.10. Dressing percentage

The effect of enzyme addition on dressing percentage was non significant. These findings are in agreement with Zanella et al. (1999), Roy et al. (2003) and Rao et al.
(2003) who reported addition of enzyme to corn-soy diet had no effect on dressing percentage of broiler chickens.

Feeding of high fibre diet is known to increase the relative weights of gizzard and intestine, which indirectly affects the dressing percentage. The explanation for the relatively higher intestinal weights of the birds fed diet rich in NSP (wheat based) probably lies in the increased digesta viscosity, reduced intestinal mobility and the resultant increase in pathogenic microbial activity that stimulate intestinal growth (Bernes et al., 2002). The influence of increased viscosity in stimulating the growth of anaerobic microflora is documented. The numerical improvement in the dressing percentage of broiler chickens fed diets with enzyme may also be due to decreased intestinal viscosity and microbial load of broilers in the present study.

5.11. Mortality

Results of cumulative mortality at sixth week did not show difference among the treatments. The percent mortality among different treatment groups is within the acceptable limit. Postmortem examination of dead birds revealed the etiology to be independent of dietary origin. The results on mortality are in accordance with Mohandas and Devegowda (1993), Prakash and Devegowda (1996), Rao and Devegowda (1996) and Zanella et al. (1999), who reported enzymes supplementation had no effect on livability. In contrary to these findings, Rajmane (1992), Pack and Bedford (1997) and Jackson et al. (2004) recorded higher survivability in the broilers fed diets with enzyme addition compared to control groups. The reduction in mortality with enzyme addition may be due to elimination or deactivation of activity of antinutritional factors present in the feed ingredients.

5.12. Matrix value for enzyme complex

It has been understood that enzymes improve the digestibility and availability of nutrients. However, more often than not, enzymes are supplemented as a top dressing to a formulated diet. Knowledge of the level of improvement of different nutrients by supplementation of enzymes at a specific level would help diet reformulation at a much higher accuracy for the best efficiency and economy. The current experiment compared
the availability of nutrients vis-à-vis both enzyme supplementation and diet reformulation to assess the efficiency of enzymes in improving nutrient utilization as well as to evaluate the applicability of manufacturer provided nutrient matrix of enzyme. However, protein was not reduced in the reformulated diet. The digestibility coefficient of crude protein was considered to arrive at matrix value of protein for enzyme complex. Similarly, the improvement in the digestibility and availability of nutrients with the addition of enzyme complex was reported by Marsman \textit{et al.} (1997); Zanella \textit{et al.} (1999) and Kocher \textit{et al.} (2002). The observations made in this study are indicating that enzyme complex improve digestibility and availability of nutrients and matrix value of enzyme complex can be effectively used in the diet reformulation to have better performance and economy.