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Effect of dietary supplementation of *Azadirachta indica* leaf meal on egg-nutrient profile and production economics of vanaraja laying hens

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Abstract
An experiment was conducted to study the effect of dietary supplementation of *Azadirachta indica* leaf meal (NLM) on egg-nutrient profile and production economics of Vanaraja laying hens for a period of 12 weeks. One hundred twenty, Vanaraja laying hens of 24 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 @ 50g/ quintal) and in rest of the 3 treatment groups T_2, T_3 and T_4, the birds were fed with NLM at levels of 0.25, 0.5 and 1.0 per cent, respectively. The nutrient composition of egg did not show any significant difference among the different treatment groups. Also, amino acid profile did not differ significantly among the treatment groups. In production economics, the cost of feed/egg produced did not vary significantly ($P>0.05$) between the different dietary groups Thus, from the present findings NLM up to 1% level in the diet of Vanaraja laying hens is beneficial in terms of egg quality characteristics and production performance when compared to antibiotic powder supplementation.

Keywords: Neem leaf meal, production economics, egg nutrient composition, vanaraja, amino acid

Introduction
Rearing of poultry birds for commercial production of egg and meat is one of the India's finest pioneering industries. Chicken earned a unique tag for the best converter of agricultural by products and nutritionally poor feed stuffs into superior quality meat and egg [1]. The reputation of poultry meat is augmenting for the last three decades and is presently accounting for more than half of the total meat consumed in the country. However, the commercial poultry activity is resolute to a few regions of our country and that too mainly focused on urban and semi-urban areas. But the rural poultry sector picture remains untouched [2]. Vanaraja is a dual-purpose chicken variety developed by the ICAR- Directorate of Poultry Research, Hyderabad, India, aimed at rural communities where it can be reared in backyard on natural, scavenged food with minimal supplementation [3]. These birds are suitable for the backyard farming systems and feeding them with *Azadirachta indica* (Neem) leaf meal (NLM) would reduce the feed cost with enhancement in their immunity status, production performances and welfare. Thus, the major cause of concern among the poultry farmers are the feed cost and diseases encountered in due course of rearing for which reckless usage of antibiotics are added for its prevention leading to antibiotic resistance and its residual effect [4]. The naturally occurring semi-synthetic or synthetic compounds with antimicrobial activity is called antibiotics and are most extensively used drugs in the poultry industry [5]. These drugs are administered parenterally or intravenously, topically, and orally [6]. Owing to the greater nutritional demand from the ever growing population has led to intensification of poultry production within the available space and time. As the intensity per unit space increased for the poultry, the chances of getting disease and its spread has rocketed many folds than the normal rearing condition. In this context, farmers are forced to use antibiotics as a token on prophylactic measure as well as in the form of growth promoter [7]. The extensive use of antibiotics, however, has been banned in developed countries, but the situation is quiet contrary in developing or underdeveloped countries due to inadequate safety standards and regulations or in some cases these standards or regulations do not even exist [6]. If these antibiotics used as feed supplement is not absorbed, or if they are metabolized by the animal, it would be harmless to products and does not cause a problem. But, that is not what always happens. Hence, unsafe drug residues tend to accumulate in various concentrations in the

edible parts of antibiotic fed birds [8]. Ingestion of tissues and organs (meat, offals, eggs, etc.) containing drug remnants above safe maximum residual levels leads to numerous health hazards and vulnerabilities [9] directly as initiation of hypersensitive or allergic reactions, cutaneous eruptions, dermatitis, alteration of intestinal microflora etc., indirectly as carcinogens, teratogens, development of antibiotic resistances among microbial strains, and often leads to drug toxicity in human beings [10]. Thus, it's high time to replace the antibiotics with photobiotics which will provide the same beneficial effects without any harmful effects and NLM is an ideal candidate for the same.

Neem leaves have been found to be useful in relieving pain, fevers, infections and other complaints that it has been called the "village pharmacy" [11]. Generally, Neem leaves contain (on dry matter basis) 185.1 to 206.8 g/ kg crude protein, 174.2 to 245.7 g/ kg crude fiber, 24 to 45 g/ kg ether extract, 406 to 502 g/ kg nitrogen free extract and 65 to 72 g/kg ash [12]. In addition, neem leaves also have role in improving the immune system of the body. Increase in antibodies against Infectious bursal disease and Newcastle disease viruses had been reported by incorporation of Neem in poultry feeds [13]. Neem leaves exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties [13, 14, 16]. Owing to these properties, neem leaf meal has been tried as a feed additive in animals. Neem leaves contain compounds with proven antimicrobial activity [17, 18]. The antimicrobial activity of extracts of neem leaves against micro-organisms as *Staphylococcus spp., Streptococcus spp., Pseudomonas spp.* and *Escherichia coli*, and some fungal strains have been reported [18, 19, 20]. Antimicrobial studies on the effects of neem leaves and their extracts on cultured micro-organisms in vitro have also been carried out [20]. The present study focuses on the effect of dietary supplementation of *Azadirachta indica* leaf meal (NLM) on egg-nutrient profile and production economics of Vanaraja laying hens reared under intensive system of rearing.

Materials and Methods

Stock, diets and husbandry

One hundred twenty (120), Vanaraja laying hens of 24 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. Experiment was conducted for 12 weeks. These birds were placed in 12 pens having floor space of 18 sq. ft. each. Rice husk was used as litter material. NLM was prepared by sun drying, followed by grinding the leaves. In control group T_1, the birds were fed with antibiotic powder – oxytetracycline (OTC) @ 50g/ quintal and in rest of the 3 treatment groups T_2, T_3 and T_4, the birds were fed with Neem leaf meal (NLM) at levels of 0.25, 0.5 and 1.0 per cent, respectively (Table. 1). A measured quantity of feed was given on daily basis. The birds were given restricted amount of 125g of feed per bird for first six weeks, followed by 130g of feed for next six weeks. Standard management practices were followed and clean drinking water was made available *ad lib.* Throughout the experiment.

Nutrient Composition of Eggs

For analyzing the egg composition 12 eggs (three eggs per replicate) were randomly chosen from each dietary treatment from the eggs laid during the last three consecutive days of the experiment. The protein and fat content of the eggs were analyzed by following the method of [21] official method of analysis. The amino acid analysis of eggs was done at EVONIK, Mumbai, India.

Economics

The effect of providing different dietary supplementation with the antibiotic powder and various concentration of NLM levels on the cost of feed per egg produced during the experimental period was calculated. Cost of ingredients in local market was taken into account for calculation of feed cost (Table 2). The feed cost per bird and for egg produced was calculated basing on the feed cost (Table 3) and quantity of feed consumed during the experimental period.

Statistical Analysis

The data obtained from the experiment were statistically analyzed according to [22]. The data were subjected to analysis of variance (ANOVA) and Duncan Multiple Range (DMR) Test [23] to test the difference between treatments means, wherever necessary.

Results and Discussion

The effect of dietary supplementation of NLM on nutrient composition of eggs of Vanaraja laying hens for the duration of 25-36 weeks is being depicted in the Table 4. There was no significant effect of dietary supplementation of NLM on nutrient composition of eggs of Vanaraja laying hens for the period of 25-36 weeks. The analyzed protein and fat per cent of eggs varied from 41.55 (T_2) to 48.96 (T_4) and 40.73 (T_2) to 47.16 (T_4) respectively on dry matter basis.

The effect of dietary supplementation of NLM on the Amino acid profile of eggs of Vanaraja laying hens for the duration of 25-36 weeks has been depicted in Table 5. The amino acid composition of dried eggs (after hydrolysis) from Vanaraja laying hens fed with NLM as dietary supplement did not vary significantly. NLM supplementation up to 1 per cent level in the diet of Vanaraja laying hens had no influence on egg nutrient composition or amino acid profile. To our knowledge, there is no available information in literature on the influence of NLM on egg nutrient composition in general and amino acid profile in particular. The results of the present study suggested that dietary inclusion of NLM up to 1 per cent level did not affect the egg nutrient composition and amino acid profile. The cost of feed per egg produced of Vanaraja laying hens during the dietary supplementation of NLM is presented in Table 6.Considering the composition of four rations the cost per kg feed was Rs 21.14 (T_1), Rs 20.61 (T_2), Rs 20.57 (T_3) and Rs 20.49 (T_4) during entire experimental period. During 25 to 30 weeks of age the feed consumption per bird was 5.25 kg in each treatment group. The cost of feed consumed per bird was Rs 110.99, Rs 108.20, Rs 107.99, and Rs 107.57 in different treatment groups T_1, T_2, T_3 and T_4, respectively. The egg production per bird and the cost of feed per egg did not show any significant difference ($P>0.05$) between the different treatment groups. During 31 to 36 weeks of age the feed consumption per bird was 5.46 kg in each treatment group. The cost of feed consumed per bird was Rs 115.42, Rs 112.53, Rs 112.31, and Rs 111.88 in different treatment groups T_1, T_2, T_3 and T_4, respectively. The egg production per bird and the cost of feed per egg produced did not show any significant difference ($P>0.05$) between the different treatment groups.

During 25 to 36 weeks of age the feed consumption per bird

was 10.71 kg in each treatment group. The cost of feed consumed per bird was Rs 226.41, Rs 220.73, Rs 220.30 and Rs 219.45 in different treatment groups T_1, T_2, T_3 and T_4, respectively. The egg production per bird showed no significant difference (P>0.05) between the different treatment groups. The highest mean values were observed among the treatment groups T_1 and T_4. The cost of feed per egg revealed no significant difference (P>0.05) between the different treatment groups and all the mean values were comparable among themselves. The cost of feed per egg produced was lowest in T_3 (Rs. 3.68) followed by T_1 (Rs. 3.80), T_2 (Rs. 3.83) and T_4 (Rs. 3.89) in increasing order.

The cost of feed per egg produced was comparable among all the treatment across the study. These findings of the present study revealed that NLM can be included in the diet of Vanaraja laying hens up to 1per cent level without affecting the cost of production. In agreement with the findings of the present study, [24] reported similar cost of production (kg feed/ kg egg) due to dietary inclusion of NLM up to 2.5 per cent. The same researchers, however, reported higher feed cost when NLM was included beyond 2.5 per cent (5, 7.5, and 10 per cent). In another study with the commercial broiler chickens, [25] reported optimum performance and economic benefits by including NLM up to 2 per cent in the diet.

Table 1: Ingredient and nutrient composition of experimental diets (% air dry basis) with Neem leaf meal

Ingredients	Parts per quintal			
	T_1	**T_2**	**T_3**	**T_4**
Yellow Maize	58.46	58.26	58.01	57.51
Soyabean meal	20.7	20.7	20.7	20.7
Deoiled rice bran	10.2	10.2	10.2	10.2
Shell grit	8.7	8.7	8.7	8.7
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	0.052	0.052	0.052	0.052
Toxin Binder	0.052	0.052	0.052	0.052
Oxytetracycline	0.05	-	-	-
NLM	-	0.25	0.5	1
Total	100	100	100	100
Nutrient composition (Calculated value)				
ME(kcal/kg)	2601	2609	2617	2625
CP (%)	15.99	16.04	16.09	16.14
Lysine (%)	0.79	0.70	0.81	0.90
Methionine (%)	0.31	0.33	0.35	0.35
Calcium (%)	3.23	3.24	3.25	3.24
Phosphorous (%)	0.35	0.35	0.35	0.35

*TraceMin CB (Venky's India Private Limited, Pune).
Composition: Each 1 kg Trace Min CB contains Manganese: 90g, Zinc: 80 g, Iron: 90.0g, Copper: 15.0g, Iodine: 2.0g, Selenium: 300mg.

Table 2: Cost of feed ingredients

Sl. No.	Ingredients	Cost (Rs)/kg
1	Maize	16.00
2	Soya Bean Meal	42.00
3	Deoiled Rice Bran	12.00
4	Shell Grit	4.00
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

Table 3: Cost of experimental diet with Neem leaf meal

Treatment No.	Treatments	Feed (Rs)/kg
T_1	Diet with 50 g/quintal OTC	21.14
T_2	Diet with 0.25% NLM	20.61
T_3	Diet with 0.5% NLM	20.57
T_4	Diet with 1.0% NLM	20.49

OTC-Oxytetracycline
NLM-Neem leaf meal

Table 4: Effect of dietary supplementation of Neem leaf meal on the nutrient composition of egg (on dry matter basis)

Parameters (%)	Treatments				SEM	P value
	50 g/ quintal OTC(T_1)	0.25% NLM (T_2)	0.5% NLM (T_3)	1% NLM (T_4)		
Dry matter	94.41	92.83	94.66	94.48	0.845	0.714
Protein	46.48	46.55	47.29	48.96	0.516	0.618
Ether extract	44.80	45.73	45.46	46.16	0.498	0.814

SEM – Standard Error of Mean
OTC-Oxytetracycline
NLM-Neem leaf meal

Table 5: Effect dietary supplementation of Neem leaf meal on the amino acid profile of eggs (on dry matter basis)

Parameters (%)	Treatments				SEM	P value
	50 g/ quintal OTC (T_1)	0.25% NLM (T_2)	0.5% NLM (T_3)	1% NLM (T_4)		
Methionine	1.45	1.23	1.47	1.58	0.014	0.573
Cystine	1.01	0.84	1.05	1.16	0.094	0.622
Methionine +Cystine	2.46	2.07	2.53	2.73	0.023	0.446
Lysine	3.04	2.72	3.15	3.20	0.021	0.326
Threonine	2.08	1.84	2.13	2.23	0.084	0.684
Arginine	2.77	2.50	2.82	3.02	0.092	0.521
Isoleucine	2.46	2.17	2.51	2.61	0.048	0.638
Leucine	3.95	3.49	4.03	4.12	0.024	0.557
Valine	2.95	2.58	2.99	3.08	0.023	0.478
Histidine	1.14	0.98	1.15	1.18	0.008	0.359
Phenyl alanine	2.36	1.99	2.37	2.45	0.126	0.635
Glycine	1.51	1.31	1.53	1.59	0.020	0.798
Serine	3.23	2.87	3.31	3.49	0.041	0.483
Proline	1.78	1.54	1.78	1.88	0.018	0.536
Alanine	2.57	2.22	2.61	2.74	0.027	0.582
Aspartic acid	4.59	3.98	4.65	4.83	0.036	0.428
Glutamic acid	5.86	5.12	5.94	6.11	0.037	0.472

SEM – Standard Error of Mean
OTC-Oxytetracycline
NLM-Neem leaf meal

Table 6: Cost of feed/egg produced (Rs.) of Vanaraja laying hens during different laying period with the dietary supplementation of Neem leaf meal

Particulars	Treatments				SEM	P value
	50 g/ quintal OTC (T_1)	0.25% NLM (T_2)	0.5% NLM (T_3)	1% NLM (T_4)		
Cost of feed (Rs/kg)	21.14	20.61	20.57	20.49		
25-30 weeks period						
Feed consumption per bird (kg)	5.25	5.25	5.25	5.25		
Cost of feed consumed per bird (Rs)	110.99	108.20	107.99	107.57		
Egg production per bird (No.)	29.42	28.58	29.17	27.83	0.256	0.485
Cost of feed per egg produced (Rs)	3.77	3.79	3.70	3.87	0.152	0.211
31-36 weeks period						
Feed consumption per bird (kg)	5.46	5.46	5.46	5.46		
Cost of feed consumed per bird (Rs)	115.42	112.53	112.31	111.88		
Egg production per bird (No.)	30.83	29.50	32.17	29.42	0.224	0.277
Cost of feed per egg produced (Rs)	3.74	3.81	3.49	3.80	0.156	0.275
25-36 weeks period						
Feed consumption per bird (kg)	10.71	10.71	10.71	10.71		
Cost of feed consumed per bird (Rs)	226.41	220.73	220.30	219.45		
Egg production per bird (No.)	59.55	57.62	59.84	56.46	0.688	0.189
Cost of feed per egg produced (Rs)	3.80	3.83	3.68	3.89	0.117	0.109

SEM – Standard Error of Mean
OTC-Oxytetracycline
NLM-Neem leaf meal

Conclusion

From the findings of the present study, it can be concluded that NLM up to 1 per cent level in the diet of Vanaraja laying hens is beneficial in terms of nutrient composition of egg and cost economics of egg production.

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