Effect of dietary supplementation of Antimicrobial peptide on production performance, egg quality and serum biochemical parameters of laying hens

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BHUBANESWAR-751003
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Shastri J, M.V.Sc. (Livestock Production and Management) Thesis, 2020.

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A THESIS SUBMITTED TO
THE ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE

IN

LIVESTOCK PRODUCTION AND MANAGEMENT

By

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CERTIFICATE-I

This is to certify that the thesis entitled **"Effect of dietary supplementation of Antimicrobial peptide on production performance, egg quality and serum biochemical parameters of laying hens"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science (Livestock Production and Management)** to the Odisha University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Jaya Shastri** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

Babu

CHAIRMAN

ADVISORY COMMITTEE

CERTIFICATE-II

This is to certify that the thesis entitled **"Effect of dietary supplementation of Antimicrobial peptide on production performance, egg quality and serum biochemical parameters of laying hens"** submitted by **Jaya Shastri** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **Master of Veterinary Science (Livestock Production and Management)** has been approved/disapproved by the student's advisory committee and the external examiner.

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ACKNOWLEDGEMENT

Firstly, I must express my heartiest gratitude to almighty for showering many blessing upon me throughout my life; I owe my gratitude to all those people who have made untold contribution towards the completion of my thesis, some identified below and others with apologies, perhaps unintentionally overlooked.

I m exhilarated to acknowledge and express my deepest gratitude to my beloved guide and major advisor, **Dr. L.K. Babu,** Professor, Department of Livestock Production and Management, and Dean, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar who generously formulated and finalized the programme and providing necessary facilities, to carry out the research work smoothly. I wish to express my sincere gratitude to him from the core of my heart for his valuable and critical suggestions, scholarly guidance, active persuasion, constant inspiration, affectionate personal attention, inexhaustible encouragement, constructive criticism, keen interest and supervision, which served as a constant source of inspiration throughout the course of my study and work. It’s his vigour and hunger to perform in adverse situation, which has inspired me to thrive for excellence and nothing less. His attitude of living every moment as it comes and converting them to new possibilities, correlating ideas and understanding the obvious has helped me to come a long way. It is matter of great pride to get an opportunity to work under the guidance of an able, affectionate and highly intellectual academician of his calibre.

I avail this opportunity in life to express my heartfelt gratitude to **Dr. A.K. Panda,** Ph.D., Principal Scientist, CIWA, Bhubaneswar and member of advisory committee for his scientific skill, meticulous guidance, necessary suggestions, diligent help, continuous inspiration, critical appreciation and whole hearted cooperation in analysis of gargantuan, experimental data, selfless support during entire course of this study. I shall forever remain an admirer of his keen interest in work, depth of knowledge, approach to subject, his affection for students has made a great difference in pursuit of this venture. No words can convey my gratitude to him, for the enormous effort for my research work and during the compilation of the thesis.

I express my sincere gratification and indebtedness to **Dr. B. Panigrahi,** Ph.D. Professor, Department of Livestock Production and Management, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar and member of Advisory Committee for his meticulous guidance, valuable suggestions, proficient knowledge, encouragement, kind cooperation and diligent help and blessing throughout the course of work and study period.

I am privileged to express my gratitude to **Dr. S.K. Mishra,** Professor, Department of Animal Nutrition college of veterinary science and animal husbandry, Odisha University of Agriculture and Technology, Bhubaneswar and member of advisory committee for his support, diligent help whenever and wherever required. It is great privilege to thank all the members of advisory committee who were always there to guide and support me

I also take this opportunity to convey my sincere regard to my revered teachers **Dr. G.P. Mohanthy, Dr. K. Behera, Dr. S. Kanungo, Dr. (Mrs.) Jessy Bagh,** Department of Livestock Production and Management, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar for their encouragement and generous willingness to render all possible help and guidance throughout the post graduate programme.

I am thankful to the **Director,** ICAR-Central Institute for Women in Agriculture (CIWA), Bhubaneswar for providing the necessary facilities to carry out biological trials of my experiment at the renowned institute.

I am also thankful to my Departmental Colleagues and seniors **Dr. Gayathri didi, Dr. Shubhashree didi, Dr. Sarbajith bhai, Dr. Girija, Dr. Pravanjan, Dr. Guru, Dr. Pratik, Dr. Rojalin** and my juniors **Dr. Harika, Dr. Sukanya** and **Dr. Babita** for their support and unreserved help during the course of my study.

A special thanks to all the staff members of Department of Livestock Production and Management, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar- **Sri. J.N. Rout, Sri N. Gochhayat and Sri K. Sethi** for their active co-operation during my research work.

I want to convey my sincere thanks to **Dr. Tanuja, Dr. Charles Jeeva, Dr. Aananth Sarkar, Dr. Jyoti Nayak, Sadangi Sir, Monalisa Didi, Manoj Bhai, Bapi Bhai, Babuli Bhai, Gobardhan Bhai** and other staffs of CIWA, Bhubaneswar for their constant and valuable help during the course of my study.

I am very much thankful to the **Chief Librarian** and staffs of **Central Library**, Odisha University of Agriculture and Technology, Bhubaneswar for supporting me with concerned material and journal during my entire experimental period.

My vocabulary utterly fails in expressing my accolade to my revered parents and family members for their unconditional support, encouragement and prayer. Words are not enough to express my cordial regards, love and devotion to my respected father **Mr. J.K. Shastri,** a visionary, whose foresightedness and blessing made this venture successful one. My Beloved Mother **Mrs. Premlata Shastri,** has always acted as an endless source of love and affection with constant motivation, encouragement in each and every sphere of my life, I deeply express my sincere regards to my elder sister **Bhoomika Shastri,** younger brothers **Derik Shastri** and **Ashish Shastri** whose continuous inspiration encouragement and affection boosted up my moral during the period of study & each and every sphere of my life.

Finally I am very much thankful to each and everyone, who were important to the successful realization of the thesis, as well as expressing my apology if I forgot to mention any.

Place: Bhubaneswar

Jaya Shastri

(Dr. Jaya Shastri)

Dated: 20-10-2020

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LIST OF ABBREVIATIONS

%	:	Percentage
@	:	At the rate of
ALP	:	Alkaline Phosphatase
ALT	:	Alanine Transaminase
AMPs	:	Antimicrobial Peptides
ANOVA	:	Analysis of Variance
AST	:	Aspartate Transaminase
BIS	:	Bureau of Indian Standard
BWG	:	Body Weight Gain
CP	:	Crude Protein
CRD	:	Complete Randomized Design
DM	:	Dry Matter
DMR	:	Duncan Multiple Range
EM	:	Egg Mass
EW	:	Egg Weight
FCR	:	Feed conversion ratio
Fig.	:	Figure
g	:	Gram
g/dl	:	Gram per decilitre
g/kg	:	Gram per kilogram
HHEP	:	Hen Housed Egg Production
hrs	:	Hours
HU	:	Haugh Unit
kg	:	Kilogram
kg/q	:	kilogram per quintal
ME	:	Metabolizable Energy
mg/dl	:	Milligram per decilitre
mg/kg	:	Milligram per kilogram
ml	:	Millilitre
mm	:	Millimetre
ºC	:	Degree centigrade
OTC	:	Oxytetracycline
rpm	:	Revolution per minute
Rs	:	Rupees
SBM	:	Soya Bean Meal
SEM	:	Standard error of mean
TEP	:	Total Egg Production
TP	:	Total Protein
U/L	:	Enzyme unit per litre
W	:	Weight
µL	:	Micro litre

ABSTRACT

The experiment was done to study the effect of dietary addition of Antimicrobial peptide on production performance, egg quality and serum biochemical parameters of vanaraja laying hens (26-34 weeks). One hundred twenty (120), Vanaraja laying hens of 26 weeks of age were randomly selected and distributed them into four groups of 30 birds each group was having three replicates of 10 birds in each pen in a complete randomized design. The diets was supplemented with antibiotic – oxytetracycline (OTC) @ 50g/ quintal in control group (T_1) and in the remaining 3 treatment groups (T_2, T_3 and T_4), antimicrobial peptide (AMP) were supplemented at the concentration of 10, 15 and 20 g/quintal of feed, respectively. Each diet was fed to 3 replicates of 10 birds during the experimental period of 8 weeks. The birds were maintained in deep litter system of housing with standard managemental practices. Dietary supplementation of AMP significantly increased ($P<0.005$) the body weight of vanaraja laying hens at 34 wks of age at all the levels of supplementation (100-200 mg/kg diet). The body weight gain also improved significantly ($P<0.001$) due to dietary supplementation of AMP compared to the antibiotic supplemented group (oxytetracycline, 500 mg/kg diet). The hen housed egg production, egg weight; egg mass and feed efficiency did not differ significantly due to dietary supplementation of AMP. Dietary supplementation of AMP increased the albumen per cent and decreased yolk percent in eggs compared to that of antibiotic supplementation. Haugh unit increased significantly due to supplementation of AMP at all the levels of supplementation. Dietary supplementation of AMP in the diet significantly ($P<0.05$) increased the serum protein concentration compared to antibiotic supplementation. The concentration of serum albumin, globulin, creatinine, uric acid, calcium and phosphorus did not differ remarkably by dietary addition of AMP. Dietary supplementation of AMP @ 150 mg/kg diet significantly reduced the serum cholesterol concentration. The serum alkaline phosphatase concentration reduced significantly at all the levels of AMP supplementation compared to antibiotics supplemented group. The cost of feed per egg produced in 100, 150 and 200 mg/kg diet AMP supplemented group was reduced marginally by 2.01, 4.21 and 1.65%, respectively compared to antibiotic supplemented group. It is concluded that dietary supplementation of antimicrobial peptide (AMP), cecropin II could replace antibiotics for eliciting optimum performance in laying hens.

CHAPTER-I

INTRODUCTION

Poultry for commercial production of egg and meat is one of the India's fastest growing industries. Focused principally by powerful need it has extended, united and internationalised since from past decade in population of all income levels. Poultry industry is growing @ 8-10% per year. India with annual production **(**BAHS, 2019) of around 103.32 billion eggs and 4.06 MT of poultry meat, rank 3rd in egg production and 5th in broiler production respectively, in world. The egg production has increased by 8.5% (compared to previous year) and meat production from poultry which contributes about 50% of total meat production and increased by 7.85 % over previous year.

Poultry is one of the most widespread food industries worldwide and it helps in managing the challenges of food security in an efficient manner in lower productive country having scarcity of food. Poultry sector has been a productive sector in most of the country because of its contribution towards the improvement of human condition by providing quality nutrient in the form of meat & egg. In developing country this sector also empowered women providing better economic stability through backyard poultry farming. In addition to this the poultry manures are widely used in agriculture because of its high organic content & eco friendly attributes. Among these, feed accounts for 60-70 % production cost in broilers, 75-80 percent for layers and become most imp factor in economic viability of raising poultry birds.

In today's generation poultry products such as egg, meat is widely acceptable food because of presence of high quality proteins and micronutrients. Chickens, therefore, have received more attention in Indian planning, research and development process. Improved technology with standard management protocol helps the poultry producers & nutritionist to combat various challenges evolved in this industry. Maintaining significant profit with better quality of product and attain good response from human & environment is a major challenges in poultry sector. Under intensive system of poultry production birds are exposed to considerable stresses, which adversely affect their production performances. To avoid this kind of problem now a days synthetic feed additives which blended with various antibiotics are used to maintain proper gut health by eliminating detrimental microorganism & subsequently witness better feed efficiency & growth in poultry production. The world witnessed 63151 tons of antibiotic consumption in food animal production in 2010 (Boeckel *et al.,* 2015).abandoned use of antibiotics may treat the disease in poultry efficiently but also deteriorate the health status of human being as it producing antibiotic resistance. On the whole developed and developing countries facing serious health problem and human death because of antibiotic resistance.

It has been estimated that antibiotic resistance will be the leading cause of death by the year 2050 in world witnessing 10 millions death every year (O'Neill *et al.,* 2014). Meticulous and judicious use of antibiotic may able to maintain the standard human food chain by reducing the bacterial resistance and antibiotic residue in meat and egg. In view of the above, intensive research has been focused on the development of alternative strategies to maintain gut health and performance in intensive poultry production systems. One such alterative to antibiotics is Anti microbial peptides (AMPs).

Antimicrobial peptides are biological molecules having size less than 10kDa and pose both primary and secondary structure, are potent peptides against wide range of microbes such as bacteria, fungi & virus. It is different from the traditional antibiotics in combating microbial infection through the mechanism of action on host targets rather than microbial targets. So the chance of development of antibiotic resistance is very less which is a major advantage upon conventional antibiotic. Multiple mechanisms are manifested by AMPs like it can kill harmful microorganism and having low tendency to develop bacterial resistance. Besides this it ensures, eubiosis, intestinal integrity by maintaining the population of good bacteria in the host and improves intestinal health, increases laying rate and improves egg shell quality which is the best alternative mechanism of AMPs towards microbial protection.

It has been reported that AMPs elicited beneficial effects on growth traits (Shan *et al.,* 2007; Wang *et al.,* 2011) nutrient digestibility (Jin *et al.,* 2008a,b) increased egg production and improved egg quality (Chen *et al.,* 2020) and immune response (Shan *et al.,* 2007; Tang *et al.,* 2009). The AMPs also have potency to kill the bacteria which is resistant to multiple drugs (Zhang *et al.*, 2005). Only a limited study is available on the use of AMP as a potential alternative to antibiotics in poultry.

The resent study was therefore proposed with the below objectives:

1. To study the effect of dietary supplementation of anti microbial peptides (AMPs) on production performance of laying hens
2. To evaluate the effect of different concentration of AMPs on egg quality and haemato-biochemical parameters

CHAPTER-II

REVIEW OF LITERATURE

The literature pertaining to the activity of AMPs against microbes and its effect through dietary supplementation on production performance, egg quality traits, growth performance, intestinal morphology, nutrient digestibility, and immune status in chicken and serum biochemical profiles of vanaraja laying hens have been reviewed in this chapter.

a) Antimicrobial peptides and its broad spectrum activity

Over the last 10 years, the fast appearance of multidrug resistant pathogens has turn into a worldwide concern, because of which there is need to find out alternative antibacterial substance that can be utilize in food animals. Antimicrobial peptides (AMPs) obtained either through microbes or certain chemical synthesis can be best alternative to overcoming the bacterial resistance as they have natural antimicrobial properties and having low tendency to develop resistance by microraganism (Wang *et al.*, 2016).

Mechanism of action of Antimicrobial peptides is by destabilizing the membrane integrity of pathogen either inhibits its metabolic processes or by alter the host innate immune system (Brogden 2005; Brown and Hancock, 2006). Antimicrobial peptides cover cecropin, histatins, cathelicidin, cathepsin, chymase, defensins, lactoferrin, and many others. Several study described the function of antimicrobial peptides in innate immunity, for example killing a wide spectrum microorganisms like gram-negative and gram-positive bacilli, fungi, and viruses and even tumour cells. (Boman, 1995; Oppenheim *et al.*, 2003). In addition to this one another major function of Mammalian antimicrobial peptides is act as a bridge between innate and acquired immune system. Unlike the many traditional antibiotics these peptides are mostly bactericidal in place of bacteriostatic. (Agerberth *et al.*, 1991; Chertov *et al.*, 1996; Chan and Gallo, 1998; Korthuis *et al.*, 1999).

Presently, various peptides have been come across from plants, animals, and insects. Significantly, some of the AMPs were isolated from blood, neutrophil, leucocytes, skin, tongue, and intestine of swine (Agerberth *et al.,* 1991; Selsted *et al.,* 1992). And also from urogenital tract, epithelial cells lining, tracheobronchial tree. (Hancock and Scott, 2000; Oppenheim *et al.,* 2000), naturally Antimicrobial peptides are produced by particular metabolic pathways and are encoded by genes.

In the recent year, considerable attention has been given to evaluate (AMPs) as novel antimicrobial agents in poultry production (Choi *et al.,* 2013; Wang *et al.,* 2007). Past studies suggested that AMPs have satisfactory influence on host animal which ensure the better intestinal health with stable microbial load by eradicating unfavourable microorganism such as clostridium and favouring propagation of good bacteria like bifido bacterium and lactobacillus (Tang *et al.,* 2009). Sound impact of AMPs on performance are because of their antimicrobial and immunomodulating activity, their by enhancing digestibility of nutrient and health of the animal (Wang *et al.,* 2016).

Addition of AMPs in the diet of poultry and other animals not only ensure diseases prevention and fall in morbidity, But also improve growth performance by prolonging nutrients utilization (Cromwell *et al.,* 2001). Continuous use of antibiotic growth promoters increases drug-resistant which make prohibition on the use of such AGP globally and look for new means of preventing bacterial resistance. In this circumstances, Yoon *et al.* (2012) believed that AMPs are thought to be ultimate candidates owing to their broad spectrum of activity against pathogen, and low tendency to develop bacterial resistance.

Nitisinprasert *et al.* (2000) and Nakphaichit *et al.* (2011) found Lactobacillus reuteri KUB-AC5 which had isolated from chicken intestine functions as antimicrobial peptides and showed broadspectrum activity against both Gram-positive and Gram-negative bacilli, fungus, viruses, and even carcinogenic cells.

b) Effect of dietary supplementation of AMPs on production performance, egg quality parameters

Abdulrahim *et al*. (1996) found that Feeding of lactobacillus in the diet of layer bird increased the egg production, feed conversion ratio and reduced concentration of Cholesterol in egg in this way we can anticipate that the increasing the number of good bacteria by feeding of AMPs may helps in increasing the egg production, egg quality, feed conversion ratio and reducing the cholesterol level in egg yolk.

Zhang *et al*. (2016a) studied the effect of adding antimicrobial peptides in the diet of laying hens. He randomly selected 480 hy-line brown commercial layers with similar egg production rate and divided them into 2 groups. One was the control group, and another was experimental group, each group has 3 replicates of 80 chickens. The study showed that the egg production rate was increased by 2.98% of hens fed with @0.01 L/kg cecropin antimicrobial peptide. The egg cholesterol content of the test group was 18.61% which was lesser to control group, and significant ($P<0.05$) influence was observed. The levels of different amino acids such as the content of methionine and tyrosine in the eggs were increased by 18.18% and 5.12%, respectively.

A study was conducted by Chen *et al.* (2020) total 360 laying hens (72 weeks old) were equally allocated into 3 groups with four replicates having 30 birds in each group. The 1^{st} group kept as control having no supplementation on basal diet, and for the 2^{nd} and 3^{rd} group diet were supplemented with @ 50 mg/kg, 100 mg/kg AMP in basal diet respectively. It was marked that AMP supplementation showed a significant increase in egg production, egg quality and decreased feed/egg ratio ($p < 0.05$).

c) Effect on growth performance, intestinal morphology and immune status

Dietary supplementation of antimicrobials in food animals alters the presence of microflora in the GIT (Anderson *et al*., 1999).Which could influence the nutrient availability, shrinks detrimental bacteria, and elicit an immune response (Cromwell, 2001), Stimulate host for utilization of amino acids as a source of energy, together with reduced ammonia excretion, (Veraeke *et al.*, 1979; Henderickx *et al.*, 1979).

Bao *et al.* (2008), used pig antibacterial peptides (PABP) as AMPs. He did one experiment on 300 day old chick by divided them into different groups in CRD to explore the impact of PABG on performance of broiler birds. AMPs provided them either through drinking water @ 20-30 mg/l or through feed 150-200 mg/kg diet. He observed improved growth performance, increased feed intake, increased serum concentration of alkaline phosphatase, liver enzymes, secreting IgA, and boost goblet cell numbers. Hence PABP supplementation beneficially affects the growth performance by improving the intestinal capacity to absorb nutrients and boost immunity.

Wang *et al.* (2009) did experiment on specific-pathogenfree (SPF) chickens by fed them with swine gut antimicrobial peptides (SGAMP) which was considered as peptides. He showed that dietary treated group with AMPs promote the expression of secretory IgA at different sites in the intestinal tract and markedly increased the number of mast cells and intraepithelial lymphocytes in the duodenum and ileum where as goblet cells were improved significantly in duodenum and jejunum ($P < 0.05$).

Ohh *et al.* (2009) used potato protein (PP) and refined potato protein (RPP) as an antimicrobial peptide in the diet of broilers. AMPs were incorporated various concentration in the diet of broilers and it was found that higher dose of PP (AMPs) was directly proportional to body weight gain, feed intake, and feed conversion ratio where as growth performance was not affected by the feeding of RPP (AMPs).

Choi *et al.* (2012) conducted an experiment on day old chick were randomly classified in to 4 treatment groups, with 4 replicates of 20 in each replicates. It was observed that chick fed with @ 60 mg/kg AMP-P5 has showed superior growth performance, feed conversion ratio, overall body weight gain, and retention of DM and Nitrogen. In addition, increasing levels of AMP-P5 linearly ($P<0.05$) improved growth performance and FCR of birds and reduce intestinal coliforms in broilers.

A study was carried by Wen *et al.* (2012) to know the effect of antimicrobial peptide, [cecropin A (1-11)-D (12-37)-Asn (CADN)] instead of antibiotic growth promoter (AGP) as an alternative in food animals. He took 1500(14d) indigenous male chickens separated them into five groups and 5 replicates with sixty birds in each, and provided them basal feed with a AMPs at 0, 2, 4, 6 and 8ml/kg, respectively in all treatment groups. He observed that AMPs increased body weight gain, FCR and improved nutrient utilisation for both growers and finishers; it decreased harmful microbes and maintains health of birds. Therefore CADN could be a potential alternative to AGP in broiler feeds.

Wu *et al.* (2012) were conducted an experiment to conclude the function of cecropin (AMPs) on piglets health and production which was already manifested by E. Coli K88 by assigning them into different treatment groups. He observed that cecropin treated groups showed improved performance by regulating their immune system and energy retention, lower concentration of immunoglobulins like IgA (secretory) in jejunum and IgA, IgM, IL- 1β, IL-6 in serum.

Park *et al.* (2013) was taken 320 broiler birds and divided them in to 4 treatment groups according to their initial body weight and he was reported significant increased in overall body weight gain, feed conversion ratio, gross energy, crude protein and mean retention time of dry matter in GIT, of birds fed with AMPs (90 mg/kg AMP-A3).

Bacillus subtilis PB6 (AMPs) having broad spectrum activity against different variety of microbial species (*Campylobacter* and *Clostridium*) which was originated from healthy chicken gut could be an alternative to antimicrobial growth promoters. This AMPs containing 5 × 10(11) cfu/kg was used to fed broiler bird @ 500 g/t of feed by Jayaraman *et al.* (2013) in in-vitro and he observed average body weight gain and FCR was increased in AMPs treated groups. AMPs not only controlled the infection but also maintain the health of broiler birds.

Yuan *et al.* (2014) conducted an experiment on 450, one-year-old Gushy chickens with similar body weight, which were randomly divided into 5 groups. Birds in control group were supplemented with basal feed, while those in treatment groups were fed with basal diet+500 mg/kg antibiotics and 100 mg/kg, 200 mg/kg, 500 mg/kg cecropin (AMPs), respectively. The results were showed as follows. The basal diet supplemented with 200 mg/kg cecropin significantly increased average daily weight gain and decreased feed conversion rate of chickens aged 1-21 d (P<0.05). 200-500 mg/kg cecropin significantly raised the mucosal thickness and the height of villus to depth of crypt ratio of intestine (P<0.05). The higher dose @ 500 mg/kg antimicrobial peptides significantly increased the number of *Lactobacillus*, and boosted the contents of immune globulin A, immune globulin G and immune globulin M in serum (P<0.05). The basal diet supplemented with 200-500 mg/kg antimicrobial peptides significantly improved the contents of complement 4 (C4) in serum (P<0.05).

The AMPs used in the study of Xiong *et al.* (2014) was a mixture of lactoferrin, cecropin, defensin and plectasin. They found that dietary supplementation with AMPs 2 and 3 g/kg of feed in the feed of piglet Showed constructive impact on growth rate, daily feed intake, and FCR. The morbidity and mortality rate was decreased, and chances of occurrence of infection were decreased.

The in vivo and vitro study was done by Wang *et al.* (2015) he took 252 birds divided them into 5 groups in completely randomized design, he took lincomycin as antibiotic a side and in another side sublancin as AMPs, his treatment groups consisting of negative control (uninfected group), infected control. 3 infected group was provided with sublancin (AMPs) @ 2.88, 5.76, or 11.52 mg activity/L of water respectively and one infected group was fed with lincomycin at 75 mg activity/L of water (positive control). He observed that concentration of minimum inhibition was significantly greater for AMPs (sublancin) than that of antibiotic (lincomycin), sublancin was required in very less concentration for control of infection his conclusion was that AMPs can kill microbes in very low concentration, and it may be a potent alternative substance to control infection.

Daneshmand *et al.* (2019) did an experiment to know the advantageous impact of an antimicrobial peptide (AMPs) cLF36, in broiler chickens affected with *E.Coli*. He allocated 360 chicks in 4 groups of 6 replicates, in CRD. His experiment revealed improved microflora population, immunity against infection, and eradicates *E. coli* concentration there by maintains the number of good bacteria in E. coli-challenged chickens fed @ 20 mg AMP (cLF36)/kg.

A study was conducted by Wang *et al.* (2020a) here he used microcin J25 (MccJ25) as an antimicrobial peptide. Birds were selected randomly and divided them into five groups of 12 replicates with 52 birds in each replicate. His different treatment groups involving control, challenge (0 mg/kg), different level of AMPs groups (0.5 and 1mg/kg), and antibiotic groups (20 mg/kg).They found that the AMPs treated group showed better feed utilization, improved growth performance, inhibits the growth of total anaerobic bacteria and *E. coli* in faeces, and minimize the concentration of TNF-α, IL-1β, and IL-6 compared with challenge group.

c) Effect of antimicrobial peptides (AMPs) on serum biochemical indices.

Shan *et al.* (2007) reported significant difference in peripheral lymphocyte and spleen lymphocyte proliferation which was enhanced by 36% ($p<0.01$), and 258% ($p<0.01$) respectively, improved concentration of serum immunoglobulins like IgG, IgA, IgM and serum iron values by 20%, 13%, 15%, 29% and 22% respectively, in the weanling piglets fed with lactoferrin (AMPs) @ 1g/kg of LF.

Tang *et al*. (2009) found that addition of AMPs cipB-LFC-LFA @ 100 mg/kg (C+L) in the diet of pig challenged with enterotoxigenic *Escherichia coli*, increased revival from diarrhoea, significantly improved serum glutathione peroxidase, total antioxidant content (T-AOC), liver GPx, POD, superoxide dismutase and total Fe-binding capacity, IgA, IgG and IgM levels ($P< 0.05$), increased concentration of good bacteria ($P < 0.05$), minimise the concentration of *E. coli* in the small intestine.

A study involving four hundred and eighty, 210-day-old Hisex (laying) hens, which were categorised into 4 groups has 4 replicates of 30 hens in each replicate, with one group fed with the basal diet as control, and others fed with the basal diet supplemented with antimicrobial peptide (AMPs) extract at 200, 400, 600 mg/kg, Zhou *et al.* (2012) reported significantly increased egg yolk weight percentage by 9.88% and 10.52% ($P<0.05$) of laying hens fed antimicrobial peptides AMPs @ 200 and 400mg/kg diet respectively, and super oxide dismutase (SOD) activity in the serum and laying rate were significantly increased by 5.91% ($p<0.05$) and 1.97% of hens fed with 600 mg/kg and 200 mg/kg respectively.

Hu *et al.* (2017) reported increased average daily gain and feed efficiency, increased activity of alkaline phosphatase, elevated intestine intraepithelial lymphocytes, greater ratio of secreting IgA, diminished histological lesions in intestine, a lesser amount of goblet cell number, and minor ratios of proliferating cell nuclear antigen, HSP-70, and glucose-6-phosphatase ($P < 0.05$ or $P < 0.01$) in broilers fed with swine gut intestinal antimicrobial peptides (SGAMP).

In the experiment with piglets (28d) were challenged with mycotoxin deoxynivalenol (DON), Xiao *et al.* (2013a) investigated the beneficial effects of composite antimicrobial peptide (CAP). It was found that the dietary supplementation of AMPs (CAP) was positively enhanced feed efficiency ($P < 0.05$), increased antioxidation capacity, diamine oxidase activity in the liver, increased concentrations of ALP, ALT, AST and serum catalase content, The numbers of platelets and thrombocytocrit were also higher, and lessen the risk of organ damage, Hence it was indicated that CAP could be a possible alternative to AGP.

CHAPTER-III

MATERIALS AND METHODS

Location of the experiment

To study the "**Effect of dietary supplementation of Antimicrobial peptide on production performance, egg quality and serum biochemical parameters of laying hens"** the research work was done in the Department of Livestock Production and Management, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, during the year 2020 and the experiment was done at the ICAR-Central Institute for Women in Agriculture (CIWA), Bhubaneswar.

Materials and facilities utilized during the experimental period

One hundred twenty (120), Vanaraja laying hens of 26 weeks were randomly divided into four groups. Each group was having three replicates with 10 birds in each replicate pen. As a whole the birds were kept in 12 pens with a floor area of 18 square feet each (6ftx3ft). The birds were kept under deep litter system of housing. The feed was prepared in Central Institute for Women in Agriculture (CIWA), Bhubaneswar for feeding the birds.

Period of experiment

The trial of 8 weeks was conducted from 2nd week of January, 2020 to 1st week of March 2020.

Experimental programme

To study the "Effect of dietary supplementation antimicrobial pepetides on the performance of Vanaraja laying hens" was studied for a period of 8 weeks as per the experimental design presented in Table 1.

One hundred twenty (120), Vanaraja laying hens of 26 weeks were distributed into four groups of 30 in each group Further each group was divided into three replicates of 10 birds in each. The birds in control group, were fed the diets supplemented with antibiotic – oxytetracycline (OTC) @ 50g/ quintal (T_1) and in the remaining 3 treatment groups (T_2, T_3 and T_4), birds were fed with diet supplemented with antimicrobial peptides at the concentration of 10, 15 and 20 g/quintal of feed, respectively. Each diet was supplemented to 3 replicates of 10 birds during the experimental period of 8 weeks. The design of the experiment conducted during the study has been presented in Table 1.

Table 1. Experimental design

Treatment no.	No of birds	Treatments
T_1	30	Basal diet with Oxytetracycline @ 50 g/quintal (Control)
T_2	30	Basal diet with Antimicrobial peptide @ 10 g / quintal of feed
T_3	30	Basal diet with Antimicrobial peptide @ 15 g/ quintal of feed
T_4	30	Basal diet with Antimicrobial peptide @ 20 g/ quintal of feed

Antimicrobial peptide (cecropin II)

The antimicrobial peptide used in the present study was Cecropin II isolated from *Bacillus subtilis* having both primary and secondary structure. For primary structure it has 37 amino acid with a molecular weight of 3.8 KDalton and secondary structure consists of 78.38% of amphiphatic helix and 21.62% random coil. The beneficial effects of AMP is, it ensures, eubiosis, intestinal integrity and improves intestinal health, increases laying rate and improves egg shell quality.

Feed preparation and Experimental feeding

The experimental feed was prepared in Central Institute of Women in Agriculture. To meet the required diet all the the required ingredients were weighed and mixed thoroughly to meet the energy, protein and other nutrient requirements of the birds and kept in a clean and dry place to be offered to birds daily. The nutrient composition of experimental diets with AMPs as well as proximate composition is presented in Table 2, 3, respectively.

Restricted amount of feed weighing 125g of per bird per day was offered for 8 weeks. Feed was given daily (arrived from the equation basing on ME requirement). In the early morning weighed quantities of feed was offered once and drinking water was provided twice daily.

Table 2.Ingredients and nutrient composition of diets (% as fed basis)

Ingredients	**Quantities per quintal**			
	T_1	**T_2**	**T_3**	**T_4**
Maize	58.8	58.8	58.8	58.8
Soyabean meal	20.8	20.8	20.8	20.8
Deoiled rice bran	9.66	9.70	9.70	9.69
Shell grit	8.8	8.8	8.8	8.8
Dicalcium phosphate	1.15	1.15	1.15	1.15
DL - methionine	0.08	0.08	0.08	0.08
Common salt	0.40	0.4	0.4	0.4
Vitamin B complex	0.02	0.02	0.02	0.02
Vitamin ABDK	0.02	0.02	0.02	0.02
Mineral mixture*	0.12	0.12	0.12	0.12
Choline	0.05	0.05	0.05	0.05
Toxin Binder	0.05	0.00	0.00	0.00
Oxytetracycline	0.05	0.00	0.00	0.00
AMPs	0.00	0.01	0.015	0.020
TOTAL	100	100	100	100
Nutrient composition (Calculated value)				
ME(kcal/kg)	2602	2602	2602	2602
CP (%)	16.01	16.01	16.01	16.01
Lysine (%)	0.78	0.78	0.78	0.78
Methionine (%)	0.34	0.34	0.34	0.34
Calcium (%)	3.22	3.22	3.22	3.22
Phosphorous (%)	0.34	0.34	0.34	0.34

*Trace Min CB (Venky's India Private Limited, Pune).

Table 3. Proximate composition of experimental diets

Proximate composition (%)	**Treatments**			
	(T_1)	**(T_2)**	**(T_3)**	**(T_4)**
Dry matter	91.24	91.04	91.20	91.14
Crude protein	16.01	15.99	16.02	16.05
Ether extract	3.54	3.52	3.56	3.55
Total ash	6.78	6.74	6.72	6.76

The ME requirements of the birds were arrived through the following equation

Daily ME requirement = ME for maintenance + ME for activity + ME for growth + ME for egg production.

The ME requirement (kcal) of birds was calculated as follows,

$$\text{ME (maintenance)} = \frac{83 \times \text{BW (kg)}^{0.75}}{82}$$

ME (activity) = 50% of ME (maintenance)

ME (growth) = 2.1 kcal/ g gain (2.1 kcal energy require for 1g growth)

ME (egg production) = 84 x % egg production (one egg contain 84 kcal energy)

The daily feed requirement was calculated from the equation,

$$\frac{\text{ME requirement (kcal)}}{\text{ME content of the feed (kcal)}} \times 100 = \text{feed requirement (g/day)}$$

General Management

At the beginning, the sheds were thoroughly cleaned and disinfected followed by proper drying. Blowlamp was utilized to kill the leftover litter materials and microorganisms. The walls from all sides up to one foot height and floors were painted with lime. Five per cent formalin was sprayed inside the house and around the experimental shed. Rice husk was spread at a thickness of 5 cm. Feeders and Waterers were provided as per the requirements of the birds inside the pen. Weighed quantity of feed was offered once in the morning and drinking water twice a daily. The litter materials were stirred properly and wet litter was replaced with fresh litter material. At weekly interval required light was provided with adequate ventilation facility

Methods of analysis

A. Production Performances

a. Recording of body weight:

At the beginning of the experiment the body weight of the birds was recorded (26^{th} week) and finally at the end of the experiment (34^{th} week). The body weight gain was calculated by deducting the previous body weight from the final body weight measured.

b. Egg production:

The egg production of the bird was recorded daily on individual pen basis The hen housed egg production (HHEP) was calculated by the formula as total no of eggs produced to the total no of birds housed at beginning of the experiment multiplied by 100 and is represented as per cent.

c. Feed consumption and calculation of FCR:

Weighed quantity of feed was given daily. The feed intake was derived by subtracting the left over feed at the end of each period from the total quantities offered during that period to the birds in each replicate. The average feed consumption was recorded as gram / bird / day. The FCR of the bird was calculated as the gram feed required to produce gram egg mass.

d. Egg weight

The eggs laid by the bird during the last five days of each four weeks period on individual pen basis were utilized to measure egg weight.

e. Egg mass

The egg mass was calculated as follows,

$$\text{Egg mass per day} = \frac{\text{Number of egg produced} \times \text{average egg weight}}{\text{Number of birds} \times \text{duration (days)}}$$

B. Egg quality parameters

The egg quality was studied on fourth and eighth week of the experimental period. Twelve eggs were selected at random from each treatment group during the last 3 days of each 28 days for egg quality parameters. The details of the procedure are given below

a. Parameter of unopened egg

(i) Egg weight: Egg weight was measured by an electronic balance.

b. Parameters of opened eggs

After breaking the eggs the contents were transferred over a plate kept on a table and the following parameters were measured.

i. **Albumen height:** The height of the albumen was measured with the help of spherometer at three different places.

ii. **Albumen weight:** The weight of albumen was calculated by separating the albumen from the yolk and taking weight in a previously weighed petridish.

Albumen weight = (Weight of petridish + Albumen) – Weight of petridish.

iii. **Albumen per cent:**

$$\text{Albumen \%} = \frac{\text{Weight of albumen}}{\text{Weight of egg}} \times 100$$

iv. **Haugh unit (HU):** According to Haugh (1937)

$$HU = 100 \log (H + 7.57 - 1.7\, W^{0.37})$$

Where H = Average height of albumen (mm)

W = Average weight of egg (g)

v. **Yolk weight:** The weight of yolk was calculated as.

Yolk weight = (Weight of petridish + Yolk) – Weight of petridish.

vi. **Yolk percent:** The yolk percent was calculated as follows

$$\text{Yolk \%} = \frac{\text{Weight of the yolk}}{\text{Weight of the egg}} \times 100$$

vii. **Shape index:** The shape should not be too round or too elongated and is measured in terms of shape index.

$$\text{Shape index} = \frac{\text{Breadth of egg (cm)}}{\text{Length of egg (cm)}} \times 100$$

viii. **Albumen index:** The albumen index was derived as:

$$\text{Albumen index} = \frac{\text{Albumen height}}{\text{Albumen diameter}} \times 100$$

ix. **Yolk index:**

$$\text{Yolk index} = \frac{\text{Yolk height}}{\text{Average width of Yolk}} \times 100$$

x. **Shell thickness:** After removing the shell membrane the shell thickness were measured by a screw gauge taking two pieces from both narrow and broad end. The shell thickness was calculated by taking the average of the three measurements.

xi. **Shell per cent:** The shell per cent was calculated by the formula

$$\text{Shell \%} = \frac{\text{Weight of the dried shell}}{\text{Weight of the egg}} \times 100$$

xii. **Shell weight:** After pouring the contents of the eggs completely on the slab, the egg shells were kept overnight in a hot air oven for drying and on the next day weights were recorded by an electronic balance.

xiii. **Egg composition:** For calculation of egg composition three eggs per replicate were taken from each dietary treatment from the eggs laid during the last three consecutive days of experiment. The protein and fat content of the eggs were analyzed by following the methods of AOAC (1995) official method of analysis. **The amino acid analysis of eggs was done at EVONIK, Mumbai, India.**

C. Serum biochemical profile

a. Collection of blood

From 3 birds of each replicate pen blood samples were collected on 34th week of age.

Blood of about 3-5ml was collected from the wing vein with 23 gauze needle in all asceptic manners the serum samples so obtained were kept at -10ºC for further analysis.

b. Estimation of serum biochemical parameters

The serum biochemical parameters were estimated by following the procedures mentioned in the reagent kit for each parameter supplied by Crest Biosystems, a division of Coral Clinical Systems, Goa, India by using Biochemistry autoanalyzer.

i. **Total protein:** The concentration of total protein (g/dl).was estimated by Biuret method as per the procedure supplied by Crest Biosystems, a division of Coral Clinical Systems, Goa, India.

ii. **Albumin:** The serum albumin concentration (g/dl). was estimated by BCG (Bromo-Cresol Green) method as per the procedure supplied by Crest Biosystems, Goa, India

iii. **Globulin:** The globulin concentration of serum (g/dl) was determined as:

Concentration of globulin (g/dl) = Concentration of total protein (g/dl) – Concentration of albumin (g/dl)

iv. **Phosphorous:** The concentration of phosphorous (mg/dl).was estimated by Molybdate U.V. method as per the procedure supplied by Crest Biosystems, Goa, India.

v. **Calcium:** The concentration of calcium (mg/dl.) was estimated by O-Cresol phtalein cromogenic (OCPC) method as the procedure described in the reagent kit supplied by Crest Biosystems, Goa, India.

vi. **Triglycerides:** The concentration of triglyceride (mg/dl.) was estimated by GPO- Trinder method as per Crest Biosystems, Goa, India.

vii. **Total cholesterol:** The concentration of cholesterol (mg/dl.) was estimated by CHOD/ PAP method Crest Biosystems, Goa, India.

viii. **Uric acid:** The concentration of uric acid (mg/dl.) was estimated by Modified Berthelot method Crest Biosystems, Goa, India.

ix. **Creatinine:** The concentration of creatinine (mg/dl.) was estimated by Modified Jaffe's Kinetic method Crest Biosystems, Goa, india.

x. **Aspartate transaminase (AST) Serum glutamic- oxaloacetic transaminase ((SGOT):** The concentration of AST (units/litre) was estimated by Modified IFCC method Crest Biosystems, Goa, India.

xi. **Alanine transaminase (ALT)/ Serumglutamic-pyruvic transaminase (SGPT):** The concentration of ALT(units/litre) was estimated by Modified IFCC method as per the procedure mentioned in the reagent kit supplied by Crest Biosystems, a division of Coral Clinical Systems, Goa, India

xii. **Alkaline phosphatase:** The concentration of alkaline phosphatase (units/litre) was estimated by PNPP Kinetic method (Crest Biosystems, a division of Coral Clinical Systems, Goa, India).

D. Proximate analysis of feed

The feed was properly mixed and dried and four feed samples collected for proximate analysis.

i. Dry matter

Moisture content of a feed sample was determined by heating the sample in an hot air oven at a temperature of 100 to 105 ºC in a moisture cup following standard procedure of dry matter analysis.

$$\text{Moisture \% in feed} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where, W_1=Weight of the empty moisture cup in grams

W_2= Weight of the moisture cup with feed sample before drying in grams

W_3- Weight of the moisture cup with the dried sample in grams

Dry matter (%) of feed = 100 – Moisture % of feed

ii. Total ash

The inorganic residue represent the total mineral matter left after heating the smoke free sample at a temperature of 550 ºC to 600 ºC for 2 to 3 hours taken. Standard procedure was adopted for determination of ash.

iii. Ether extract

The ether extract represents a fraction of the feed generally composed of esters of fatty acids with glycerol, free fatty acids, sterol, phospholipids; chlorophyll, fat soluble vitamins, other pigments, volatile oils, waxes, resins etc. The fraction is extracted from the moisture free sample with petroleum ether (60 to 80 ºC) by Soxhlet apparatus. The ether extract was then calculated by the formula.

W_1 – Weight of dried sample

W_2- Weight of empty oil flask

W_3- Weight of the oil flask after drying

Ether Extract % = $(W_3-W_2) / W_1 \times 100$

iv. Estimation of Crude Protein (CP)

Protein content of feed was determined by Kjeldahl method. Feed was digested in kjeldahl flask with concentrated sulphuric acid with digestion mixture. The digested material was quantitatively estimated for nitrogen following the standard procedure of distillation and titration and multiplied by the 6.25 to get the protein percentage.

v) Economics of production

The cost of feed ingredients was taken into account for calculating the cost of experimental diet (Table 4 and Table 5). Abd basing on which the cost of feed per egg produced was calculated.

Table 4.Cost of feed ingredients used in the experiment

Sl. No.	Ingredients	Cost (Rs per kg)
1	Maize	17.00
2	Soya Bean Meal	37.00
3	Deoiled Rice Bran	12.00
4	Stone Grit	5.00
5	Dicalcium phosphate	35.50
6	Methionine	350.00
7	Salt	7.00
8	ABDK vitamin	200.00
9	Vitamin B complex	200.00
10	Choline	80
11	Oxytetracycline	1000.00
12	Toxin Binder	200.00
13	Mineral mixture	70.00
14	Antimicrobial peptide (Cecropin II)	3300.00

Table 5.Cost of different rations

Treatment No.	Treatments	Feed (Rs per kg)
T_1	Basal diet with Oxytetracycline @ 50 g/quintal (Control)	20.90
T_2	Basal diet with Antimicrobial peptide @ 10 g / quintal of feed	20.75
T_3	Basal diet with Antimicrobial peptide @ 15 g/ quintal of feed	20.90
T_4	Basal diet with Antimicrobial peptide @ 20 g/ quintal of feed	21.05

Statistical analysis

The statistical analysis of the data was done according to Snedecor and Cochran (1994).

Fig. 3.1: Preparation of the pen before keeping the bird

Fig. 3.2: Leg banding of birds for identification

Fig. 3.3: Preparation of feed

Fig. 3.4: Mixing of feed

Fig. 3.5: Weighing of bird

Fig. 3.6: Keeping birds in different pens

Fig. 3.7: Providing feed to birds in feeder

Fig. 3.8: Feeding by birds

Fig. 3.9: Marking of collected eggs

Fig. 3.10: Weighing of egg

Fig. 3.11: Breaking of egg

Fig. 3.12: Poured egg content after breaking

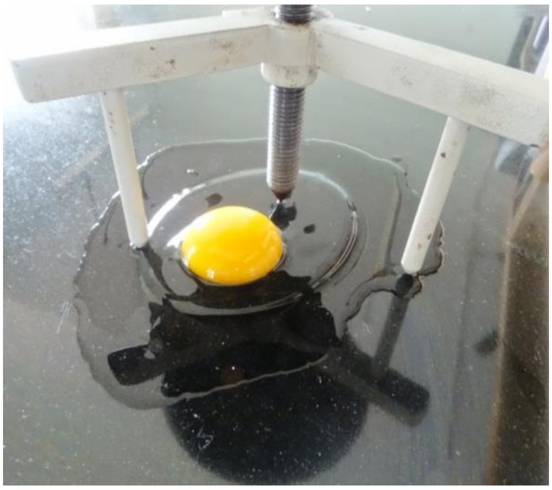


Fig. 3.13: Measuring height of albumen by spherometer

Fig. 3.14: Weighing of yolk

Fig. 3.15: Drying of egg shells

Fig. 3.16: Weighing of egg shell

Fig. 3.17: Screw gauge for measuring egg shell thickness

Fig. 3.18: Collection of blood

Fig. 3.19: Keeping blood samples in centrifuge machine

Fig. 3.20: Separation of serum sample and marking

CHAPTER-IV

RESULTS

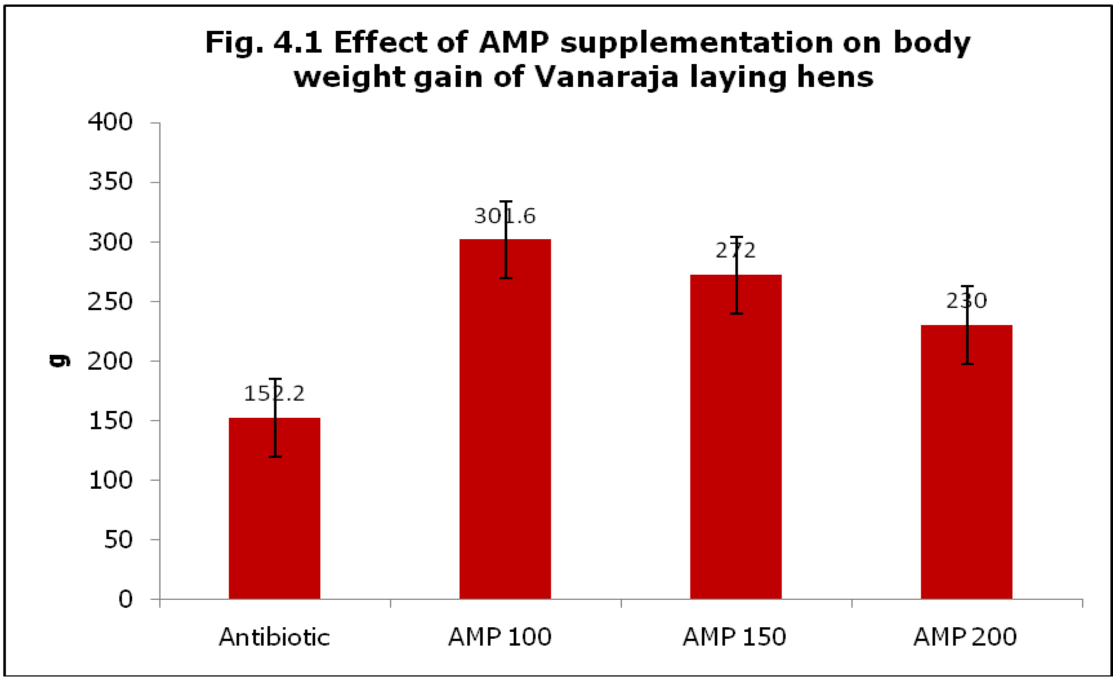
Body weight and Body weight gain

The results on body weight and body weight gain of Vanaraja laying hens as influenced by dietary supplementation of antimicrobial peptide (AMP) is given in Table 6. The early body weight of laying hens in the starting of the experiment was comparable among the treatments and ranged from 2259 to 2378g. The body weight and body weight gain increased in all the dietary groups during the experimental period of eight weeks (26-34 weeks). Dietary supplementation of AMP significantly increased ($P<0.005$) the body weight of laying hens at 34 weeks of age at all the levels of supplementation (100-200 mg/kg diet). However, no difference in body weight could be found due to different dietary levels of AMP supplementation (100, 150 & 200 mg/kg diet). The body weight gain also improved significantly ($P<0.001$) due to dietary supplementation of AMP compared to the antibiotic supplemented group (oxytetracycline, 500 mg/kg diet).

Table 6. Effect of Antimicrobial peptide (cecropin II) supplementation on body weight of Vanaraja laying hens (26-34 wks)

Parameters	Oxytetracycline (500mg/kg diet)	AMP (mg/kg diet)			SEM	P value
		100	150	200		
Initial body weight (g) at 26 weeks	2270	2259	2378	2324	19.85	0.119
Body weight (g) at 34 weeks	2422 ^{a}	2560^{b}	2651^{b}	2554^{b}	23.59	0.005
Body weight gain (g) 26-34 weeks	152.2 ^{a}	301.6^{b}	272.0^{b}	230.0^{b}	14.11	0.001

$^{a, b}$ Means with different superscript in a row differ significantly; SEM-Standard error of mean



Production performances

The production performance of Vanaraja laying hens in the course of experimental period of 8 weeks is presented in Table 7. The hen housed egg production (%), egg weight (g) and egg mass (g/day) did not differ significantly among the dietary groups due to supplementation of AMP compared to that of antibiotic supplemented group. The feed consumed per bird per day was similar across the dietary groups and was around 127.5 g/day. The feed conversion ratio as expressed by g feed required to produce g egg also did not differ remarkably due to dietary addition of AMP in Vanaraja laying hens.

Table 7. Effect of Antimicrobial peptide (cecropin II) supplementation on production performance of Vanaraja laying hens during phase I (26-34 weeks)

Parameters	Oxytetracycline (500mg/kg diet)	AMP (mg/kg diet)			SEM	P value
		100	150	200		
Hen housed egg production (%)	48.98	49.46	50.95	50.01	0.628	0.775
Egg weight (g)	51.31	51.26	50.46	50.30	0.408	0.798
Egg mass (g/day)	25.10	25.32	25.71	25.14	0.229	0.831
Feed consumed (g/day)	127.5	127.5	127.5	127.5	--	--
Feed conversion ratio (g feed/g egg)	5.088	5.035	4.959	5.075	0.047	0.818

Egg quality

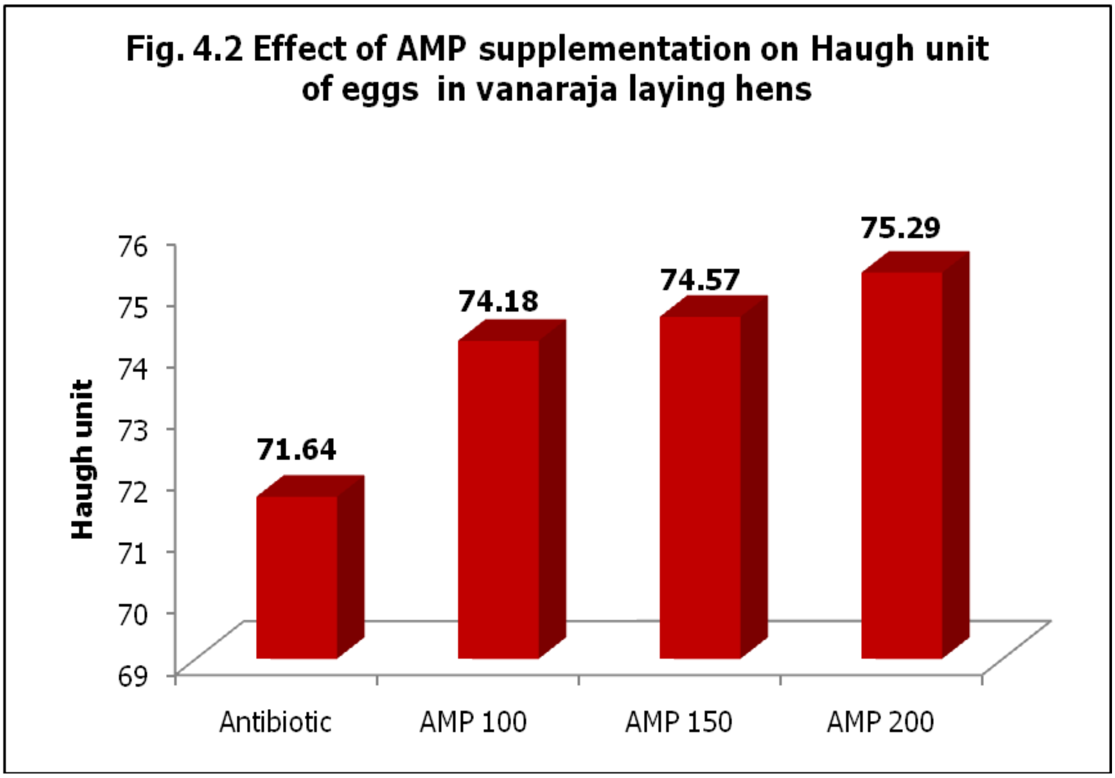
The effect of dietary supplementation of AMP on egg quality parameters of Vanaraja laying hens is presented in Table 8. Egg weight was comparable among the dietary groups. Dietary supplementation of AMP increased the albumen per cent in eggs compared to that of antibiotic supplementation. However, significant improvement in albumen percent in eggs was noticed at AMP supplementation @ 200mg/kg diet compared to that of antibiotic supplementation (oxytetracycline, 500mg/kg diet). It was intermediate at 100 and 150 mg/kg diet AMP supplemented dietary groups. The yolk per cent in eggs have shown the reverse trend to that of albumen percent in eggs. The yolk percent in eggs reduced linearly with increase in the levels of dietary supplementation of AMP and was found to be significantly ($P<0.05$) lower at 200mg/kg diet supplementation compared to that of antibiotic supplemented group. The egg shell per cent varied from 9.47 to 9.82 and was comparable among the dietary treatment groups.

Table 8. Effect of Antimicrobial peptide (cecropin II) supplementation on egg quality parameters of Vanaraja laying hens at the end of experiment (34 weeks)

Parameters	Oxytetracycline (500mg/kg diet)	AMP (mg/kg diet)			SEM	P value
		100	150	200		
Egg weight (g)	52.01	51.71	52.10	51.35	0.306	0.830
Albumen (%)	58.30^{a}	60.09^{ab}	60.48^{ab}	62.14^{b}	0.439	0.011
Yolk (%)	31.87^{b}	30.4^{ab}	29.91^{ab}	28.36^{a}	0.461	0.050
Eggshell (%)	9.48	9.47	9.60	9.82	0.088	0.494
Albumen Index	6.92	7.24	7.51	7.01	0.278	0.158
Yolk Index	34.91	35.19	34.59	35.11	0.298	0.899
Shape Index	74.42	73.75	75.19	75.62	0.576	0.681
Haugh Unit	71.64^{a}	74.18^{b}	74.57^{b}	75.29^{b}	0.164	0.048
Eggshell thickness (mm)	0.353	0.348	0.341	0.342	0.003	0.516

$^{a, b}$ Means with different superscript in a row differ significantly;

SEM-Standard error of mean



The other egg quality parameters such as albumen index, yolk index and shape index did not influence significantly due to dietary supplementation of AMP in the diet of Vanaraja laying hens. However, Haugh unit increased significantly (P<0.05) due to supplementation of AMP at all the levels of supplementation. No difference in Haugh unit could be observed due to various levels of dietary supplementation of AMP (100 to 200 mg /kg diet) in the diets of Vanaraja laying hens. The eggshell thickness varied from 0.341 to 0.353 mm and was comparable among the dietary groups.

Serum biochemical parameters

The serum biochemical parameters of laying hens as influenced by dietary supplementation of AMP are given in Table 9. Dietary supplementation of AMP in the diet significantly (P<0.05) increased the serum protein concentration compared to antibiotic supplementation. However, no difference in serum protein concentration could be noticed by enhancing the concentration of AMP from 100 mg/kg diet to 200 mg/kg diet. The concentration of albumin, globulin, creatinine, uric acid, calcium and phosphorus did not differ significantly due to dietary supplementation of AMP in Vanaraja laying hens. Dietary supplementation of AMP @ 100 mg/kg diet had no change on serum cholesterol concentration but increasing the dietary levels of AMP to 150 mg/kg diet significantly (P<0.01) reduced the serum cholesterol concentration. Further increase in the levels of AMP supplementation to 200mg/kg diet did not have any additional advantage in reducing serum cholesterol to further lower levels.

Table 9. Effect of Antimicrobial peptide (cecropin II) supplementation on serum biochemical indices of Vanaraja laying hens (34 weeks)

Parameters	**Oxytetracycline (500mg/kg diet)**	**AMP (mg/kg diet)**			**SEM**	**P value**
		100	**150**	**200**		
Total protein (g/dl)	5.11^{a}	5.94^{b}	6.04^{b}	6.14^{b}	0.124	0.050
Albumin (g/dl)	2.01	2.34	2.42	2.48	0.064	0.184
Globulin (g/dl)	3.10	3.60	3.62	3.66	0.044	0.176
Creatinine (mg/dl)	1.60	1.64	1.62	1.66	0.022	0.684
Uric acid (mg/dl)	6.24	6.28	6.18	6.11	0.044	0.768
Calcium (mg/dl)	12.24	13.02	13.14	12.98	0.024	0.050
Phosphorus (mg/dl)	5.14	5.08	5.16	5.04	0.032	0.815
Triglyceride (mg/dl)	432.2	444.7	422.4	434.8	4.722	0.640
Cholesterol (mg/dl)	184.8^{b}	180.2^{b}	160.6^{a}	164.4^{a}	1.745	0.014

$^{a, b}$ Means with different superscript in a row differ significantly; SEM-Standard error of mean

Serum enzyme activities

The effect of AMP supplementation on activities of serum enzymes of Vanaraja laying hens is presented in Table 10. Dietary supplementation of AMP reduced the serum alkaline phosphatase concentration significantly (P<0.05) at all the levels compared to antibiotics supplemented group. No difference in the activities of alkaline phosphatase could be noticed due to the levels of AMP supplementation. The concentration of serum aspartate amino transferase and alanine amino transferase did not influenced by the dietary supplementation of AMP in the diet. The concentration of serum aspartate amino transferase and alanine amino transferase did not influenced by the dietary supplementation of AMP in the diet.

Table 10. Effect of Antimicrobial peptide (cecropin II) supplementation on serum enzyme activities of Vanaraja laying hens

Parameters	Oxytetracycline (500mg/kg diet)	AMP (mg/kg diet)			SEM	P value
		100	150	200		
ALP (mg/dl)	428.2^{b}	402.7^{a}	398.8^{a}	392.2^{a}	4.54	0.051`
ALT (mg/dl)	38.42	42.24	36.72	40.02	2.47	0.684
AST(mg/dl)	146.24	142.46	138.02	144.84	3.48	0.861

$^{a, b}$ Means with different superscript in a row differ significantly;

SEM-Standard error of mean

ALT- Alanine Transaminase, AST-Aspartate Transaminase, ALP- Alkaline Phosphatase

Economics

The cost of feed per egg produced of Vanaraja laying hens due to dietary supplementation of antimicrobial peptide (cecropin II) during the experimental period (26-34 wks) is shown in the table 11. Considering the composition of four rations the cost per kg feed was Rs 20.90 (oxytetracycline, 500 mg/kg diet), Rs 20.75 (100 mg AMP/kg diet), Rs 20.90 (150 mg AMP/kg diet) and Rs 21.05 (200 mg AMP/kg diet) during entire experimental period.

Table 11. Cost of feed/egg produced (Rs.) of Vanaraja laying hens during (26-34) weeks laying period

Particulars	OTC (500mg/kg diet)	AMP (mg/kg diet)			SEM	P value
		100	150	200		
Cost of feed (Rs/kg)	20.90	20.75	20.90	21.05	---	---
Feed consumption per bird (kg)	7.14	7.14	7.14	7.14	--	--
Cost of feed consumed per bird (Rs)	149.23	148.16	149.23	150.30		
Egg production per bird (No.)	27.43	27.70	28.53	28.00	0.352	0.776
Cost of feed per egg produced (Rs)	5.46	5.35	5.23	5.37	0.071	0.771

The egg production of the different dietary groups was comparable during the experimental period. The cost of feed per egg produced in the antibiotic supplemented group was Rs 5.46. The cost of feed per egg produced in 100, 150 and 200 mg/kg diet AMP supplemented group was Rs 5.35, Rs.5.23 and Rs.5.37, respectively. Dietary supplementation of AMP reduced the feed cost per egg in the entire AMP supplemented groups. The feed cost was reduced by 2.01, 4.21 and 1.65%, respectively. But the reduction in feed cost was found to be non-significant.

CHAPTER-V

DISCUSSION

The present study was conducted to evaluate the effect of antimicrobial peptide (AMP) on production performance, eggshell quality and serum biochemical parameters of Vanaraja laying hens.

Body weight and Body weight gain

Dietary supplementation of AMP significantly increased the body weight and body weight gain during the experimental phase of eight weeks (26-34 wks) compared to the antibiotic supplemented group. Concurrent to the finding of the current study, Yuan *et al.* (2014) observed significantly increased in average daily weight gain of Gushi chicken fed diet supplemented with 200-500 mg/kg cecropin. In another study, Choi *et al.* (2012) observed that dietary addition of 60 mg AMP-P5/kg in Ross broiler chickens improved the growth performance, higher overall (d 0–35) body weight gain and increased nutrient retention of dry matter and nitrogen. In the current study, birds of all the dietary group gained body weight implied that there was positive energy balance. However, supplementation of AMP in the diet enhanced the body weight gain significantly compared to that of antibiotic supplemented group which is in agreement with Wen and He (2012). The finding of the present study suggested that AMP can be used as an alternative to antibiotic in the diet of laying hens in optimizing body weight gain during 26 to 34 weeks of age.

Production performances

The production performance parameters like hen housed egg production, egg weight, egg mass and feed conversion ratio were not influenced due to dietary addition of AMP (100 - 200 mg/kg diet) as compared to that antibiotic (oxytetracycline, 500mg/kg diet) supplemented group. In contrast to the observation of the present study, Chen *et al.* (2020) found significantly increased in egg production and decreased feed/egg ratio in Brown laying hens due to dietary supplementation of AMP (50-100 mg/kg diet). On the other hand, Zhang *et al*. (2016a) observed only a marginal improvement of 2.98% in the egg production rate due to dietary supplementation of cecropin antimicrobial peptide in brown laying hens. Enhancement in nutrient digestibility and health due to antimicrobial and immune-modulating activity of AMPs has been reported by Wang *et al.,* (2016). In the present study, the production performance of the hens of AMP supplemented group were at par with antibiotic supplemented group implied that AMP can be a potential alternative to antibiotic in the diet of laying hens.

Egg quality

The albumen concentration increased and yolk concentration decreased in eggs due to dietary supplementation of AMP @ 200mg/kg diet compared to that of antibiotic supplementation (oxytetracycline, 500mg/kg diet). Haugh unit increased significantly ($P<0.05$) due to supplementation of AMP at all the levels of supplementation. The findings on egg quality of this study suggested that AMP in the diet of laying hens could be beneficial in improving the overall quality of the eggs. Similar to the findings of the present study, Chen *et al.* (2020) reported significantly improvement in egg quality parameters by supplementation of AMP @ 50 to 100 mg/kg diet. Zhang *et al*. (2016) also reported higher methionine and tyrosine content by 18.18% and 5.12%, respectively, another measure of egg quality due to dietary supplementation of AMP in brown laying hens. Although few researchers reported improvement in egg shell thickness and higher eggshell percentage due to dietary supplementation of AMP, no such results could be observed in the present study.

Serum biochemical parameters

Serum biochemical indices are indicators of nutritional status of an individual. Dietary supplementation of AMP in Vanaraja laying hens significantly ($P<0.05$) increased the serum protein concentration compared to antibiotic supplementation. It has been reported that antimicrobial peptides were found to increase the apparent total tract digestibility of dry matter, crude protein and gross energy in weaning piglets and broilers (Yoon *et al.,* 2012; Choi *et al.,* 2013). Wen and He (2012) found that dietary addition with an antimicrobial peptide, a cecropin hybrid (artificial), enhanced the apparent digestibility of crude fat, higher nitrogen retention and enhanced apparent metabolizable energy in broiler chicken. Although, nutrient digestibility study was not carried out in the present study, but it could be hypothesized that the higher serum protein concentration in the AMP supplemented group could be due to higher digestibility of protein. The concentration of albumin, globulin, creatinine, uric acid, calcium and phosphorus did not differ significantly due to dietary supplementation of AMP in Vanaraja laying hens. Dietary supplementation of AMP @ 150 or 200 mg/kg diet significantly ($P<0.01$) reduced the serum cholesterol concentration. Earlier studies advise that AMPs beneficially affect the host animal by boosting its gut health and making a favorable microbial gut ecology in that could suppresses detrimental microorganisms (Yoon *et al.,* 2012). The antimicrobial peptide lactoferrin significantly decreased the total viable counts of Escherichia coli and Salmonella, and improved the Lactobacillus and Bifidobacterium counts in the small intestine of pigs compared with the control group (Wang *et al.,* 2007). Reduced concentration of cholesterol due to AMP feeding could be attributed to reduction in absorption and/or synthesis of cholesterol in the gastro-intestinal tract (Mohan *et al.,* 1995), utilization of cholesterol by microbes for their own metabolism (Goodling *et al.,* 1987), de-conjugation of bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis (Abdulrahim *et al.,* 1996).

Serum enzyme activities

The concentration of serum aspartate amino transferase and alanine amino transferase did not influenced by the dietary supplementation of AMP in the diet. The activity of AST and ALT are of great importance in clinical conditions of myocardial infection and hepatitis (La Due *et al.,* 1954). Liver and heart are rich in transaminases, due to abnormal condition results in great extent destruction of cells which could result in liberation of the enzyme into circulating blood stream, thereby increasing the concentration in serum. In the current study no such abnormality could be observed in both AMP and antibiotic supplemented group. Dietary supplementation of AMP reduced the serum alkaline phosphatase concentration significantly ($P<0.05$) at all the levels of AMP supplementation compared to antibiotics supplemented group. Alkaline phosphatase activity is an indicator of stress and the lower concentration of alkaline phosphatise activity in serum of laying hens fed diets supplemented with AMP suggested that AMP could reduce the stress in laying hens.

Economic efficiency

The egg production of the different dietary groups was comparable during the experimental period. Dietary supplementation of AMP reduced the feed cost per egg in the entire AMP supplemented groups. The feed cost was reduced by 2.01, 4.21 and 1.65%, respectively. Supplementation of AMP in the diet of Vanaraja laying hens not only maintained the egg production but also lower the cost of production marginally compared to the antibiotic supplemented group. This finding of the present study further suggested that AMP can be used in the diet of laying hens as an alternative to antibiotics.

The advantageous effects of AMPs on growth performance are mostly due to their antimicrobial and immunomodulating activity, which lead to higher promoting digestibility and improved health (Wang *et al.,* 2016). In the present study, body weight gain increased, serum protein concentration was higher and cholesterol concentration was lower, Haugh unit an indicator of egg quality improved and albumen concentration in egg increased due to dietary supplementation of AMP in the diet of Vanaraja laying hens. Thus it could be suggested that antimicrobial peptide used in the present study Cecropin II isolated from *Bacillus subtilis* could be used as a possible alternative to antibiotic in the diet of laying hens. Since the experiment was conducted with limited number of birds and of a shorter duration, large scale comprehensive field trial is needed to validate the findings of the present study before its commercial exploitation.

CHAPTER-VI

SUMMARY AND CONCLUSION

The research work entitled "Effect of dietary supplementation of Antimicrobial peptide on production performance, egg quality and serum biochemical parameters of laying hens" was accomplished for a phase of 8 weeks (26-34 weeks) to know the effect of dietary supplementation of antimicrobial peptide (cecropin II) in Vanaraja laying hens on body weight changes, egg production, feed consumption, egg weight, egg mass, feed conversion ratio, egg quality traits, serum biochemical parameters and economics of production. **One hundred twenty, Vanaraja laying hens of 26 weeks of age were randomly selected and separated into four treatment groups involving 30 birds each with three replicates of 10 in each group in a complete randomized design.** The diets was supplemented with antibiotic – oxytetracycline (OTC) @ 50g/ quintal in control group (T_1) and in the remaining 3 treatment groups (T_2, T_3 and T_4), diet were supplemented with antimicrobial peptides at the concentration of 10, 15 and 20 g/quintal of feed, respectively. Each diet was fed to 3 replicates of 10 birds during the experimental period of 8 weeks. The birds were maintained in deep litter system of housing with standard managemental practices.

Following results were observed from the study

Body weight

The initial body weight of laying hens in the beginning of the experiment was comparable among the treatments. The body weight and body weight gain increased in all the dietary groups during the experimental period of eight weeks (26-34 weeks). Dietary supplementation of AMP significantly increased ($P<0.005$) the body weight of laying hens at 34 weeks of age at all the levels of supplementation (100-200 mg/kg diet). However, no difference in body weight could be found due to different dietary levels of AMP supplementation (100, 150 & 200 mg/kg diet). The body weight gain also improved significantly ($P<0.001$) due to dietary supplementation of AMP compared to the antibiotic supplemented group (oxytetracycline, 500 mg/kg diet).

Production performances

The hen housed egg production (%), egg weight (g) and egg mass (g/day) did not differ significantly among the dietary groups due to supplementation of AMP compared to that of antibiotic supplemented group. The feed consumed per bird per day was similar across the dietary groups and was around 127.5 g/day. The feed conversion ratio as expressed by g feed required to produce g egg also did not differ significantly due to dietary supplementation of AMP in Vanaraja laying hens.

Egg quality

Egg weight was comparable among the dietary groups. Dietary supplementation of AMP increased the albumen percent in eggs compared to that of antibiotic supplementation. However, significant improvement in albumen per cent in eggs was noticed at AMP supplementation @ 200mg/kg diet compared to that of antibiotic supplementation (oxytetracycline, 500mg/kg diet). It was intermediate at 100 and 150 mg/kg diet AMP supplemented dietary groups. The yolk percent in eggs have shown the reverse trend to that of albumen percent in eggs. The yolk percent in eggs reduced linearly with increase in the levels of dietary supplementation of AMP and was found to be significantly ($P<0.05$) lower at 200mg/kg diet supplementation compared to that of antibiotic supplemented group. The egg shell percent varied from 9.47 to 9.82 and was comparable among the dietary treatment groups. Haugh unit improved significantly ($P<0.05$) due to supplementation of AMP at all the levels of supplementation. No difference in Haugh unit could be observed due to various levels of dietary supplementation of AMP (100 to200 mg /kg diet) in the diets of Vanaraja laying hens.

Serum biochemical parameters and enzyme activities

Dietary supplementation of AMP in the diet significantly ($P<0.05$) increased the serum protein concentration compared to antibiotic supplementation. However, no difference in serum protein concentration could be noticed by enhancing the concentration of AMP from 100 mg/kg diet to 200 mg/kg diet. The concentration of albumin, globulin, creatinine, uric acid, calcium and phosphorus did not differ significantly due to dietary supplementation of AMP in Vanaraja laying hens. Dietary supplementation of AMP @ 100 mg/kg diet had no change on serum cholesterol concentration but increasing the dietary levels of AMP to 150 mg/kg diet significantly ($P<0.01$) reduced the serum cholesterol concentration. Further increase in the levels of AMP supplementation to 200mg/kg diet did not have any additional advantage in reducing serum cholesterol to further lower levels.

Dietary supplementation of AMP reduced the serum alkaline phosphatase concentration significantly ($P<0.05$) at all the levels compared to antibiotics supplemented group. No difference in the activities of alkaline phosphatase could be noticed due to the levels of AMP supplementation. The concentration of serum aspartate amino transferase and alanine amino transferase did not changed by the dietary addition of AMP in the diet.

Economics

Taking into consideration the composition of four rations the cost per kg feed was Rs 20.90 (T_1), Rs 20.75 (T_2), Rs 20.90 (T_3) and Rs 21.05 (T_4) during entire experimental period. The egg production of the different dietary groups was comparable in the course of experimental period. The cost of feed per egg produced in the antibiotic supplemented group was Rs 5.46. The cost of feed per egg produced in 100, 150 and 200 mg/kg diet AMP supplemented group was Rs 5.35, Rs.5.23 and Rs.5.37, respectively. Dietary supplementation of AMP reduced the feed cost per egg in the entire AMP supplemented groups. The feed cost was reduced by 2.01, 4.21 and 1.65%, respectively. But the reduction in feed cost was found to be non-significant.

CONCLUSION

Following conclusions might be drawn in view of the results obtained from the present experimental study.

- The body weight gain improved significantly ($P<0.001$) due to dietary supplementation of antimicrobial peptide (cecropin II) compared to the antibiotic (oxytetracycline) supplemented group.
- The hen housed egg production, egg weight; egg mass and feed efficiency did not differ significantly due to dietary supplementation of AMP in Vanaraja laying hens.
- Dietary supplementation of AMP increased the albumen per cent and decreased yolk per cent in eggs compared to that of antibiotic supplementation. Haugh unit increased significantly due to supplementation of AMP at all the levels of supplementation. No difference in Haugh unit could be observed due to various levels of dietary supplementation of AMP in Vanaraja laying hens.
- Dietary supplementation of AMP in the diet significantly ($P<0.05$) increased the serum protein concentration compared to antibiotic supplementation. The concentration of albumin, globulin, creatinine, uric acid, calcium and phosphorus did not differ significantly due to dietary supplementation of AMP in Vanaraja laying hens. Dietary supplementation of AMP @ 150 mg/kg diet significantly reduced the serum cholesterol concentration.
- The concentration of serum aspartate amino transferase and alanine amino transferase did not influenced by the dietary supplementation of AMP in the diet. However, dietary supplementation of AMP reduced the serum alkaline phosphatase concentration significantly at all the levels compared to antibiotics supplemented group.
- The cost of feed per egg produced in the antibiotic supplemented group was Rs 5.46. The cost of feed per egg produced in 100, 150 and 200 mg/kg diet AMP supplemented group was Rs 5.35, Rs.5.23 and Rs.5.37, respectively and was reduced marginally by 2.01, 4.21 and 1.65%, respectively.
- It is concluded that dietary supplementation of antimicrobial peptide (AMP) cecropin II could replace antibiotics for eliciting optimum performance in laying hens.

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