INFLUENCE OF FEED ENZYMES ON NUTRIENT AVAILABILITY AND PRODUCTION PERFORMANCE OF LAYING HENS

By

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THESIS

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DECLARATION

I here by declare that this thesis entitled "INFLUENCE OF FEED ENZYMES ON NUTRIENT AVAILABILITY AND PRODUCTION PERFORMANCE OF LAYING HENS" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "INFLUENCE OF FEED ENZYMES ON NUTRIENT AVAILABILITY AND PRODUCTION PERFORMANCE OF LAYING HENS" is a record of research work done independently by **Sri. B. Satyamoorthy** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

Poultry industry in India has grown into a dynamic agri-business in the last two decades. India ranks as the sixth largest country in the world in egg production (Anon, 1994a). Despite the rapid growth, the country's poultry production is inadequate, as the per capita availability of eggs is less than 32 per annum against the minimum requirement of 180 (Patnaik, 1994). The top five egg producing states in India are Andhra Pradash, Tamil Nadu, Maharashtra, Punjab and Haryana. Kerala has not yet emerged as a commercial producer of eggs. A peculiar situation of per capita availability of 51 eggs and the consumption of 62 eggs per annum exists in the state, the excess demand being met from the neighbouring states (Unni, 1995). About 85 per cent of people of Kerala are non-vegetarians and hence there is tremendous scope for further expansion of the industry in the state. Further, poultry rearing is a viable enterprise for improving rural economy.

Feeds accounts for 65 to 75 per cent of the total cost of livestock and poultry production. The shortage of feeds and fodders is one of the major constraints in the animal husbandry sector in the country, the extent of shortage in quantity being 12 and 19 per cent for dry fodder and green forage respectively, while concentrates availability being just sufficient to meet the requirements (Anon,1994b). Regarding nutrient availability, the situation is still worse with a deficit of about 55 per cent of digestible crude protein and 20 per cent each of dry matter and total digestible nutrients. About nine million tonnes of feed ingredients may be required to feed the present poultry population in the country (Devegowda, 1993). The projected growth in this sector is likely to increase the requirement of feeds by several million tonnes.

A level of efficiency has already been achieved in poultry industry and a further improvement in efficiency can only be marginal. Feed is one single factor where all emphasis can be made to develop and capitalise on cost efficiency. Satisfying the consumer demands for quality eggs and meat at competitive prices is the target for the industry at present. Ours is an economy operating with shortages and poor purchasing power. Use of alternate feed sources in livestock and poultry production has become a need of the day. The greatest challenge faced by feed manufacturers to day is how to cope up with the shortage of essential feed ingredients and how to market out the feed at competitive price, at the advent of the steep rises in price of all feed items. Intensive research is going on for identifying newer feed resources, substitutes and appropriate technology for their better and maximum utilisation. One of the promising ways to maximise nutrient availability from alternate feed resources is perhaps through the exploration of biotechnological avenues. One such avenue is enzyme biotechnology. Enzymes often called the "work horses" of biotechnology industry, have not been fully exploited by the feed industry.

Modern poultry producers have long been users of additives for bettering feed quality and health of birds for enhancing productivity. Increased awareness on the probable health hazards from growth promoting drugs and chemical substances in foods perhaps forced the poultry men to turn to biological products such as biostimulators, probiotics, yeast cultures or to feed enzymes as biological tools to augment production in a normal way.

Information accrued on enzymes has lead to an awareness of its importance and the problems and possibilities of its application in livestock and poultry industry (Chesson, 1993). Commercial feed enzymes are crude extracts of microbial, fungal and biotechnological products. A wide range of enzymes are commercially available (Hotten, 1991). Often multi-enzymes bring about better response in practical feeding operations. About 85 to 95 per cent of poultry feeds consist of plant materials, a few of which have higher dietary fibre. Many cereals and their by-products have a portion of their energy locked up in the form of non-starch polysaccharides (NSP) derived from plant cell walls. The significance of non-starch polysaccharides in animal feed is well documented (Jeroch, 1993; Muller, 1993; Ramasubba Reddy, 1993) as these are reported to block the accessibility of digestive enzymes to other nutrients viz. . protein and starch, and also act as anti-nutritional factors unless degraded. Degradation of NSP involves enzyme digestion which could be accomplished either by host enzymes or those elaborated by micoflora in the gut. Poultry do not produce enzymes like cellulase, hemicellulase and betagluconase which are required for digestion of plant cell wall materials and for the breakdown of certaiin antinutritional substances found in feed raw materials. Feed enzymes release the structural barrier in the form of cell wall carbohydrates (Chesson 1987). The enzymes from gastrointestinal microflora in the lower digestive tract are less efficient in digesting NSP

Moreover, birds have shorter feed transit period which may reduce the extent of digestion in the gut, necessitating the use of exogenous enzymes.

The potential benefits from feed enzymes are thus on two primary areas such as supplementing endogenous enzymes and/or acting specifically on anti-nutritional substances in specific raw materials such as rye, barley, oats, wheat, jowar or certain plant protein supplements. The enzyme additives are known to reduce the viscosity of intestinal contents, thereby augmenting the absorption of nutrients from the gastrointestinal tract (Bedford *et al.*, 1994). The sticky droppings problem when certain cereal grains were included in diets was overcome by enzyme usage in feeds (Raghavan, 1990 and Petterson and Aman, 1992). Crude extracts of bacterial or fungal origin also contain phytase which is useful in splitting phytin phosphorus (Simons and Versteegh, 1990; Cantor *et al.*, 1994 and Pointillart, 1994). The key uses of feed enzymes are in young animals where the digestive capabilities are under-developed or in sick animals and in those under stress or at high level of production where the digestive capacity is limited (Wenk, 1994). In short, feed enzymes offer means of using economical ingredients in feeds without sacrificing nutrient availability for productive performance.

Earlier experiments with exogenous enzymes in hens have failed to bring positive results (Berg, 1959 and 1961). Continuing developments in product formulation, stability of products and introduction of new enzyme preparations further improved the results in layers. The enzyme biotechnology in animal production has been thoroughly documented (Mul, 1989 ; Inborr, 1990; Low and Longland 1990; Graham, 1991 and Flhenakis and Kyriazakis, 1993). So far, there are no reports of nutritional or physiological harmful effects from using enzymes. Feed enzyme technology, although, still in infancy, holds a major promise for upgrading the nutritive value of a range of feed ingredients. Feed enzymes are nutritionally beneficial and financially attractive.

The present investigation was envisaged to ascertain the effect of the feed enzymes, viz., cellulase and protease, singly or in combination in a standard and a less dense layer ration, on nutrient availability and on production performance of laying hens and also to arrive at its economic feasibility.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Enzymes as additives have attracted substantial interest from feed industry as a novel means of improving animal performance. Despite reports on enzymes in feeds as early as 1925 (Clickner and Follwell, 1925), only recently the use of feed enzymes became extensive and research activities to improve the desirable characteristics of the product intensified. Many types of enzymes have been studied as additives including proteases, lipases, phytases and polysaccharidases.

Animals differ widely in their digestive capabilities. No animal can degrade the cell wall components of plants, for which they rely upon micro-organisms. The animal system in ruminants is specifically adapted for this function and is different from those in monogastric and avian systems. Efforts are going on extensively to enhance productivity of various species of livestock and poultry with enzyme additives (Jeroch and Muller, 1992; Campbell and Bedford, 1992 and Farrell and Martin, 1993). Enzyme additives are either used directly in feed mixtures or provided in drinking water, or used for pre-treatment of raw materials. The enzyme technology is fast advancing to elaborate products with specific characteristics regarding activity range and stability in feeds. The effect of feed enzymes were mainly studied in broilers on barley or corn based diets with milo or other millets. Only limited research reports are available on the use of dietary enzymes for laying hens, of which most reports deal with an effect on egg production. For a thorough understanding of the importance of enzymes in poultry production and the effects of supplementation on nutrient availability and production performance of birds, the literature has been reviewed under separate heads.

2.1 Egg production, egg weight and egg quality

Berg *et al.* (1956) reported a positive response on egg production when diets formulated with barley was supplemented with enzymes. Nelson and Hutto (1958) showed that the addition of enzymes to barley rations increased egg production and hatchability of eggs. Ely (1963) reported that laying hens fed typical commercial corn /milo layer rations containing fungal and bacterial enzymes exhibited only marginal response until after they reached maximum rate of egg production. Gleaves and Dewan (1970) found that a fungal enzyme in corn and milo rations improved egg production significantly. Tishenkova and Serikov (1987) observed that supplementation of diet containing barley and wheat with the enzyme *Tsellovidirin* G3x increased egg yield. Aimonen and Nasi (1991) found that good quality oats with a multi-enzyme premix could substitute barley in the diet of laying hens without any negative effect on laying performance. Graham (1991) reported a modest but significant increase in egg production from the application of multi-enzyme additive to barley based diets for layers. Jeroch (1991) observed no enzyme effect from multi-enzymes used with whole or partly husked barley, however, with extracts having beta-glucanase and cellulase activities the laying performance could be improved. Wyatt and Goodman (1993) reported that eventhough barley cultivars may react differently as grain source, supplementing the diets containing barley with enzymes may produce positive response on performance of laying hens. Purushothaman and Natanam (1995) evaluated the replacement of maize with little millet with enzyme and reported that addition of enzyme did not influence the age at first egg, but hen-day production was improved.

Incorporating enzymes in layer diets brought about positive effects on egg production. Dovgan *et al.* (1972) observed that amylorizin P10x supplementation increased egg yield in hens. Iotsyus *et al.* (1974) assessed the effect of aminosubtilin GZx and protosubtilin GZx on laying hens and showed that egg production was increased by 5 to 7 per cent with enzymes. Patel and Mc Ginnis (1985) reported a significant increase in egg yield by autoclaving and enzyme supplementation of raw guar meal diet. Nasi (1988) reported that a multi-enzyme product said to contain cellulase, beta-glucanase and protease improved egg production significantly. Kuchta *et al.* (1991) studied the effect of pectinolytic enzymes in commercial diets for laying hens with enzyme and/ or antibiotic and recorded highest egg production with diet containing enzyme and antibiotics. Enzymes possibly improved the persistency of laying. Sharma and Katoch (1993) assessed the effect of adding Novozyme sp-243, a fibre degrading enzyme in the diet in 26 weeks old layers and obtained a numerical but not significant increase in egg production.

Bustany and Elwinger (1988) reported that cross-bred layers fed ground or whole barley with beta-glucanase for a period of 20 to 80 weeks of age showed no significant effect on performance. Richter *et al.* (1990) found that when hybrid Leghorn layers of different age groups were given diets with rye at increasing levels with beta-glucanase or alpha-amylase for varying periods, laying performance was not improved.

Adams (1989) obtained an increase of 7.2 per cent in egg yield using kemzyme containing alpha-amylase, beta-glucanase and protease activity in the feed. The egg size was not affected by enzyme in feed. Mohandas and Devegowda (1991) and Jayanna and Devegowda (1991) recorded that enzyme supplementation of diets containing varying levels of energy and protein for commercial layers improved egg yield numerically in case of low energy feeds, but egg weight was not affected. Prakash and Devegowda (1993) obtained significantly higher egg production when enzymes were incorporated in high fibre diets. The egg weight was not affected by enzyme supplementation. Brufau *et al.* (1994) observed an improvement in egg size with enzyme in barley based diet in young layers.

Tolokonnikov and Berezhnova (1975) reported that supplementation of diets for hens and cocks with Amylosubtilin G3 mostly having beta-amylase activity and protosubtilin G3x with proteolytic activity increased egg yield and improved egg grading and the biological quality of eggs. Baranauskas (1988) reported that there was no adverse effect on egg quality or on visceral organ of hens fed on diet containing ground poultry excreta supplemented with feed fat and enzyme preparations.

Berg (1959) reported that fungal and bacterial enzyme preparations or malt barley in barley based layer ration did not affect rate of lay, hatchability of fertile eggs or egg quality characterstics. Berg (1961) noticed no significant improvement in rate of lay or egg quality traits by bacterial enzymes in barley containing layer diets. Wetscherek and Zollitsch (1991) reported that use of enzyme (Polans) did not improve the egg production and egg quality until the birds attain 45 weeks of age. Aimonen and Rauva (1991) noticed that almost all physical and chemical egg quality traits were significantly affected by hen's age in a linear and gradatic manner. Enzyme supplementation had small but significant negative effect on all the egg quality traits except the percentage of cracked eggs. Yolk colour points were higher on diets with the enzyme.

Blum and Sauveur (1973) observed that Fradiase, a proteolytic enzyme derived from *Streptomyces fradiae*, in diets with two protein levels with or without animal protein did not differ much between treatments in egg production, enzyme produced positive response in egg weight while the effect on egg quality was variable. Korniewicz *et al.* (1980) assessed the influence of the enzymatic preparation 'Promase' on the laying performance of hens and hatchability of eggs using New Hampshire birds receiving different levels of total protein with animal protein and reported a significantly lower egg production. Addition of promase to feeds without animal protein increased egg production by about 4 per cent. There was no enzyme effect on egg weight or egg quality. Benabdeljelil and Arbaoui (1994) found that barley level in diet or enzyme supplementation did not affect egg production and high barley levels in diet had no consistent dietary effects on egg weights, but reduced yolk colour. Brenes *et al.* (1993) studied the effect of beta-glucanase/ pentosanase supplementation of diet containing less commonly used grains and reported no beneficial effect on egg weight, or on egg quality characteristics. Miles *et al.* (1985) noticed no significant benefit in egg production or on egg weight due to addition of enzyme to corn soya bean diet.

2.2 Feed intake and feed efficiency

White *et al.* (1981) recorded improved feed efficiency when a cellulase degrading enzyme was added to barley ration fed to chicks. Hijikuro and Takemasa (1982) reported that in young male White Leghorn chicks receiving a high barley diet as the only grain source cellulase addition significantly increased feed conversion efficiency (P< 0.01). Jeroch (1991) noticed an improvement in feed conversion rate in White Leghorn type layers with enzyme preparation in a ration containing barley. The effect of enzyme feed supplement at different levels on performance of broiler diets was studied by Kadam *et al.* (1991) and noticed a better feed intake and feed efficiency in broilers. Jeroch *et al.* (1993) with Bergazym (cellulolytic and hemi-cellulolytic enzymes) in broiler diets with rye concluded that enzyme supplementation improved feed efficiency in a linear manner. Francesch *et al.* (1994) recorded that *Trichoderma viridae* enzymes (cellulase, beta-glucanase and xylanase) improved feed conversion efficiency in growing chicks.

Berg (1959) observed that corn diet was superior to barley based diet without enzyme in terms of feed efficiency in caged layers and that enzymes improved feed consumption. Gleaves and Dewan (1970) showed that fungal enzyme in corn and milo diets did not influence the feed intake in laying hens, but improved feed efficiency in corn diet. Rexen (1981) reported that enzyme supplementation of diet significantly improved feed utilisation ($P<0.01$). Baranauskas (1988) showed significantly increased feed conversion efficiency in laying hens with enzyme supplementation. Nasi (1989) recorded improved feed conversion rate in the laying period with multi-enzyme preparation in diets with barley and oats. Aimonen and Nasi (1991) observed that feed conversion ratio was significantly improved with multi-enzyme premix (Avizyme) in a diet with barley and oats in laying hens. Brenes *et al.* (1993) reported beneficial effect on feed intake in layers from enzyme supplementation of various cereal based rations.

Berg (1961) noticed that enzyme preparation was of no value in reducing feed consumption during laying period. Miles *et al.* (1985) found no significant benefit in feed conversion from enzyme supplementation of cereal based diets in laying hens. The grain source, rather than enzyme influenced feed consumption and feed conversion rate. Brufau *et al.* (1994) observed that feed intake and feed conversion efficiency were not affected by enzyme supplementation of barley based diet.

2.3 Body weight gain

Kumprecht *et al.* (1990) reported an increased body weight of broiler chicken using a cellulase enzyme in feed mixtures. Morkunas *et al.* (1991) obtained an increase in body weight gain by 3 per cent and yield of first grade meat by 2.9 per cent with an enzyme premix in feed mixtures for broilers. Richter *et al.* (1991) noted that feed enzymes had the most pronounced effects when broiler chicks were fed with rye based diets. Pettersson and Aman (1992) reported improved body weight gain with enzyme supplementation of diet in broilers. Zobac *et al.* (1992) reported that enzyme preparations having amylases and cellulases are effective supplements in poultry feeds in as much as these increased live weight gain in broiler chickens. Brenes *et al.* (1993) noticed that enzymes improved weight gain significantly in broilers when hulled barley was included in the diet ($P < 0.05$). Creswell *et al.* (1995) reported that an enzyme additive to a sorghum based diet containing 25 per cent Canola meal improved broiler weight gains.

Bhatt *et al.* (1991) evaluated the influence of supplementation of a standard diet without or with different levels of a fibre degrading enzyme in broiler chicks and reported that body weight gains were significantly ($P<0.05$) higher with enzyme supplements. Jeroch *et al.* (1993) showed that the enzyme preparations significantly improved performance of broiler chicks and the extent of positive response with enzyme was dependent upon the proportion of barley in the diet and on the age of the chickens.

Balloun and Baker (1957) based on the results obtained in chicks with diets containing varying levels of dietary protein and enzyme concluded that baby chick has a well developed proteolytic enzyme system at hatching time and the enzyme supplementation did not improve growth. Willingham *et al.* (1958) reported a highly significant improvement in growth of chicks with enzyme supplementation of barley containing diets. Leong *et al.* (1961) showed that the determining factor in the response to enzyme supplementation of chick rations was the presence or absence of barley rather than the level of crude fibre in the ration. Anderson and Warnick (1964) observed that addition of crude enzyme preparations to rations containing guar by-products showed increased growth rate of chicks. Rexen (1981) reported that chicks fed on barley based diets with cellulase and/ or pectinase or protease showed increased growth upto 7 per cent ($P<0.05$). Hijikuro and Takemasa (1982) observed that in male White Leghorn chicks given a high barley diet addition of cellulase significantly increased weight gain. Heger *et al.* (1984) showed that Mikrozym, a bacterial protease in commercial feed significantly increased body weight gain in chick and the effect of Mikrozym was greater from 28 to 49 days of age than from 0 to 28 days. Alisheikhov *et al.* (1988) stated that inclusion of multi-enzyme in diets for egg-line chickens improved daily body weight gains. Muramatsu *et al.* (1992) using male Single Comb White Leghorn chickens reported improved body weight gain with fungal enzyme. Brenes *et al.* (1993) found that there was improvement in weight gain of chicken receiving hull-less barley and oats based diets with enzyme combination and on wheat diet, there was no response with enzyme addition. Marquardt *et al.* (1994) recorded that feed enzyme preparations considerably improved ($P<0.05$) weight gain in chicks when added to diets containing barley , wheat and rye, but not when added to the maize diet. Purushothaman and Natanam (1995) reported that little millet (*Panicum miliare*) with enzyme or yeast culture resulted in improvement of body weight gain in growers.

Berg (1961) reported an increased growth rate in White Leghorn chicks upto 8 weeks of age, no improvement during the second stage of growth and some indication of increased body weight gain during the laying period. Gleaves and Dewan (1970) found that the fungal enzyme fed with both corn and milo increased body weight gain of hens. Berezhnora (1979) evaluated the effect of enzyme preparations with amylolytic and proteolytic action in White Russian pullets and showed that the enzyme preparation increased growth and development ensuring better body conformations with normal physiological status. Tishenkova and Serikova (1987) conducted experiments in chicks and laying hens with enzyme preparation and recorded that addition of enzyme increased percentage body weight gain in chicks and in hens feed enzymes reduced cost per weight gain.

Berg (1959) reported that birds fed with corn diet gained more in body weight ($P<0.01$) than those fed any of the barley rations and addition of enzymes to the barley feeds did not increase weight gain. Benabdeljelil and Arbaoui (1994) recorded no significant difference in live body weight in hens from enzyme supplementation of barley based diets.

2.4 Excreta moisture

Berg (1959) observed that in a barley based diet fungal enzyme brought about significant decrease in litter moisture in White Leghorn pullets, while the bacterial enzyme reduced litter moisture slightly, but not significantly. Anderson and Warnick (1964) reported that enzyme preparation when added to rations containing guar meal, guar gum or locust gum reduced the sticky droppings problem. Gohl *et al.* (1978) observed that the sticky droppings and poor performance in poultry when fed with barley could be overcome by either water treatment or heat treatment of barley or by addition of betagluconase. The problem of wet droppings associated with feeding of oats to broilers could be overcome by incorporation of enzyme preparations rich in beta-glucanase activity (Broz and Frigg, 1986 and Campbell *et al.* 1987). Inclusion of endo beta-xylase (pentosanase) in rye based diets has been reported to reduce the incidence of sticky droppings markedly and to improve litter quality (Pettersson and Aman 1988, 1989; Bed ford *et al.* 1991). Mohandas and Devegowda (1991) and Jayanna and Devegowda (1991) observed that diets containing varying levels of energy and protein supplemented with cellulolytic or proteolytic enzymes singly or in combination when fed to commercial layers resulted in more drier litter condition.

2.5 Nutrient availability.

Leong *et al.* (1962) reported that addition of fungal enzyme supplement increased the metabolisable energy of western pearled barley. Potter *et al.* (1965) registered an increase of 18 per cent in metabolisable energy of western grown barley by the presence of fungal enzymes. Moran *et al.*(1969) reported that the metabolisable energy of rye grain was increased when supplemented with a crude fermentation product of Aspergillus species. Kuzmicky *et al.* (1978) using broiler chicks reported that the nitrogen corrected metabolisable energy (MEn) value of wheat bran treated with a commercial cellulolytic enzyme, pectinol 41P was increased by 32 per cent (1612 to 2132 kcal/ kg). Broz (1987) observed an increase in bioavailable energy when rye containing diets were supplemented with different enzymes. Bhatt *et al.* (1991) showed that the efficiency of feed utilisation was significantly ($P<0.05$) higher with a fibre degrading enzyme, Novozyme in broiler chicks and the beneficial effect of feed enzyme was clear in first three weeks and later faded away. Friesen *et al.* (1991) found that the addition of *Trichoderma viridae* (Cellulase) to a diet with high rye content resulted in an increase in the dietary metabolisable energy by 23 per cent compared with the unsupplemented diet. Friesen *et al.* (1992) demonstrated that the nutritive value of different cereal grains and their varieties could be improved by the addition of crude fungal extracts to the diets of young chicks. The anti-nutritives, beta-glucans in barley and oats, and pentosans in rye had the most pronounced effect on nutrient digestibility. Wantia (1993) reported that feed enzymes in the diets for broiler cocks based on rye or wheat enhanced true metabolisable energy by 1.1 and 6.3 per cent respectively. Marquardt *et al.* (1994) reported that enzyme supplementation of the diets containing barley, rye and wheat significantly improved the apparent metabolisable energy of diets by 12, 10 and 4 per cent respectively in growing Leghorn chicks. Apparent protein and dry matter digestibilities were both improved by 6 and 9 per cent in diets with barley and rye respectively. Vranjes *et al.* (1994) showed that enzyme complex Roxazyme-G (beta-glucanase, xylanase, amylase, pectinase) when added to a commercial diet for broiler chicks containing wheat improved energy metabolisability, fat, nitrogen and fibre utilisation, but the difference was not significant. Almirall *et al.* (1995) recorded higher nutrient digestibilities with enzyme supplementation of corn and barley diets in broiler chicks and in one year old cocks, the improvement being more in young chicks. Reddy (1995) reported that ragi is a potential energy source for broiler chicken and the amylase supplementation did not enhance the metabolisable energy of ragi, but increased the dry matter digestibility.

Dovgan *et al.* (1972) estimated the effect of enzyme preparation amylorizin P10x in ration for hens and observed an increase in total sugars, glucose, and amino nitrogen and a reduction in protein in the duodenal contents. Saunders *et al.*(1972) observed that various commercial cellulolytic enzymes increased dry matter and protein digestibility of wheat bran. Bran treated with pectinol P increased dry matter digestibility from 37 per cent to 52 per cent. Herstad and Mc Nab (1975) reported that the digestibility and metabolisable energy values of a North American variety of barley were significantly improved by enzyme supplements containing amylase. Supplementation of poultry feeds with appropriate enzymes partially degraded the endosperm cell wall polysaccharides in the feeds, leading to a more complete absorption of nutrients such as protein, starch and fats in the anterior intestine (Hesselman, 1983 and Bedford, 1991). Hesselman and Aman (1986) stated that the pentosans may impede the nutrient uptake in the small intestine in a way similar to that shown for mixed beta-D-glucans (beta-glucans) the major cell wall constituent in the barley endosperm. Baranauskas (1988) noticed an increase in energy utilisation in hens fed on a diet containing ground poultry excreta supplemented with feed fat and the enzyme preparations. Jongbloed and Kemme (1990) made an attempt to quantify the effect of plant phytase activity on phosphorus digestibility and reported that the presence of phytase in wheat enhanced phosphorus digestibility in wheat from 31 to 49 per cent. Simons and Versteegh (1990) reported that the availability of phosphorus was improved considerably when microbial phytase was added and it also improved the availability of calcium. Slominski and Campbell (1990) reported that when laying hens were fed diet containing 40 per cent commercial canola meal with enzyme non-starch polysaccharide digestibility was increased to 37 per cent. Rotter *et al.* (1990) reported that enzyme supplementation increased bioavailable energy and apparent protein digestibility of barley. Aimonen and Nasi (1991) found that supplementation of diets containing barley with Avizyme, a multi-enzyme premix improved the apparent metabolisable energy in adult cocks and apparent crude fat digestibility showed minor effects when crude fibre content of the diet was less than 8 per cent. Kuchta *et al.* (1991) observed that hens fed a basal diet with enzymes and antibiotic resulted in higher nitrogen retention. Sharma and Karoch (1993) registered significantly higher apparent digestible fibre and cellulose with a fibre degrading enzyme in layer diet but with no improvement on metabolisability co-efficients of proximate principles except ether extract. Wyatt and Goodman (1993) recorded that barley cultivars may react differently and suggested that some factors, probably other than just total beta-glucan in the barley might be interacting to alter digestion and dietary energy utilisation in diets for laying hens, enhancing energy bioavailability. Pointillart (1994) stressed the importance of cereal phytases since a high dietary phytase might lead to a greater absorption of phosphorus. Improved phosphorus utilisation was generally accompanied by improved calcium retention. Creswell and Haddengraham (1995) demonstrated that certain enzyme combinations improve the energy value and increase digestible protein and amino acids in wheat and barley.

Gohl *et al.* (1978) reported that use of beta-glucanase or water treatment of barley did not significantly influence the nutritional value of the medium viscosity barley in chicks, but enzyme treatment of micronised or autoclaved barley increased nutritional values. Hijikuro and Takemasa (1982) noticed that addition of commercial cellulase to a diet containing 60 per cent barley significantly increased metabolisable energy of barley in male White Leghorn chicks. Petterson and Aman (1989) reported that the metabolisable energy of rye diet was increased significantly ($P<0.05$) by supplementation with a pentosanase preparation with concurrent improvements ($P<0.05$)in the ileal digestibilities of organic matter, crude protein and starch. The effect of enzyme supplementation of different wheat fractions were studied by Steenfeldt (1991) in adult roosters and reported that enzyme treatment increased digestibility of ether extract and AMEn significantly ($P<0.01$) in the bran fraction, where the content of non-starch polysaccharides (NSP) is very high. Digestibility of protein and carbohydrates were not significantly affected. Annison (1992) found that commercial enzyme used in a wheat based broiler diet significantly raised apparent metabolisable energy so also starch digestibility and apparent pentosan digestibility in comparison with control. Kiiskinen (1993) reported that supplementation of several industrial by-products mainly originating from grains with enzymes in adult cockerels did not significantly improve their metabolisable energy.

2.6 Intestinal viscosity

White *et al.* (1981) observed that barley beta-glucan in a corn based diet increased the viscosity of the chick intestinal contents and the culture filtrate with beta-glucanase activity reduced the intestinal viscosity nearer to that of control diets. Bedford *et al.* (1994) studied the effect of enzyme supplementation on gut viscosity in a trial with broiler chicks and demonstrated that suppplementation of barley based diets with appropriate enzymes reduced intestinal viscosity thus improving the efficiency of digestive enzymes

2.7. Cost-benefit analysis

Rexen (1981) suggested that better feed utilisation could be obtained by use of enzyme in feed mixture only if the feed was compounded with less-digestible feed ingredients. Inborr (1990) noticed that instead of single activity enzyme product multi-enzyme products specifically designed for application to particular feed types were more cost-effective in commercial practice. Kadam *et al.* (1991) reported that enzyme supplements in broiler feed was cost-effective under tropical conditions in India. Graham and Inborr (1991) reported that use of appropriate microbial enzymes in feeds has given more formulation flexibility to poultry producers and to reduce feed cost.

Baranauskas (1988) recorded a significantly reduced cost of feeds in hens with enzyme supplementation. Iotsyus *et al.* (1974) reported that aminosubtilin GZx and protosubitilin GZx in layer diets reduced the feed cost by 5 to 6 per cent. Morkunas *et al.* (1991) reported that use of enzyme premix in diets for broiler chicken at the rate of 3 kg /tonne has reduced the feed cost per kg gain by 3 per cent.

MATERIALS AND METHODS

MATERIALS AND METHODS

An investigation was carried out in the Department of Nutrition, College of Veterinary and Animal Sciences, Mannuthy, to find out the influence of feed enzymes on nutrient availability and production performance of laying hens.

3.1 Experimental materials

3.1.1 Birds

One hundred IWN strain of Single Comb White Leghorn pullets of 20 weeks of age procured from the Mannuthy centre of All India Co-ordinated Research Project on Poultry for Eggs, formed the experimental subjects.

3.1.2 Rations

The feed ingredients required for the study were procured from the local market. The standard layer ration (SLR) was computed as per BIS (1993) for chicken layers. The less dense ration (LDR) was formulated with less percentage of crude protein and low level of metabolisable energy than the standard layer ration. A few of the feed items such as jowar, wheat bran, rice bran (deoiled) and sun flower cake (undecorticated) with high dietary fibre or non-starch polysaccharides were incorporated in the above rations. The ingredient composition and the chemical composition of the two rations are set out in Tables 1 and 2 respectively. The layer rations were compounded every month as per the ingredient composition. Cellulase (Jaysons cellulase, activity 30 FPU per gram) and protease (activity 45,000 to - 48,000 IU) were added at the rate of 0.06 per cent and 0.02 per cent of the feed respectively.

3.2 Experimental methods

3.2.1 Housing of birds.

The experiment was conducted at the Mannuthy centre of AICRP on Poultry for Eggs. The cage house, the cages, the feed trough and water channel were cleaned thoroughly and disinfected one week prior to housing of pullets. The pullets were weighed and wing badged.

3.2.2 Experimental design

The pullets were allotted randomly to five dietary treatments, viz., T_1, T_2, T_3, T_4 and T_5 so as to have almost the same initial average body weight in all the groups (Table 3).

Table 3. **Distribution of dietary treatments**

Dietary treatment	No. of birds	Rations	Enzyme treatment	Level of enzyme inclusion (%)
T_1	20	SLR	Nil	-
T_2	20	SLR	Cellulase & Protease	0.06 & 0.02
T_3	20	LDR	Cellulase	0.06
T_4	20	LDR	Protease	0.02
T_5	20	LDR	Cellulase & Protease	0.06 & 0.02

3.2.3 Management

Feed and water were provided *ad libitum* throughout the experiment. Routine managemental procedures were followed for the entire period of the experiment. The duration of the experiment was five 28-day periods.

3.2.4 Microclimate

The dry and wet bulb readings and the ambient temperature were recorded at 7 a.m., 1 p.m. and 4 p.m. each day, throughout the experimental period. The average day-light hours were calculated from available data. No artificial lighting was provided to the birds.

3.2.5 Body Weight

The body weight of the birds were recorded at the commencement and at the end of the experiment.

3.2.6 Feed intake and feed efficiency

Feed intake of the birds in different treatment groups was recorded at weekly intervals and the average daily feed intake and feed efficiency for egg number (kg feed/ dozen egg) and egg mass (kg feed/ kg egg) were calculated.

3.2.7 Egg production

Individual egg production records were maintained and percentage hen-housed and hen-day egg production were calculated. All eggs produced in last three days of each period were weighed. Egg quality traits were assessed based on eggs collected for three consecutive days at 39 weeks of age of the birds. The height and width of albumen and yolk, and shell thickness were measured and albumen and yolk indexes and Haugh unit scores were calculated.

The microbial load on eggs from each treatment group was estimated using eggs collected from six birds at random from each treatment for three days. Soon after laying, each egg was transferred to a 250 ml beaker containing 10 ml of sterile saline solution. From the solution in beaker 0.5ml was poured on agar plate (Pour plate technique) and incubated for 24 hrs. The microbial load on the eggs was calculated by counting the colonies developed on agar plates.

3.2.8 Metabolism trial

Towards the end of the experiment a three day metabolism trial was conducted using six birds randomly selected from each treatment group. Total collection method was employed. Water was provided *ad libitum*. The excreta collected from each bird, each day was stored in a deep freezer for analysis.

3.2.9 Chemical analysis

The chemical composition and the fibre fractions of the different rations and excreta collected from the metabolism trial were estimated as per the procedures in AOAC (1990) and Van-Soest and Wine (1967) respectively. The content of nitrogen was analysed using Tecator Kjeltec system. Nitrogen content of excreta was determined in fresh material. Uric acid nitrogen in the droppings was determined (Marquardt, 1983) and the nutrient digestibilities were calculated.

The gross energy in feed and the excreta samples were estimated using adiabatic digital bomb calorimeter. From the data collected, the apparent metabolisable energy of different dietary treatments was calculated.

3.2.10 Intestinal viscosity

At the end of the experiment, four birds from each treatment group were randomly selected and sacrificed to determine the viscosity of intestinal contents. The intestine was ligatured at the junction of duodenum and jejunum and at the ileo-caecal junction. The intestinal contents from the ligatured portion of the intestine were collected in a test tube and centrifuged at 6000 rpm. The supernatant fluid was used for estimation of viscosity by Ostwald viscosity meter (Oser, 1965).

3.2.11 Livability

The mortality from each treatment group was recorded. Post-mortem examination was conducted in each case to find out the cause of death.

3.2.12 Cost-benefit analysis

From the cost of feed, the quantity of feed consumed and number of eggs produced by birds in each treatment group, the cost-benefit analysis was carried out.

3.2.13 Statistical analysis

The data collected on various parameters were statistically analysed as per the methods of Snedecor and Cochran (1967).

Table 1. Percentage ingredient composition of the experimental rations

Ingredients	Standard layer ration (SLR)	Less dense ration (LDR)
Yellow maize	24.00	19.00
Jowar	20.00	20.00
Groundnut cake (exp)	12.00	7.00
Gingelly oil cake	5.00	5.00
Sunflower cake	10.00	10.00
Wheat bran	8.00	13.00
De-oiled rice bran	8.00	13.00
Unsalted dried fish	6.00	6.00
Shell grit	5.00	5.00
Common salt	0.25	0.25
Mineral mixture*	1.75	1.75
Vitamin mixture **	0.01	0.01
Calculated chemical composition:		
Crude protein %	18.1	16.7
ME kcal /kg of feed	2630	2515
Cost of the feed /100kg	Rs. 493.50	Rs. 469.53

*** Mineral mixture composition:**

Calcium 32%, Phosphorus 6%, Magnesium 100ppm, Iron 0.1%, Iodine 100 ppm, Copper 100 ppm, Manganese 2700 ppm.

**** Vitamin mixture composition:**

Vitamin A - 82,000 IU, Vitamin B2 - 50 mg, Vitamin D3 - 12,500 IU & Vitamin K - 10 mg / g

Table . 2 Percentage chemical composition of the experimental rations (dry matter basis)

Nutrients	Standard layer ration* (SLR)	Less dense ration* (LDR)
Dry matter	91.5±0.80	91.6±0.85
Crude protein	18.2±0.16	16.1±0.11
Ether extract	4.5±0.12	4.0±0.20
Crude fibre	6.7±0.17	7.6±0.23
NFE	58.5±0.64	60.3±0.59
Total ash	12.1±0.53	12.0±0.45
Acid insoluble ash	3.6±0.09	4.1±0.09
Calcium	3.0±0.08	3.0±0.04
Phosphorus	1.0±0.03	1.1±0.04
NDF	22.6±0.16	23.8±0.37
ADF	11.2±0.32	13.2±0.27

* Average of six samples

Plate 1. Birds in the feeding experiment with enzymes in progress.

Plate 2. Birds in the feeding experiment with enzymes - metabolism trial.


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RESULTS

RESULTS

The influence of feed enzymes on nutrient availability and production performance in laying hens was assessed by conducting a feeding trial in Single Comb White Leghorn pullets of 20 weeks of age for a period of 20 weeks. The results obtained are presented in this chapter.

Microclimate

The mean temperature and relative humidity during different periods in the experiment from October 1994 to February 1995 are presented in Table 4.

4.1 Egg production, egg weight and egg quality

The data on percentage hen-housed and hen-day egg production for different treatment groups during the five periods are set out in Tables 5 and 6 and the statistical analysis of the data on weekly basis in Tables 7 and 8 respectively. Total number of eggs produced by the birds in different treatment groups were analysed statistically and the data are presented in Table 9.

The age of the birds in days at 10, 50, and 95 per cent production are depicted in Fig..1 and hen-housed and hen-day egg production expressed as per cent in Fig. 2 and 3 respectively.

The egg weight records of the birds in the five treatment groups for the five periods are given in Table 10 and their statistical analysis in Table 11.

The data on egg quality traits such as yolk index, albumen index, Haugh unit score and shell thickness are presented in Tables 12, 13, 14, and 15 respectively. Table 16 includes the summarised data on egg quality and Table 17, its statistical analysis.

4.2 Feed intake and feed efficiency

Feed intake per week and the period-wise daily average feed intake of birds in different treatment groups are given in Tables 18 and 19 respectively and its statistical analysis data in Table 20.

The data on feed efficiency for egg number (dozen egg) and egg mass (kg) during different periods are set out in Tables 21 and 22 respectively. Feed efficiency for egg number from 27th week onwards is represented in Table 21a and Fig. 4. Feed efficiency (egg mass) from third period onwards is graphically represented in Fig. 5.

The feed efficiency for producing dozen eggs from 27th week onwards was statistically analysed by 't' test and data given in Table 23.

4.3 Body weight gain

Body weight gain records of the birds in five treatment groups are set out in Table 24. The above data were statistically analysed and data presented in Table 25.

4.4 Excreta moisture

The moisture contents of droppings collected during the three day metabolism trial are given in Table 26 and the statistical analysis in Table 27. Table 28 contains the values on microbial load on the eggs from different treatment groups.

4.5 Nutrient availability

The data on apparent metabolisable energy of feeds from different treatments are set out in Table 29 and graphically represented in Fig. 6. The statistical analysis of data is presented in Table 30.

The digestibility coefficients for crude protein, ether extract, NFE and fibre fractions are presented in Tables 31, 32, 33 and 34 respectively and graphically represented in Fig. 7. The statistical analysis of the data on nutrient digestibility are given in Table 35.

4.6 Intestinal viscosity

The data on the viscosity of intestinal contents of birds sacrificed at the end of the experiment are presented in Table 36.

4.7 Livability

The data on mortality of birds during the experimental period are presented in Table 37.

4.8 Cost-benefit analysis

The cost of experimental rations without and with enzymes are presented in Table 38 and the economics of enzyme supplementation in Table 39.

4.9 Overall performance

The performance of birds in different dietary treatments in respect of egg production is set out in Table 40.

Table 4. Meteorological data for the experimental period (Oct.'94 to Feb.- '95)

h

Months (28 days period)	Temperature °C			Relative Humidity %			Day light in hrs/ min.
	7 a.m	1 p.m	4 p.m	7 a.m	1 p.m	4 p.m	
Oct.- Nov.	25.4	30.8	29.9	80	64	70	11:53
Nov.- Dec.	25.5	30.3	28.9	70	56	59	11:28
Dec.	23.8	31.0	29.6	66	46	49	11:17
Jan.	23.2	30.6	30.5	68	48	49	11:24
Jan.- Feb.	24.7	33.2	32.5	91	58	59	11:38

Table 5. Influence of feed enzymes on percentage hen- housed egg production

Age in weeks	Dietary treatments				
	T1	T2	T3	T4	T5
21 - 24	11.96	10.36	8.39	11.25	7.86
25 - 28	66.43	77.14	62.68	62.68	49.64
29 - 32	88.93	91.61	86.07	92.86	84.29
32 - 36	87.50	89.82	86.07	87.68	85.18
37 - 40	83.04	85.36	82.14	84.46	87.86
Overall	67.57	70.68	64.50	67.82	62.86

Table 6. Influence of feed enzymes on percentage hen-day egg production

Age in weeks	Dietary treatments				
	T1	T2	T3	T4	T5
21 - 24	11.96±1.22	10.36±2.12	8.39±1.31	11.25±1.08	7.86±1.04
25 - 28	66.43±2.77	76.61±2.70	62.86±4.21	62.86±4.41	49.64±3.89
29 - 32	88.93±1.01	91.61±1.26	89.30±1.47	92.86±0.79	87.37±1.50
32 - 36	87.50±1.40	92.99±1.11	85.03±1.80	91.05±1.34	89.66±1.45
37 - 40	83.04±0.79	89.66±1.16	92.06±1.23	88.91±1.09	92.29±0.88
Overall	67.57	71.91	67.31	69.03	64.54

Table 8. Influence of feed enzymes on hen-day egg production - ANOVA

Source	d.f	s.s	m.s.s	F
Treatment	4	257.190	64.297	0.13 NS
Error	95	48035.330	505.635	
Total	99	48292.520		

NS Not significant

Table 9. Influence of Feed enzymes on egg production - ANOVA

Source	d.f	s.s	m.s.s	F
Treatment	4	1631.16	407.79	1.026 NS
Error	95	37770.55	397.585	
Total	99	39401.71		

NS Not significant

Fig. 1. Influence of feed enzymes on age at different levels of production (days)

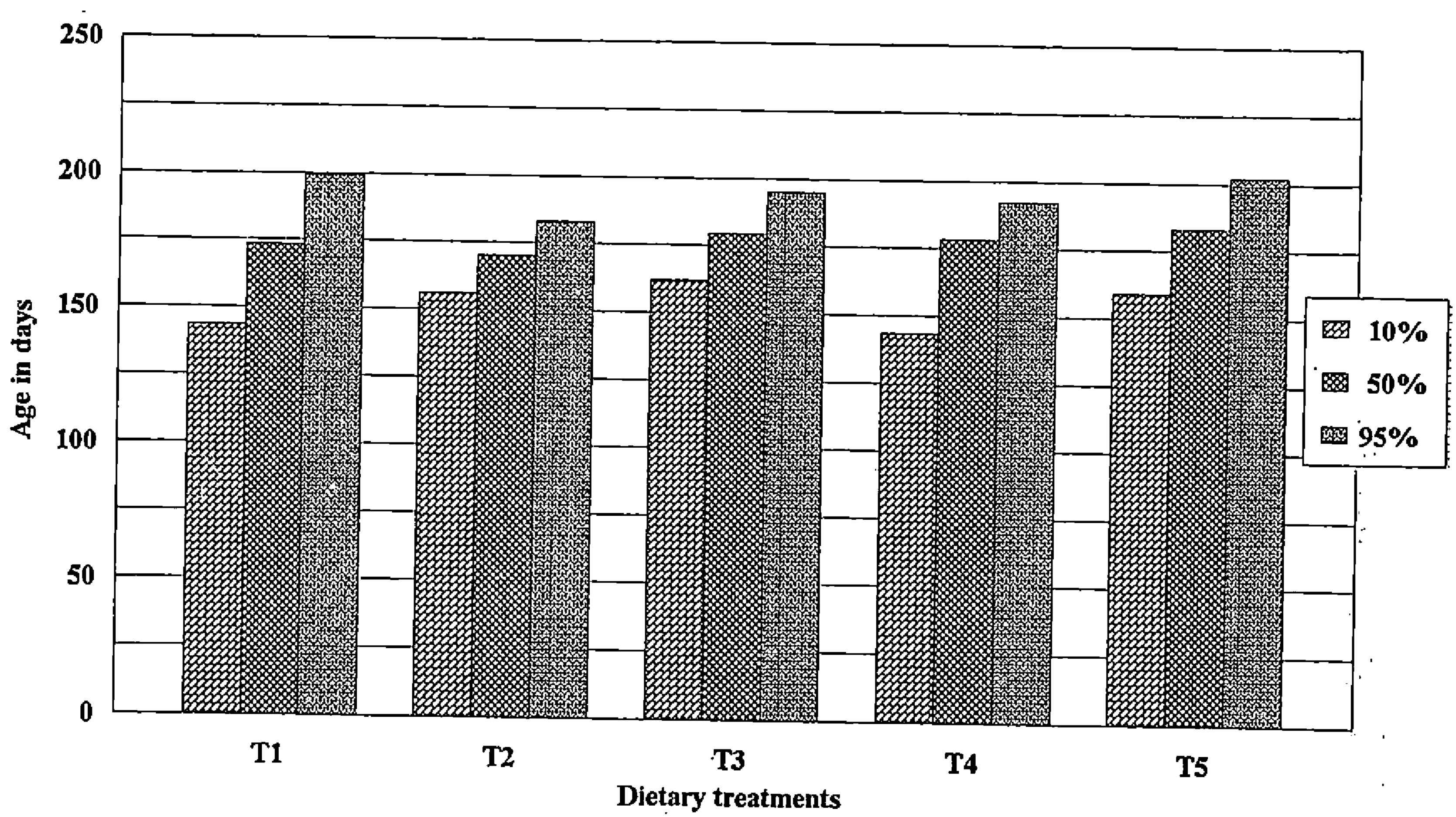

Fig. 2. Influence of feed enzymes on percentage hen-housed egg production(week)

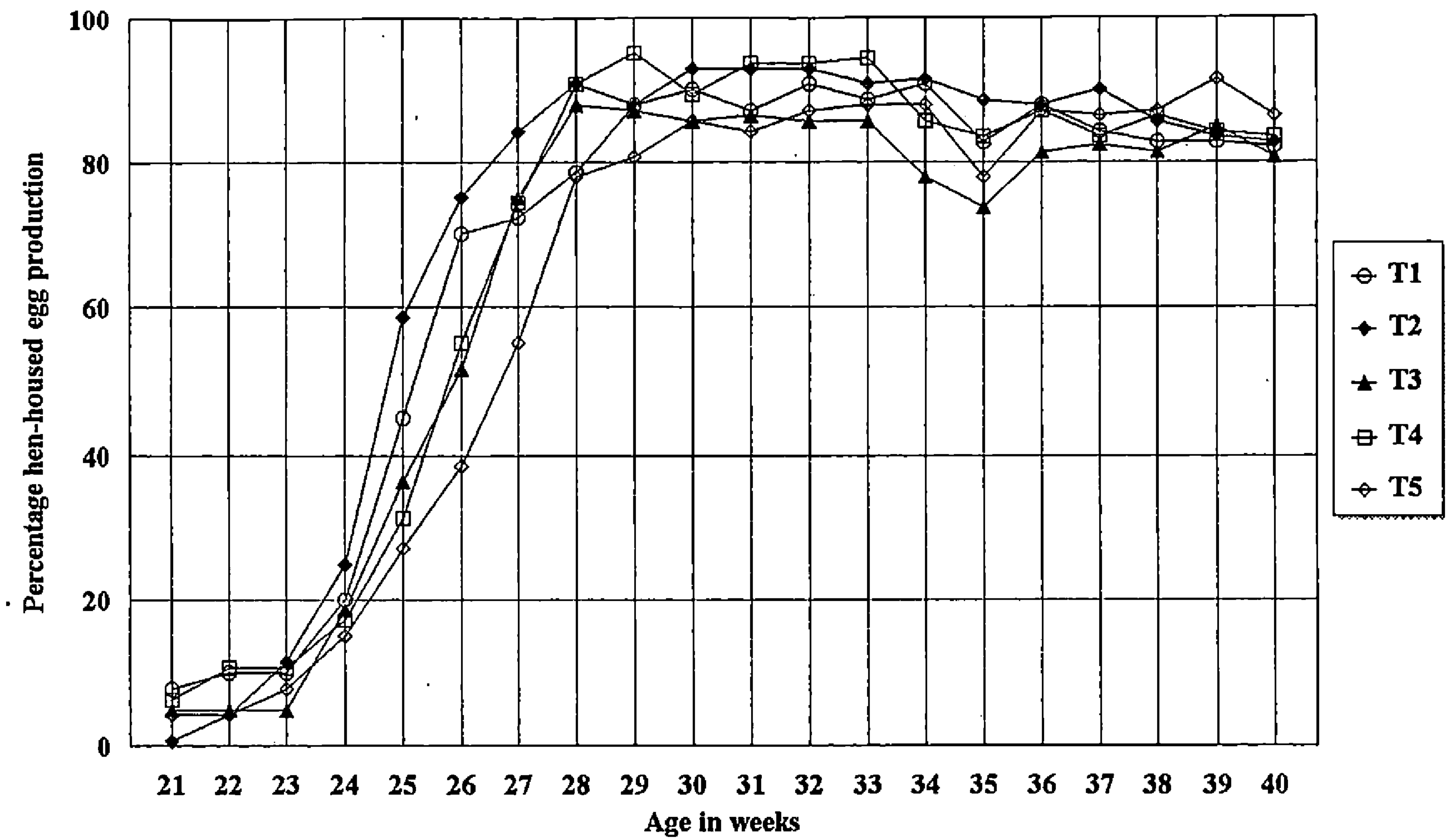

Table 10. Influence of feed enzymes on egg weight (g)

Age in weeks	Dietary treatments				
	T1	T2	T3	T4	T5
21 - 24	45.4±1.61	48.3±0.78	49.1±1.35	46.5±1.12	44.0±1.32
25 - 28	52.0±0.99	52.6±0.67	52.3±0.76	51.5±0.84	50.7±1.06
29 - 32	54.3±1.07	53.0±0.75	54.2±0.56	54.7±0.84	53.9±0.83
32 - 36	55.6±0.67	56.8±0.74	55.9±0.64	56.3±0.75	55.5±0.70
37 - 40	55.5±1.14	56.6±0.74	55.7±0.88	56.2±0.82	55.6±0.60
Mean±	52.56	53.46	53.44	53.04	51.94
SE	1.90	1.56	1.26	1.85	2.17

Table 11. Influence of feed enzymes on egg weight - ANOVA

Source	d.f	s.s	m.s.s	F
Treatment	4	18.216	4.554	0.376 NS
Error	94	1138.09	12.107	
Total	98	1156.306		

NS Not significant

Table 12. Influence of feed enzymes on egg quality-Yolk index

Bird No.	Dietary treatments				
	T1	T2	T3	T4	T5
1	0.40	0.44	0.38	0.43	0.42
2	0.40	0.40	0.41	0.43	0.41
3	0.40	0.43	0.39	0.42	0.40
4	0.37	0.38	0.42	-	0.41
5	0.41	0.39	0.41	0.41	0.42
6	0.40	-	0.38	0.43	0.42
7	0.37	0.42	0.43	0.36	0.41
8	0.39	0.42	0.41	-	-
9	0.40	0.40	0.43	0.40	0.44
10	0.39	0.43	-	0.42	0.43
11	0.40	-	0.41	0.42	0.40
12	-	0.41	0.44	0.39	0.41
13	0.42	0.41	0.41	0.42	0.35
14	0.34	0.35	-	0.40	0.38
15	0.41	0.42	0.41	0.39	0.42
16	0.44	0.40	-	0.40	0.40
17	0.41	0.43	0.38	0.41	0.43
18	0.41	-	0.43	0.38	0.42
19	0.41	0.40	0.42	0.38	0.43
20	0.40	0.37	0.39	0.40	0.43
Mean±SE	0.40±0.005	0.41±0.006	0.41±0.004	0.41±0.004	0.41±0.005

Table 13. **Influence of feed enzymes on egg quality-albumen index**

Bird No.	Dietary treatments				
	T1	T2	T3	T4	T5
1	0.08	0.11	0.08	0.10	0.07
2	0.06	0.10	0.07	0.07	0.07
3	0.09	0.10	0.08	0.09	0.10
4	0.11	0.08	0.10	-	0.08
5	0.09	0.09	0.11	0.09	0.05
6	0.10	-	0.05	0.06	0.05
7	0.08	0.09	0.07	0.06	0.07
8	0.10	0.09	0.10	-	-
9	0.07	0.07	0.05	0.06	0.05
10	0.08	0.07	-	0.10	0.07
11	0.10	-	0.07	0.08	0.06
12	-	0.07	0.08	0.08	0.05
13	0.09	0.07	0.07	0.08	0.09
14	0.08	0.06	-	0.08	0.08
15	0.08	0.08	0.08	0.04	0.10
16	0.06	0.08	-	0.05	0.04
17	0.04	0.11	0.06	0.08	0.11
18	0.06	-	0.09	0.07	0.09
19	0.07	0.04	0.05	0.06	0.07
20	0.08	0.09	0.07	0.05	0.08
Mean±SE	0.08±0.004	0.08±0.005	0.07±0.004	0.07±0.004	0.07±0.005

Table 14. **Influence of feed enzymes on egg quality-Haugh unit score**

Bird No.	Dietary treatments				
	T1	T2	T3	T4	T5
1	80.50	93.50	79.00	86.33	74.00
2	73.00	89.50	74.33	77.00	73.33
3	83.33	87.00	80.00	85.67	85.33
4	91.00	79.67	87.67	-	81.00
5	84.67	85.00	89.33	83.00	65.00
6	87.67	-	65.00	69.00	65.33
7	79.00	84.67	75.50	73.50	74.33
8	87.00	87.50	86.33	-	-
9	77.67	78.33	69.67	73.00	66.33
10	79.00	74.00	-	86.00	79.00
11	88.67	-	77.67	78.00	73.00
12	-	75.67	79.67	79.33	68.67
13	84.00	76.50	75.67	82.00	84.50
14	78.00	68.00	-	77.33	79.00
15	82.00	79.67	80.67	61.67	85.50
16	73.00	81.67	-	66.33	52.33
17	60.67	88.33	71.67	81.33	89.50
18	73.00	-	85.50	74.33	85.00
19	78.00	62.33	65.00	75.50	72.50
20	79.00	84.50	74.00	65.67	78.00
Mean±SE	79.96±1.62	80.93±1.94	77.45±1.76	76.39±1.72	75.35±2.11

Table 15. Influence of feed enzymes on egg quality-shell thickness (mm)

Bird No.	Dietary treatments				
	T1	T2	T3	T4	T5
1	0.51	0.46	0.45	0.51	0.42
2	0.47	0.46	0.55	0.49	0.44
3	0.43	0.55	0.50	0.50	0.47
4	0.49	0.45	0.48	-	0.45
5	0.49	0.54	0.52	0.49	0.46
6	0.46	-	0.50	0.52	0.45
7	0.57	0.54	0.46	0.43	0.51
8	0.55	0.51	0.46	-	-
9	0.62	0.44	0.49	0.46	0.53
10	0.57	0.45	-	0.51	0.48
11	0.52	-	0.46	0.47	0.44
12	-	0.51	0.43	0.52	0.47
13	0.55	0.47	0.47	0.48	0.48
14	0.49	0.61	-	0.54	0.55
15	0.54	0.53	0.58	0.50	0.54
16	0.52	0.47	-	0.47	0.54
17	0.55	0.51	0.46	0.48	0.57
18	0.50	-	0.45	0.45	0.54
19	0.51	0.48	0.50	0.54	0.53
20	0.49	0.46	0.55	0.46	0.58
Mean±SE	0.52±0.01	0.49±0.01	0.49±0.01	0.49±0.01	0.50±0.01

Table 16. **Influence of feed enzymes on egg quality (summarised data)**

Parameters	Dietary treatments				
	T1	T2	T3	T4	T5
Yolk index	0.40 ±0.005	0.41 ±0.006	0.41 ±0.004	0.41 ±0.004	0.41 ±0.005
Albumen index	0.08 ±0.004	0.08 ±0.005	0.07 ±0.004	0.07 ±0.004	0.07 ±0.005
Haugh unit score	79.96 ±1.62	80.93 ±1.94	77.45 ±1.76	76.39 ±1.72	75.35 ±2.11
Shell thickness (mm)	0.52 ±0.01	0.49 ±0.01	0.49 ±0.01	0.49 ±0.01	0.50 ±0.01

Table 17. Influence of feed enzymes on egg quality - ANOVA

source	d.f	m.s.s				F value			
		Yolk index	Albumen index	Haugh units	Shell thickness	Yolk index	Albumen index	Haugh units	Shell thickness
Treatments	4	0.0005	0.0005	100.98	0.0025	1.25 NS	1.667 NS	1.651 NS	1.319 NS
Error	85	0.0004	0.0003	62.96	0.0018				
Total	89								

NS Not significant

Table 18. Influence of feed enzymes on feed intake (kg per week)

Age in weeks	Dietary treatments				
	T1	T2	T3	T4	T5
21	13.4	12.8	12.8	13.6	13.4
22	15.2	13.7	13.2	13.6	14.6
23	15.0	15.0	14.6	14.4	14.1
24	16.6	15.7	14.8	13.3	14.0
25	14.9	17.4	16.8	16.0	15.5
26	15.8	15.7	14.9	16.0	16.0
27	15.8	15.7	14.9	16.0	16.0
28	14.9	16.6	16.1	16.5	16.7
29	16.8	17.9	17.7	18.0	16.8
30	15.8	16.9	17.9	17.1	17.4
31	17.0	17.5	17.3	18.6	17.8
32	18.3	18.5	17.2	18.3	17.0
33	17.9	17.5	16.5	17.3	17.7
34	17.1	16.2	15.1	15.9	15.5
35	17.3	17.3	16.7	17.2	16.1
36	17.2	17.5	17.6	18.2	18.6
37	17.6	17.0	16.6	17.7	17.2
38	17.0	16.3	16.3	17.5	16.7
39	16.6	15.9	15.4	17.3	16.6
40	16.2	15.0	14.8	17.2	16.4
Total	326.4	326.1	317.2	329.7	324.1

Table 19. Influence of feed enzymes on feed intake (g /bird/day)

Age in weeks	Dietary treatments				
	T1	T2	T3	T4	T5
21 - 24	107±4.68	102±4.63	99±3.57	98±1.69	100±1.76
25 - 28	109±1.86	116±2.92	112±3.36	115±0.89	114±1.76
29 - 32	121±3.67	126±2.41	129±1.09	128±2.32	127±2.85
32 - 36	124±1.28	126±2.63	125±5.14	127±3.91	127±5.36
37 - 40	120±2.13	120±3.14	125±3.29	131±0.83	125±1.28
Mean±	117	119	118	120	119
SE	3.31	4.50	5.62	6.12	5.27

Table 20. Influence of feed enzymes on feed intake- 't' test values

Treatments	't' values
T1 VsT2	0.6216 NS
T1 VsT3	0.4075 NS
T1 VsT4	0.9435 NS
T1 VsT5	0.5311 NS
T2 VsT3	0.1297 NS
T2 VsT4	0.3638 NS
T2 VsT5	0.8068 NS
T3 VsT4	0.4524 NS
T3 VsT5	0.7111 NS
T4 VsT5	0.9847 NS

NS Not Significant

Table 21. Influence of feed enzymes on feed efficiency (egg number)

Age in weeks	Dietary treatments				
	T1	T2	T3	T4	T5
21 - 24	10.78±1.65	11.83±35.04	14.14±4.15	10.46±2.37	15.30±4.97
25 - 28	1.98±0.27	1.82±0.23	2.14±0.55	2.21±0.63	2.77±0.67
29 - 32	1.64±0.05	1.66±0.04	1.75±0.02	1.66±0.02	1.75±0.03
32 - 36	1.70±0.04	1.63±0.04	1.77±0.04	1.68±0.06	1.71±0.07
37 - 40	1.74±0.02	1.61±0.17	1.65±0.05	1.77±.02	1.63±0.03
Overall	2.07	1.98	2.11	2.08	2.21

Table 21a. Influence of feed enzymes on feed efficiency (egg number)/week

Age in weeks	Dietary treatments				
	T1	T2	T3	T4	T5
27	1.88	1.61	1.70	1.85	2.49
28	1.63	1.59	1.57	1.56	1.84
29	1.64	1.75	1.74	1.62	1.78
30	1.50	1.56	1.79	1.64	1.74
31	1.67	1.62	1.72	1.70	1.81
32	1.73	1.71	1.72	1.68	1.66
33	1.73	1.65	1.65	1.57	1.73
34	1.62	1.52	1.66	1.59	1.51
35	1.79	1.67	1.74	1.76	1.77
36	1.68	1.71	1.84	1.79	1.83
37	1.79	1.62	1.73	1.82	1.72
38	1.76	1.64	1.69	1.74	1.64
39	1.72	1.63	1.54	1.76	1.61
40	1.69	1.57	1.54	1.76	1.63
Mean±SE	1.70±0.02	1.63±0.02	1.69±0.02	1.70±0.02	1.77±0.06

Fig. 4. Influence of feed enzymes on feed efficiency (egg number)

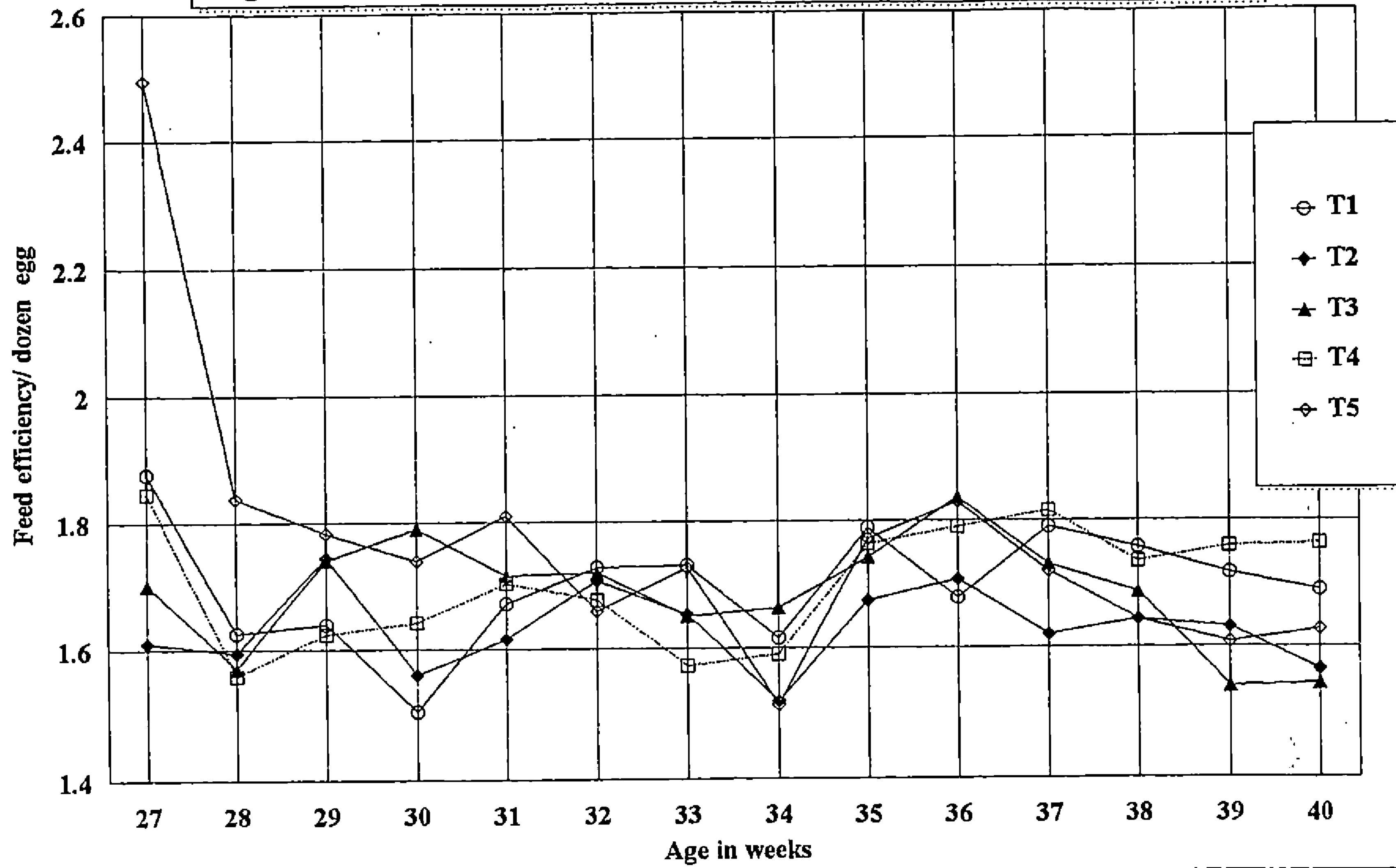

Table 22. Influence of feed enzymes on feed efficiency (egg mass)

Age in weeks	Dietary treatments				
	T1	T2	T3	T4	T5
21 - 24	19.79	20.42	24.01	17.55	28.72
25 - 28	3.17	2.88	3.42	3.60	4.55
29 - 32	2.49	2.60	2.68	2.53	2.71
32 - 36	2.55	2.40	2.64	2.48	2.56
37 - 40	2.61	2.37	2.46	2.62	2.45
Overall	3.18	2.86	3.26	3.18	3.40

Fig.5 Influence of feed enzymes on feed efficiency (egg mass)

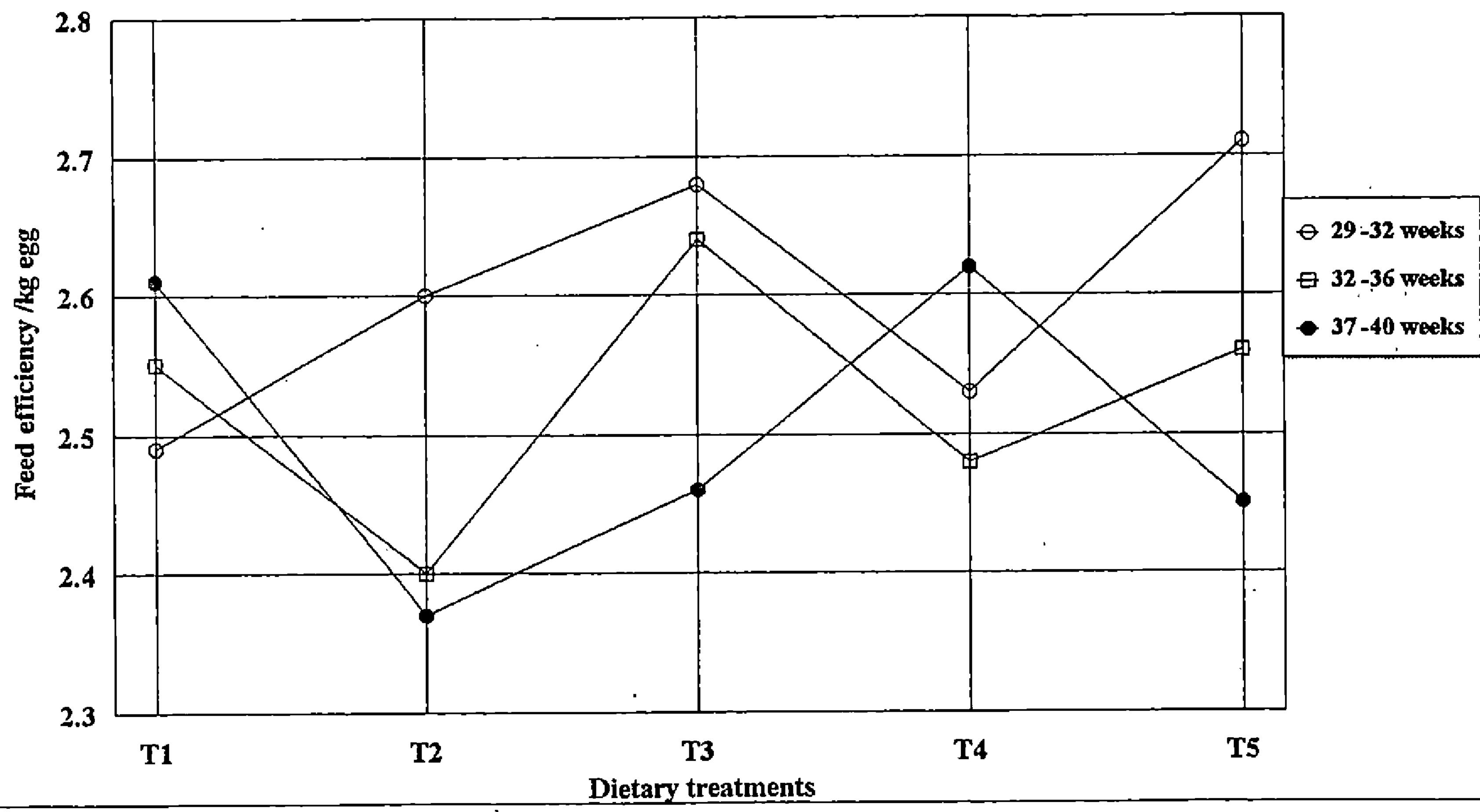

Table 23. **Influence of feed enzymes on feed efficiency (egg number) 't' test values**

Treatments	't' values
T1 VsT2	2.7053 *
T1 VsT3	0.4026 NS
T1 VsT4	0.0313 NS
T1 VsT5	1.2337 NS
T2 VsT3	2.4682 *
T2 VsT4	2.4807 *
T2 VsT5	2.2107*
T3 VsT4	0.4753 NS
T3 VsT5	1.3320 NS
T4 VsT5	1.7550 NS

* Significant ($P<0.05$)

NS Not Significant

Table 24. Influence of feed enzymes on body weight gain (kg)

Bird No.	Dietary treatments				
	T1	T2	T3	T4	T5
1	0.22	0.58	0.56	0.32	0.63
2	0.50	0.39	0.68	0.48	0.55
3	0.40	0.78	0.50	0.55	0.34
4	0.46	0.36	0.52	died	0.40
5	0.40	0.64	0.62	0.42	0.47
6	0.45	0.61	0.42	0.74	0.44
7	0.82	0.55	0.69	0.47	0.60
8	0.43	0.57	0.71	0.77	died
9	0.31	0.61	0.49	0.77	0.56
10	0.65	0.65	died	0.56	0.70
11	0.57	0.16	0.36	0.46	0.45
12	1.22	0.67	0.46	0.63	0.57
13	0.83	0.74	0.62	0.69	0.56
14	0.67	0.45	0.23	0.27	0.46
15	0.48	0.67	0.16	0.50	0.41
16	0.67	0.48	died	0.52	0.73
17	0.54	0.59	0.49	0.40	0.50
18	0.68	died	0.61	0.67	0.79
19	0.70	0.55	0.52	0.24	0.43
20	0.36	0.61	0.49	0.52	0.06
Mean±SE	0.57±0.05	0.56±0.03	0.51±0.03	0.53±0.04	0.51±0.04

Table 25. Influence of feed enzymes on body weight gain- ANOVA

Source	d.f	s.s	m.s.s	F
Treatment	4	0.064	0.016	0.552 NS
Error	90	2.63	0.029	
Total	94	2.694		

NS Not significant

Table 26. Influence of feed enzymes on excreta moisture (percentage)

Bird No.	Dietary treatments				
	T1	T2	T3	T4	T5
1	77.0	77.0	78.4	70.1	56.5
2	75.6	67.5	75.6	75.7	68.1
3	75.5	67.5	70.4	77.8	78.1
4	76.4	78.1	50.8	70.1	74.1
5	74.9	76.5	70.3	74.9	75.9
6	82.6	77.9	75.9	77.0	66.0
Mean±	**77.0**	**74.1**	**70.3**	**74.3**	**69.8**
SE	**1.2**	**2.1**	**4.1**	**1.4**	**3.3**

Table 27. Influence of feed enzymes on excreta moisture - ANOVA

Source	d.f	s.s	m.s.s	F
Treatment	4	91.233	22.808	1.42 NS
Error	25	401.584	16.063	
Total	29	492.817		

NS Not significant

Table 28. **Influence of feed enzymes on Microbial count on eggs** (dilution: 20 times)

	Dietary treatments				
	T1	T2	T3	T4	T5
No. of samples	11	11	9	11	8
Microbial count on eggs	67.27	59.55	54.33	49.00	56.50
	±8.480	±7.180	±6.811	±4.602	±6.033

Table 29. Influence of feed enzymes on nutrient availability-apparent metabolisable energy (kcal/ kg of feed)

Bird No.	Dietary treatments				
	T1	T2	T3	T4	T5
1	2661	2752	2505	2559	2555
2	2668	2815	2537	2468	2391
3	2556	2629	2487	2608	2727
4	2715	2812	2487	2537	2500
5	2686	2694	2535	2564	2519
6	2685	2556	2428	2484	2770
Mean±	**2662**	**2710**	**2497**	**2537**	**2577**
SE	**22.50**	**42.32**	**16.40**	**21.47**	**58.91**

Fig. 6. Influence of feed enzymes on nutrient availability

Apparent metabolisable energy (kcal./ kg feed)

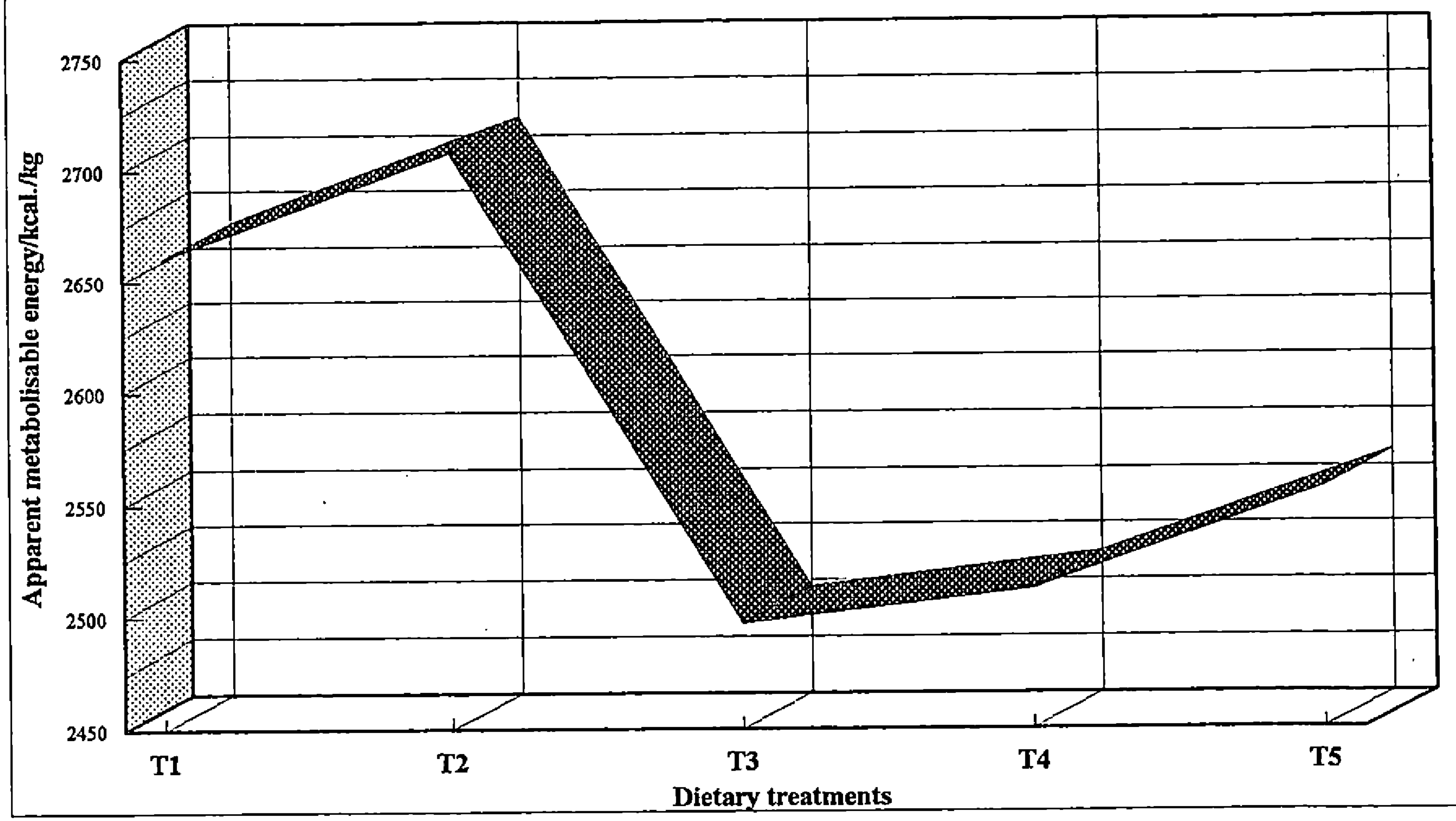

Table 30. Influence of feed enzymes on nutrient availability - apparent metabolisable energy - 't' test vaules

Treatments	't' values
T1 VsT2	0.9980 NS
T3 VsT4	0.4242 NS
T3 VsT5	1.3164 NS
T4 VsT5	0.9650 NS

NS Not Significant

Table 31. Influence of feed enzymes on nutrient availability crude protein: digestibility coefficients

Bird No.	Dietary treatments				
	T1	T2	T3	T4	T5
1	80.4	79.4	68.2	86.0	82.5
2	73.4	80.8	80.8	72.7	78.7
3	76.4	76.6	73.8	81.0	76.6
4	82.4	82.8	72.4	78.8	82.4
5	78.8	80.2	77.9	74.7	78.3
6	81.8	85.4	75.9	79.5	85.3
Mean±	78.9	80.9	74.8	78.8	80.6
SE	1.4	1.2	1.8	1.9	1.3

Table 32. Influence of feed enzymes on nutrient availability - ether extract: digestibility coefficients

Bird No.	Dietary Treatments				
	T1	**T2**	**T3**	**T4**	**T5**
1	85.3	79.4	69.0	75.8	81.1
2	82.1	88.6	81.0	73.5	71.8
3	84.5	76.2	73.4	79.3	84.4
4	82.5	81.2	79.7	78.6	81.9
5	84.0	85.6	69.5	83.1	80.8
6	84.2	80.3	69.0	81.2	84.7
Mean±	**83.8**	**81.9**	**73.6**	**78.6**	**80.8**
SE	**0.5**	**1.8**	**2.2**	**1.4**	**1.9**

Table 33. Influence of feed enzymes on nutrient availability-NFE: digestibility coefficients

Bird No.	Dietary treatments				
	T1	T2	T3	T4	T5
1	77.7	74.8	74.4	75.2	73.4
2	76.2	78.7	77.9	69.6	59.1
3	77.8	81.4	74.2	76.8	72.4
4	72.0	69.3	72.3	73.2	69.1
5	72.0	79.7	74.5	74.4	73.4
6	71.9	74.0	64.7	70.1	78.8
Mean±	74.6	76.3	73.0	73.2	71.0
SE	1.2	1.8	1.8	1.2	2.7

Table 34. Influence of feed enzymes on nutrient availability-fibre fractions: digestibility coefficients

Bird No	Dietary treatments									
	T1		T2		T3		T4		T5	
	NDF %	ADF %	NDF %	ADF %	NDF %	ADF %	NDF %	ADF %	NDF %	ADF %
1	25.1	14.6	17.4	18.7	12.9	11.8	8.8	8.9	12.5	9.6
2	14.8	7.8	11.6	8.0	18.6	17.1	1.4	6.0	13.4	10.8
3	14.4	6.5	23.0	12.0	14.5	13.3	26.9	18.8	10.3	10.9
4	18.9	11.7	18.1	7.6	6.4	6.3	10.6	10.1	15.3	13.0
5	13.1	12.1	20.8	13.9	10.4	10.0	14.8	11.7	10.2	9.9
6	22.9	15.2	17.6	13.8	12.9	11.1	6.4	4.9	15.7	13.6
Mean±	18.2	11.3	18.1	12.3	12.6	11.6	11.5	10.1	12.9	11.3
SE	2.01	1.44	1.57	1.7	1.66	1.46	3.58	2.03	0.97	0.67

Fig. 7. Influence of feed enzymes on nutrient availability

Digestibility coefficients

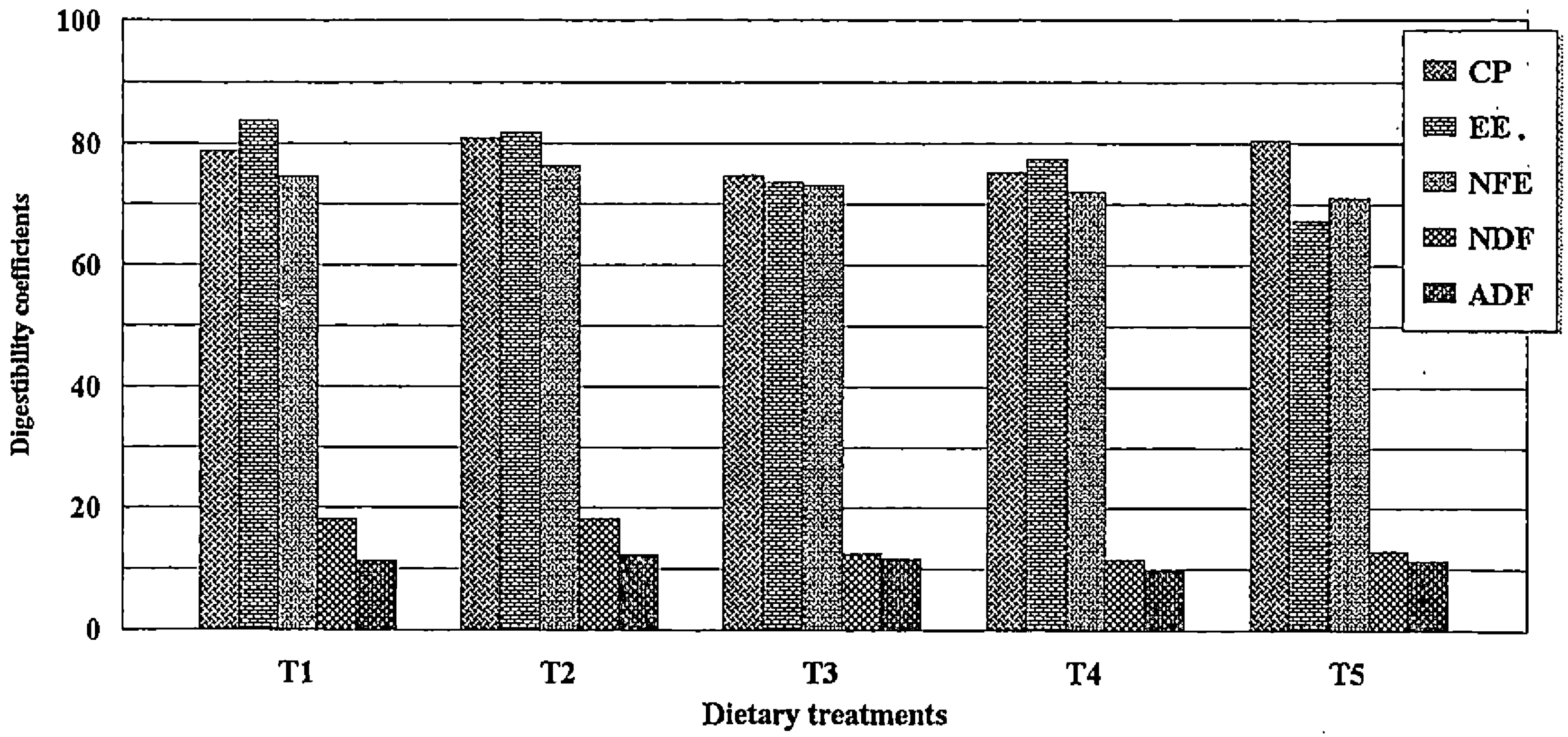

Table 35. **Influence of feed enzymes on nutrient availability -ANOVA**

source	d.f	m.s.s					F value				
		CP	EE	NFE	NDF	ADF	CP	EE	NFE	NDF	ADF
Treatments	4	24.06	45.68	10.73	50.3	5.3	1.68 NS	1.183 NS	1.22NS	2.37NS	0.455 NS
Error	25	14.28	38.61	8.81	21.22	11.6					
Total	29										

NS Not significant

Table 36. Influence of feed enzymes on intestinal viscosity (Ostwald method)

Bird No.	Dietary treatments				
	T1	T2	T3	T4	T5
1	3.38	2.10	1.59	1.70	1.70
2	3.69	1.80	1.85	1.80	1.45
3	3.01	1.81	1.74	1.89	1.64
4	2.97	1.05	1.96	1.45	1.57
Mean±	**3.26**	**1.69**	**1.79**	**1.71**	**1.59**
SE	**0.09**	**0.09**	**0.08**	**0.03**	**0.07**

Table 37. Mortality during the experimental period

Age in weeks	Dietary treatments				
	T1	T2	T3	T4	T5
21 - 24	-	-	-	-	-
25 - 28	-	-	-	-	-
29 - 32	-	-	1	-	1
32 - 36	-	1	1	1	-
37 - 40	-	-	-	-	-
Total	-	1	2	1	1
Rate - %	-	5	10	5	5

Table 38. Cost of rations without or with feed enzymes

Ingredients	Cost/100kg	Cost of rations (Rs.)				
	Rs.	T1	T2	T3	T4	T5
Yellow maize	473.00	113.52	113.52	89.87	89.87	89.87
Jowar	395.00	79.00	79.00	79.00	79.00	79.00
Groundnut-oil cake (exp)	729.00	87.48	87.48	51.03	51.03	51.03
Gingelly oil cake	804.48	40.22	40.22	40.22	40.22	40.22
Sunflower cake	310.00	31.00	31.00	31.00	31.00	31.00
Wheat bran	434.48	34.76	34.76	56.48	56.48	56.48
De-oiled rice bran	290.00	23.20	23.20	37.70	37.70	37.70
Unsalted dried fish	781.00	46.86	46.86	46.86	46.86	46.86
Shell grit	399.48	19.97	19.97	19.97	19.97	19.97
Common salt	155.00	0.39	0.39	0.39	0.39	0.39
Mineral mixture	560.00	9.80	9.80	9.80	9.80	9.80
Vitamin mixture /kg	720.00	7.20	7.20	7.20	7.20	7.20
Cellulase/kg	122.00		7.32	7.32		7.32
Protease/kg	1000.00		20.00		20.00	20.00
Total		493.40	520.72	476.85	489.53	496.85

Table 39. Economics of production

Particulars	Dietary treatments				
	T1	T2	T3	T4	T5
1. Total feed intake (kg)	326.4	326.1	317.2	329.7	324.1
2. Total no. of eggs	1892	1979	1806	1899	1760
3. Feed consumed per egg (g) 20-40 weeks	172.5	164.9	175.6	173.2	184.1
4. Feed consumed per egg (g) 27-40 weeks	141.5	136.0	140.6	141.6	145.6
5. Cost of feed/kg	4.93	5.21	4.77	4.90	4.97
6. Cost of feed/ egg (paise)	85.00	85.90	83.80	84.90	91.50

Table 40. **Influence of feed enzymes on overall performance of laying hens**

Parameters	Dietary treatments				
	T1	T2	T3	T4	T5
Hen - housed egg production %	67.57	70.68	64.5	67.82	62.86
Hen-day egg production %	67.57	71.91	67.31	69.03	64.54
Age at 10 % egg production (day)	143	156	162	143	159
Age at 50 % egg production (day)	173	170	179	178	183
Age at 95 % egg production (day)	199	183	195	192	202
Feed efficiency - egg number (kg/dozen egg)	2.07	1.98	2.11	2.08	2.21
Feed efficiency - egg mass (kg/kg egg)	3.18	2.86	3.26	3.18	3.4
Initial body weight (kg)	1.2	1.2	1.2	1.2	1.2
Final body weight (kg)	1.76	1.67	1.71	1.72	1.7
Egg weight (g)	52.56	53.46	53.44	53.04	51.94
Yolk index	0.40	0.41	0.41	0.41	0.41
Albumen index	0.08	0.08	0.07	0.07	0.07
Haugh unit score	79.96	80.93	77.45	76.39	75.35
Shell thickness (mm)	0.52	0.49	0.49	0.49	0.5
Mortality (%)	0	5	10	5	5

DISCUSSION

DISCUSSION

Results obtained during the course of the present investigation are discussed below under separate heads.

5.1 Egg production, egg weight and egg quality

The total number of eggs produced by birds in different dietary treatments namely, standard layer ration without enzymes (T_1) and with cellulase and protease (T_2), less dense ration with either cellulase (T_3), or with protease (T_4) or with cellulase and protease (T_5) during the experimental period of 20 weeks were 1892, 1979, 1806, 1899 and 1760 respectively. In overall percentage hen-housed egg production, the highest value was recorded in T_2 (70.68) and the lowest in T_5 (62.86), the descending order in this respect being T_2, T_4, T_1, T_3 and T_5 (Table 5). The birds in T_2 produced 3.11 per cent more eggs than those in T_1. In comparison to the less dense ration with enzymes, birds in T_4 were almost similar to T_1; while T_5 and T_3 were having 4.71 and 3.07 less hen-housed eggs respectively than T_1. The pattern of egg production during the five laying cycles showed that in commencing laying T_1 was the first among treatments. T_2 picked up production at a faster rate and during the second cycle topped the list. During the next cycle T_4 recorded the highest production and in the fourth cycle T_2 for the second time occupied the top position. In the last laying cycle of the experiment, T_5 registered the highest value among treatments. When the highest production in this respect in each treatment group was critically viewed, it was found that in all treatments except T_5 the birds reached their top level of production in the third period, 29 to 32 weeks of age, while T_5 attained that level during the fifth period.

It was observed that the birds in T_5 were lagging in egg production till the fifth cycle, probably because the enzyme combinations (cellulase and protease) employed at the levels of 0.06 and 0.02 per cent along with less dense ration inhibited the full expression of the genetic potential of the birds in the early phase of egg production. On the other hand, the enzyme combination in standard layer ration (T_2) and protease in less dense ration (T_4) at the above levels of inclusion had a beneficial effect on egg production.

The age at 10 per cent hen-housed egg production was 143 days each in T_1 and T_4 where as T_2, T_5 and T_3 reached the same level of production at 156, 159 and 162 days of age (Table 39 & Fig. 1). The birds in treatments T_1 to T_5 attained 50 per cent production during 173, 170, 179, 178 and 183 days, and 95 per cent level at 199, 183, 195, 192 and 202 days of age respectively. A critical assessment of the above data showed that the birds in T_1 and T_4 were the first to commence laying, whereas in attaining 50 and 95 per cent production level, the above groups were 3 & 16 days, and 8 & 9 days respectively later than T_2. The graphic representation of weekly hen-housed egg production (Fig. 2) showed that the birds in the five dietary treatments reached their peak production during 32, 30, 28, 29 and 39 weeks of age in the order of T_1, T_2, T_3, T_4 and T_5, the level of production observed being 90.71, 92.86, 87.86, 95.0 and 91.43 per cent respectively. Though, the birds in T_3 showed their peak production in 28 weeks of age, the level of production at peak was 2.85 per cent less than that of T_1. Birds in T_2 and T_4 were definitely superior in this regard in as much as the birds reached their peak earlier and the level was higher to the extent of 2.15 and 4.29 per cent than in T_1. The birds in T_5 showed a slight improvement over T_1 in this regard. Afterwards, the production curve in all treatments showed almost a plateau indicating high level of production at the end of the experiment.

As regards the percentage hen-day egg production, the trend was almost similar to percentage hen-housed egg production. In overall value, T_2 recorded the highest (71.91), the descending order being T_2, T_4, T_1, T_3 and T_5 (Table 6). In overall hen-day percentage egg production, T_2 recorded the highest among treatments and recorded higher values than T_1 to the extent of 4.34. When considering less dense ration with enzymes, T_4 showed a slight improvement of 1.46 per cent over T_1 where as T_3 and T_5 exhibited a reduction in this parameter to the extent of 0.26 and 3.03 per cent respectively in comparison with that in T_1. Perusal of the data on period-wise percentage hen-day egg production showed that during the five laying cycles, the birds in different treatments registered the highest position among treatments in the order of T_1, T_2, T_4, T_2 and T_5 respectively, T_3 never attaining that position. During the first period, in all treatments production was low. Second period exhibited a boost in egg number in all groups that was continued in the third period with the birds in T_1 (88.93) and T_4 (92.86) touching their highest production level in that period, while the birds in T_2 (92.99) reaching that level during the fourth and those in T_3 (92.06) and T_5 (92.29) in the fifth cycle.

The pattern of hen-day egg production depicted in Fig. 3 indicated that after an initial slag, the production in different dietary treatments (T_1, T_2, T_3, T_4 and T_5) shot up and attained the peak in the order of 32, 34, 31, 29 and 32 weeks of age, the peak production being 90.71, 95.30, 90.98, 95.00 and 92.48 percentage respectively. At 32 weeks of age, the level of production in enzyme treated groups (T_2, T_3, T_4 and T_5) were 92.7, 90.2, 93.5 and 91.7 respectively. The percentage increase in peak production recorded for the enzyme treated groups, T_2, T_3, T_4 and T_5, over the control, T_1 were 4.59, 0.27, 5.21 and 1.67 respectively. The above figures clearly indicate a positive response on egg production as a result of enzyme supplementation.

The statistical analysis of the data on both percentage hen-housed and hen-day egg production and also the total egg production during the experimental period indicated that there is no significant difference among treatments (Table 7, 8 & 9). In other words, the numerical differences among treatments registered in hen-housed and hen-day egg production percentage and total egg number were not substantial indicating that the less dense ration with enzymes were almost similar to standard layer ration without or with enzyme with respect to egg production parameters.

The successful use of enzymes in broiler industry stimulated interest in the application of feed enzymes to diets for laying hens. Recently, the use of enzymes became extensive and several investigators reported that addition of single or multi-enzymes to layer diets constituted with less commonly used cereal grains numerically improved egg yield (Tishenkova and Serikov, 1987; Kuchta *et al.*, 1991; Jayanna and Devegowda, 1991 and Sharma and Katoch, 1993). Ely (1963) stated that enzyme supplementation resulted in only a marginal increase in egg production until the birds reached maximum rate of lay. Dovgan *et al.* (1972) reported an increase in egg production to the extent of 20.9 per cent with amylolytic enzyme where as Iotsyus *et al.* (1974) obtained only 5 to 7 per cent increase with both amylolytic and proteolytic enzymes. Adams (1989) recorded 7.2 per cent increase in egg production in mature layers with multi-enzyme preparation. Wetscherek and Zollitsch (1991) found that egg production was not improved with feed enzymes until 45 weeks of age. A positive response to enzyme supplementation on egg production was noticed by Purushothaman and Natanam (1995).

Gleaves and Dewan (1970) recorded a significantly higher egg production when diets were supplemented with fungal enzymes . Nasi (1988) and Graham (1991) reported a significant increase in egg production with multi-enzymes preparation.

Earlier reports by Berg (1959 & 1961) claimed no benefits from feed enzymes in laying hens in as much as enzymes did not affect rate of lay, egg quality traits, egg weight , fertility or hatchability of eggs. In many studies no enzyme effect on egg production was reported (Korniewicz *et al.*, 1980; Miles *et al.*, 1985 and Richter *et al.*, 1990). Jeroch (1991) observed no effect with multi-enzymes in barley based diets, however, enzyme with beta-glucanase or cellulase activities improved egg production. The above findings suggest the need for selecting ingredient specific enzymes in practical feeding of poultry.

The average weight of eggs produced by birds in dietary treatments T_1 to T_5 during the experimental period were 52.56, 53.46, 53.44, 53.04 and 51.94g respectively (Table 10). The differences in egg weight among the treatments were marginal and were not significant statistically (Table 11). The lowest egg weight was recorded in T_5 and the highest in T_2. An analysis of the period-wise data indicated that the weight of eggs increased from the first period to the fifth period as the age of the birds increased. Among the treatments, the maximum egg weight recorded in T_2 was 56.8 g, the maximum in other treatment groups, T_1, T_3, T_4 and T_5 being 55.6, 55.9, 56.3 and 55.6 g respectively.

Tolokonnikov and Berezhnova (1975) reported an increased egg weight with proteolytic enzyme in diets constituted with different cereal grains. A significantly higher egg weight was recorded by Blum and Sauveur (1973) with enzyme supplementation of high protein diet. Brufau *et al.* (1994) suggested that addition of enzymes to barley based diets appeared to improve egg size in young layers. Adams (1989) found that use of specific enzymes in diets for laying hens, though increased egg yield, did not affect the egg size.

From an assessment of egg quality traits in Tables 12, 13, 14 and 15, and the summarised data in Table 16, it could be found that in yolk index, the enzyme treated groups (T_2, T_3, T_4 and T_5) had uniformly higher average values (0.41) than the untreated group T_1 (0.40). In case of albumen index, the ration with a better nutrient content namely the standard layer ration without or with enzymes, recorded higher values (0.08); while less dense rations showed lower values (0.07). In Haugh units, the descending order of the scores was 80.93, 79.96, 77.45, 76.39 and 75.35 in treatments T_2, T_1, T_3, T_4 and T_5 respectively. Egg from birds on standard ration without or with enzymes had a higher value than from less dense ration with enzymes. The maximum shell thickness was recorded in T_1 (0.52 mm), then T_2 (0.50 mm) and all others had equal values of 0.49 mm. No obvious abnormalities of egg shell, albumen or yolk were observed in any of the eggs from the treatments. Yolk colour was found more or less uniform in all eggs. As a whole, the enzyme treated groups had better grading in certain quality traits while in certain others, these were having poor scores. Statistical analysis of data on egg quality characteristics (Table 17) showed that the differences noticed among treatments in this regard were not significant.

Blum and Sauveur (1973) reported that enzyme supplementation improved yolk colour on low protein diet and reduced the Haugh units. Baranauskas (1988) found no adverse effect on egg quality when enzymes were used in feeds containing poultry excreta. A similar observation was made by Berg (1959 & 1961), where enzyme additives did not affect egg quality characteristics. Most authors did not find any adverse effect on egg quality traits by use of feed enzymes (Wetscherek and Zollitsch, 1991; Korniewicz *et al.*, 1980 and Brenes *et al.*, 1993). Aimonen and Rauva (1991) reported a small but significant negative effect on all egg quality traits except the percentage of cracked eggs with multiple enzymes preparation.

5.2 Feed intake and Feed efficiency

The total feed consumed during the experimental period of 20 weeks by birds in the different treatment groups namely, T_1 & T_2 (standard layer ration without or with cellulase and protease) and T_3, T_4 & T_5 (less dense rations either with single or both enzymes) were 326.4, 326.1, 317.2, 329.7 and 324.1 kg respectively (Table. 18). The average daily feed intake during the experiment for the different dietary treatments ranged from 117 to 120 g , the lowest feed intake being recorded in birds receiving standard layer ration without enzymes (T_1) and the highest in those receiving less dense ration with protease (T_4). When the period-wise average feed intake was assessed, the range during the five periods was found to be 107 to 131 g. The maximum average daily feed intake recorded in different periods by each treatment group was 124, 126, 129, 131 and 127 g respectively for T_1, T_2, T_3, T_4 and T_5. A comparatively higher feed intake was observed in those birds which commenced laying at an early date and in those which were at a high level of production than the others.

The feed per egg calculated from 27^{th} week of age, when the birds had attained uniformly a high level of production, was 142, 136, 141, 142 and 146 g respectively in T_1, T_2, T_3, T_4 and T_5 (Table 39). The lowest quantity of feed for egg recorded in T_2 and was 4.2 per cent less than that in T_1. T_3 required 0.7 per cent less, and T_5, 2.8 per cent more than T_1, while T_4 and T_1 were similar in this respect. There was positive influence of enzymes on feed intake per egg in both T_2 and T_3. The above results clearly indicated that enzymes in LDR except the enzyme combination (T_5) were successful in reducing the quantity of feed required per egg or in equalising the amount to that of the control (T_1). The feed per egg when calculated for the entire period of the experiment were 173, 165, 176, 173 and 184 g in T_1 to T_5 respectively (Table 39). The trend was almost similar to those calculated from 27^{th} week of age, T_2 recording the lowest followed by T_1, T_4, T_3 and T_5. T_3 and T_5 required 1.7 and 6.4 per cent respectively more feed per egg than the control (T_1), whereas T_2 consumed 4.6 per cent less feed per egg and T_4 and T_1 were similar with respect to feed intake per egg produced. Perusal of the results suggested a positive effect of feed enzymes on feed intake in dietary treatment groups, T_2 and T_4.

The statistical analysis of data on weekly feed intake by 't' test showed that the differences recorded were not significant (Table 20). The ration having less crude protein and low level of metabolisable energy when supplemented with enzymes, feed intake was comparable to the control. On the other hand, standard layer ration with both enzymes was found to be superior among the five treatments.

Overall feed efficiency (egg number) was calculated by taking into consideration the total feed consumed and the total number of eggs produced during the entire period of the experiment, the range of the values being 1.98 to 2.21 (Table 21 & Fig. 4). The highest feed efficiency among the five treatments was recorded by birds in T_2 and the descending order in efficiency was T_1, T_4, T_3 and T_5. During the first two laying cycles, the feed efficiency was uniformly low since the birds were picking up egg production. The efficiency data in third period showed that T_1 was superior to all others, while in fourth period T_2 and T_4 superseded T_1 in this regard and in the last period all treatments except T_4 were superior to T_1. The average feed efficiency for egg number per week from 27th week of age for each treatment group was 1.70, 1.63, 1.69, 1.70 and 1.77 respectively in T_1, T_2, T_3, T_4 and T_5. (Table 21a). SLR with cellulase and protease (T_2) was superior among the treatments in this parameter followed by LDR with cellulase (T_3). LDR with protease was similar to control (T_1), LDR with enzyme combination (T_5) being last in the lot. The maximum feed efficiency in different treatments were during 30th, 34th, 39th, 28th and 34th weeks with values 1.50, 1.52, 1.54, 1.56 and 1.51 respectively for five treatments, T_1 to T_5.

The overall feed efficiency values for egg mass were 3.18, 2.86, 3.26, 3.18 and 3.40 in T_1 , T_2, T_3, T_4 and T_5 respectively (Table 22 and Fig. 5). As in the case of feed efficiency for egg number, the trend was the same, the highest efficiency being seen in T_2, followed by T_1, T_4, T_3 and T_5 in the descending order. From the analysis of the data on period-wise efficiency (egg mass), the highest recorded for each treatment was in third period for T_1 (2.49), in fourth for T_4 (2.48) and in fifth period for T_2 (2.37), T_3 (2.46)and T_5 (2.45). All the enzyme treated groups were better in this regard than the control (T_1), the percentage improvement over T_1 being 4.8, 1.2, 0.4 and 1.6 in T_2, T_3, T_4 and T_5 respectively. Egg weight was found to be increased as age advanced and this probably may be the factor that influenced feed efficiency for egg mass.

The data on feed efficiency for egg number per week from 27th week of age of birds in the five dietary treatments (Table 21a) were statistically analysed by 't' test (Table 23). T_2 had significantly higher feed efficiency than T_1, T_3, T_4 and T_5 ($P< 0.05$). Standard layer ration with both enzymes (T_2) was significantly superior to control (T_1) and to less dense ration with enzymes (T_3, T_4 & T_5) in feed efficiency for egg number. The standard layer ration though formulated as per BIS for chicken layers, contained a few of the ingredients, viz., jowar, de-oiled rice bran, wheat bran and undecorticated sunflower cake which were less commonly used in poultry rations because of known anti-nutritive factors. Probably the feed enzymes helped to break the anti-nutritive substance in these ingredients and removed the barriers for nutrient absorption in the ration. The cellulolytic and proteolytic enzymes in less dense ration improved feed utilisation and the feed efficiencies for egg production in these treatments were not significantly different from that in control. Cellulase was more effective than protease in improving feed efficiency, whereas enzyme combination was less effective in this regard. Berg (1959) and Nasi (1989) reported that feed enzymes improved feed efficiency in layers, while Berg (1961) could not record any enzyme effect in reducing feed intake during laying period. Brenes *et al.* (1993) obtained beneficial effect on feed intake with enzymes. Gleaves and Dewan (1970) found that fungal enzyme did not influence feed intake, but improved feed efficiency in laying hens.

Miles *et al.* (1985) noticed no significant benefit in feed conversion ratio from enzyme supplementation in layers. A significantly better feed conversion ratio with multi-enzyme supplementation was registered by Baranaukas (1988) and Aimonen and Nasi (1991). Mohandas and Devegowda (1991) and Jayanna and Devegowda (1991) reported beneficial effects from enzyme supplementation in rations with low energy in terms of feed intake and feed efficiency. Prakash and Devegowda (1993) recorded statistically significant improvement in feed efficiency from enzyme supplementation in high fibre diets. More recently, Brufau *et al.* (1994) showed that feed intake and feed efficiency were not affected by enzyme supplementation. The inconsistent reports on feed intake and feed efficiency indicated that feed enzymes are ingredient specific and requires further research for selecting the appropriate enzymes in feed mixtures.

5.3 Body weight gain

Mean body weight gain during the experimental period of 20 weeks in the five treatment groups namely T_1, T_2, T_3, T_4 and T_5 was 0.57, 0.56, 0.51, 0.53 and 0.51 kg respectively (Table 24). The weight gains recorded were more or less uniform in all treatments in as much as the differences among treatments being in the range of 10 to 60 g. The less dense ration with single or both enzymes were also promoting body weight gain though at a lower rate than the treatments on standard layer ration (T_1 and T_2). Probably feed enzymes have positively influenced nutrient absorption from less dense ration and brought about a gain in body weight. The marginal differences noticed among treatments in body weight gain were not statistically significant (Table 25).

Berg (1959) did not record any increase in body weight with enzyme in hens. Gleaves and Dewan (1970) obtained increased body weight gain in hens from supplementation of corn milo diets with fungal enzyme. Berezhnora (1979) also reported a positive effect of enzymes on growth and weight gain of pullets whereas Benabdeljelil and Arbaoui (1994) found no significant difference in body weight in hens with feed enzyme supplementation.

5.4 Excreta moisture

The percentage moisture content of excreta (Table 26) was lowest in T_5 (69.8) and highest in T_1 (77). The enzyme supplementation of the two rations (SLR and LDR) reduced the moisture content of the droppings, the less dense ration with enzymes exhibiting a better effect than the standard layer ration with enzymes. The reduction in percentage moisture content of droppings in enzyme treated groups (T_2, T_3, T_4 and T_5) from that of untreated

(T_1) was in the range of 2.7 to 7.2. Sticky droppings is a problem when certain less commonly used cereals and their by-products and a few of the protein supplements are incorporated in poultry rations and adversely affects the cleanliness of the eggs produced, which is of economical importance. The presence of non-starch polysaccharides especially from certain cereal grains are not properly digested and absorbed because of the anti-nutritive factors in them and results in sticky droppings, which adhere to the surface of the eggs.

The data on the microbial load on eggs from different dietary treatments (Table 28) showed that eggs from enzyme treated groups (T_2, T_3, T_4 and T_5) had less number of micro-organisms on the surface than those from untreated group (T_1).

Statistical analysis of the data on excreta moisture failed to show any significant difference among treatments (Table 27). The above results are in accordance with the findings of several investigators that enzyme treatment of diets containing certain cereals and protein supplements reduce the moisture content of excreta and the incidence of sticky dropping problem (Anderson and Warnick, 1964; Gohl *et al.*, 1978; Pettersson and Aman, 1988 and 1989, and Bedford *et al.*, 1991). Chesson (1993) had reviewed the incidence of sticky dropping problem rather extensively. Berg (1959) reported a significant decrease in litter moisture with a fungal enzyme in diets for pullets, but only a slight reduction in litter moisture with bacterial enzyme.

5.5 Nutrient availability

The gross energy contents of standard layer ration and less dense ration were 3782 and 3614 kcal/kg of feed respectively. The apparent metabolisable energy estimated for the five treatment groups viz., standard layer ration (T_1), standard layer ration with cellulase and protease (T_2), less dense ration with cellulase (T_3), less dense ration with protease (T_4) and less dense ration with cellulase and protease (T_5) were 2662, 2710, 2497, 2537 and 2517 kcal/ kg respectively. (Table 29). The percentage availability of energy from the two rations without or with enzymes for birds in treatments T_1, T_2, T_3, T_4 and T_5 respectively were 70.4, 71.7, 69.1, 70.2, 71.3. The use of enzymes resulted in marginal improvement in energy utilisation by the birds in T_2 and T_5 where as that for T_3 was slightly less than the energy utilisation by birds in T_1, T_1 and T_4 being almost similar in this respect. The standard layer ration without and with enzymes were analysed statistically by 't' test and it was found that the difference observed between the two treatments in apparent metabolisable energy was not significant. The less dense rations with cellulase, protease, or both enzymes were compared statistically by 't' test and found that they were not statistically different in the content of apparent metabolisable energy. The enzyme effect on energy utilisation by birds in the treatment groups was not substantial.

The crude protein content of SLR was 18.2 and that of LDR 16.1 per cent (Table 2). The average crude protein digestibility for the rations fed to birds in T_1 to T_5 were 78.9, 80.9, 74.8, 78.8, and 80.6 per cent respectively.

Use of feed enzymes viz., cellulase and protease in standard layer ration improved the crude protein digestibility by 2 per cent. In the case of less dense ration with enzymes, the highest crude protein digestibility was recorded where both cellulase and protease enzymes were supplemented (T_5) followed by the ration where only protease (T_4) was used and the last being cellulase supplemented (T_3) ration. The difference in digestibility of crude protein recorded was 4.0 and 5.8 per cent more respectively in T_4 and T_5 than in T_3. In the two rations treated with cellulase and protease (T_2 & T_5) the digestibility of crude protein was almost similar suggesting a possible effect of feed enzymes on crude protein digestibility. When only one enzyme was supplemented, the extent of improvement in digestibility of protein in the ration was less and protease was found to be better in this respect than cellulase.

SLR and LDR contained 4.5 and 4.0 per cent in respect of ether extract. The mean digestibility of ether extract was 83.8 (T_1), 81.9 (T_2), 73.6 (T_3), 78.4 (T_4), and 78.8 (T_5). Less dense ration with cellulase (T_3) recorded the lowest digestibility and the maximum was in standard layer ration (T_1). In standard layer ration with enzymes (T_2) the digestibility of ether extract was slightly less than that in the control (T_1). The less dense ration with protease (T_4) and that with both enzymes (T_5) were similar in the digestibility of ether extract.

NFE contents of SLR and LDR were 58.5 and 60.3 per cent respectively. Digestibility coefficients of NFE ranged from 73 to 76.3 with marginal difference among treatments, the highest being recorded in standard layer ration with both enzymes (T_2) and the lowest in less dense ration with cellulase (T_3).

NDF and ADF in SLR were 22.6 and 11.2 and those in LDR were 23.8 and 13.2 per cent respectively. The digestible NDF in treatment groups T_1 to T_5 were 18.2, 18.1,12.6, 11.5 , 12.9 per cent and ADF 11.3, 12.3, 11.6, 10.1 and 11.3 per cent respectively. Between standard layer ration (T_1) and that with enzymes (T_2) there were no appreciable changes in the digestibility of NDF, whereas in less dense ration with enzymes the digestibility of this fibre fraction was 5.3 to 6.7 per cent less than that in SLR. Among the LDR with either single or both enzymes, the one with protease had the lowest digestibility. The other two treatments were having almost similar digestibility of NDF. The digestible portion of ADF was low in all cases the differences recorded among groups being only to the extent of 0.7 to 2.2 per cent. The above data indicated that protease was less efficient than cellulase in digesting the fibre fractions.

Statistical analysis of the digestibility coefficients of crude protein, ether extract, NFE, NDF and ADF among the treatment groups (T_1 to T_5) showed that the differences recorded were not significant (Table 35) even though crude protein digestibility in certain enzyme treated groups was improved.

A great deal is known about dietary sources of nutrients for poultry but the available information is quite limited on many aspects of digestion and absorption in poultry. The capacity of fowl to digest and absorb carbohydrates is not same in all circumstances and is highly adaptable and multifaceted (Moran, 1985). The unstirred water layer in the lumen of the intestine restrict saccharides and deny access to the carbohydrases which are finalising carbohydrate digestion in intestine. Supplementation of poultry feed with appropriate enzymes was shown to complete the absorption of nutrients such as protein, starch and fats in the anterior intestine (Hesselman, 1983 and Bedford, 1991). Influence of enzymes in improving the metabolisable energy and/or nutrient digestibility of feeds containing certain cereal grains and by products in laying hens was reported by several authors (Saunders *et al.* 1972, Rotter *et al.* 1990 and Aimonen and Nasi 1991). A few others recorded significantly higher positive response with enzyme supplementation in apparent metabolisable energy and/ or nutrient digestibility (Hersted and McNab 1975, Petterson and Aman 1989, Steenfeldt 1991 and Annison 1992).There were also reports in literature stating no enzyme effect on energy availability and/or digestibility of nutrients (Gohl *et al.* 1978, Steenfeldt, 1991 and Kiiskinen 1993). Vranjes and Wenk (1995) studied the interaction of an enzyme complex and a feed antibiotic in barley containing diet on metabolisable energy and egg production parameters of laying hens and reported that enzymes positively influenced AME content of the feed, organic matter utilisation and NDF digestibility ($P<0.01$), where as enzyme antibiotic combination reduced the enzyme effect.

Cellulase and protease enzymes used in the present study, at the levels of inclusion 0.06 and 0.02 per cent respectively improved apparent metabolisable energy and digestibility of crude protein. Further work is warranted for better understanding of the scope of enzyme technology in commercial laying operations.

5.6 Intestinal viscosity

The mean values for intestinal viscosity in the five dietary treatments T_1, to T_5 were 3.26, 1.69, 1.79, 1.71 and 1.59 respectively. The viscosity of intestinal contents of birds fed with the enzyme treated rations, viz., T_2, T_3, T_4 and T_5 were reduced to the extent of 48.2, 45.1, 47.5 and 51.2 per cent respectively in comparison with standard layer ration. The reduction in the viscosity of intestinal contents recorded in the present study suggested a marked influence of feed enzymes on the gut viscosity. Further detailed study is required in this aspect to explain the enzyme effect on intestinal viscosity.

Reported literature on the effect of feed enzymes on viscosity of intestinal contents are mostly related to experiments carried out with chicks. Lyons and Jacques (1987) reported that beta-glucan and pentosan become soluble in the gut and act as a viscous barrier to nutrient absorption from the gut epithelium resulting in poor performance by birds and sticky droppings. Feed enzymes reduce the intestinal viscosity and alleviate the problem. White *et al.* (1981) observed that when beta-glucan was added to corn diet fed to chicks, the intestinal viscosity was increased three fold and when beta-glucan and a filtrate containing fungal enzyme were added, the viscosity was reduced to that of the control diet. Bedford *et al.* (1994) found a reduction in gut viscosity when an appropriate enzyme was added to barley based diets in broiler chicks. Campbell and Bedford. (1992) in a review, stated that young chicks exhibited the greatest response to enzyme induced reduction in intestinal viscosity.

5.7 Livability

There was no mortality among birds receiving the standard layer ration (T_1) and the mortality rate in enzyme treated groups (T_2, T_3, T_4 and T_5) ranged from 5 to 10 per cent. The cause of death was fungal infection of crop (Candidiasis) in T_2 and T_4 and heat stroke in T_3 and T_5. The mortality rate in all treatment groups except T_3 was within permitted limits in commercial production. In T_3 two birds died during the test period. The cause of death of birds could not be attributed to enzyme treatment. Moreover, so far there are no reports of harmful effects from use of feed enzymes.

5.8 Cost benefit analysis

The estimated cost per 100 kg of standard layer and less dense layer ration was Rs. 493.40 and Rs. 469.53 respectively, the less dense ration being cheaper to the extent of Rs. 23.87. The cost of feed inclusive of enzyme cost per 100 kg in the five dietary treatments respectively were Rs. 493.40, 520.72, 476.85, 489.53 and 496.85 (Table 38). The enzyme cost alone amounted to Rs.7.32 to Rs. 27.32 in experimental groups (T_2, T_3, T_4 & T_5).

The feed cost per egg in different treatments for the present experiment was 83.8 (T_3), 84.9 (T_4), 85.0 (T_1), 85.9 (T_2) and 91.5 (T_5) paise (Table 39). Eventhough, the birds in dietary treatment T_2 produced more number of eggs and had significantly higher feed efficiency for egg number than T_1, the increase in feed cost was due to enzyme cost. The cost of feed per egg in treatment groups T_3 and T_4 was less than that in T_1, while T_5 recorded the highest value in this regard. The birds in T_5 were showing an initial setback in egg production, the highest hen-housed production in this group being obtained during the fifth laying cycle. The cost of feed per egg from ingredients and additives except enzyme for the dietary treatments T_1 to T_5 were 85.0, 81.3, 82.4, 81.3 and 86.4 paise respectively. Comparatively high feed cost per egg was recorded in the dietary treatments in the present experiment since the experiment was in the early laying phase during which the birds commenced laying, reached the peak and was at high level of production at the end of the experiment (Table 40). More over, the cost of feed ingredients were also comparatively higher (Table 38).

The feed cost per egg after the birds reached uniformly a high level of production (27^{th} week), the above values for the dietary treatments T_1 to T_5 were 70.1, 70.9, 67.1, 69.4 and 72.4 paise respectively. T_3 recorded the minimum cost per egg , the other treatments in the ascending order were T_4, T_1, T_2 and T_5. The feed cost per egg had markedly reduced in T_2 and T_5 in this assessment, since the birds in these groups produced more eggs in subsequent laying cycles. At a selling price of Rs. 1/ egg the net returns over feed cost from an egg was 3.8 and 0.7 paise less in T_3 and T_4 than the control (T1), while the same was 0.8 and 2.3 paise more in T_2 and T_5 respectively. In order to have a clear picture on cost benefit, the experiment should have been continued for the entire laying period. Hence, a conclusive opinion could not be formed from the present study based on cost of feed and net returns. Prakash and Devegowda (1993) reported that enzyme supplementation to high and very high fibre diets in commercial layers are economical compared to low and medium fibre diets.

5.9 Overall performance

During the experimental period of 20 weeks, the White Leghorn pullets used for the study had an overall hen-housed egg production percentage ranging from 64.50 to 70.68 (Table 40). Addition of cellulase and protease to a standard layer ration (BIS, 1993) improved the hen-housed egg production percentage by 3.11 than the control. With respect to the less dense ration with enzymes it was found that birds receiving LDR with protease (T_4) was almost similar to the control (T_1), while LDR with cellulase or LDR with cellulase and protease produced 3.1 and 4.7 per cent respectively less hen-housed egg numbers. An assessment of attainment of various stages of egg production by birds in different treatment groups indicated that in initiating egg production birds receiving SLR and those on LDR plus protease were superior to other groups, but in picking up production and reaching the top level of production SLR with enzyme combination (T_2) was superior followed by LDR plus protease (T_4) and LDR plus cellulase (T_3). A similar trend was also prevailing in percentage hen-day egg production. There were no marked differences in egg weight and egg quality characteristics among treatments. The numerical differences noticed in egg production parameters were not statistically significant. Enzyme supplementation of SLR (T_2) and LDR (T_3, T_4 and T_5) enhanced egg production in pullets.

The standard layer ration with enzyme combination (T_2) was significantly superior to the control group (T_1) and to LDR with enzymes(T_3, T_4 and T_5) in feed efficiency (egg number). Probably the feed enzymes helped to break the anti-nutritive factors in the feed ingredients and improved feed utilisation in the ration. In promoting body weight gain LDR with enzymes was not statistically different from SLR without or with enzymes suggesting a positive influence of enzymes in sub-standard rations. The energy availability and digestibility of crude protein were enhanced with feed enzymes. There was reduction in intestinal viscosity when the birds were fed with enzyme supplemented diets.

The above findings revealed that feed enzymes have positive influence on egg production and feed utilisation, especially when unconventional or non-standardised feed ingredients were incorporated in the rations. Further detailed studies are warranted for choosing the appropriate enzymes and appropriate levels of inclusion in feed mixtures as the enzymes are mostly raw material specific. Moreover, the proper time for enzyme supplementation based on the stage of egg production has to be worked out for optimum benefits from enzymes in commercial production. Based on the present investigation it is concluded that there is tremendous scope for enzymes in feed industry.

SUMMARY

SUMMARY

An investigation was carried out using one hundred IWN strain of Single Comb White Leghorn pullets of 20 weeks of age to determine the influence of feed enzymes, viz., cellulase and protease at levels of 0.06 and 0.02 per cent respectively, in a standard and a less dense layer ration, on nutrient availability and production performance of laying hens. The pullets were allotted randomly to five dietary treatments (T_1, T_2, T_3, T_4 and T_5) of 20 birds each so as to have almost the same average initial body weight and maintained for a period of 20 weeks. The dietary treatments were standard layer ration (SLR) without or with cellulase and protease (T_1 and T_2), and less dense ration (LDR) with either cellulase (T_3), with protease (T_4) or with both enzymes (T_5). The standard layer ration was formulated as per BIS 1993, with crude protein 18 per cent and ME 2600 kcal/kg and less dense ration with CP 16 per cent and ME 2500 kcal/kg of feed. A few feed ingredients, such as jowar, deoiled rice bran, wheat bran and undecorticated sun flower cake were incorporated in both the layer rations. The birds were housed in individual cages and routine managemental procedures were followed for the entire period of the experiment. No artificial lighting was provided to the birds. Data on feed intake, egg production and egg weight were recorded throughout the experiment. Towards the end of the experiment egg quality studies were carried out using three days collection. A metabolism trial was conducted at the end of the experiment in six birds from each treatment group. The percentage hen-housed and hen-day egg production, egg weight,

shell thickness, feed intake, feed efficiency for egg number and egg mass, body weight gain, excreta moisture, apparent metabolisable energy, digestibility of nutrients and intestinal viscosity were determined to evaluate the enzyme effect on nutrient availability and laying performance of birds. The cost-benefit analysis was also carried out based on the results of the study.

The salient observations made during the course of the present study and the inferences drawn from the data recorded are summarised below:

1. In overall hen-housed egg production during the experimental period the birds fed on standard layer ration with cellulase and protease were superior by 3.11 per cent to those on standard layer ration without enzymes (control). Birds receiving less dense ration with protease were almost similar to the control, while those with cellulase and with both the enzymes were inferior by 3.07 and 4.71 per cent respectively to those on control ration.

2. As regards percentage hen-day egg production, the trend was almost similar to hen-housed production. Birds receiving SLR with cellulase and protease recorded the highest percentage among treatments, the production being higher to the extent of 4.34 than the control, while LDR with protease showed a slight improvement of 1.46 per cent over the control where as LDR with cellulase and that with both enzymes exhibited a reduction in this parameter to the extent of 0.26 and 3.03 respectively. The data on egg

production indicated that the group fed on SLR with cellulase and protease enzymes performed better than control while LDR with protease was almost similar to control. However, the other two LDR treatment groups were inferior to control in egg production. The numerical differences recorded in egg production parameters among treatments were not significant statistically.

3. The period-wise egg weight records for the five treatments showed that as age advanced the egg weight also increased in all treatments and the minor differences noticed in egg weight records among treatments were not statistically significant.

4. The egg quality traits such as yolk and albumen indexes, Haugh unit scores and shell thickness recorded with respect to the different treatments were not statistically different from that of the control.

5. The average daily feed intake of the birds in five dietary treatments ranged from 117 to 120 g, the lowest quantity being recorded in the control and the highest in those receiving LDR with cellulase and protease. The statistical analysis of the data on weekly feed intake between treatment groups showed no significant differences.

6. The highest feed efficiency for egg number as well as for egg mass was registered by birds in SLR with both enzymes while the lowest was in LDR with both enzymes. The feed per egg was lower in SLR with both enzymes and in LDR with protease than in other treatment groups. Statistical analysis

of the data on feed efficiency (egg number) revealed that the treatment group fed on SLR with both enzymes had significantly higher feed efficiency than the control and LDR with enzymes ($P < 0.05$). Between the other treatment groups there was no significant differences.

7. The body weight gains obtained in the five dietary treatments were almost similar.

8. The enzyme supplementation of both SLR and LDR reduced the moisture content of excreta, the effect being more in LDR treatment groups. However, the reduction in moisture level recorded among treatments was not significantly different. The microbial load on the surface of eggs was uniformly low in enzyme treated groups than the control, the lowest number being recorded in those fed with LDR plus protease and the highest in the control.

9. The availability of apparent metabolisable energy from SLR and LDR without or with enzymes was in the range of 69.1 to 71.7 per cent. The combination of enzymes in both rations marginally improved energy utilisation than the control. Between the LDR treatments with enzymes, protease was having better effect on energy utilisation than cellulase. However, there were no significant differences in apparent metabolisable energy values with respect to different treatment groups studied.

10. Enzyme treatment of SLR increased the crude protein digestibility by 2 per cent, while the increase was to the extent of 5.8 and 4.0 per cent respectively

in LDR with both enzymes and LDR with protease compared to LDR with cellulase. Though not significant statistically, the enzyme combination in both SLR and LDR exhibited a better response than either protease or cellulase, and between the two enzymes, protease was better in this regard. The digestibility coefficients of other nutrients were more or less similar in the treatments, the differences recorded being not significant statistically.

11. Reduction in viscosity of intestinal contents was observed when enzymes were included in the rations.

12. The feed cost per egg estimated in the study was comparatively high since the enzyme additives were rather expensive. The same calculated after the birds attained a high level of production was 3.0 and 0.7 paise less in the treatment groups fed on LDR with cellulase and that with protease respectively than the control. The treatment groups with both enzymes were found to be less economical than the control.

From a critical evaluation of the results obtained in the present study it was inferred that feed enzymes had positive influence on feed efficiency and a probable effect on egg production, feed intake and on nutrient availability. The above findings warrant further detailed studies in this regard using appropriate enzymes at appropriate levels, as feed enzymes are ingredient specific.

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INFLUENCE OF FEED ENZYMES ON NUTRIENT AVAILABILITY AND PRODUCTION PERFORMANCE OF LAYING HENS

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ABSTRACT OF A THESIS

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ABSTRACT

The influence of feed enzymes, viz., cellulase or protease supplemented at levels of 0.06 or 0.02 per cent respectively, on nutrient availability and on production performance was evaluated in a standard and a less dense layer ration using one hundred IWN strain of Single Comb White Leghorn pullets of 20 weeks of age for an experimental period of 20 weeks. A standard layer ration was formulated as per BIS, 1993. with 18 per cent crude protein and 2600 kcal of ME per kg of feed and a less dense layer ration with 16 per cent crude protein and 2500 kcal of ME per kg of feed. A few feed ingredients such as jowar, deoiled rice bran, wheat bran and undecorticated sunflower cake were included in both rations. A numerical improvement was noticed in percentage hen-housed and hen-day egg production in enzyme treated groups, however, the increase in egg number was not statistically significant. Egg weight and egg quality characteristics such as yolk and albumen indexes, Haugh unit scores and shell thickness were not affected by enzyme treatment. A positive influence of the enzymes on feed intake by birds was observed in as much as the feed intake with less dense ration supplemented with enzymes was not different statistically from those with the control ration. There was positive enzyme effect on feed per egg in treatment groups on standard layer ration with both enzymes and in less dense ration with protease. Feed efficiency for egg production (egg number) was significantly higher in birds fed standard layer ration with cellulase and protease than those on other treatments ($P<0.05$). There was

improvement in digestibility of crude protein and utilisation of energy, even though the differences noted were not significant statistically. The enzyme treatment of rations reduced moisture content of excreta. The microbial load on the surface of eggs was comparatively low in enzyme treated groups. The intestinal viscosity was reduced with enzyme addition in feed mixtures. The cost-benefit analysis indicated that but for the prohibitive enzyme cost, the feed enzymes were nutritionally beneficial. Based on the present investigation it is concluded that there is tremendous scope for enzymes in feed industry, especially at the present context of feed shortages and the emphasis for the utilisation of alternative feed resources in poultry rations.