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Effect of Dietary Supplementation of Antimicrobial Peptide on Production Performance, Egg Quality and Serum Biochemical Parameters of Laying Hens

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ABSTRACT

Background: Antibiotic resistance has become more and more serious problem with worldwide human health. 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 Antimicrobial peptides (AMPs). The present study was as conducted to study the effect of dietary supplementation of Antimicrobial peptide on production performance, egg quality and serum biochemical parameters of laying hens.

Methods: One hundred twenty (120), Vanaraja laying hens of 26 weeks of age were selected at random and divided into four groups of 30 each with three replicates of 10 in each group in a complete randomized design. Four experimental diets were fed to birds in which T_1 (control- antibiotic powder-oxytetracycline @ 50 g/ quintal) and in rest of the 3 treatment groups T_2, T_3 and T_4, the birds were fed with, antimicrobial peptide (AMP, cecropin II) at the concentration of 10, 15 and 20 g/quintal of feed, respectively.

Result: 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). Haugh unit increased significantly due to supplementation of AMP at all the levels of supplementation. It is concluded that dietary supplementation of antimicrobial peptide (AMP), cecropin II could replace antibiotics for eliciting optimum performance in laying hens.

Key words: AMP (Cecropin II), Antibiotics, Egg quality, Laying hens,Production performance, Serum biochemical profile.

INTRODUCTION

Poultry products such as egg and meat supply high quality readily digested protein and, energy and are a compact source of readily available micronutrients. Chickens, therefore, have received more attention in Indian planning, research and development process. The major challenges facing in the poultry industry are maintaining significant profit with better quality product and attain good response from human and environment. Under intensive system of poultry production birds are exposed to considerable stresses, which adversely affect their production performances. In such conditions, antimicrobial feed additives such as antibiotics and synthetic antimicrobial agents are frequently used to suppress or eliminate detrimental organisms in the intestine, thereby civilizing growth and feed effectiveness in conventional livestock and poultry production. 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, 2014). Concurrent with the success of antibiotics for treating infections, the emergence and speedy diffusion of antibiotic-resistant bacteria poses considerable risks for human health. 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 antimicrobial peptides (AMPs).

Antimicrobial peptides are biological molecules having size less than 10 kDa and pose both primary and secondary structure, are potent peptides against wide range of microbes such as bacteria, fungi and 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 (Zhang *et al.* 2005). Besides this it ensures,

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eubiosis, nutrient digestibility (Jin *et al.* 2008) by maintaining the population of good bacteria in the host and improves intestinal health, increases egg production and improved egg quality (Chen *et al.,* 2020) and immune response (Shan *et al.* 2007; Tang *et al.* 2009). The present study was conducted to study the effect of dietary supplementation of antimicrobial peptide on production performance, egg quality and serum biochemical parameters of laying hens.

MATERIALS AND METHODS

Stock, diets and husbandry

One hundred twenty (120), laying hens (Vanaraja) of 26 weeks of age were selected at random and divided into four groups of 30 each with three replicates of 10 in each group in a complete randomized design. As a whole the birds were kept in 12 pens with a floor area of 18 square feet each (6ft×3ft). The birds were kept under deep litter system of housing. Rice husk was used as litter material. A basal diet based on maize-soybean-deoiled rice bran was prepared. Subsequently four experimental diets were prepared as T1-diet with (OTC) @ 50 g/ quintal and rest of the 3 treatment groups T_2, T_3 and T_4, the diet with antimicrobial peptides at the concentration of 10, 15 and 20 g/quintal of feed, respectively (Table 1). The antimicrobial peptide used in the present study was Cecropin II isolated from *Bacillus subtilisis* (Subtide, INTAS Pharmaceuticals Ltd. India). A measured quantity of feed was given on daily basis. Standard management practices were followed and clean drinking water was made available *ad lib* throughout the experimental period of 8 weeks. The experiment protocol was approved by the institute animal ethics committee and experiment was conducted utilizing the experimental facility at ICAR-Central Institute for Women in Agriculture, Bhubaneswar, Odisha during 2020.

Parameters studied

Body weight, egg production and egg weight

Individual body weight of the bird was recorded at the beginning (26 wk) and subsequently at the end (34 wk) of the experiment. Egg production was recorded daily on individual basis and percent hen day egg production (HDEP) was calculated. The average feed consumption was recorded as g/hen/day and feed conversion ratio was calculated as g feed consumed per g egg mass produced. All the eggs laid during the last five consecutive days of every 28 days period were collected to measure the egg weight.

Egg quality

Twelve eggs were randomly chosen in each treatment from the eggs laid during the last three consecutive days of each 28-day period to measure the shell weight, shell thickness

Table 1: Ingredient and nutrient composition of experimental diets (% as such basis).

Ingredients	Parts 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	10.4	10.4	10.4	10.4
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	0.4	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	0.05	0.05	0.05
Oxytetracycline	0.05	0.05	0.05	0.05
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).

Composition: Each 1 kg trace min CB contains Manganese: 90 g, Zinc: 80 g, Iron: 90.0 g, Copper: 15.0 g, Iodine: 2.0 g, Selenium: 300 mg AMP-Antimicrobial peptide.

and Haugh unit (HU). The cleaned egg-shells were dried for 24 h, weighed and expressed as % of whole egg. The shell thickness was measured at two different locations (broad and narrow ends) using a screw gauge.

Serum bio-chemical parameters

On 34th week of experimental feeding, around 3 ml of blood was collected from brachial vein from 12 birds in each dietary treatment. Subsequently serum was separated and the concentrations of total protein, albumen, globulin, calcium, phosphorous, cholesterol, creatinine uric acid triglycerides and enzymes (alanine transaminase, aspartate transaminase and alkaline phosphatase), were estimated in the serum by auto-analyzer using diagnostic kits (Coral Clinical Systems, Goa, India).

Statistical analysis

The data obtained from the experiment were statistically analyzed according to Snedecor and Cochran (1994). The data were subjected to analysis of variance (ANOVA) and Duncan multiple range (DMR) test (Duncan, 1955) to test the difference between treatments means, wherever necessary.

RESULTS AND DISCUSSION

The effect of dietary supplementation of AMP on body weight and body weight gain of laying hens is presented in Table 2. 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 dietary levels of AMP supplementation (100, 150 and 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). Concomitant to the finding of the present 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 supplementation of 60 mg AMP-P5/kg in Ross broiler chickens improved the growth performance, higher overall (0-35 d) body weight gain and increased nutrient retention of dry matter and nitrogen. Wen and He (2012) reported that dietary supplementation with an antimicrobial peptide, a cecropin hybrid (artificial), increased the apparent digestibility of crude fat, increased nitrogen retention and improved apparent metabolizable energy in broiler chickens, which could be the reasons for higher body weight gain. In the current study, birds of all the dietary grouped 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.

The production performance of laying hens during the experimental period of 8 weeks is presented in Table 3. 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 contrast to the findings of the present study, Chen *et al.* (2020) reported significantly increased in egg production and decreased feed/egg ratio in Brown laying hens due to

Table 2: Effect of antimicrobial peptide (cecropin II) supplementation on body weight body weight gain of laying hens (26-34 wks).

Parameters	Oxytetracycline (500 mg/kg diet)	AMP (mg/kg diet)			SEM	P value
		100	150	200		
Body weight (g), 26 wk	2270	2259	2378	2324	19.85	0.119
Body weight (g), 34 wk	2422[a]	2560[b]	2651[b]	2554[b]	23.59	0.005
Body weight gain (g), 26-34 wks	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.

AMP- Antimicrobial peptide.

Table 3: Effect of antimicrobial peptide (cecropin II) supplementation on production performance of laying hens.

Parameters	Oxytetracycline (500 mg/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

SEM-Standard error of mean.

AMP-Antimicrobial peptide.

dietary supplementation of AMP (50-100 mg/kg diet). On the other hand, Zhang *et al*. (2016) 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. The beneficial effects of AMPs on performance are mostly due to their antimicrobial and immunomodulating activity, their by enhancing nutrient digestibility and health (Wang *et al.* 2016).

The effect of dietary supplementation of AMP on egg quality parameters of laying hens is presented in Table 4. Dietary supplementation of AMP increased the albumen percent in eggs compared to that of antibiotic supplementation. However, significant improvement in albumen percent in eggs was noticed at AMP supplementation @ 200 mg/kg diet compared to that of antibiotic supplementation (oxytetracycline, 500 mg/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 decreased 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 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. 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. 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. The eggshell thickness varied from 0.341 to 0.353 mm and was comparable among the dietary groups.

The various serum biochemical parameters (albumin, globulin, phosphorus, uric acid and creatinine) studied except serum total protein, calcium and serum cholesterol concentrations were not influenced due to dietary supplementation of AMP in the diet of laying hens (Table 5). In the present study Dietary supplementation of AMP in the diet significantly (P<0.05) increased the serum protein and calcium concentration compared to antibiotic supplementation. However, no difference in serum protein and calcium concentration could be noticed by enhancing the concentration of AMP from 100 mg/kg diet to 200 mg/kg diet. Dietary supplementation of AMP @ 100 mg/kg diet had no influence 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 200 mg/kg diet did not have any additional advantage in reducing serum cholesterol to further lower levels. Previous studies suggest that AMPs beneficially affect the host animal by improving its intestinal health and creating the microbial ecology that suppresses harmful microorganisms like Clostridium and by favoring proliferation of beneficial microorganisms, *e.g*., *Lactobacillus and Bifidobacterium* (Yoon *et al.* 2012). In pigs, the antimicrobial peptide lactoferrin significantly reduced the total viable counts of Escherichia coli and Salmonella, and increased the *Lactobacillus* and *Bifidobacterium* counts in the small intestine compared with the control group (Wang *et al,* 2007).

Dietary supplementation of AMP in the diet of laying hens did not influence the activities of AST and ALT enzymes in serum (Table 6). But, dietary supplementation of AMP reduced the serum alkaline phosphatase (ALP) concentration significantly (P<0.05) at all the levels compared to antibiotics supplemented group. The activity of AST and ALT in the serum is of diagnostic significance in clinical conditions of myocardial infection and hepatitis

Table 4: Effect of antimicrobial peptide (cecropin II) supplementation on egg quality parameters of laying hens (34 wks).

Parameters	Oxytetracycline (500 mg/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; (P<0.01).

SEM- Standard error of mean.

AMP- Antimicrobial peptide.

Table 5: Effect of antimicrobial peptide (cecropin II) supplementation on serum biochemical indices of laying hens.

Parameters	Oxytetracycline (500 mg/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[a]	13.02[b]	13.14[b]	12.98[b]	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; (P<0.01).

SEM- Standard error of mean.

AMP- Antimicrobial peptide.

Table 6: Effect of antimicrobial peptide (cecropin II) supplementation on serum enzyme activities of laying hens.

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

[a,b]Means with different superscript in a row differ significantly; (P<0.05).

SEM- Standard error of mean.

ALT- Alanine Transaminase, AST- Aspartate transaminase, ALP- Alkaline phosphatase.

AMP- Antimicrobial peptide.

(La Due *et al.* 1954). Since, liver and heart are rich in transaminases, large scale destruction of cells due to abnormal condition could result in liberation of the enzyme into circulating blood stream, thereby increasing the concentration in serum.

CONCLUSION

In the present study, none of the production parameters studied was affected due to dietary supplementation of AMP by replacing antibiotic powder (oxytetracycline at 50 g/quintal) in the diet of Vanaraja laying hens. Dietary supplementation of AMP increased the albumen percent 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. Dietary supplementation of AMP increased serum protein concentration and significantly reduced the serum cholesterol concentration (AMP @ 150 mg/kg diet). AMP supplementation also reduced the stress levels by lowering serum ALP activity. From the findings of the present study, it is concluded that dietary supplementation of antimicrobial peptide (AMP) cecropin II (@100 g/kg diet; 10 g/quintal) could replace antibiotics (oxytetracycline@500 mg/kg diet; 50 g/quintal) for eliciting optimum performance in laying hens.

Conflict of interest: None.

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