V. DISCUSSION

The *in vitro* trial for screening on non-starch polysaccharides of sunflower meal (SFM), deoiled rice bran (DORB) and the compounded diets and the *in vivo* trials on the effect of inclusion of SFM and DORB with enzyme supplementation A or B to layer diets in the second phase of production on performance viz egg production, egg weight, feed intake, feed efficiency, livability, intestinal viscosity and litter moisture are discussed in this chapter.

5.1 *In vitro trials*

5.1.1 Screening of nonstarch polysaccharides

5.1.1.1 Total pentosans

The pentosan contents of the ingredients utilized (maize, soybean meal, SFM and DORB) in the present study were 5.34, 4.23, 11.12 and 10.56 %. The differences in the total pentosan values among the ingredients are natural. These values are similar to those reported by Malathi and Devegowda (2001), Shivaram and Devegowda (2004) and Devegowda (2005). The linear increase of pentosans in the diet 1 to 4 is the reflection of the high pentosan contents of SFM and DORB as their inclusion rates are increased at 10, 15, 20 and 25 % in the layer rations.

5.1.1.2 Pectin content

The average pectin values were 1.03, 5.93, 4.91 and 6.12 % for maize, soybean meal, SFM and DORB and these values are on par with those reported by Malathi and Devegowda (2001), Shivaram and Devegowda (2004) and Devegowda (2005), where as Choct (1997) has quoted a low value of 1.6 for DORB which may be due to the difference in variety and geographical location where it is grown. The decreasing levels of pectin in the diets are due to low pectin content of SFM and DORB put together when compared to soybean meal.
5.1.1.3 Cellulose content

The cellulose percentage values were 3.16, 5.82, 23.52 and 15.38 for maize, soybean meal, SFM and DORB which are in concurrence with Malathi and Devegowda (2001), Shivaram and Devegowda (2004) and Devegowda (2005). The values for the diets 1 to 4 were 5.98, 7.21, 8.45 and 9.69, respectively. SFM has the highest cellulose per cent of 23.52 followed by DORB having 15.38 per cent. The increasing cellulose content of the diets 1 to 4 is represented by the increasing inclusions of SFM and DORB in the diets.

5.1.1.4 Total nonstarch polysaccharide content

The total NSP content of maize, soybean meal, SFM and DORB were 9.37, 28.54, 39.61 and 41.23% and for the diets 1 to 4 were 15.77, 16.95, 18.14 and 19.32%. The values of the ingredients are in accordance with Malathi and Devegowda (2001), Shivaram and Devegowda (2004) and Devegowda (2005). The increasing trend of total NSPs in the diets 1 to 4 is due to higher inclusions of SFM and DORB which have higher total NSPs when compared to maize and soybean meal.

The NSP analyzed values, individually and as total content are in accordance with the findings of previous research works done in this department (Malathi and Devegowda, 2001; Ramesh and Devegowda, 2004; Shivaram and Devegowda, 2004; Devegowda, 2005). The near conformity of all the samples to one standard value could be due to free trade of ingredients among the state leading to more common source of each ingredient.

5.1.2 Activity of enzymes
Activity of various enzymes in a commercial enzyme complex is an important determinant of the result obtained with application of such an enzyme. Even though different enzymes have been tried, it has been generally observed that in a common poultry diet containing cereals like maize, sorghum, ragi, etc., primarily, the arabinoxylans (pentosans) will be in considerable proportion necessitating the supplementation of xylanase enzyme on priority.

The other common ingredients of protein sources or fillers like soybean meal, groundnut meal, SFM and DORB are higher in both pectins and pentosans compelling the nutritionists to think of supplementing the pectinase enzyme also.

Further, higher cellulose levels in high fibre ingredients like SFM and DORB indicate that it will be beneficial to include cellulase also to this combination. Moreover, the high phytate contents of SFM, DORB, etc. allow the addition of phytase too into these complex enzyme mixtures. Hence most of the enzyme mixtures will have these four enzymes, with or without other enzymes like amylase, protease, lipase, etc. The enzymes A and B used in the present study were analysed for the activities. These enzymes contained xylanase, pectinase, cellulase and phytase in common.

### 5.2 Biological trial

#### 5.2.1 Hen-day egg production

Laying hens fed with increasing inclusions of SFM and DORB (10, 15, 20 and 25%) showed significant (p<0.05) reduction in egg production (89.93, 89.31, 88.94 and 88.47 %) as the level of these ingredients increased. The reduction in hen-day production at 15, 20 and 25% SFM and DORB fed birds were 0.6, 1.0 and 1.4 % as compared to the 10% SFM and DORB groups. The significant reduction in egg production in increasing levels of SFM and DORB at 15, 20 and 25% levels when compared with 10% level was due to increased fibre and decreased energy levels. The results of the present study support the findings of Mohandas and Devegowda (1993), Prakash and Devegowda (1996), Mohan et al. (2001) and
Rezaei (2001) who reported that increasing SFM levels significantly decreased the egg production. With regard to DORB, the results are in agreement with Majun and Payne (1977), Karunajeeva and Tham (1980), Warren and Farrell (1990b), Mohandas and Devegowda (1993), Farrell (1994), Mohan et al. (2001) and Raghavendra (2004) who also reported depressed egg production on increasing inclusion of DORB in layer diets.

These observations support the hypothesis that by increasing the inclusion of SFM and DORB, the nutrient density is diluted and the bird is capable of managing it to a great extent by modulating its feed intake to suit the nutrient requirements. However, minor shortcomings in the adaptation owing to the bird's or diet's limitations are inevitable and the variations observed in this experiment are also in such reasonable limits. Moreover, the fibre level of the diets also increased with the increasing levels of SFM and DORB in the diets. But in contrast to this, Deaton (1979), Mandlekar (1992), Gerendai (1998), Shivaram and Devegowda (2004) reported non-significant differences in increasing SFM inclusions probably due to isocaloric maintenance of the diets. With regard to DORB, Lodhi and Ichhaponani (1975) and Pilang et al (1982) also reported non-significant depression in hen-day egg production.

The hen-day egg production on the addition of enzyme A or B to 10, 15, 20 and 25 % SFM and DORB based diets does not differ significantly between dietary treatment groups with their respective controls without enzymes. But the hen-day production was numerically greater in all the groups fed enzyme B followed by enzyme A as compared to their respective non-enzyme controls. The numerical increase in hen-day production at 10, 15, 20 and 25% groups with enzyme addition was 0.37, 0.22, 0.30 and 0.32% and with enzyme B was 1.29, 1.07, 0.99 and 0.92%, respectively when compared with the controls without enzymes.

The failure of the enzymes to exert significant effect on egg production when compared with the respective controls without enzymes is in agreement with
Mohandas and Devegowda (1993), Esmail (1999), Meeusen and Vallet (2001), Ponnuvel et al (2001) and Shivaram and Devegowda (2004). The supplementation of enzyme A or B as expected has contributed to improve the nutrient availability at each level of SFM and DORB inclusion, as is apparent mainly from the numerical improvement in egg production in each group corresponding to the non-supplemented groups. But on the contrary, Mohan et al. (2001) reported consistent improvement in egg production with use of xylanase, pectinase and cellulase at 0.1% to SFM and DORB based diets. Similarly, Jayanna and Devegowda (1993) reported that enzyme supplementation was beneficial at low energy levels.

5.2.2 Egg weight

Neither the level of SFM and DORB nor the enzyme A or B addition has resulted in a significant change in the mean egg weights. This result is in agreement with Deaton (1979), Mandlekar (1992), Vieira (1992), Mohandas and Devegowda (1993), Gerendai (1998), Mohan et al. (2001) and Shivaram and Devegowda (2004), who also observed non-significant differences in egg weight between different levels of SFM in the diets.

With regard to DORB, the lower level of linoleic acid in the 25% level might be the reason for the numerical depression in egg weight up to 0.6 g when compared with the 10% level and these results are supported by Majun and Payne (1977) and Din (1979) who also observed non-significant differences in egg weight at different levels of DORB.

But on the contrary, Prakash and Devegowda (1996) and Rezaei (2000) reported that increasing SFM levels resulted in significant reduction of egg weight. Also Lodhi and Ichhaponani (1975) and Raghavendra (2004) reported significant reduction in egg weight upon higher inclusions of DORB in the layer diets.

Addition of enzymes resulted in non-significant numerical improvements up to 0.68 g in diets compared with and without enzyme supplementation. The
highest egg weight was 59.47g in 20% SFM and DORB diets with enzyme B and the lowest was recorded in 25% SFM and DORB groups without enzymes.

The results for egg weight with enzyme addition were in agreement with the findings of Mohandas and Devegowda (1993), Jayanna and Devegowda (1993), Prakash and Devegowda (1996), Meeusen and Vallet (2001), Mohan et al. (2001), Ponnuvel et al (2001) and Shivaram and Devegowda (2004) who also observed non-significant differences for egg weight in SFM diets. But on the contrary, Francesch (1995) reported significant improvement in egg weight by the application of 0.1% enzyme complex containing β glucanase, xylanase and pectinase.

Egg weight is a function of breed, strain and nutritional status of the bird, of which, enzyme supplementation might have influenced the nutritional status to some extent. Among the nutrients too, linoleic acid and methionine are the two nutrients of primary importance for good egg weights. Enzyme supplementation is stated to improve the digestibility of fat (emulsification of triglycerides and fat is higher at lower viscosities) and also that of protein (and hence the amino acids). Therefore, enzyme supplementation may contribute to better availability of dietary linoleic acid and methionine thereby influencing the egg size.

5.2.3 Feed intake

The inclusion of SFM and DORB in the laying hens diet caused significant (p<0.05) difference in feed intake among the dietary treatments. As the level of SFM and DORB in the diet (10, 15, 20 and 25%) increased, the feed intake also increased (110.36, 114.57, 120.82 and 127.02 g/day/bird, respectively). Inclusion of 15, 20 and 25% SFM and DORB in the diets resulted in increased feed intake by 4.21, 10.46, and 16.66 g/day/bird, respectively when compared to the 10% SFM and DORB diets.

Under the conditions of non-isocaloric dietary regime by increasing the inclusion of low nutritional value agro-industrial by products like SFM and
DORB, the feed consumption truly reflected the energy density of the diets. In this experiment, the 10% SFM and DORB diets with highest ME content diet caused lowest feed intake and the 25% diet having the lowest ME content showed the maximum feed intake.

These observations are in agreement with the findings of Deaton (1979), Vieira (1992), Mohandas and Devegowda (1993), Prakash and Devegowda (1996) who reported increased feed intake with rising dietary levels of SFM. Significant increase in feed consumption in increased levels of DORB also was observed by Majun and Payne (1977), Zombade and Ichhaponani (1983), Warren and Farrell (1990b), Mohan et al. (2001) and Raghavendra (2004) that support the findings in this experiment. On the contrary, Rezaei (2001) observed significant reduction in feed intake in an isocaloric and isonitrogenous diet with different fibre levels of SFM. Chaturvedi and Singh (2000) also observed reduction in DORB diets.

Significant (p<0.05) reduction in feed intake by 3.49, 4.17, 5.20 and 5.74 g/day/bird was observed in enzyme A and by 3.21, 4.86, 6.03 and 6.57 g/day/bird was observed in enzyme B supplemented hens fed with 10, 15, 20 and 25% SFM and DORB diets, respectively when compared to the hens fed without enzymes.

The quantum of reduction in feed intake upon enzyme supplementation was considerable throughout the six periods of study and the saving through the reduction of feed intake by the birds (in g/day/bird) would translate to savings of so many kg of feed (discussed in relative economics) by any perceivable means, is significant. The observed feed intake reduction with supplementation of enzymes has been supported by the findings of Ponnuvel et al. (2001) who reported 5 to 6 g reduction in feed intake and Shivaram and Devegowda (2004) who reported 2.5, 5.1 and 5.0 g/day/bird feed reduction in 10, 20 and 30% SFM groups upon enzyme addition and also Raghavendra (2004) who reported significant reduction of 5, 4 and 3 g/ day/ bird in FI, respectively in 10, 20 and 30% DORB fed laying hens upon enzyme supplementation.
The reduction in feed intake upon exogenous enzyme supplementation could be attributed to enhanced nutrient utilization as NSP degrading enzymes are known to reduce the viscosity of the digesta. Enzymes can access the substrates better at low viscosities due to lesser flow resistance. Further, nutrients released by the digestion process can flow back to the intestinal villi better at low viscosities than at higher viscosities. Therefore, both the process of digestion and uptake of nutrients dependent on endogenous enzymes are bettered by the supplementation of exogenous NSP degrading enzymes. Moreover, NSP degrading enzymes release the nutrients bound in the fibrinous matrix of the feed matter and also partially digest the polysaccharides releasing some mono-saccharides contributing to better energy availability. Therefore, the observed reduction in feed intake appears to be logically and practically well explained.

However, some researchers also have reported zero or negligible reduction in feed intake upon enzyme supplementation (Sharma, 1992; Mohandas and Devegowda, 1993; Jayanna and Devegowda, 1993; Jaroni et al. 1999; Meeusen and Vallet, 2001) while some have reported increased feed intake (Vranjes and Wenk, 1996; Um and paik, 1999; Ranade et al. (2004) in laying hens.

5.2.4 Feed efficiency (FE)

Increasing levels of SFM and DORB in the laying hens diet (10, 15, 20 and 25%) has a negative correlation with FE (requiring more feed per dozen of eggs and per kg of egg mass). Feed conversion being a numerical parameter dependent on feed intake and hen-day egg production (and egg weight when expressed as kg feed per kg eggs produced), observations of FE is self explanatory. FE values increased with increasing levels of SFM and DORB as the feed intake increased with simultaneous reduction in hen-day production and egg weight. This is in agreement with Mandlekar (1992), Rezaei (2001) and Shivaram and Devegowda (2004) who also observed reduction in FE with SFM inclusions.
Enzyme A or B supplementation had a positive correlation with FE (requiring less feed per dozen of eggs and per kg egg mass produced) as compared to the corresponding enzyme non-supplemented groups. Enzyme supplementation significantly improved the FE in SFM and DORB diets as feed intake reduced with simultaneous numerical increase in hen-day egg production and egg weight. These findings are in line with Mohandas and Devegowda (1993), Francesch et al (1995), Meeusen and Vallet (2001), Mohan et al. (2001) and Shivaram and Devegowda (2004) who also reported an improved FE in layers fed SFM diets. Moreover, Benabdeljelil (2001) found broilers fed enzyme supplemented diets consumed less feed than SFM non enzyme supplemented groups which lead to improvement of 5.3 to 8.6% in FE. However, Rebole (1999) and Kocher (2000) failed to observe improvement in SFM broiler diets even after enzyme addition.


With advancement of age of the hens, there was a parallel increase in the quantum of feed consumed per unit egg produced both on egg number and egg mass basis which reflected increasing feed efficiency values.

5.2.5 Intestinal viscosity

Increasing levels of SFM and DORB in the layer diets brought down the intestinal viscosity considerably. It is evident that increasing SFM and DORB (10, 15, 20 and 25%) in the diets proportionally replace and reduce the maize and soybean meal inclusion. Though the total NSP content of the diet increases due to increased inclusions of SFM and DORB, the soluble NSP fraction responsible for viscosity decreases as these two ingredients have less soluble NSPs when
compared to soybean meal which has higher soluble NSP content. Hence, the decreasing trend in viscosity was noticed (1.51, 1.49, 1.37 and 1.35 cP)

These findings are supported by Shivaram and Devegowda (2004) who reported reduction in viscosity by increasing inclusions of SFM in layer diets and Farrell and Martin (1998a) who also reported decreasing viscosity with increasing levels of DORB. However, Channegowda (2001) and Mohan et al. (2001) observed increase in viscosity with increasing levels of SFM along with Sandeep and Devegowda (2002) and Raghavendra (2004) who reported increase in viscosity with increase of DORB level.

Supplementation of enzyme A significantly (P<0.05) reduced the intestinal viscosity in the 10, 15, 20 and 25 % SFM and DORB groups from 1.51, 1.49, 1.37 and 1.35 cP to 1.42, 1.34, 1.32 and 1.30 cP and enzyme B to 1.38, 1.39, 1.29 and 1.24 cP, respectively. These data strongly suggest that enzyme supplementation has a profound influence on intestinal viscosity and can greatly reverse the viscosity increased by the soluble NSPs. The observations of the present study are in agreement with Channegowda (2001), Meeusen and Vallet (2001), Mohan et al. (2001) and Shivaram and Devegowda (2004) who also found significant reduction in intestinal viscosity in SFM based diets with enzyme supplementation and in accordance with Farrell and Martin (1998a) and Raghavendra (2004) who also reported reduction in viscosity in DORB diets through enzyme addition.

The reduction in the intestinal viscosity by the supplementation of enzymes is through break down of soluble NSPs into smaller molecules which causes the reduction in water holding capacity in turn the viscosity of the gut contents. The present study holds good the support of Ramesh and Devegowda (2004), Ramesh and Devegowda (2005a) who also reported reduction in viscosity of the intestinal contents upon enzyme supplementation.

5.2.6 Faecal moisture
Increasing levels of SFM and DORB in the layer diets (10, 15, 20 and 25\%)
brought down the faecal moisture significantly (p<0.05) as the moisture per cent
recorded were 74.71, 72.83, 66.79 and 64.83\%, respectively. The considerable
reduction in the faecal moisture is the reflection of the reduction in viscosity of the
digesta as SFM and DORB levels in the diets increased. These findings are in
accordance with Shivaram and Devegowda (2004) who also reported reduction in
faecal moisture by increasing inclusions of SFM in layer diets and Farrell and
Martin (1998a) and Raghavendra (2004) who also reported reduction in faecal
moisture with increasing levels of DORB. However, Channegowda (2001) and
Mohan et al. (2001) observed increasing faecal moisture in increasing SFM diets
and Martin and Farrell (1998) observed high faecal moisture in DORB included
diets.

Supplementation of enzyme A significantly reduced the faecal moisture in
the 10, 15, 20 and 25 \% SFM and DORB groups from 74.71, 72.83, 66.79 and
64.83 \% to 71.98, 70.21, 65.55 and 61.79 \% and enzyme B to 72.57, 66.09, 63.46
and 63.58 \%, respectively. Faecal moisture was reduced up to 4.7\% through
enzyme A and up to 9.2\% through enzyme B addition in SFM and DORB based
layer diets. The observation of the present study are in agreement with
Channegowda (2001), Meeusen and Vallet (2001), Mohan et al. (2001) and
Shivaram and Devegowda (2004) who also found significant reduction in faecal
moisture in SFM based diets upon enzyme supplementation and in accordance
with Farrell and Martin (1998a) who also reported reduction in faecal moisture in
DORB diets through enzyme addition.

Many researchers have suggested the reduction in moisture content of the
droppings and consequent reduction in ammonia levels and fly menace as one of
the major benefits associated with enzyme supplementation in layer diets. The
proposed mode of action is also clear. High viscosity of the ingesta reduces the
rate of its passage in the intestine. Presence of high viscosity material and the
consequent growth and colonization processes associated with bacteria irritate the
mucosa and result in excessive secretion of water and salts. The colonization efforts by the bacteria result in localized damages to the intestinal villi and walls thereby reducing the absorption of water and other nutrients. Moreover, the high viscous materials swell up and form a jelling with the water available in the intestine and pass out in the faeces without getting absorbed leading to increase in the faecal moisture. Enzyme supplementation to diets would reduce the adverse effects such as increased intestinal viscosity and bacterial colonization thereby resulting in a dryer dropping condition.

The present study also holds good the support of Jayanna and Devegowda (1993), Mohandas and Devegowda (1993), Rao and Devegowda (1996), Ponnuvel and Jalaludeen (1999), Kocher (2002) and Ramesh and Devegowda (2004) who reported reduction in the faecal moisture upon enzyme supplementation.

5.2.7 Livability

No mortality was recorded during the entire experimental period suggesting that neither SFM and DORB inclusion nor the enzyme supplementation had effect on livability. Similar findings were reported by Jayanna and Devegowda (1993), Mohandas and Devegowda (1993), Rao and Devegowda (1996), Jayashree (2000), Devendran (2001), Mohan et al. (2001), Raghavendra (2004) and Shivaram and Devegowda (2004).

5.2.8 Cost effectiveness

Cost of the layer diets with 10, 15, 20 and 25% inclusions of SFM and DORB were rupees 6.91, 6.35, 5.73 and 5.34, respectively. The cost of feed decreased considerably with the increasing inclusions of SFM and DORB. Even though these feeds recorded low cost by the inclusions of the cheap agro industrial by products SFM and DORB, considerable performance reduction also accompanied.
It is evident from the present study that supplementation of either enzyme A or B to these SFM and DORB diets are economical and cost effective. A net profit of 91, 156, 142 and 149 rupees per ton of feed was generated through enzyme A supplementation and 114, 200, 223 and 215 rupees per ton of feed through enzyme B supplementation along with considerable improved laying performance. These cost savings were mainly because of the reduced feed intake up to 5.74g through enzyme A and up to 6.57 g through enzyme B without causing any reduction in performance.

The economical benefits derived from enzyme supplementation is supported by Graham (1996b) who supported that although attempts to reduce the feed cost by utilizing cheap ingredients resulted in performance reduction, the addition of enzyme improves the value of feed, resulting in a similar performance to that of normal more expensive feed formulation that result in savings of 3 to 5 dollars per ton of feed. Similarly, Zhang (1999) observed 2 dollars saving per ton of feed and Bhat (1998), Osei and Oduro (2000), Devendran (2001), Mohan et al. (2001) and Ponnuvel (2001) also reported profits by enzyme supplementation.

5.2.9 Overall performance due to enzyme supplementation

The overall performance (Table 19) of laying hens fed varying levels of SFM and DORB with enzyme A or B supplementation revealed that merely increasing the levels of SFM and DORB (10, 15, 20 and 25%) resulted in significant (P<0.05) reduction in performance parameters like egg production, feed intake, feed efficiency per dozen and kilo of eggs, intestinal viscosity and litter moisture along with numerical reduction in egg weight. However, in the same diets with enzyme A or B supplementation improvement in all the performance parameters was observed.

As the daily feed intake was reduced by 5.74 g/bird/day through supplementation of enzyme A and 6.57 g/bird/day through enzyme B without causing any performance reduction, enzyme A supplementation generated 91, 156,
142, and 149 rupees per ton of feed and enzyme B 114, 200, 224 and 215 rupees, respectively.

Enzyme B supplementation showed more beneficial effects over enzyme A in all the performance parameters observed in the entire period of study. It is presumed that the higher activities of pectinase by 485 U/g and cellulase by 96 U/g along with an extra activity of 486 U/g of amylase in enzyme B resulted in more beneficial effect when compared with enzyme A as these diets had a range of 2.04 to 2.15 per cent pectin in them along with higher cellulose content up to 9.69 per cent. Moreover, the NSP bound starch released by these NSP degrading enzymes would have been well acted upon by the exogenous amylase addition that resulted in improved performance.

It is confirmed by the present study that enzyme supplementation is economical and cost effective and paves way for higher inclusions of low cost agro-industrial by products without performance reduction when compared with normal expensive layer diets.