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**SUPPLEMENTATION OF PROTEASE ON THE
PRODUCTION PERFORMANCE OF JAPANESE
QUAILS (*Coturnix coturnix japonica*) FED LOW
PROTEIN DIET**

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**Thesis submitted in partial fulfilment of the
requirement for the degree of**

Master of Veterinary Science

**Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur**

2005

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DECLARATION

I hereby declare that this thesis entitled “ **SUPPLEMENTATION OF PROTEASE ON THE PRODUCTION PERFORMANCE OF JAPANESE QUAILS (*Coturnix coturnix japonica*) FED LOW PROTEIN DIET** ” 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.

Mannuthy
31.8.05


SHEENA GRACE KOSHY

CERTIFICATE

Certified that the thesis entitled “**SUPPLEMENTATION OF PROTEASE ON THE PRODUCTION PERFORMANCE OF JAPANESE QUAILS (*Coturnix coturnix japonica*) FED LOW PROTEIN DIET**” is a record of research work done independently by **Dr. Sheena Grace Koshy**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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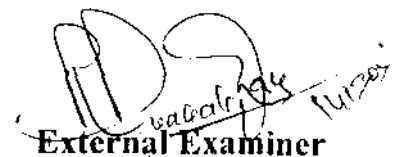
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Introduction

1. INTRODUCTION

Poultry farming in India is now recognized as an organized and scientifically based industry and a potential tool to fight poverty and malnutrition. India, one among the developing countries in the world, is experiencing gross *undernutrition and malnutrition in human sector particularly with animal proteins* (Narayanankutty, 1987). Today, India ranks as the worlds fifth egg producer with a production of 35,000 million eggs (Anon, 2004). But the per capita availability is only 33 eggs while the Indian Council of Medical Research recommends 182 eggs per person per annum. Hence there is considerable scope for increasing egg production. Quails are one of the most efficient biological machines for converting feed into animal protein of high biological value. Quail eggs are renowned for richness in vitamins, essential amino acids, unsaturated fatty acids, phospholipids and macro and micro elements. Quail eggs can be included in the diet of children, pregnant mothers, geriatrics and convalescing patients and essential for all social and economic groups in society to whom energy and animal protein availability are meagre. The importance of quails in the poultry scenario of India is well recognized and quail production has registered a remarkable progress in the country since its introduction during mid seventies. Quails are inherently endowed with rapid growth, short generation interval, early sexual maturity, high rate of egg production, requirement of less floor space, short incubation period and less susceptibility to diseases.

Quail production requires less investment, gives quick returns and higher profits and hence can be adopted by rural masses quickly. The increased *production of quail meat and egg make their availability easier and cheaper and will promote the consumption of those nourishing products by weaker sections of the society.* But the most important constraint encountered in present commercial quail production is the increased feed cost and it accounts for 60 to 70 per cent of *the cost of production.* *The production levels of the conventional ingredients are*

not increasing proportionately to meet the demand for feed. Among the different nutrients in the feed, protein is the costliest item and Japanese quails require a high protein diet during their growth and reproduction. The high protein diet facilitates their fast growth and earlier and persistent egg production. Vegetable protein sources such as soyabean contains protease inhibitors, lectins and tannins which reduce the birds' performance.

Poultry nutritionists are now engaged in the application of biotechnology in poultry research to increase the efficiency and to reduce the unit cost of the feed. One of the fields of applying the concepts of biotechnology is the enzyme dietary inclusion. Use of enzymes in poultry feed is a practice with increasing interest and this enables nutritionists to formulate less dense and less expensive rations. The supplementation of exogenous enzymes has been suggested to compensate the digestibility losses due to enzyme inhibition. The benefits of using enzyme in poultry diets include not only enhanced bird performance and feed efficiency, but also less environmental problems due to reduced output of excreta.

Inclusion of exogenous protease can partially hydrolyse the dietary protein and enhance the protein utilization (Yu *et al.*, 2002). 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 digestion capacity is limited. Earlier experiments with exogenous enzymes in hens have failed to bring positive results. But the continuing developments in product formulation, stability of products and introduction of new enzyme preparations further improved the results in layers. In many of the studies reported proteolytic enzymes are found included as multienzyme preparations in broilers and laying hens. Little or no previous work has been published in the literature on the efficacy of protease, a proteolytic enzyme for improving the protein digestibility in Japanese quails. Moreover, the

proteolytic activity of proventriculus of adult quail was reported to be considerably lower than that of ducklings and chicks (Beena and Philomina, 2000).

In the present study, an attempt was made to explore the effects of supplementation of protease to a low protein diet on protein digestibility, and production performance of laying Japanese quails and also to arrive at its economic feasibility.

Review of Literature

2. REVIEW OF LITERATURE

The literature on the effects of enzyme supplementation are reviewed in this chapter. References on Japanese quails are very meagre and hence other poultry species are dealt with.

Enhanced protein hydrolysis was demonstrated for several of protease enzyme preparations studied in the *in vitro* incubation system (Simbaya *et al.*, 1996).

There is considerable interest in the application of multienzyme products containing cellulase, betaglucanase, alpha amylase, lipase and protease in poultry nutrition (Thirumalai, 2002).

2.1 BODY WEIGHT

2.1.1 Chicks

Balloun and Baker (1957) observed that addition of pepsin to chick diet did not improve the body weight. They have also reported that ficin, a proteolytic enzyme decreased the body weight of chick.

Rexen (1981) reported that addition of protease to one week old cockerels improved the body weight at three weeks (567 vs 536 g).

The addition of papain with chick mash did not improve the body weight (Thirumalai *et al.*, 1991).

2.1.2 Layers

Gleaves and Dewan (1970) observed an increase in body weight of hen supplemented with a fungal enzyme containing protease.

The mean body weight gain of Leghorn hen supplemented with protease in the diet from 20 to 40 weeks of age was 0.53 kg and that of the control was 0.57 kg (Satyamoorthy, 1995).

Body weight at 60 weeks of age was not affected by the addition of an enzyme mixture containing protease to Leghorn hen (Jaroni *et al.*, 1999a).

Rosen (2000) reported that in 1925, scientists first reported that a commercial enzyme product containing protease had improved the live weight gain of White Leghorn chicken (955 vs 927 g).

There was no significant effect on weight gain by the supplementation of Beetazyme containing protease to growing pullet diet (Nandi *et al.*, 2005a).

2.1.3 Broilers

Supplementation of a multienzyme preparation containing protease significantly improved the body weight of broilers compared to control (1.007 vs 0.873 kg) (Arora *et al.*, 1991).

The supplementation of a multienzyme preparation containing protease to broiler diets improved the body weight (Brenes *et al.*, 1993).

Pillai *et al.* (1995) found that the addition of 0.2 per cent protease significantly improved body weight of broilers (1506 vs 1471 g).

An overall improvement in weight gain by 4.50, 5.43 and 9.01 per cent was recorded for broilers fed with 0.5, 1.0 and 1.5 g of the multienzyme preparation Ventrigoold (Dhar *et al.*, 1996a).

There was no significant effect of protease supplementation on the body weight gain of broilers (Simbaya *et al.*, 1996).

Suresha *et al.* (1996) found that there was an improvement in the body weight of broilers supplemented with enzyme preparation containing protease compared to the control.

The addition of an enzyme preparation Innozym Alpha L to the broiler diet increased body weight (Yalda and Forbes, 1996).

According to Babu and Devegowda (1997), supplementation of Nutrizyme containing 48,00,000 IU /g of protease to broilers did not improve the final body weight.

Benabdeljelil (1997) found that body weight gain of broilers was improved by four to 11 per cent by the addition of a multienzyme preparation containing protease.

According to Daveby *et al.* (1998), enzyme supplementation had no significant effect on body weight of broiler chicken.

The supplementation of multienzyme preparation containing protease @ 1 g/kg feed of broilers showed significant improvement (9.4%) in body weight (Deshmukh *et al.*, 2000).

According to Preston *et al.* (2000) enzyme mixture with protease did not improve the live weight gain of broilers.

Vinil *et al.* (2000) found that supplementation of an enzyme mix with protease @100 and 150 g /100 kg in broiler ration did not vary the body weight.

The supplementation of an enzyme mixture containing protease to broiler diet with 75 per cent fish meal replaced by sesame cake protein on isonitrogenous basis significantly increased body weight compared to unsupplemented group (Majumder and Samanta, 2001).

According to Nahas and Lefrancois (2001) significant differences were noted between control and enzyme treated group of broilers for body weight during finisher period (2365 vs 2448 g).

According to Café *et al.* (2002) the addition of 0.1 per cent Avizyme containing protease to the broilers significantly improved the body weight at 16, 35 and 49 days of age compared to birds fed unsupplemented diets.

Ghazi *et al.* (2002) reported that body weight of broiler chicks of seven to twenty eight days of age was improved when protease treated soyabean meal was fed.

Broilers fed multienzyme preparation containing protease supplemented ration gained 88.79 g more body weight as compared to those fed control ration (Bhat, 2002).

Medhi *et al.* (2002) observed that supplementation of Anizyme containing 250,000 U protease/kg to broiler diet did not influence the total body weight gain.

Gracia *et al.* (2003) reported that when enzyme mix containing 19,000 protease units/g was supplemented to broiler diet, weight gain was 28.9 g/day compared to 25.5 g/day for the control.

Supplementation of enzyme preparation with 497 HUT and 1397 HUT protease per kg feed separately, significantly improved body weight gain of broilers at 42 days of age as 1661 and 1689 g respectively compared to 1599 g for the control (Rao *et al.*, 2003).

According to Odetallah *et al.* (2003), when low protein diet supplemented with a broad spectrum protease enzyme PWD-1 Keratinase was fed to broiler chicks, there was a significant increase in body weight at 26 days of age (1,025 and 1,032 vs 965 g for 0.10 and 0.15 per cent of enzyme vs low protein diet respectively).

Supplementation of Maxigest containing protease at the rate of 50 g/kg of feed to broiler diets decreased the days to reach two kilogram body weight from 42 days to 38 days (Pal *et al.*, 2004).

Feed restricted broilers fed a protease supplemented diet showed a higher percentage weight gain than non supplemented birds (Pineiro *et al.*, 2004).

According to Ramesh and Devegowda (2005) supplementation of Allzyme SSF™ containing protease significantly improved the body weight gain of broilers (2165 vs 2086 g).

Ranade *et al.* (2005) observed that broiler chicks fed diet with 10 per cent reduction in CP with supplementation of Maxigest GB containing protease @ 500 g/T had similar live weight as that of the control.

2.1.4 Japanese Quails

Panda *et al.* (1980) reported that the body weight of Japanese quail at six weeks is 123.29 g.

Edwin *et al.* (2004) reported that supplementation of 0.05 per cent non starch polysaccharide hydrolysing (NSP) enzyme to Japanese quail resulted in 5.75 per cent increase in body weight gain at fifth week over control group (206.49 vs 195.26 g).

2.1.5 Ducks

According to Hong *et al.* (2002) the addition of an enzyme preparation containing 12,000 units of protease/g to White Pekin ducks showed a six to eight per cent increase in body weight gain.

2.1.6 Other Species

According to Rexen (1981) the supplementation of papain to the diet of rats aged four weeks did not increase the body weight.

By the supplementation of an enzyme mixture containing protease to pre-weaned lambs, no significant difference was observed in weaning weight (Bhatt and Bhatia, 1999).

2.2 AGE AT SEXUAL MATURITY (ASM)

Berg (1961) found that the addition of an enzyme mix at zero to eight weeks did not significantly affect the ASM.

Chidananda *et al.* (1986) found the ASM of Japanese quail reared under cage system as 53 days and age at 50 per cent production as 77 days.

Panda and Mohapatra (1989) reported that the average ASM is about 50 days in Japanese quails.

Age at 55 per cent production of White Leghorn hen supplemented with a diet containing protease was found to be lesser than that of the unsupplemented group (Satyamoorthy, 1995).

According to Bhat *et al.* (2005) supplementation of a multienzyme mixture containing 2400 units of protease /g to pullet diet @1g/ kg feed had no significant effect on ASM.

Nandi *et al.* (2005a) observed that supplementation of Beetazyme containing protease @ 50g/100 kg to growing pullets during nine to twenty weeks of age did not produce any significant difference in ASM.

2.3 EGG PRODUCTION

The addition of Agrozyme to layer ration did not improve the rate of lay significantly (Berg, 1961).

According to Ely (1963) pullets just coming into production usually exhibited only marginal response until after they reached their maximum rate of production by the supplementation of bacterial and fungal enzymes.

Gleaves and Dewan (1970) noted an improvement in egg production of hen supplemented with a fungal enzyme.

Shrivastav and Johri (1993) found the hen day production percentage of Japanese quail fed diet containing 27 per cent crude protein (CP) up to three weeks and 24 per cent from four to five weeks as 68.6.

Satyamoorthy (1995) reported the hen housed and hen day egg production percentage of White Leghorn hen supplemented with less dense ration containing protease from 20 to 40 weeks of age as 67.82 and 69.03 and for those with standard layer ration as 67.57 for both.

The addition of a multienzyme product containing cellulase, betaglucanase and protease significantly improved the egg production in the laying period (Nasi, 1998).

Jaroni *et al.* (1999a) found that the egg production of Leghorn hen fed diet with an enzyme mixture containing protease was not significantly increased.

Supplementation of Avizyme 1500 containing protease to pullet diet increased the egg per hen housed by 2.7 to 6 per cent (Cook *et al.*, 2000).

According to Ranade *et al.* (2004b) the hen day egg production percentage of layers supplemented with enzyme mixture containing protease with 10 per cent reduction in CP was 71.91 and that of control was 62.16.

Numerical decrease in egg production was observed in pullets aged 21 to 36 weeks by replacing maize by apple pomace at 10 and 15 per cent levels with supplementation of multienzyme mixture containing protease @1g/kg feed compared to the control (Bhat *et al.*, 2005).

2.4 EGG WEIGHT

According to Berg (1961) egg weight was not affected by the addition of Agrozyme to layer ration.

Panda and Mohapatra (1989) reported an approximate egg weight of 6.7 to 10.7 g for Japanese quails.

The average egg weight of Japanese quail from 6 to 20 weeks was found to be 10.14g (Shukla *et al.*, 1994).

According to Satyamoorthy (1995) average weight of eggs produced by Leghorn hen supplemented with protease containing enzyme mix was 53.04 and that of control was 52.56 g.

Jaroni *et al.* (1999a) reported that the egg weight of Leghorn hen was significantly increased by the supplementation of an enzyme mixture containing protease (63.1 vs. 62.4 g).

Marginal deficiency of protein or any essential aminoacid cause reduction in egg size (Palod and Tiwari, 2004).

According to Ranade *et al.* (2004a and b) there was no significant difference in egg weight of layers supplemented with Maxigest L containing protease compared with nonsupplemented group.

According to Bhat *et al.* (2005) numerical but statistically nonsignificant increase in egg weight was observed in the pullet group in which maize was replaced by apple pomace at 10 per cent level with supplementation of enzyme mixture containing protease.

2.5 FEED CONSUMPTION

According to Gleaves and Dewan (1970) feed intake was not influenced by the supplementation of a fungal enzyme to corn milo layer rations.

According to Rexen (1981) there was no significant difference in feed consumption by the addition of protease and papain to the diet of one week old cockerels and four week old rats respectively.

According to Morris and Njuru (1990) feed intake of the layer chicks was not significantly affected by dietary protein content.

White Leghorn chicks supplemented with papain @ 1.25 g /60 kg mash showed no improvement in feed consumption (Thirumalai *et al.*, 1991).

Shukla *et al.* (1993) reported the feed consumption of laying Japanese quail as 28.89 g/bird/day.

According to Pillai *et al.* (1995) the supplementation of 0.2 per cent protease had no significant effect on feed consumption of broilers (2986 vs 3001g)

Satyamoorthy (1995) reported that daily feed intake of White Leghorn hen supplemented with less dense ration containing protease was 120 g and those with standard layer ration was 117 g.

Feed consumption was not influenced by the addition of 0.5, 1 and 1.5 g of the enzyme Salfeed supplement containing 1000 units of protease / kg to broiler diet (Dhar *et al.*, 1996b).

According to Simbaya *et al.* (1996) supplementation of protease G at the rate of 0.02 g/kg to broiler chicks enhanced the feed intake.

According to Vinil *et al.* (2000) the addition of multienzyme mix containing protease @ 100 and 150 g/100 kg in broiler ration had no significant effect on feed intake.

Babu and Devegowda (1997) found that there was a reduction in feed intake of broilers supplemented with enzyme mixture containing protease during early period of life (0 to 4 weeks) compared to the control (1217 and 1410 g respectively).

According to Benabdeljelil (1997) the amount of feed consumed was increased by two to four per cent by the addition of multienzyme preparation containing protease.

According to Bhatt and Bhatia (1999) the daily feed intake was not influenced by supplementation of a multienzyme preparation containing protease to pre weaned lambs.

Enzyme supplementation containing protease did not have any significant effect on feed consumption (Preston *et al.*, 2000).

Nahas and Lefrancois (2001) found superior feed intake in enzyme treated broiler group as compared to non supplemented group (4102 vs 3944 g).

When protease treated soyabean meal was fed to broiler chicks from seven to twenty days of age feed intake was improved (Ghazi *et al.*, 2002).

Bhat (2002) observed that the addition of multienzyme mixture containing 2400 units of protease @ 1 g/kg feed to broiler diet increased the feed consumption compared to control (1735 vs 1683 g).

According to Medhi *et al.* (2002) supplementation of Anizyme containing protease to broiler diets did not affect the feed consumption.

There was no significant difference in feed intake of broilers supplemented with enzyme mixture containing protease (Gracia *et al.*, 2003).

The feed intake of broilers was increased to 3136 g when supplemented with enzyme mixture containing protease compared to 2964 g for the control up to 42 days (Rao *et al.*, 2003).

Feed consumption of broilers supplemented with an enzyme mixture Maxigest (GB) containing protease was 3.05 and that of control was 3.68 kg/bird (Pal *et al.*, 2004).

Feed consumption of layers supplemented with Maxigest L containing protease was similar to that of control (110.57 and 110.39 g/bird/day respectively) (Ranade *et al.*, 2004a).

According to Ranade *et al.* (2004b) the total feed consumption of layers fed enzyme mix containing protease with 10 per cent reduction in CP was more than that of control.

Supplementation of Beetazyme containing protease to growing pullets did not produce any significant difference in feed intake (Nandi *et al.*, 2005a).

2.6 FEED EFFICIENCY (FE)

Feed efficiency of cockerels was significantly improved by the addition of enzyme protease compared to the control. (1.93 vs 2.02) (Rexen, 1981).

Arora *et al.* (1991) reported that the addition of a multienzyme preparation containing protease to broiler diet showed better feed efficiency of 2.85 compared to 3.28 for the control.

According to Chesson (1993) enzyme addition must be able to demonstrate a sufficiently substantial improvement in feed efficiency or the quality of the product to cover the cost of supplementation and to provide an increased margin for the producer.

According to Shukla *et al.* (1993) the feed efficiency/kg egg of laying Japanese quail was 3.09.

Pillai *et al.* (1995) reported that 0.2 per cent protease supplementation significantly improved feed efficiency of broilers upto six weeks (1.98 vs 2.03).

According to Satyamoorthy (1995) the overall feed efficiency values for egg mass of White Leghorn hen supplemented with a diet containing protease from 20 to 40 weeks of age was similar to the control group (3.18).

Feed efficiency was improved by 1.5 to 4.5 per cent by the supplementation of enzyme mix containing protease to broiler diet (Dhar *et al.*, 1996b).

According to Suresha *et al.* (1996) there was an improvement in feed efficiency of broilers supplemented with a commercial enzyme containing protease at levels of 0.75 and 1.50 per cent to the feed.

According to Babu and Devegowda (1997) the feed efficiency of broilers fed enzyme mixture containing protease was significantly improved compared to control (1.429 vs. 1.632).

Benabdeljelil (1997) reported that feed efficiency was improved by 7.5 per cent by the supplementation of multi enzyme preparation containing protease at a level of 0.05 per cent to broiler diet.

According to Daveby *et al.* (1998), enzyme supplementation had no significant effect on feed efficiency of broiler chicken.

Addition of a multienzyme product containing cellulase, betaglucanase and protease significantly improved the feed efficiency late in the laying period (Nasi, 1988).

Cook *et al.* (2000) reported that supplementation of 0.075 per cent of Avizyme containing protease reduced the feed per dozen egg of pullets by 3.5 to 4 per cent.

According to Deshmukh *et al.* (2000) addition of multi enzyme preparation containing protease to broiler diet significantly improved feed efficiency.

Rosen (2000) reported that in 1925, scientists first reported that a commercial enzyme product containing protease had improved the feed efficiency in White Leghorn chicken (1.93 vs 2.01).

Addition of multienzyme mix containing protease @ 100 and 150 g/kg in broiler ration revealed no significant variation in feed efficiency (Vinil *et al.*, 2000).

Feed efficiency was improved by the supplementation of an enzyme mixture containing 20 g/kg of proteolytic enzyme to broiler diets with 75 per cent of fish meal replaced by sesame cake protein on isonitrogenous basis, compared to non supplemented group (Mejumder and Samanta, 2001).

Nahas and Lefrancois (2001) reported that by the supplementation of enzyme mix containing protease to broiler diet, feed efficiency was found to be better compared to control (0.585 vs 0.602).

According to Bhat (2002) addition of multi enzyme mix containing protease to broilers improved the feed efficiency as 2.07 compared to 2.25 in supplemented and unsupplemented group respectively.

Medhi *et al.* (2002) reported that feed efficiency of broilers fed diets containing multi enzyme mix with protease was not improved compared to control.

Feed efficiency of broilers was significantly improved (1.49 compared to 1.65 for control) by supplementing enzyme mix containing 19,000 protease units/g (Gracia *et al.*, 2003).

Feed efficiency was not influenced by the supplementation of enzyme mixture containing protease to broiler diet (Rao *et al.*, 2003).

Pal *et al.* (2004) reported feed efficiency of broilers supplemented with enzyme mix containing protease as 1.43 and that of control group as 1.86.

Ranade *et al.* (2004a) reported the feed efficiency of layers fed diet with protease containing enzyme preparation as 1.51 and that of control as 1.50.

Ranade *et al.* (2004b) reported that feed efficiency (kg feed/dozen egg) of layers supplemented with enzyme mix containing protease was significantly better than the control (1.97 vs 2.23).

Supplementation of canola meal diets with protease G @ 0.02 g/kg dietary level to broilers had no effect on feed efficiency (Simbaya *et al.*, 1996).

Use of Beetazyme containing protease with different combinations of cereals and oil seed meals to the diet of growing pullets did not affect the feed efficiency significantly (Nandi *et al.*, 2005a).

According to Saraswat *et al.* (2005) feed efficiency of broiler chicks was improved by 3.0, 4.5 and 9.0 per cent by the supplementation of multienzyme preparation containing protease @ 0.10, 0.15 and 0.20 per cent respectively.

2.7 EGG QUALITY

Berg (1961) observed no significant difference in Haugh unit by the supplementation of Agrozyme to layer ration.

Shukla *et al.* (1993) found the albumen index and shell thickness of eggs of Japanese quail as 0.105 and 0.177 mm respectively.

Satyamoorthy (1995) reported the albumen and yolk indices of eggs produced by White Leghorn hen supplied with less dense ration containing protease as 0.07 and 0.41 and that with standard layer ration as 0.08 and 0.40. He further reported the shell thickness of White Leghorn hen supplemented with protease containing diet as 0.49 and that of control as 0.52.

Ranade *et al.* (2004a) reported that albumen, yolk and shape indices of eggs of laying chicken supplemented with multienzyme preparation containing protease was more than that of control.

According to Ranade *et al.* (2004b) the supplementation of an enzyme mix containing protease with a reduction of 10 per cent in CP to layer diet did not

significantly influence the shape, albumen and yolk indices. But the shell thickness was significantly higher in layers supplemented with enzyme mix containing protease (0.33 vs 0.30 mm).

Nandi *et al.* (2005b) reported that supplementation of Beetazyme containing protease to layer diet in which maize and soyabean meal was replaced by sorghum, ragi, niger and sesame cake in equal ratio lowered the shape and albumen indices significantly compared to the control.

2.8 SERUM PROTEIN

Narayanankutty (1987) reported the mean serum protein values of meat type Japanese quails at 5th and 6th weeks of age as 5.78 and 5.82 g per cent respectively.

Panda *et al.* (1987) found the serum protein value of Japanese quail at five weeks as 3.17 g/100 ml.

Singh *et al.* (1996) reported that the total serum protein of White Leghorn hen aged 28 weeks was 5.3 g/100 ml.

2.9 BALANCE OF NITROGEN

The addition of papain to chick ration did not improve the protein digestibility (Thirumalai *et al.*, 1991).

Mahagna *et al.* (1995) reported that broiler diet supplemented with 0, 250 and 1000 µg/kg of enzyme preparation containing protease had no effect on digestibility of protein except for the highest level of enzyme supplementation, which improved slightly the digestibility of amino acid.

Satyamoorthy (1995) reported that crude protein digestibility coefficient of White Leghorn hen supplemented with less dense ration containing protease was 78.8 and those with standard layer ration was 78.9.

The supplementation of a commercial enzyme preparation, Ventrigold containing protease to broiler diet did not affect the per cent utilization of protein (Dhar *et al.*, 1996a).

The per cent utilization of protein was not found to be significantly affected by the addition of enzyme Salfeed containing protease in the broiler diet, although a positive balance of nitrogen was observed (Dhar *et al.*, 1996b).

Protein digestibility was significantly increased by the addition of an enzyme mixture to the broiler diet (Yalda and Forbes, 1996).

Caine *et al.* (1997) found that the treatment of soyabean meal with protease @ one microgram per gram to weaned pigs did not improve the digestibility of amino acid.

According to Marsman *et al.* (1997), protease treatment improved apparent ileal digestibility of CP (85.2 vs 83.7 %) of broiler chicks compared with no enzyme treatment.

Jaroni *et al.* (1999b) found that the supplementation of an enzyme preparation containing protease to White Leghorn hen significantly increased the protein digestibility at 50 weeks of age.

According to Zanella *et al.* (1999) supplementation of an enzyme mixture containing protease to broiler diet improved the ileal crude protein digestibility by 2.9 per cent.

Barbour *et al.* (2002) reported that digestion of pressed turkey feathers with an enzyme mixture containing protease resulted in an increase in nutritional value of protein in poults.

Ghazi *et al.* (2002) reported that soyabean meal pretreated at 500° C for two hours with protease when fed to broiler chicks improved apparent nitrogen digestibility.

Apparent nitrogen retention was greater in the ducks that received enzyme preparation containing protease at the rate of 0.5g/kg diet than in ducks that fed diet without enzyme (Hong *et al.*, 2002).

Kocher *et al.* (2002) reported that the addition of an enzyme mixture containing protease to broiler diet at five times the recommended dosage improved ileal protein digestibility.

Yu *et al.* (2002) found that the inclusion of bromelin, a proteolytic enzyme did not significantly increase the ileal protein digestibility in either the growing or finishing broiler diet.

2.10 LIVABILITY

Berg (1961) found no significant effect on livability by supplementation of Agrozyme containing protease to layer ration.

Livability of hen was increased by addition of a fungal enzyme (Gleaves and Dewan, 1970).

According to Pillai *et al.* (1995) mortality rate in broilers fed protease treated feed was very low and similar to the control group.

Satyamoorthy (1995) reported that mortality of hen supplemented with a diet containing protease was within permitted limits.

The supplementation of multienzyme preparation containing protease to broiler diet lowered the mortality rate (Benabdeljelil, 1997).

The supplementation of Avizyme 1500 containing protease to 18 week old pullets significantly improved the livability by three to four per cent (Cook *et al.*, 2000).

Café *et al.* (2002) found that mortality at 16, 35 and 42 days was significantly less among the broilers supplemented with enzyme mix containing protease.

According to Rao *et al.* (2003), livability was unaffected by supplying multienzyme preparation containing protease to broiler diet.

The per cent of mortality was lower in broilers supplemented with enzyme mixture containing protease compared to the control (Medhi *et al.*, 2003).

Ranade *et al.* (2004b) found that the mortality was not affected by the supplementation of multienzyme mix containing protease to layers.

Bhat *et al.* (2005) reported that mortality was not affected by the supplementation of multienzyme mixture containing protease @ 1g/kg feed of pullet diets.

2.11 ECONOMICS

According to Satyamoorthy (1995) cost of feed /egg of White Leghorn hen was unaffected by the supplementation of protease .

Babu and Devegowda (1997) found that the supplementation of an enzyme mixture containing protease to broilers was found to be economical compared to control (margin over feed cost, rupees/bird at six weeks – 29.13 and 28.13 respectively) at 12.5 per cent fiber level.

Cook *et al.* (2000) observed that hen fed Avizyme containing protease had a return above the enzyme cost.

Bhat (2002) observed that the profit per bird was found to be higher (Rs.2.91) in the multienzyme containing protease supplemented group than the control .

Enzyme supplementation containing protease facilitated the inclusion of ajar seed meal in the broiler diets replacing maize up to five per cent level with economic advantage (Medhi *et al.*, 2002).

Feed cost per bird of those supplemented with multienzyme preparation containing protease was two rupees less than the control diet (Pal *et al.*, 2004).

Cost of production per egg of layers supplemented with multienzyme preparation containing protease was similar to that of control (92.4 and 92.9 paise respectively) (Ranade *et al.*, 2004a).

The supplementation of enzyme mixture containing protease to layer diet with 10 per cent reduction in CP reduced the cost of production of eggs for 19 paise as compared to the control (Ranade *et al.*, 2004b).

Cost of feeding was not affected significantly by the supplementation of Beetazyme containing protease to the diet of growing pullet during nine to twenty weeks of age (Nandi *et al.*, 2005a).

Saraswat *et al.* (2005) reported that the feed cost to produce one kg live weight was reduced to 12.40 per cent due to incorporation of 0.20 per cent of multienzyme containing protease to broiler diet.

Materials and Methods

3. MATERIALS AND METHODS

An investigation was carried out in the Department of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy to find out the effect of supplementation of protease on the laying performance of Japanese quails fed low protein diet from December 2004 to May 2005.

3.1 EXPERIMENTAL MATERIALS

3.1.1 Birds

One hundred and sixty Japanese quails of six weeks of age procured from University Poultry Farm (UPF) were used for the experiment.

3.1.2 Rations

The feed ingredients procured from UPF, Mannuthy were used for preparing the experimental rations. The experiment was conducted using two rations, standard layer ration and low protein layer ration. The standard quail layer ration was formulated as per the recommendations for laying Japanese quails by the Central Avian Research Institute, Izatnagar. It contained 22 per cent crude protein (CP) and 2650 kcal/kg of metabolisable energy. The low protein quail layer ration was formulated with a low percentage of crude protein (CP 18) and same level of metabolisable energy as the standard layer ration. The ingredients used were yellow maize, soyabean meal, rice polish, ground nut cake and unsalted dried fish. The ingredient composition of feed is given in Table 1. The proximate composition of the rations were estimated according to AOAC (1990) and the per cent proximate composition of nutrients in the rations are presented in Table 2.

Table 1. Ingredient composition of experimental rations, %

Sl. No.	Ingredients	Standard quail layer ration	Low protein quail layer ration
1	Yellow maize	37.00	40.00
2	Ground nut cake (exp)	15.00	11.00
3	Soyabean meal	22.00	10.00
4	Rice polish	10.00	21.00
5	Unsalted dried fish	8.00	10.00
6	Shell grit	6.00	6.00
7	Mineral mixture*	1.75	1.75
8	Salt	0.25	0.25
	Total	100.00	100.00
	Added per 100 kg of feed		
1	Nicomix A+B ₂ +D ₃ +K(g)**	15	15
2	Nicomix B+E (g)***	20	20

* Supermin P mineral mixture without salt (Kwality Agrovet Industries, Salem)

Composition: Calcium – 30.0%, Phosphorus – 9.0%, Iron – 0.2%, Iodine – 0.01%, Zinc = 0.05%, Manganese – 0.4%, Copper – 0.4%, Flourine (max) – 0.05%, Acid insoluble ash (max) – 2.5% and moisture – 3%.

Composition per gram

** Nicomix A+B₂+D₃+K (Nicholas Piramal India Ltd., Mumbai)

Vitamin A – 82,500 IU, Vitamin B₂ – 50 mg

Vitamin D₃ – 12,000 IU, Vitamin K – 10 mg

*** Nicomix B+E (Nicholas Piramal India Ltd., Mumbai)

Vitamin B₁ – 4 mg, Vitamin B₆ – 8 mg, Vitamin B₁₂ – 40 mcg, Niacin – 60 mg, Calcium pantothenate – 40 mg, Vitamin E – 40 mg

Table 2. Chemical composition of experimental rations (on dry matter basis), %

Parameters	Standard quail layer ration	Low protein quail layer ration
Analysed values ¹		
Moisture	8.50	9.08
Crude protein	22.11	18.17
Ether extract	4.41	5.80
Crude fibre	2.75	4.57
Nitrogen free extract	58.71	59.26
Total ash	12.02	12.20
Acid insoluble ash	3.70	3.90
Calcium	3.60	3.59
Available phosphorus	0.81	0.87
Calculated values		
ME (kcal/kg)	2651	2660
Lysine (%)	1.03	1.00
Methionine (%)	0.37	0.37

1. Average of six samples

3.1.3 Enzyme

The enzyme protease with an activity of 1500 IU per gram was added @ 0.02 and 0.04 per cent to the low protein layer ration. The protease used was the food grade proteolytic enzyme papain isolated from the latex of *Carica papaya* fruit supplied by Zeus Biotech. Ltd., Bombay.

3.2 EXPERIMENTAL METHODS

3.2.1 Housing of Birds

The experiment was conducted at University Poultry Farm, Mannuthy. The cage house, the cages, the feed and water troughs were thoroughly cleaned and disinfected one week prior to housing of birds. The birds were housed at five weeks of age for cage adaptation.

3.2.2 Experimental Design

At six weeks of age, the birds were weighed and ten birds of almost the same initial average body weight were randomly allotted to each replicate (Table 3). There were four treatment groups, with four replicates for each. They were fed experimental diets from six to twenty six weeks of age and the experimental duration of twenty weeks was divided into five, 28 day periods and data were collected for each period of each replicate. Weekly data are presented from seven to twenty six weeks of age.

3.2.3 Management

The birds were provided with feed and water *ad libitum* throughout the experimental period and were maintained under cage system of management.

Table 3. Distribution of dietary treatments

Dietary treatment	Number of replicates	Number of birds in each replicate	Rations	Enzyme treatment	Level of enzyme inclusion (%)
T0	4	10	Standard protein (22%) quail layer ration	Nil	0.00
T1	4	10	Low protein (18%) quail layer ration	Nil	0.00
T2	4	10	Low protein (18%) quail layer ration	Protease	0.02
T3	4	10	Low protein (18%) quail layer ration	Protease	0.04

3.2.4 Meteorological Parameters Inside the House

The dry and wet bulb readings and maximum and minimum temperature were recorded daily at 8.00 a.m. and 2.00 p.m. throughout the experiment period.

3.2.5 Body Weight

Body weight at the beginning and end of the experiment *ie.*, at 6 and 26 weeks of age (BW 6 and BW 26) were recorded individually to the nearest 10 g.

3.2.6 Age at Sexual Maturity (ASM)

The age at first egg and 50 per cent production (days) were recorded in each replicate and from the data mean age at sexual maturity and 50 per cent production were determined.

3.2.7 Egg Production

Egg production was recorded daily from six to twenty six weeks of age. It is expressed as total egg production, hen housed and hen day egg production in number and per cent replicate wise and period wise.

3.2.8 Egg Weight

The individual weight of all eggs produced during the last three days of each 28 day period was measured to the nearest 0.01 g. The mean egg weight was calculated for each replicate.

3.2.9 Feed Consumption

The weight of feed issued was recorded for each replicate period wise. The balance feed available at the end of each period was also noted. From this data, period wise mean daily feed consumption per bird was worked out for each replicate.

3.2.10 Feed Efficiency

Feed efficiency per dozen eggs was calculated from the feed consumed and number of eggs produced during each period for each replicate.

3.2.11 Egg Quality

Five eggs were collected at random from each replicate during the last three days of each period for egg quality studies. Length and breadth of eggs, width of albumen and diameter of yolk were measured using Vernier calipers. Height of albumen and yolk were measured using Ame's tripod stand micrometer. The shell thickness was measured using shell thickness measuring gauge to the nearest 0.001 mm. The shape, albumen, yolk indices and internal quality unit (IQU) were recorded by using the following formulae.

$$\text{Shape Index} = \text{Breadth/length} \times 100$$

$$\text{Albumen Index} = \text{Height of thick albumen/width of thick albumen}$$

$$\text{Yolk Index} = \text{Height of yolk/diameter of yolk}$$

$$\text{IQU} = 100 \log (H + 4.18 - 0.8989 W^{0.6674})$$

Where H= Height of thick albumen in mm and

W=Weight of egg in g

3.2.12 Serum Protein

At the end of 26th week of age, blood samples were collected from two birds in each replicate by severing the jugular vein for the estimation of serum protein. The serum protein was estimated by modified Biuret method.

3.2.13 Balance of Nitrogen

At the end of the experiment, a three day metabolism trial was conducted using one bird selected randomly from each replicate. They were housed individually in cages with facilities for feeding, watering and excreta collection. The total amount of feed consumed was recorded and water was provided *ad libitum*. Excreta was collected over 24 hour period for three consecutive days by total collection method as described by Summers *et al.* (1976). The excreta was weighed each day and samples were taken in air tight containers and were stored in a deep freezer till they were analysed.

3.2.14 Chemical Analysis

The chemical composition of experimental rations and the nitrogen content of the excreta samples were determined in fresh material as per the procedure described by AOAC (1990). From the data obtained on the total intake and outgo of nitrogen during the metabolism trial, the per cent of nitrogen retained was calculated.

3.2.15 Mortality

Daily mortality was recorded and the total percentage of different treatments during the experimental period was calculated.

3.2.16 Economics

Economics of the experiment was calculated from the cost of feed ingredients, quantity of the feed consumed and number of eggs produced during each period by each replicate.

3.3 STATISTICAL ANALYSIS

Data collected on various parameters were statistically analysed by Completely Randomised Design (CRD) as described by Snedecor and Cochran (1985). Means were compared by Least Significant Difference (LSD) test using MSTAT-C.

Results

4. RESULTS

The effect of supplementation of protease on the production performance of laying Japanese quails was assessed by conducting a feeding trial for a period of 20 weeks. The results obtained are presented in this chapter.

4.1 CLIMATIC PARAMETERS

The mean maximum and minimum temperatures and per cent relative humidity during different periods of experiment (December 2004 to May 2005) are presented in Table 4. During the experimental period, the maximum temperature ranged from 32.5 to 36.5°C and the minimum temperature ranged from 22.0 to 24.9°C. The per cent relative humidity in the morning varied from 67.4 to 89.2 while in the afternoon it ranged from 33.2 to 59.6. The maximum and minimum temperature was highest during 23-26 weeks and lowest during 7 to 10 weeks of age. The maximum per cent relative humidity in the morning and afternoon was also observed during 23-26 weeks of age.

4.2 BODY WEIGHT

The mean body weight at the beginning and end of the trial and body weight gained by the birds during the experimental period among the treatment groups are shown in Table 5.

The mean initial body weight was maximum for T3 (174.5g) followed by T0 (172.24g), T2 (167.75g) and T1 (166.96g). But finally T0 (205.16g) reached the highest body weight and descending order was T2 (203.81g), T3 (202.23g) and T1 (196.20g).

The mean body weight gain during the experimental period among the different treatment groups viz., T0, T1, T2 and T3 were 32.92, 29.24, 36.06 and 27.73 g respectively. The birds supplemented with 0.02 per cent enzyme gained

Table 4. Mean climatic parameters during different periods of the experiment

Age in weeks	Temperature (°C)		Relative Humidity (%)	
	Maximum	Minimum	8 a.m.	2 p.m.
7-10 weeks	32.5	22.0	67.4	39.3
11-14 weeks	33.9	23.0	75.1	38.7
15-18 weeks	34.7	22.7	74.7	33.2
19-22 weeks	35.0	24.7	84.1	46.6
23-26 weeks	36.5	24.9	89.2	59.6
Mean	34.52	23.5	78.1	43.5

Table 5. Mean (\pm SE) body weight at the end of 6 and 26 weeks of age of Japanese quails maintained on different dietary treatments, g

Treatments	BW 6	BW 26	BW gain
T ₀	172.24 \pm 4.07	205.16 \pm 6.38	32.92
T ₁	166.96 \pm 4.41	196.20 \pm 2.85	29.24
T ₂	167.75 \pm 2.01	203.81 \pm 4.91	36.06
T ₃	174.50 \pm 4.46	202.23 \pm 5.85	27.73

maximum body weight during the experimental period while those birds fed 0.04 per cent protease was poorest in body weight gain. T0 was second highest followed by T1.

Statistical analysis of the data pertaining to the mean body weight revealed no significant differences among treatment groups.

The mean body weight gain of birds maintained on different dietary treatments are graphically represented in Fig 1.

4.3 AGE AT SEXUAL MATURITY AND 50 PER CENT PRODUCTION

The mean age at first egg and 50 per cent production are presented in Table 6. The mean age at first egg ranged from 44-48 days. The birds fed low protein diet supplemented with 0.04 per cent enzyme (T3) reached sexual maturity earlier followed by T1, T0 and T2. The mean age at sexual maturity of different treatment groups are statistically similar. The mean age at 50 per cent production for the different treatment groups viz. T0, T1, T2 and T3 were 58, 58, 60 and 56 days respectively. The trend was almost similar to age at sexual maturity with T3 the earliest and T2 the most delayed to reach age at 50 per cent production. The mean age at 50 per cent production also showed no significant difference between the treatment groups.

The mean age at first egg and 50 per cent production are depicted in Fig.2.

4.4 EGG PRODUCTION

Total number of eggs produced by the birds of different treatment groups during the five periods are set out in Table 7. The values were 3232, 2710, 2903 and 2415 for T0, T1, T2 and T3, respectively. The highest egg production was recorded by T0. Birds fed low protein diet supplemented with 0.02 per cent protease (T2) ranked second and T3 was lowest in total egg production.

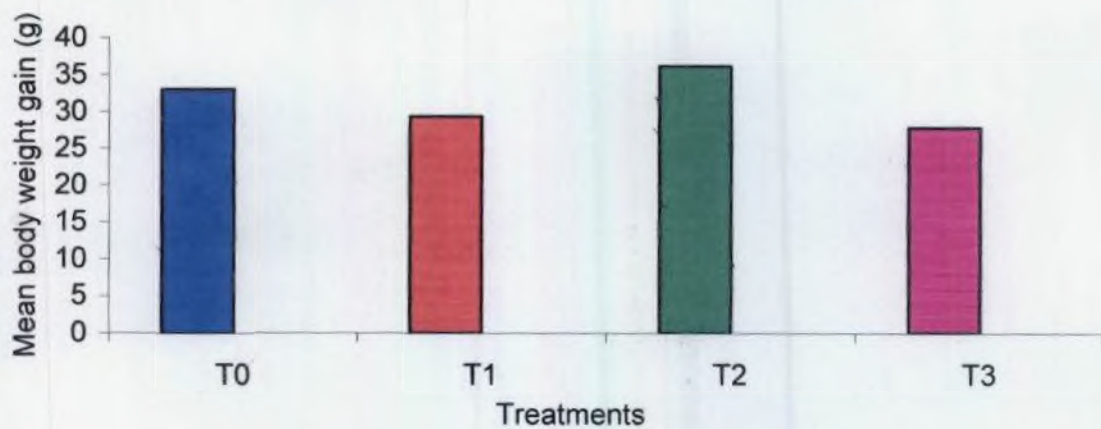


Fig.1. Mean body weight gain (g) of Japanese quails maintained on different dietary treatments.

Table 6. Mean (\pm SE) age at sexual maturity and 50% production of Japanese quails maintained on different dietary treatments, days

Treatments	Age at sexual maturity	Age at 50 per cent production
T ₀	47 \pm 2.87	58 \pm 1.31
T ₁	45 \pm 2.74	58 \pm 1.89
T ₂	48 \pm 1.93	60 \pm 3.07
T ₃	44 \pm 2.80	56 \pm 1.18

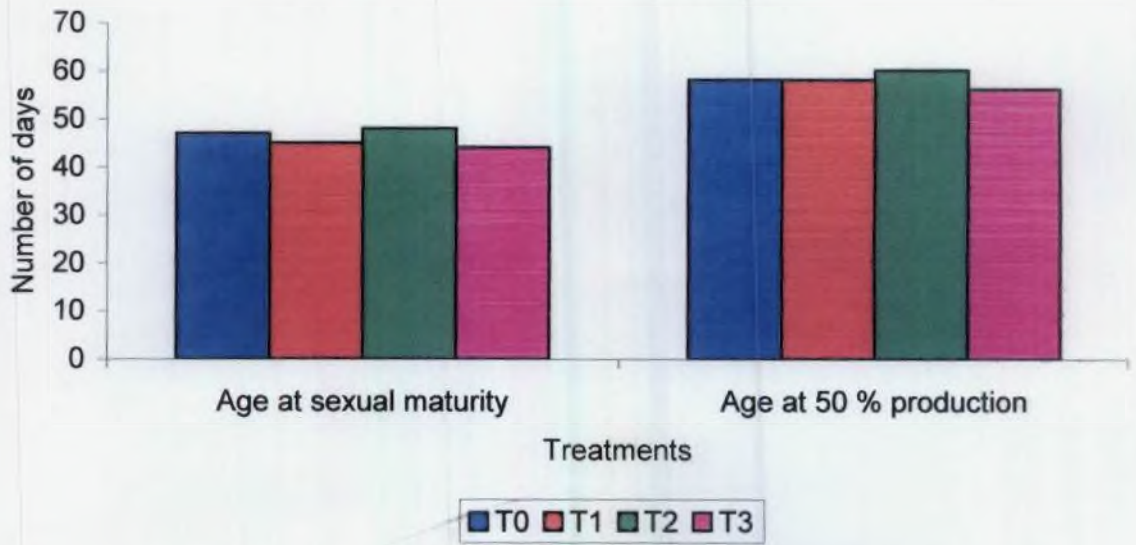


Fig.2. Mean age at sexual maturity and 50% production (days) of Japanese quails maintained on different dietary treatments

Table 7. Total egg production of Japanese quails maintained on different dietary treatments

Treatments	Total egg production					
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	7-26 weeks
T ₀	388	901	822	737	384	3232
T ₁	391	697	669	587	366	2710
T ₂	354	776	783	609	381	2903
T ₃	363	669	622	475	286	2415

The data on mean values of hen housed and hen day egg number for different treatment groups during the entire experimental period are set out in Table 8 and 9 respectively. Overall hen housed egg number were 80.80, 67.75, 72.58 and 60.38 and overall hen day egg number were 85.08, 74.94, 77.75 and 67.44 respectively for T0, T1, T2 and T3. Mean hen housed egg number was highest for T0 during the entire experimental period except during 7-10 weeks of age where T1 ranked top. The trend was same for mean hen day egg number also.

The data on mean values of percentage hen housed and hen day egg production for different treatment groups during the experimental periods are given in Table 10 and 11 respectively. Overall per cent hen housed egg production were 57.71, 48.39, 51.84 and 43.13 and overall per cent hen day egg production were 60.77, 53.53, 55.53 and 48.17 respectively, for T0, T1, T2 and T3. The birds fed standard layer ration (T0) showed the highest hen housed egg production per cent during the entire experimental period except with slight differences during 7-10 and 15-18 weeks of age. The birds supplemented with 0.04 per cent protease was lowest in hen housed egg production per cent during the entire experimental period except 7 to 10 weeks of age. But the birds fed 0.02 per cent protease reached the top in hen housed egg production per cent during 15-18 and second during 11-14 and 23-26 weeks of age.

In overall per cent hen day egg production also T0 stood first, followed by T2, T1 and T3. Except during the initial period where T1 was the top scorer, the birds fed standard layer ration were best in hen day egg production per cent. The birds supplemented with 0.02 per cent protease was next in hen day egg production per cent during 11-14, 15-18, 19-22 and 23-26 weeks of age. Statistical analysis of the total egg production, hen housed and hen day egg number and hen housed and hen day egg production per cent revealed no significant differences among the treatment groups. Total egg production, hen housed and hen day egg number are graphically represented in Fig.3, 4 and 5 respectively.

Table 8. Mean hen housed egg number of Japanese quails maintained on different dietary treatments

Treatments	Hen housed egg number					
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	7-26 weeks
T0	9.70	22.53	20.55	18.43	9.60	80.80
T1	9.78	17.43	16.73	14.68	9.15	67.75
T2	8.85	19.40	19.58	15.23	9.53	72.58
T3	9.08	16.73	15.55	11.88	7.15	60.38

Table 9. Mean hen day egg number of Japanese quails maintained on different dietary treatments

Treatments	Hen day number					
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	7-26 weeks
T0	9.70	22.53	21.82	20.31	10.67	85.08
T1	9.82	19.36	19.11	16.77	10.46	74.94
T2	8.85	20.21	21.58	16.92	10.58	77.75
T3	9.12	18.65	17.77	13.82	8.41	67.44

Table 10. Mean hen housed egg production of Japanese quails maintained on different dietary treatments,%

Treatments	Hen housed per cent					
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	7-26 weeks
T ₀	34.64	78.20	73.39	69.86	42.13	57.71
T ₁	34.91	64.02	68.86	60.89	37.65	48.39
T ₂	31.61	69.26	73.99	59.33	38.13	51.84
T ₃	32.41	63.01	63.28	47.78	31.53	43.13

Table 11. Mean hen day egg production of Japanese quails maintained on different dietary treatments, %

Treatments	Hen day per cent					
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	7-26 weeks
T ₀	34.64	80.45	78.30	72.96	38.92	60.77
T ₁	35.07	71.60	68.86	60.45	37.65	53.53
T ₂	31.61	72.13	77.31	60.51	38.11	55.53
T ₃	32.51	64.22	63.28	48.62	30.26	48.17

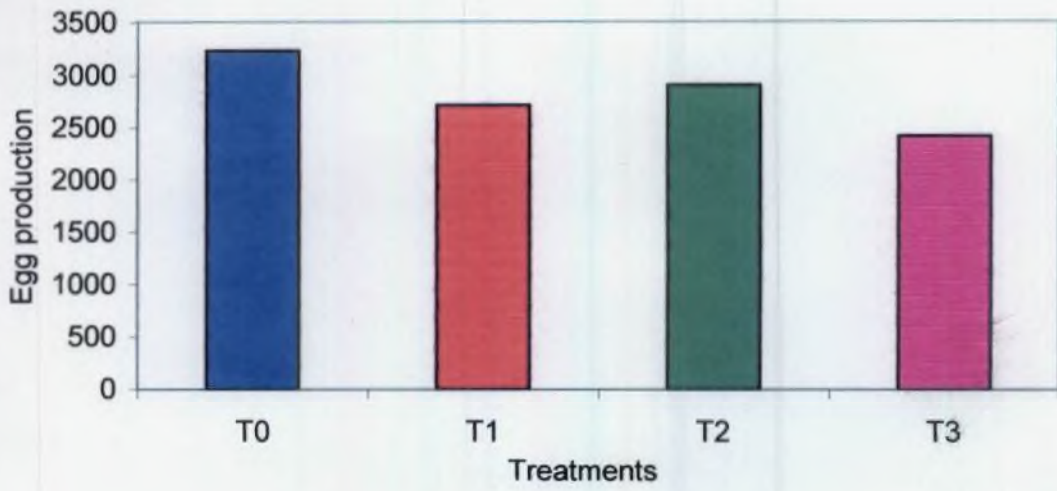


Fig.3. Total egg production of Japanese quails maintained on different dietary treatments

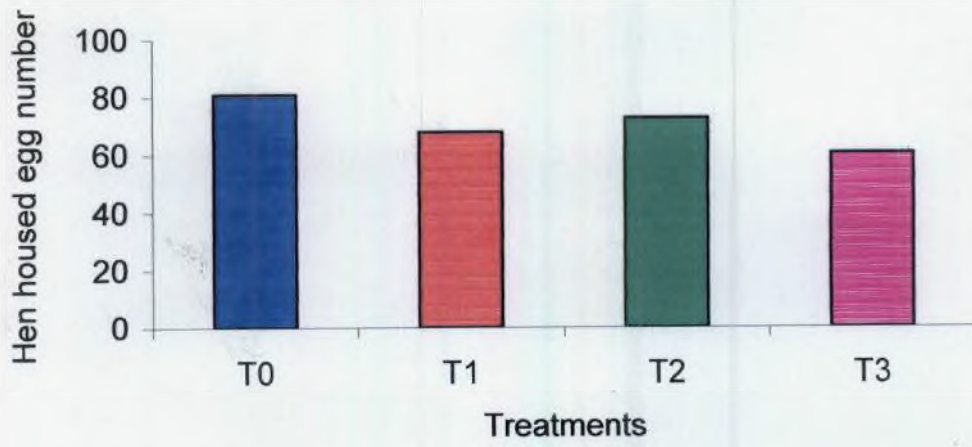


Fig.4. Overall hen housed egg number of Japanese quails maintained on different dietary treatments

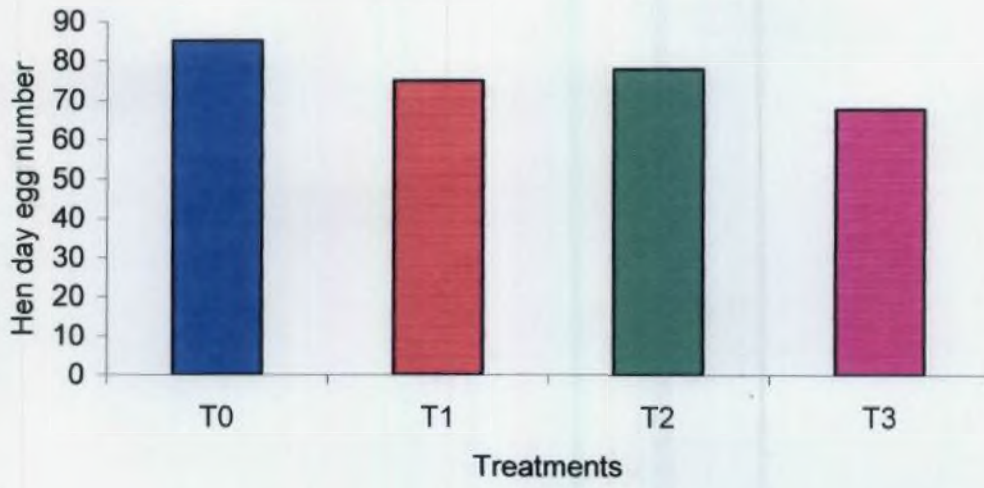


Fig.5. Overall hen day egg number of Japanese quails maintained on different dietary treatments

4.5 EGG WEIGHT

The mean egg weight of the birds in the four treatment groups from 6 to 26 weeks of age are presented in Table 12.

The highest overall egg weight was recorded by T0 (11.14 g) followed by T3 (10.72 g), T1 (10.61 g) and T2(10.40g). The egg weight of T0 was significantly higher ($p<0.05$) than the low protein groups during 19 to 22 weeks of age and numerically higher than low protein groups during the remaining periods of experiment. The birds supplemented with 0.04 per cent protease was higher in overall egg weight compared to other groups except T0 and those supplemented with 0.02 per cent protease was lowest in overall egg weight. No significant difference was observed in egg weight during the other four periods among the treatment groups.

The mean overall egg weight values of different treatment groups are graphically presented in Fig. 6.

4.6 FEED CONSUMPTION

The mean daily feed intake per bird during each period among the different treatment groups are given in Table 13.

The overall daily feed intake per bird among the four treatment groups viz., T0, T1, T2 and T3 during the whole experimental period were 29.15, 29.41, 28.48 and 28.60g, respectively. The birds fed standard layer ration (T0) and those birds fed low protein layer ration (T1) were more or less similar in mean daily feed consumption during all the periods. Only slight numerical differences were observed in mean daily feed consumption of enzyme supplemented groups during the entire experimental period.

The analysis of variance of the data on feed intake showed that there was no significant difference in daily feed intake among the treatment groups. The

Table 12. Mean (\pm SE) egg weight of Japanese quails maintained on different dietary treatments, g

Treatments	Egg weight					Overall
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	
T ₀	10.65 \pm 0.30	11.04 \pm 0.42	11.15 \pm 0.23	11.27 ^a \pm 0.10	11.58 \pm 0.11	11.14 \pm 0.25
T ₁	10.41 \pm 0.21	10.34 \pm 0.12	10.85 \pm 0.05	10.5 ^b \pm 0.11	10.94 \pm 0.16	10.61 \pm 0.16
T ₂	9.86 \pm 0.11	10.18 \pm 0.13	10.36 \pm 0.2	10.74 ^{ab} \pm 0.24	10.90 \pm 0.20	10.40 \pm 0.23
T ₃	10.39 \pm 0.25	10.53 \pm 0.27	10.82 \pm 0.55	10.64 ^b \pm 0.2	11.23 \pm 0.25	10.72 \pm 0.29

Means bearing different superscripts with in the same column differed significantly ($p < 0.05$)

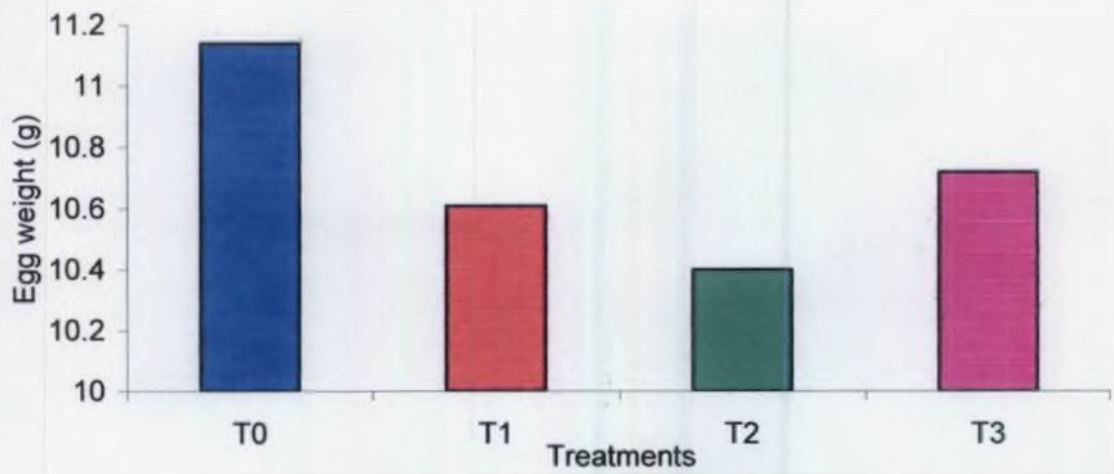


Fig .6. Overall egg weight (g) of Japanese quails maintained on different dietary different treatments

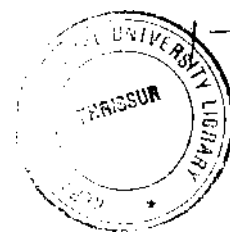


Table 13. Mean (\pm SE) daily feed consumption of Japanese quails maintained on different dietary treatments, g

Treatments	Feed consumption					Overall
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	
T ₀	24.23 \pm 0.37	28.16 \pm 0.46	30.80 \pm 0.75	31.26 \pm 0.84	31.28 \pm 0.50	29.15 \pm 1.34
T ₁	24.61 \pm 0.83	28.19 \pm 0.40	30.63 \pm 0.69	31.88 \pm 0.75	31.75 \pm 0.22	29.41 \pm 1.35
T ₂	23.76 \pm 1.21	27.65 \pm 0.27	29.87 \pm 1.28	30.66 \pm 0.64	30.45 \pm 0.38	28.48 \pm 1.37
T ₃	23.68 \pm 0.28	27.96 \pm 0.43	30.16 \pm 0.44	30.66 \pm 0.72	30.53 \pm 0.44	28.60 \pm 1.27

mean daily feed intake per bird as influenced by protease supplementation is graphically presented in Fig .7.

4.7 FEED EFFICIENCY

The data on mean feed efficiency (kg feed per dozen eggs) among the treatment groups during the five periods is presented in Table 14. The overall feed efficiency of 7 to 26 weeks of age was superior for T0 (0.57) followed by T2 (0.61), T1 (0.65) and T3 (0.73). The birds supplemented with 0.02 per cent enzyme was slightly better than low protein treated group during the entire experimental period except during 7 to 10 weeks of age. The poorest mean feed efficiency was obtained for the birds supplemented with 0.04 per cent protease during 15 to 18, 19 to 22 and 23 to 26 weeks of age.

Feed efficiency of the treatment groups during the five periods were statistically similar.

4.8 EGG QUALITY

The data on egg quality traits such as, shape, albumen, yolk indices, IQU and shell thickness are presented in Table 15, 16, 17, 18 and 19, respectively.

The overall mean values for shape index of the eggs of the birds maintained on different dietary treatments were almost same (Table 15). The mean shape index values of the four treatment groups during the entire experimental period were statistically similar.

The overall mean albumen index of the eggs of T0, T2 and T3 were same (0.103) (Table 16). The overall mean albumen index of the birds fed low protein diet was lower (0.101) than the other groups. The mean albumen index of the eggs of four treatments during the entire experimental period did not differ statistically.

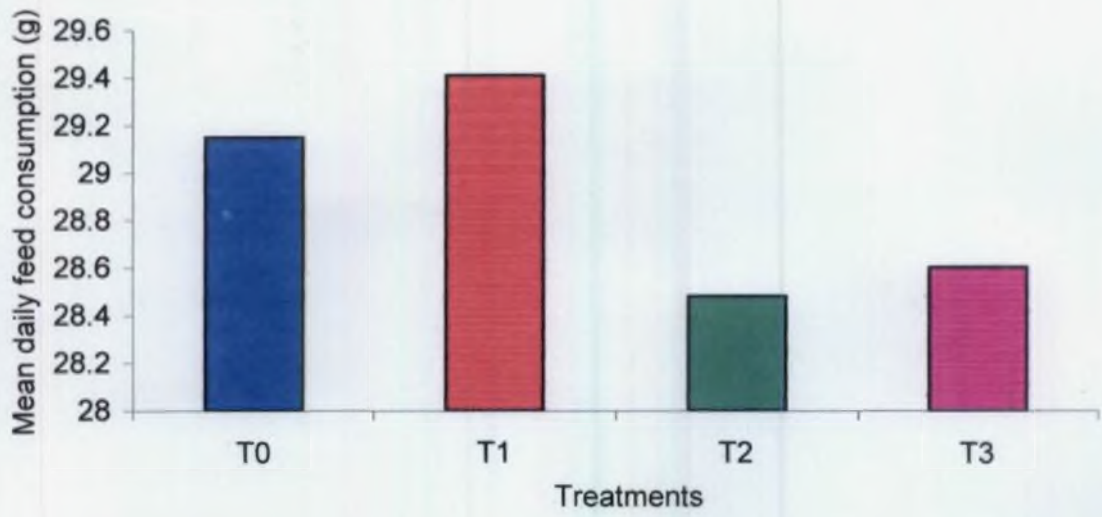


Fig. 7. Overall daily feed consumption (g) of Japanese quails maintained on different dietary treatments

Table 14. Mean (\pm SE) feed efficiency per dozen eggs of Japanese quails maintained on different dietary treatments

Treatments	Feed efficiency per dozen eggs					Overall
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	
T ₀	0.84 \pm 0.06	0.42 \pm 0.02	0.47 \pm 0.03	0.52 \pm 0.06	0.99 \pm 0.18	0.57 \pm 0.13
T ₁	0.84 \pm 0.16	0.50 \pm 0.01	0.53 \pm 0.04	0.63 \pm 0.05	1.02 \pm 0.12	0.65 \pm 0.12
T ₂	0.90 \pm 0.27	0.46 \pm 0.04	0.46 \pm 0.04	0.62 \pm 0.07	0.96 \pm 0.10	0.61 \pm 0.15
T ₃	0.88 \pm 0.13	0.50 \pm 0.03	0.57 \pm 0.03	0.75 \pm 0.09	1.19 \pm 0.20	0.70 \pm 0.15

The overall mean yolk index values of the eggs of T0 and T2 were higher (0.45) than that for T1 and T3 (0.44) (Table 17). Statistical differences were not observed in mean yolk index of eggs of the treatment groups during the experimental period.

The highest overall mean IQU (54.59) was obtained for the eggs of the birds fed standard layer ration (Table 18). The enzyme treated bird groups obtained second and third values of 53.71 (T3) and 53.27 (T2). The birds fed low protein diet had lowest IQU. The IQU values of the eggs during the entire experimental period were statistically nonsignificant.

The overall mean shell thickness of the eggs of T0, T2 and T3 was 0.189 and that of T1 was 0.186 mm. Statistical difference were not observed in the mean shell thickness of the eggs of the birds maintained on different dietary treatments during all the periods.

4.9 SERUM PROTEIN

The mean serum protein values of different dietary treatments are set out in Table 20 and graphically represented in Fig 9. The mean values were 5.51, 4.74, 4.55 and 4.74 g per cent for T0, T1, T2 and T3 respectively.

The results of statistical analysis of the data showed that serum protein of the birds fed standard layer ration was significantly higher ($p < 0.05$) than the other treatment groups. Serum protein values of other treatment groups were statistically similar.

4.10 BALANCE OF NITROGEN

The data on the retention percentage of nitrogen for different treatment groups are given in Table 21. The birds maintained on low protein layer ration had the highest retention percentage followed by T3, T2 and those birds fed standard layer ration showed the lowest retention percentage of nitrogen.

Table15. Mean (\pm SE) shape index of the eggs of Japanese quails maintained on different dietary treatments

Treatments	Shape index					Overall
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	
T ₀	78.32 \pm 0.33	78.65 \pm 0.17	78.69 \pm 0.74	79.66 \pm 0.76	78.42 \pm 0.79	78.75 \pm 0.53
T ₁	78.23 \pm 0.59	78.53 \pm 0.26	77.62 \pm 0.55	79.44 \pm 0.51	78.88 \pm 0.30	78.54 \pm 0.46
T ₂	78.26 \pm 0.26	78.19 \pm 0.42	78.51 \pm 0.28	79.31 \pm 0.85	78.63 \pm 0.40	78.57 \pm 0.44
T ₃	78.12 \pm 0.32	78.95 \pm 0.32	78.50 \pm 0.19	79.38 \pm 0.33	78.50 \pm 0.13	78.69 \pm 0.29

Table16. Mean (\pm SE) albumen index of the eggs of Japanese quails maintained on different dietary treatments

Treatments	Albumen index					Overall
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	
T ₀	0.104 \pm 0.01	0.104 \pm 0.00	0.100 \pm 0.00	0.104 \pm 0.00	0.105 \pm 0.00	0.103 \pm 0.00
T ₁	0.101 \pm 0.01	0.102 \pm 0.00	0.096 \pm 0.00	0.101 \pm 0.00	0.105 \pm 0.00	0.101 \pm 0.00
T ₂	0.104 \pm 0.01	0.102 \pm 0.00	0.100 \pm 0.00	0.103 \pm 0.00	0.106 \pm 0.00	0.103 \pm 0.00
T ₃	0.105 \pm 0.01	0.101 \pm 0.00	0.100 \pm 0.00	0.105 \pm 0.00	0.102 \pm 0.00	0.103 \pm 0.00

Table 17. Mean (\pm SE) yolk index of the eggs of Japanese quails maintained on different dietary treatments

Treatments	Yolk index					Overall
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	
T ₀	0.45 \pm 0.02	0.45 \pm 0.01	0.45 \pm 0.01	0.47 \pm 0.01	0.45 \pm 0.01	0.45 \pm 0.01
T ₁	0.44 \pm 0.01	0.44 \pm 0.01	0.46 \pm 0.01	0.45 \pm 0.01	0.43 \pm 0.01	0.44 \pm 0.01
T ₂	0.44 \pm 0.01	0.45 \pm 0.01	0.46 \pm 0.01	0.46 \pm 0.01	0.45 \pm 0.01	0.45 \pm 0.01
T ₃	0.44 \pm 0.01	0.43 \pm 0.01	0.45 \pm 0.01	0.46 \pm 0.00	0.44 \pm 0.01	0.44 \pm 0.01

Table 18. Mean (\pm SE) IQU of the eggs of Japanese quails maintained on different dietary treatments

Treatments	IQU					Overall
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	
T ₀	53.52 \pm 0.95	55.16 \pm 2.10	54.09 \pm 1.04	54.88 \pm 1.26	55.28 \pm 2.20	54.59 \pm 1.30
T ₁	52.11 \pm 0.67	52.80 \pm 1.11	51.61 \pm 0.79	54.30 \pm 0.67	52.00 \pm 1.66	52.56 \pm 0.94
T ₂	52.79 \pm 0.54	53.39 \pm 0.46	52.97 \pm 1.30	53.20 \pm 0.70	53.93 \pm 0.61	53.27 \pm 0.64
T ₃	53.25 \pm 0.76	54.46 \pm 1.18	53.55 \pm 1.26	52.77 \pm 0.30	54.5 \pm 1.67	53.71 \pm 0.96

Table 19. Mean (\pm SE) shell thickness of the eggs of Japanese quails maintained on different dietary treatments, mm

Treatments	Shell thickness					Overall
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	
T ₀	0.189 \pm 0.01	0.187 \pm 0.01	0.187 \pm 0.00	0.192 \pm 0.00	0.186 \pm 0.00	0.188 \pm 0.00
T ₁	0.188 \pm 0.01	0.189 \pm 0.01	0.190 \pm 0.01	0.190 \pm 0.00	0.189 \pm 0.00	0.189 \pm 0.00
T ₂	0.189 \pm 0.01	0.188 \pm 0.01	0.187 \pm 0.01	0.190 \pm 0.00	0.187 \pm 0.00	0.188 \pm 0.00
T ₃	0.180 \pm 0.01	0.186 \pm 0.01	0.187 \pm 0.01	0.189 \pm 0.01	0.189 \pm 0.01	0.186 \pm 0.00

Table 20. Mean (\pm SE) serum protein values of Japanese quails maintained on different dietary treatments, g%

Treatments	Replicates				Mean (\pm SE)
	R1	R2	R3	R4	
T0	5.35	4.97	5.90	5.80	5.51 ^a \pm 0.22
T1	4.85	4.55	5.00	4.55	4.74 ^b \pm 0.11
T2	4.25	5.05	4.25	4.65	4.55 ^b \pm 0.19
T3	4.70	4.60	5.00	4.65	4.74 ^b \pm 0.09

Means bearing different superscripts with in the same column differed significantly ($p < 0.05$).

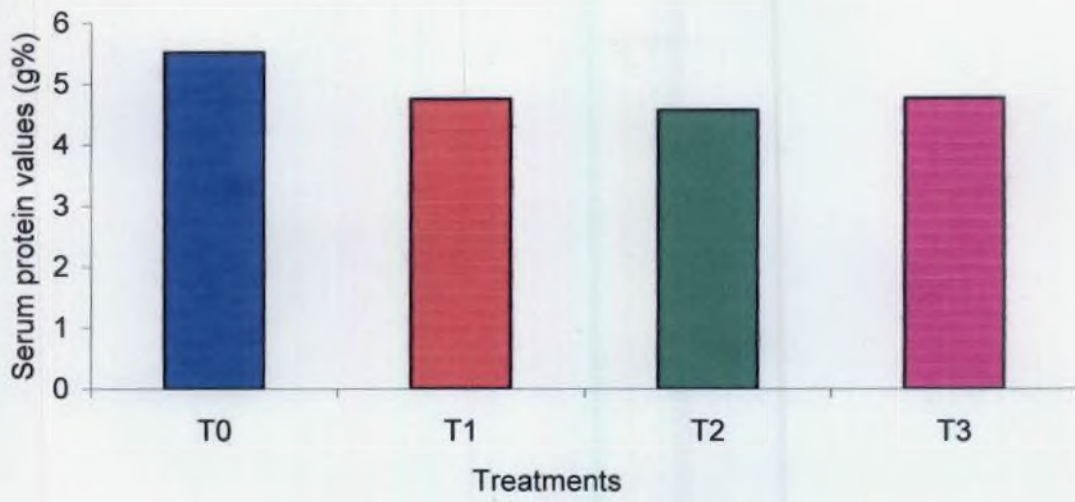


Fig.8. Mean serum protein values (g%) of Japanese quails maintained on different dietary treatments

Table 21. Mean (\pm SE) retention of nitrogen of Japanese quails maintained on different dietary treatments, %

Treatments	Replicates				Mean (\pm SE)
	R1	R2	R3	R4	
T0	61.27	60.57	64.78	51.16	59.45 \pm 2.91
T1	64.97	65.16	62.13	68.87	65.08 \pm 1.55
T2	60.18	62.47	65.43	68.11	64.05 \pm 1.73
T3	61.49	71.96	61.11	63.20	64.44 \pm 2.55

The mean values among the treatment groups were 59.45, 65.08, 64.05 and 64.44 per cent for T0, T1, T2 and T3 respectively.

The results of statistical analysis of the data showed that there was no significant difference among the treatment groups.

4.11 MORTALITY

Mortality pattern of birds in the different treatment groups are shown in Table 22. Altogether 20 birds died during the course of study and the percentage of mortality ranged from a minimum of 10.0 to a maximum of 17.5. Mortality was less in T0 and T2. Necropsy of the dead birds were conducted to detect the cause of death which did not show any signs that are attributable to treatment effect.

4.12 ECONOMICS

In order to assess the economics of supplementation of protease enzyme in low protein diet, the cost of different rations used in the study was calculated based on the price of feed ingredients which prevailed at the time of experiment and are presented in Table 23. Cost of rations computed for different treatments viz., T0, T1, T2 and T3 were 9.55, 8.41, 8.48 and 8.55 rupees per kg respectively.

The economics for different dietary treatments set out in Table 24 indicated that feed cost for the production of one egg was Rs. 0.46, 0.46, 0.43 and 0.50 for different treatments viz., T0, T1, T2 and T3 respectively. This revealed that the cost of feed/egg was the lowest in the treatment group supplemented with 0.02 per cent enzyme when compared with the other three treatment groups.

Table 22. Mortality pattern of Japanese quails during the experimental period

Treatments	Mortality (No)					7-26 weeks	
	7-10 weeks	11-14 weeks	15-18 weeks	19-22 weeks	23-26 weeks	No	%
T0	-	-	3	1	-	4	10.0
T1	1	4	-	-	-	5	12.5
T2	-	2	2	-	-	4	10.0
T3	1	4	-	2	-	7	17.5

Table 23. Cost of experimental rations (Rs /kg)

Ingredients	Cost/ kg* (Rs.)	Cost of rations (Rs.)			
		T0	T1	T2	T3
Yellow maize	6.48	2.40	2.59	2.59	2.59
Groundnut cake (exp)	13.49	2.02	1.48	1.48	1.48
Soyabean meal	13.45	2.96	1.35	1.35	1.35
Rice polish	5.47	0.55	1.15	1.15	1.15
Unsalted dried fish	10.75	0.86	1.08	1.08	1.08
Shell grit	3.23	0.19	0.19	0.19	0.19
Mineral mixture	21.90	0.38	0.38	0.38	0.38
Salt	2.23	0.01	0.01	0.01	0.01
Nicomix A+B ₂ +D ₃ +K	668.00	0.10	0.10	0.10	0.10
Nicomix B+E	374.00	0.08	0.08	0.08	0.08
Protease	350.00	0.00	0.00	0.07	0.14
Total	-	9.55	8.41	8.48	8.55

*As per the rate fixed by College of Veterinary and Animal Sciences, Mannuthy for 2004-05

Table 24. Economics of production of Japanese quails maintained on different dietary treatments

Particulars	Dietary treatments			
	T0	T1	T2	T3
Total feed intake (kg)	154.00	147.30	148.70	141.70
Total number of eggs	3232	2710	2903	2415
Feed consumed per egg (g) from 6 to 26 weeks	47.65	54.35	51.22	58.67
Cost of feed/kg (Rs)	9.55	8.41	8.48	8.55
Cost of feed/egg(Rs)	0.46	0.46	0.43	0.50

Discussion

5. DISCUSSION

5.1 CLIMATIC PARAMETERS

The data pertaining to climatic parameters during the experimental period are presented in Table 4. During the course of the experiment from December 2004 to May 2005, the mean maximum temperature ranged from 32.5 to 36.5 °C with an overall mean of 34.2°C. The mean minimum temperature ranged from 22 to 24.9°C with an overall mean of 23.5°C. The mean relative humidity ranged from 67.4 to 89.2 per cent in the morning with an overall mean of 78.1 per cent and 33.2 to 59.6 per cent in the evening with an overall mean of 43.5 per cent during the experimental period. Somanathan *et al.* (1980) reported the highest mean maximum temperature of 32.35°C during May in Mannuthy. During this experiment also the highest mean maximum temperature of 36.5°C was recorded during May.

5.2 BODY WEIGHT

Body weight gain of T0, T1, T2 and T3 during the experimental period were 32.92, 29.24, 36.06 and 27.73 g respectively. The highest body weight gain was obtained for T2 (supplemented with 0.02 per cent of the enzyme) and T3 (0.04 per cent of enzyme treated group) was the lowest in body weight gain. Thus there is a positive influence of enzyme on body weight gain at low level of supplementation (0.02%). Protease at 0.02 per cent level might have helped for better nutrient utilization from the ingredients by removing the barriers for nutrient absorption. This might have helped in increased body weight gain than that of control. But the birds could not get adjusted to the high level of enzyme. The higher level of supplementation might have depressed the endogenous enzyme production leading to poor performance.

Eventhough the analysis of variance of the data showed no significant difference in body weight gain between the birds fed standard layer ration, low

protein layer ration and low protein layer ration with enzymes there was a numerical improvement in body weight gain of 0.02 per cent protease supplemented group. According to the findings of Satyamoorthy (1995) the addition of protease to White Leghorn hen did not improve the body weight gain. Simbaya *et al.* (1996) also reported that there was no significant effect of protease supplementation in the body weight gain of broilers.

Ghazi *et al.* (2002) reported that body weight of broiler chicks was improved when protease treated soyabean meal was fed. Ranade *et al.* (2005) observed that broiler chicks fed diet having 10 per cent reduction in CP with supplementation of Maxigest GB containing protease @ 500 g/T had lower but statistically similar live weight as that of the control where as Odetallah *et al.* (2003) reported that when low protein diet supplemented with a broad spectrum protease enzyme was fed to broiler chicks, there was a significant increase in body weight at 26 days of age. Significant improvement in the body weight of broilers was also obtained by Pillai *et al.* (1995) by the addition of 0.2 per cent protease.

5.3 AGE AT SEXUAL MATURITY

The mean age at sexual maturity of different dietary treatments were 47, 45, 48 and 44 days for T0, T1, T2 and T3 respectively. Since the experiment started at 42 days, the treatments might not have affected the age at sexual maturity significantly. T3 was the first to reach age at sexual maturity and age at 50 per cent egg production. Eventhough the higher level of enzyme helped the birds to reach 50 per cent production earlier it could not maintain the egg production comparable to other treatments. But T2 with 0.02 per cent of enzyme had the most delayed age at sexual maturity and 50 per cent production. Statistical analysis of the data showed no significant effect on age at sexual maturity and 50 per cent production by the enzyme supplementation. Similar findings were recorded by Bhat *et al.* (2005) and Nandi *et al.* (2005a) who found no

significant effect on age at sexual maturity of pullets by the supplementation of multienzyme mixture containing protease.

5.4 EGG PRODUCTION

The total number of eggs produced during the experimental period by the different dietary treatments namely T0, T1, T2 and T3 were 3232, 2710, 2903 and 2415 respectively. The highest total egg production was recorded by T0 followed by T2, T1 and T3. This might be due to the higher protein requirement of Japanese quails for egg production compared to chicken. T0 maintained the maximum egg production in all the periods except during 7 to 10 weeks of age. T2 stood higher in egg production than T1 and T3 during 11-14,15-18,19-22 and 23- 26 weeks of age. This finding is in agreement with the report of Ranade *et al.* (2004b) who recorded a higher egg production for enzyme supplemented group than the groups maintained with 10 per cent reduction in CP.

The birds fed standard layer ration (T0) were highest in overall hen housed and hen day egg number followed by T2,T1 and T3. There was an increase of 2.65 hen housed eggs and 4.08 hen day eggs for the birds fed low protein layer ration supplemented with 0.02 per cent enzyme over the birds fed low protein layer ration.

The overall hen housed egg production per cent of T0, T1,T2 and T3 during the experimental period were 57.71, 48.39, 51.84 and 43.13 per cent respectively. The treatment with standard layer ration (T0) produced 5.18 per cent more hen housed eggs than T2 which was next in hen housed egg production. The overall hen housed egg production per cent of T2 was almost similar to T1. The lowest hen housed egg production per cent was recorded by T3 supplemented with 0.04 per cent of enzyme. Satyamoorthy (1995) reported similar findings where there was a numerical improvement in hen housed egg production of birds of protease treated group than the control without making any statistical significance. The birds supplemented with 0.04 per cent protease were

lowest in hen housed egg production from 11 to 14 weeks of age onwards. This may be because 0.04 per cent of enzyme inhibited the full expression of the genetic potential of the birds in all phases of egg production. On the other hand at low level (0.02%) there is not much inhibitory effect for the enzyme.

The hen day egg production per cent also revealed the same trend as hen housed egg production per cent. The hen day egg production was low in all treatments during the 7 to 10 and 23 to 26 weeks of age. T0, T1 and T3 recorded the peak hen day egg production per cent in the second period, while in the case of T2 the third period was the time for peak production. T1 ranked top in per cent hen day egg production during 7 to 10 weeks of age (first period). In all the other periods T0 was the best in hen day egg production per cent. T1 and T2 were almost similar in hen day egg production per cent with T2 slightly better than T1 during the entire experimental period except during 7 to 10 weeks of age. Compared to other treatments T3 was the poorest in hen day egg production per cent during 80 per cent of experimental period. A slight increment over T2 was observed only during 7 to 10 weeks of age.

The overall hen day production of T2 was two per cent more than T1. Satyamoorthy (1995), Cook *et al.* (2000) and Ranade *et al.* (2004b) observed numerical but statistically insignificant increase in hen day egg production by the supplementation of protease to laying hen.

It was revealed that the enzyme supplementation at lower level showed numerical improvement in total and hen housed and hen day egg production in number and per cent. Decrease in egg production was observed for the birds fed low protein diet compared to those with standard layer ration. A numerical increase of 193 eggs was obtained for the birds supplemented with 0.02 per cent enzyme compared to non supplemented group (T1). But 0.04 per cent of enzyme with low protein ration resulted in decreased egg production.

Thus it can be concluded that, there is only a numerical advantage in supplementing enzyme to low protein layer ration at 0.02 per cent level.

5.5 EGG WEIGHT

The mean egg weight of different dietary treatments during different experimental periods revealed the highest values for the birds fed standard layer ration (10.65 to 11.58 g). The other treatments T1, T2 and T3 recorded more or less similar mean egg weight as shown in Table 12. Mean egg weight of birds on standard layer ration was significantly higher than other rations during the 19 to 22 weeks of age ($p < 0.05$). There was no statistical difference in mean egg weight between the treatment groups during other periods. The birds with standard layer ration, produced eggs with more weight throughout the experimental period. These records are supported by the findings of Palod and Tiwari (2004) who reported that a marginal deficiency of protein or any essential amino acids can cause reduction in egg size.

The egg weight increased slightly with increasing age. The overall egg weight of T3 showed a numerical increase compared to T1 and T2. This might be due the lesser number of eggs produced by them. Satyamoorthy (1995) also reported that Leghorn hen supplemented with diet containing protease produced eggs of larger size compared to the control. Bhat *et al.* (2005) observed numerical but statistically insignificant increase in egg weight of pullets treated with an enzyme mix containing protease. In contrast to this Ranade *et al.* (2004b) could not find any improvement in egg weight by the supplementation of layer diet with multienzyme mix containing protease. However, the mean egg weights of birds of low protein diets with or without enzyme supplementation varied from 9.86 to 11.23 g during different periods which is higher than the range of 6.7 to 10.7 g reported by Panda and Mohapatra (1989) for Japanese quails. Also the overall egg weights for low protein diets (T1, T2 and T3) were more than 10.14 g as reported by Shukla *et al.* (1994) for Japanese quails from six to twenty six weeks of age.

5.6 FEED CONSUMPTION

The overall mean daily feed consumption per bird for the treatment groups viz., T0, T1, T2 and T3 during the whole experimental period were 29.15, 29.41, 28.45 and 28.60 g respectively. Higher feed intake was noted for T0 and T1. In contrast to this Satyamoorthy (1995) reported that daily feed intake of White Leghorn hen supplemented with less dense ration containing protease was more than that of the birds fed standard layer ration. The maximum average daily feed intake recorded in different periods by each treatment group was 31.28, 31.88, 30.66 and 30.66 g, respectively, for T0, T1, T2 and T3. There was no significant difference in mean daily feed consumption between the birds fed standard layer ration and those fed low protein layer ration without and with two levels of enzyme. These results are in accordance with the findings of Morris and Njuru (1990) who reported that feed intake of layer chicks was not significantly affected by dietary protein content.

Enzyme supplementation had no significant effect on feed consumption. The findings of Pillai *et al.* (1995), who could not obtain any significant effect in the feed consumption of broilers supplemented with 0.2 per cent protease, supports the results of present study. Thirumalai *et al.* (1991) also reported that White Leghorn chicks supplemented with papain showed no improvement in feed consumption. Nandi *et al.* (2005a) observed similar findings in growing pullets supplemented with Beetazyme containing protease. But Simbaya *et al.* (1996) reported that supplementation of protease G at the rate 0.02 g/kg to broiler chicks enhanced the feed intake. Rao *et al.* (2003) also observed that feed intake of broilers was increased by the supplementation of enzyme mixture containing protease.

The total feed intake by the different dietary treatments during the entire experimental period was 154.00, 147.30, 148.70 and 141.70 kg for T0, T1, T2 and T3 respectively (Table 24). The birds fed standard layer ration consumed more feed than the other treatment groups. These findings are in contrast with the

results of Ranade *et al.* (2004b) who reported that total feed consumption of layers fed diet containing enzyme mix with protease having 10 percent reduction in CP was more than that of the control. Comparatively higher feed intake was observed in those birds which were at a high level of production than others.

The feed consumed per egg as per Table 24 calculated for different dietary treatments were 47.65, 54.35, 51.22 and 58.67 g, respectively, for T0, T1, T2 and T3. The feed per egg for T2 was less than that of T1 and T3. Thus 0.02 per cent of enzyme supplementation had a positive influence on feed per egg compared to T1 and T3. But 0.04 per cent of enzyme supplemented group had increased feed/egg and was the highest among the treatments. Satyamoorthy (1995) also reported a similar trend of increased feed/egg for the protease supplemented group than the birds fed standard layer ration. The lowest quantity of feed/egg was obtained for those fed standard layer ration.

5.7 FEED EFFICIENCY

The overall feed efficiency per dozen eggs during the periods of study for T0, T1, T2 and T3 were 0.57, 0.65, 0.61 and 0.70 respectively. Better feed efficiency per dozen eggs was obtained by standard layer ration. This was in contrast to the findings of Satyamoorthy (1995) who reported that overall feed efficiency values for egg mass of White Leghorn hen supplemented with a diet containing protease was similar to the control group. The mean feed efficiency of different treatment groups ranged between 0.42 to 1.07, 0.5 to 1.03, 0.47 to 1.06 and 0.51 to 1.25 for T0, T1, T2 and T3, respectively. The best mean feed efficiency was noted during 11 to 14 weeks of age in all treatment groups except in T2 for which the mean feed efficiency was best during 11 to 14 and 15 to 18 weeks of age. There was no significant difference in mean feed efficiency per dozen eggs of the different treatment groups during the entire experimental period. But Rexen (1981) obtained significant improvement in feed efficiency of cockerels by the addition of protease compared to the control. Ranade *et al.* (2004b) also reported that feed efficiency per dozen eggs of layers

supplemented with enzyme mix containing protease was significantly better than the control.

5.8 EGG QUALITY

From an assessment of egg quality traits as given in tables 15,16,17,18 and 19 it could be found that the mean values of shape index for different treatment groups were same. The mean albumen index of T0, T2 and T3 was 0.103 and that of low protein diet was 0.101. But in contrast to this Satyamoorthy (1995) reported that albumen index of eggs of White Leghorn hen fed less dense ration containing protease was less than that of the eggs from the birds fed standard layer ration. The mean yolk index of the eggs from the birds fed low protein layer ration containing 0.02 per cent protease was same as that of the control (0.45). The best mean IQU was recorded by the eggs from the birds fed standard layer ration (54.59) and the mean IQU of the eggs from the enzyme supplemented groups was higher than that of the eggs of the birds fed low protein diet. The mean shell thickness values of the different treatment groups were 0.188, 0.189, 0.188 and 0.186 mm for T0, T1, T2 and T3, respectively. The highest value was for the eggs from the birds fed low protein diet with out enzyme.

Eventhough there were numerical differences, the statistical analysis of egg quality traits could not reveal any significant difference between the treatments. Though there was a reduction in protein content of treatments, T1, T2 and T3 egg quality traits were comparable with that of the birds fed standard layer ration .

Ranade *et al.* (2004b) also reported similar findings, where shape, albumen, and yolk indices of eggs from layers were not affected by the supplementation of enzyme mix containing protease with 10 per cent reduction in CP. But Nandi *et al.* (2005b) reported significant reduction in albumen and shape indices of eggs of layers fed diet in which maize and soybean used was

completely replaced by sorghum, ragi, niger and sesame cake in equal ratio along with supplementation of enzyme mix containing protease.

5.9 SERUM PROTEIN

The mean serum protein values of T0, T1, T2 and T3 were 5.51, 4.74, 4.55 and 4.74 g per cent, respectively. Those birds fed standard layer ration had significantly higher values ($p < 0.05$) compared to other groups. For the low protein rations without and with different levels of enzyme, the numerical differences were not significant. The enzyme supplementation had no significant effect on serum protein level. The value in birds fed standard layer ration was comparable to those obtained by Narayanankutty (1987) *i.e.*, 5.78 g per cent. Serum protein level reflects the protein intake.

5.10 BALANCE OF NITROGEN

The mean per cent of nitrogen retained was 59.45, 65.08, 64.05 and 64.44 for T0, T1, T2 and T3, respectively. Eventhough higher values were obtained for the birds fed low protein layer rations compared to the birds fed standard layer ration, no significant difference was observed between treatment groups. This shows that enzyme had no influence on nitrogen retention at the two levels. This supports the findings of Satyamoorthy (1995) who reported that crude protein digestibility coefficient of White Leghorn hen supplemented with less dense ration containing protease was same as that of the control. Thirumalai *et al.* (1991) also observed that the addition of papain to chick ration did not improve the protein digestibility. But Hong *et al.* (2002) reported that apparent nitrogen retention was greater in ducks receiving enzyme preparation containing protease. The nitrogen retention per cent of T0 was lowest. The low protein diets showed better retention of nitrogen.

5.11 MORTALITY

The mortality rate of different dietary treatments were 10.0, 12.5, 10.0 and 17.5 per cent, respectively, for T0, T1, T2 and T3. Mortality rate of standard layer ration and those of enzyme treated group (0.02%) was same (10). The major cause of death was egg bound condition and highest mortality per cent was during their peak production stage. The genetic predisposition of the batch might be the reason for susceptibility to egg bound condition. Though the mortality was higher in all treatment groups than the standard for laying house mortality, the cause of death of birds could not be attributed to enzyme treatment. Moreover, there are no reports of harmful effects from the use of feed enzymes.

5.12 ECONOMICS

The cost of feed inclusive of enzyme cost per kg in the four dietary treatments respectively were Rs.9.55,8.41, 8.48 and 8.55 (Table 23). The estimated cost per kg of standard protein and low protein layer ration was Rs.9.55 and Rs.8.41 respectively, the low protein layer ration being cheaper to the extent of Rs.1.14. The cost of enzyme alone in T2 and T3 were Rs.0.07 and Rs.0.14 respectively.

Cost of feed per egg of T0, T1, T2 and T3 were Rs. 0.46, 0.46, 0.43 and 0.50 respectively (Table 24). Minimum cost was recorded by T2 which produced more number of eggs consuming less feed. But for T3, even though total feed consumption was less, total number of eggs produced was very low, compared to the other treatments which led to the higher cost of feed per egg. The enzyme supplementation at 0.02 per cent had an advantage of three paise over the standard protein layer ration. Ranade *et al.* (2004b) reported a reduction of 19 paise for enzyme supplemented hen compared to the control. Thus it can be concluded that there is a positive trend in economics by the supplementation of 0.02 per cent enzyme to low protein diet in laying Japanese quails.

Summary

6. SUMMARY

An investigation was carried out using one hundred and sixty Japanese quails of six weeks of age to determine the influence of protease supplementation in low protein layer ration on the production performance and to assess the economics involved. The birds were randomly distributed into four dietary treatments with four replicates of 10 birds each. The dietary treatments consisted of standard quail layer ration (T0), low protein quail layer ration (T1) and low protein quail layer ration supplemented with 0.02 (T2) and 0.04 (T3) per cent protease. The standard quail layer ration was formulated as per the recommendations for laying Japanese quails by the Central Avian Research Institute, Izatnagar. It contained 22 per cent crude protein (CP) and 2650 kcal/kg of metabolisable energy. The low protein quail layer ration was formulated with a low percentage of crude protein (CP 18) and same level of metabolisable energy as the standard layer ration.

Feed ingredients viz. yellow maize, soyabean meal, rice polish, ground nut cake and unsalted dried fish were used for the formulation of experimental diets. The birds were housed in colony cages and reared under cage system of management. Standard managerial procedures were adopted throughout the experimental period. The duration of the experiment was twenty weeks which was divided into five 28 day periods and period wise data were collected. The body weight of the birds were recorded at the beginning and end of the experiment. Replication wise data on feed intake and egg production were recorded throughout the experiment. From the data on feed intake, feed efficiency was worked out. During the last three days of each period, replication wise individual egg weight of all eggs and egg quality traits of five eggs collected randomly from each replicate were recorded. Egg quality traits assessed were shape, albumen, yolk indices, IQU (Internal quality unit) and shell thickness. At

the end of the experimental period, a three day metabolism trial was conducted using one bird selected randomly from each replicate. Based on the data obtained from metabolism trial, the percentage of nitrogen retention was calculated. Blood samples were collected from two birds of each replicate at the end of 26 weeks of age for the estimation of serum protein. Replication wise mortality of the birds were recorded. Economics due to protease supplementation was worked out by calculating the cost of production.

The overall performance of the birds maintained on different dietary treatments are presented in table 25.

The results obtained from the trial are summarized below

(1) The mean body weight gain of the birds for different treatment groups during the entire experimental period were 32.92, 29.24, 36.06 and 27.73 g for T0, T1, T2 and T3, respectively. The gain in body weight was the highest for the birds fed low protein diet with 0.02 per cent enzyme when compared to other treatment groups. But statistical analysis showed no significant difference among the treatment groups.

(2) The mean age at sexual maturity and 50 per cent production of birds in the different treatment groups ranged from 44 to 48 and 56 to 60 days respectively. The birds supplemented with 0.04 per cent protease reached age at sexual maturity and 50 per cent production earlier. But the numerical differences between the treatment groups were not statistically significant.

(3) The total egg production during the entire experimental period was the highest in T0 followed by T2, T1 and T3 respectively. The group of birds fed low protein diet supplemented with 0.02 per cent protease produced 193 eggs more during six to twenty six weeks than the birds fed low protein layer ration. However, the birds supplemented with high level of enzyme were poor in egg production.

(4) The overall hen housed egg number was the highest for the birds fed standard layer ration (T0). The birds fed low protein layer ration supplemented with 0.02 per cent protease were the second highest followed by T1 and T3.

(5) The overall hen day egg number also expressed the same trend, with T0 being top scorer followed by T2, T1 and T3.

(6) The overall hen housed egg production of the birds fed standard layer ration (T0) was superior by 5.87 per cent to the second largest one (T2). The lowest hen housed egg production was recorded by T3, which was 14.58 per cent lower than T0. Statistical analysis revealed no significant difference in hen housed egg production during each period between the dietary treatments.

(7) The hen day egg production per cent also showed the same trend as hen housed egg production per cent. The birds receiving standard layer ration showed numerical improvement in hen day egg production per cent compared to other treatments. The production was 5.24 per cent higher than the next highest. The birds fed low protein layer ration supplemented with 0.04 per cent enzyme were lowest in hen day egg production per cent. The higher percentage of enzyme inhibited full phenotypic expression of the birds for egg production. Though statistical analysis showed no significant difference among the treatment groups, there was numerical improvement in egg production for the group of birds supplemented with 0.02 per cent enzyme.

(8) The period wise egg weight records for four treatments showed significant increase for T0 during 19 to 22 weeks of age. Even though statistically insignificant, the birds receiving standard layer ration produced eggs with more weight compared to other treatments. T1, T2 and T3 produced eggs with only marginal differences in egg weight.

(10) The overall mean daily feed intake of the birds in four dietary treatments during the experimental period were 29.15, 29.41, 28.48 and 28.60 g respectively, for T0, T1, T2 and T3 .

(11) The period wise feed efficiency per dozen eggs of the four treatments ranged between 0.42 to 0.99, 0.50 to 1.02, 0.46 to 0.96 and 0.50 to 1.19 for T0, T1, T2 and T3, respectively. Analysis of variance showed no significant difference among the treatments.

(12) The egg quality traits such as shape, albumen and yolk indices, IQU (Internal quality unit) and shell thickness recorded for different treatments were not statistically different from that of control.

(13) The mean serum protein values of T0, T1, T2 and T3 were 5.51, 4.74, 4.55 and 4.74 g% respectively. The birds receiving standard layer ration showed significantly higher values compared to the other treatments.

(14) The percentage retention of nitrogen was unaffected by the different dietary treatments. Numerically highest value was obtained by T1 (65.08). The lowest retention per cent was recorded by the birds receiving standard layer ration, T0 (59.45).

(15) Eventhough the mortality of different dietary treatments ranged from 10.0 to 17.5 per cent, protease supplementation did not influence the survivability of the birds.

(16) The feed cost per egg varied from Rs.0.43 to 0.50 for the different treatment groups. Cost of production of egg was the lowest in the birds fed 0.02 per cent protease and highest in the group supplemented with 0.04 per cent of enzyme.

Based on the results of this study, it could be inferred that the supplementation of 0.02 per cent of enzyme to a low protein layer ration is beneficial considering the cost of feed per egg.

Table 25. Overall performance of Japanese quails maintained on different dietary treatments

Particulars	T0	T1	T2	T3
Mean body weight gain, g	32.92	29.24	36.06	27.73
Mean age at sexual maturity, days	47	45	48	44
Mean age at 50% production, days	58	58	60	56
Total egg production	3232	2710	2903	2415
Over all hen housed egg number	80.80	67.75	72.58	60.38
Over all hen day egg number	85.08	74.94	77.75	67.44
Overall hen housed egg production, %	57.71	48.39	51.84	43.13
Overall hen day egg production, %	60.77	53.53	55.53	48.17
Overall mean egg weight, g	11.14	10.61	10.40	10.72
Overall mean daily feed consumption, g	29.15	29.41	28.48	28.60
Overall mean feed efficiency per dozen eggs	0.57	0.65	0.61	0.70
Overall mean egg quality traits				
(a) Shape index	78.75	78.54	78.57	78.69
(b) Albumen index	0.103	0.101	0.103	0.103
(c) Yolk index	0.45	0.44	0.45	0.44
(d) IQU	54.59	52.56	53.27	53.71
(e) Shell thickness, mm	0.188	0.189	0.188	0.186
Mean serum protein, g %	5.51	4.74	4.55	4.74
Mean retention of nitrogen, %	59.45	65.08	64.05	64.44
Mortality, %	10.00	12.50	10.00	17.50
Cost per kg feed, Rs.	9.55	8.41	8.48	8.55
Cost of feed per egg, Rs.	0.46	0.46	0.43	0.50

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**SUPPLEMENTATION OF PROTEASE ON THE
PRODUCTION PERFORMANCE OF JAPANESE
QUAILS (*Coturnix coturnix japonica*) FED LOW
PROTEIN DIET**

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ABSTRACT

The effects of different levels of protease supplementation viz., 0.02 and 0.04 per cent in low protein layer ration of Japanese quails on their production performance and economic feasibility was evaluated using one hundred and sixty laying birds for a period of twenty weeks. The birds were divided into four dietary treatment groups, viz., standard quail layer ration (T0), low protein quail layer ration (T1), low protein quail layer ration with 0.02 per cent protease (T2), and low protein quail layer ration with 0.04 per cent protease (T3). The standard quail layer ration was formulated as per the recommendations for laying Japanese quails by the Central Avian Research Institute, Izatnagar. It contained 22 per cent crude protein (CP) and 2650 kcal/kg of metabolisable energy. The low protein quail layer ration was formulated with a low percentage of crude protein (CP 18%) and same level of metabolisable energy as the standard layer ration. The body weight gain during the entire experimental period was highest for the birds fed 0.02 percent of protease compared to other treatment groups. But analysis of data revealed no significant difference between treatments. Age at sexual maturity and 50 per cent production were not at all influenced by enzyme supplementation statistically. Numerically the birds fed 0.04 per cent enzyme reached age at sexual maturity and 50 per cent production earlier. A numerical improvement was noticed in total egg production, over all hen day and hen housed egg number and overall hen housed and hen day egg production per cent in the birds fed 0.02 per cent protease with low protein layer ration compared to the non supplemented group. Egg weight and egg quality characteristics such as shape, albumen and yolk indices, internal quality unit (IQU) and shell thickness were not affected by enzyme treatment. Feed intake per bird per day and feed efficiency were not significantly influenced by the supplementation of enzyme. Only the serum protein values of standard quail layer ration was statistically more than the other groups. However, the enzyme treatment had no effect on the serum

protein values of low protein groups. The retention per cent of nitrogen was highest for the groups of birds fed low protein layer rations and lowest for those fed standard protein layer ration. The retention of nitrogen was unaffected by the enzyme treatment. The mortality was not affected by the enzyme treatment. The analysis of economics indicated least cost per egg for the birds fed 0.02 per cent enzyme (T2). The birds fed standard layer ration performed better than low protein groups without or with two levels of protease.