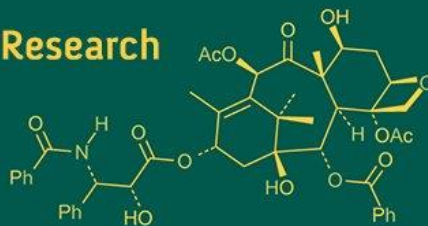
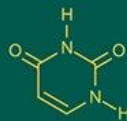
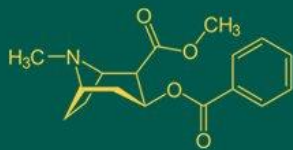


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(5): 143-146
www.biochemjournal.com
Received: 18-03-2026
Accepted: 20-04-2026

Ankesh Marode
Department of Poultry
Science, C.V.Sc. & A.H.,
NDVSU, Jabalpur, Madhya
Pradesh, India

Ambati Sai Ganesh
Department of Poultry
Science, C.V.Sc. & A.H.,
NDVSU, Jabalpur, Madhya
Pradesh, India

Laxmi Chouhan
Department of Poultry
Science, C.V.Sc. & A.H.,
NDVSU, Jabalpur, Madhya
Pradesh, India

Anil Shinde
Department of Poultry
Science, C.V.Sc. & A.H.,
NDVSU, Jabalpur, Madhya
Pradesh, India

Girraj Goyal
Department of Poultry
Science, C.V.Sc. & A.H.,
NDVSU, Jabalpur, Madhya
Pradesh, India

Nethee Deori
Department of Poultry
Science, C.V.Sc. & A.H.,
NDVSU, Jabalpur, Madhya
Pradesh, India

Corresponding Author:
Laxmi Chouhan
Department of Poultry
Science, C.V.Sc. & A.H.,
NDVSU, Jabalpur, Madhya
Pradesh, India

Effect of dietary folic acid enrichment on biochemical parameters and sensory evaluation in kadaknath birds

Ankesh Marode, Ambati Sai Ganesh, Laxmi Chouhan, Anil Shinde, Girraj Goyal and Nethee Deori

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5b.8344>

Abstract

Folate or folic acid (FA), also known as pteroylglutamic acid, is a water-soluble vitamin. Enrichment of eggs with folic acid through dietary manipulation of laying hens is a promising strategy to improve human folate intake. Limited information is available on folic acid supplementation in indigenous kadaknath laying hens. The present study was conducted for 12 weeks (25-36 weeks of age) using 120 kadaknath laying hens distributed into five dietary treatments with three replicates of eight birds each. Birds were fed a basal diet supplemented with folic acid at 4 (control), 8, 16, 32 and 64 mg/kg diet. Performance parameters, egg production, egg quality traits, egg yolk folate concentration, haematological and biochemical parameters, sensory attributes and economics of egg production were evaluated. Data were analysed using completely randomized design. Dietary folic acid supplementation had no adverse effect on body weight and feed intake. Egg production, egg mass and feed conversion ratio showed numerical improvement in folic acid-supplemented groups. Egg quality traits were maintained. Egg yolk folate concentration increased significantly with increasing dietary folic acid levels. Haemoglobin, packed cell volume and total erythrocyte count improved in supplemented groups without adverse biochemical alterations. The study demonstrated that folic acid supplementation is effective for producing folate-enriched kadaknath eggs without affecting bird performance.

Keywords: Kadaknath, folic acid, egg folate, biochemical, sensory, laying hens

Introduction

The Indian poultry sector is among the most rapidly expanding agricultural industries and plays a important role in the nation's economic development. India currently holds the second position globally in egg production. During the financial year 2024-2025, egg output recorded a growth of 4.44% compared to the preceding year ^[1]. Eggs are economical and highly nutritious, making them an ideal vehicle for the delivery of functional nutrients. In recent years, designer eggs developed through the nutritional modification of laying hen diets have attracted considerable attention, particularly among health-conscious consumers ^[11].

Folic acid plays a vital role in numerous physiological functions, including cell division, tissue growth and cellular repair ^[2]. A folic acid-rich diet is essential not only for general health but also for preventing various conditions such as cleft lip and palate, neural tube defects, autism spectrum disorders, megaloblastic anaemia and certain cancers ^[3]. The ICMR-NIN sets the RDA (Recommended Dietary Allowances) of folic acid for Indians. For adult male it is 300 µg RDA and 250 µg EAR (Estimated Average Requirements). For adult female it is 220 µg and 180 µg EAR and for pregnant women is 400 µg RDA ^[10].

Kadaknath is a popular local breed in Dhar and Jabua district of Madhya Pradesh, India, famous for its black meat, disease resistance and adaptability. The meat of kadaknath breed contains a high percentage (25.47%) of protein and have appreciable degree of resistance to diseases compared with other exotic breeds of fowl. Hence, the present study was undertaken to evaluate the effect of dietary folic acid supplementation on performance, egg quality, egg folate content and blood parameters in kadaknath laying hens ^[13].

Materials and Methods

The experiment was conducted at the Poultry Farm, All India Coordinated Research Project (AICRP) on Poultry Breeding, NDVSU, Jabalpur, Madhya Pradesh, for a period of six

months. A total of 120 kadaknath laying hens of 25 weeks of age were randomly allocated to five treatment groups with three replicates of eight birds each. The dietary treatments consisted of a basal layer ration (16% CP, 2600 kcal ME/kg) supplemented with folic acid at 4 mg/kg (T_0), 8 mg/kg (T_1), 16 mg/kg (T_2), 32 mg/kg (T_3) and 64 mg/kg (T_4). All diets were isocaloric and isonitrogenous and formulated as per ICAR (2013) standards [9].

Biochemical parameters (AST, ALT and creatinine). Sensory evaluation of boiled eggs was conducted using a nine-point hedonic scale. Statistical analysis was carried out using analysis of variance (ANOVA) under completely randomized design and treatment means were compared using Duncan's Multiple Range Test [4, 12].

Rearing of Kadaknath birds: A fortnight before the start of the experiment, a layer shed housing facility was properly cleaned, disinfected with phenyl, white-washed and dried up. The weighed quantity of the experimental ration was offered daily in each pan as per the treatment group. Waterers were washed daily and clean drinking water was provided. All management factors were kept identical for all treatment groups. The kadaknath layer birds were reared up to 12 weeks of age for the collection of data.

Experimental design and distribution of birds: A total number of 120 Kadaknath birds of 25 weeks old were selected and procured from the Poultry farm, AICRP on Poultry Breeding, Department of Poultry Science, College of Veterinary Science and Animal Husbandry, Jabalpur. For this research, 120 Kadaknath laying hens were used. There were 5 treatment groups with 3 replicates, each with 8 birds. The house was completely cleaned and disinfected. It was then kept empty for three days. Birds were housed in a cage housing system, with each bird having its own cage for the

experimental period of 12 weeks. The necessary feeding and watering arrangements were carried out in accordance with the ICAR-2013 feeding standard [9].

Kadaknath were fed a diet (ME-2600 Kcal/kg, CP-16%) between 25 and 36 weeks of age. Throughout the trial, all of the meals given to the various treatment groups were isocaloric and isonitrogenous.

Biochemical parameter: Blood samples were collected from each bird in plain vacutainers, without the addition of any anticoagulants. Serum was then separated by centrifugation at 3,000 rpm for 5 minutes. The resulting serum was carefully transferred into labelled sterilized Eppendorf vials. The serum samples were subsequently analyzed for various biochemical parameters using a semi-automatic biochemical analyzer (Erba Mannheim) and the corresponding commercial assay kits. Following biochemical parameters were estimated by using commercial kits: Aspartate transaminase (AST) (IU/L) and alanine transaminase (ALT) (IU/L), Creatinine (mg/dl), Calcium (mg/dl) and Phosphorous (mg/dl).

Sensory evaluation of eggs: A trained sensory panel of seven assessors conducted a descriptive evaluation of the characteristics of boiled eggs. Sensory attributes, including flavour and overall acceptability were evaluated using a 9-point structured scale (1 = lowest intensity, 9 = highest) [6]. The eggs were boiled for 12 minutes, cooled, peeled and served immediately for evaluation. To minimize bias, the samples were presented in a randomized order for evaluation.

Results and Discussion

Biochemical parameters

Table 1: Effect of dietary folic acid enrichment on biochemical parameters of Kadaknath birds (Mean $\pm$ SE)

Parameters	Treatments				
	T_0	T_1	T_2	T_3	T_4
AST (IU/L)	167.50 $\pm$ 8.62	159.75 $\pm$ 17.91	154.31 $\pm$ 3.99	156.65 $\pm$ 20.06	158.16 $\pm$ 5.89
ALT (IU/L)	8.61 $\pm$ 0.63	7.79 $\pm$ 1.33	3.38 $\pm$ 2.42	4.47 $\pm$ 0.53	7.41 $\pm$ 0.67
Creatinine (mg/dl)	0.49 $\pm$ 0.08	0.46 $\pm$ 0.06	0.51 $\pm$ 0.05	0.46 $\pm$ 0.03	0.41 $\pm$ 0.03
Calcium (mg/dl)	20.49 $\pm$ 0.08	20.46 $\pm$ 0.06	20.51 $\pm$ 0.05	20.46 $\pm$ 0.03	20.41 $\pm$ 0.03
Phosphorus (mg/dl)	12.72 $\pm$ 1.27	11.16 $\pm$ 1.57	13.03 $\pm$ 0.23	11.94 $\pm$ 0.39	13.11 $\pm$ 0.20

Serum Aspartate aminotransferase (AST) values for T_0 , T_1 , T_2 , T_3 and T_4 were 167.50 $\pm$ 8.62, 159.75 $\pm$ 17.91, 154.31 $\pm$ 3.99, 156.65 $\pm$ 20.06 and 158.16 $\pm$ 5.89 IU/L respectively. There were no significant differences among the treatments. The control group (T_0) recorded the numerically highest AST level, while numerically lower values were observed in folic acid-supplemented groups, particularly in T_2 . These findings indicate that dietary folic acid enrichment did not negatively affect liver function. Serum Alanine aminotransferase (ALT) values were 8.61 $\pm$ 0.63, 7.79 $\pm$ 1.33, 3.38 $\pm$ 2.42, 4.47 $\pm$ 0.53 and 7.41 $\pm$ 0.67 IU/L for T_0 , T_1 , T_2 , T_3 and T_4 respectively. ALT showed minor numerical variations among treatments, ranging from 3.38 $\pm$ 2.42 to 8.61 $\pm$ 0.63 IU/L. There were no significant differences among the treatments. Serum creatinine values were 0.49 $\pm$ 0.08, 0.46 $\pm$ 0.06, 0.51 $\pm$ 0.05, 0.46 $\pm$ 0.03 and 0.41 $\pm$ 0.03 mg/dl for T_0 , T_1 , T_2 , T_3 and T_4 respectively. Creatinine values varied from 0.41 $\pm$ 0.03 to 0.51 $\pm$ 0.05

mg/dl. Birds receiving higher levels of folic acid T_4 showed slightly lower creatinine levels compared to the T_0 .

Serum calcium values for T_0 , T_1 , T_2 , T_3 and T_4 respectively were 20.49 $\pm$ 0.08, 20.46 $\pm$ 0.06, 20.51 $\pm$ 0.05, 20.46 $\pm$ 0.03 and 20.41 $\pm$ 0.03 mg/dl. Serum calcium levels remained remarkably non-significant across all treatments, ranging from 20.41 $\pm$ 0.03 to 20.51 $\pm$ 0.05 mg/dl. Highest value observed in T_2 and lowest value observed in T_4 . Serum phosphorus values were 12.72 $\pm$ 1.27, 11.16 $\pm$ 1.57, 13.03 $\pm$ 0.23, 11.94 $\pm$ 0.39 and 13.11 $\pm$ 0.20 mg/dl for T_0 , T_1 , T_2 , T_3 and T_4 respectively. Slightly higher phosphorus levels were observed in T_2 and T_4 groups. The values remained within normal physiological limits, indicating balanced mineral metabolism irrespective of folic acid level.

The present findings are in agreement with earlier reports [7], demonstrated that dietary folic acid supplementation significantly increased plasma folate concentration without adversely affecting other blood biochemical parameters,

indicating efficient absorption and safe metabolism of folic acid [8], also reported that higher levels of folic acid and vitamin B12 did not cause any adverse biochemical changes, further supporting the safety of folic acid supplementation [5], observed increased serum creatinine, calcium and phosphorus levels when folic acid was supplemented along with vitamin C, suggesting that

combined supplementation may enhance metabolic activity. However, in the present study, folic acid alone did not markedly alter creatinine or mineral levels, which may be attributed to differences in bird type, age and dietary combinations.

Sensory evaluation

Table 2: Effect of dietary folic acid enrichment on sensory evaluation of the Kadaknath eggs (Mean $\pm$ SE)

Parameters	Treatments				
	T ₀	T ₁	T ₂	T ₃	T ₄
Flavour (1-9)	7.25 $\pm$ 0.25	7.00 $\pm$ 0.32	8.21 $\pm$ 0.18	7.46 $\pm$ 0.18	5.75 $\pm$ 0.41
Yolk colour (1-9)	7.25 $\pm$ 0.31	7.00 $\pm$ 0.53	8.15 $\pm$ 0.16	7.87 $\pm$ 0.26	5.75 $\pm$ 0.52
Overall acceptability (1-9)	7.20 $\pm$ 0.32	6.87 $\pm$ 0.35	8.13 $\pm$ 0.16	7.37 $\pm$ 0.26	5.87 $\pm$ 0.47

Kadaknath eggs flavour scores were 7.25 $\pm$ 0.25, 7.00 $\pm$ 0.32, 8.21 $\pm$ 0.18, 7.46 $\pm$ 0.18 and 5.75 $\pm$ 0.41 for T₀, T₁, T₂, T₃ and T₄ respectively. Eggs from birds in treatment T₂ recorded the highest flavour score, indicating superior flavour compared to the control group and other treatments. Treatments T₁ and T₃ were comparable to the control, suggesting that moderate folic acid supplementation did not adversely affect egg flavour. In contrast, T₄ showed a noticeable reduction in flavour score, indicating that higher levels of folic acid enrichment may negatively influence flavour score.

Egg yolk colour scores were 7.25 $\pm$ 0.31, 7.00 $\pm$ 0.53, 8.15 $\pm$ 0.16, 7.87 $\pm$ 0.26 and 5.75 $\pm$ 0.52 for T₀, T₁, T₂, T₃ and T₄ respectively. The highest yolk colour score was observed in T₂, closely followed by T₃. The control and T₁ treatments recorded intermediate scores, while T₄ again showed the lowest score. The enhanced yolk colour in T₂ and T₃ may be attributed to improved nutrient utilization and pigment deposition associated with optimal folic acid supplementation.

Overall acceptability scores for T₀, T₁, T₂, T₃ and T₄ were 7.20 $\pm$ 0.32, 6.87 $\pm$ 0.35, 8.13 $\pm$ 0.16, 7.37 $\pm$ 0.26 and 5.87 $\pm$ 0.47 respectively. Treatment T₂ achieved the highest overall acceptability score, indicating strong consumer preference. T₃ and the control group were comparable and fell within the “liked moderately” category. Eggs from T₁ were slightly less preferred, whereas T₄ showed the lowest overall acceptability (5.87 $\pm$ 0.47), suggesting reduced consumer appeal at higher folic acid levels.

Overall, the results demonstrated that dietary folic acid enrichment had a clear influence on sensory attributes of Kadaknath eggs. Moderate folic acid supplementation, particularly in T₂, improved flavour, yolk colour and overall acceptability, followed by T₃, T₁, T₀, while excessive supplementation (T₄) appeared to adversely affect sensory quality.

Conclusion

Dietary folic acid supplementation significantly increased plasma folate concentration without adversely affecting other blood biochemical parameters, indicating efficient absorption and safe metabolism of folic acid. Folic acid supplementation can be recommended as a nutritional strategy for producing functional eggs from indigenous poultry and moderate folic acid supplementation, particularly in T₂, improved flavour, yolk colour and overall acceptability as compared to T₃, T₁, T₀ and T₄.

Acknowledgment

We express my sincere gratitude to my Principle Investigator of Poultry farm, AICRP on Poultry Breeding, Department of Poultry Science, College of Veterinary Science and Animal Husbandry, Jabalpur for providing me with the opportunity to undertake this research work.

References

1. BAHs. Basic Animal Husbandry Statistics-2025. New Delhi: Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India; 2025. p. 7-8.
2. Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory JF III, Mills JL, *et al.* Biomarkers of nutrition for development folate review. The Journal of Nutrition. 2015;145(7):1636-1680.
3. Czeizel AE, Dudas I, Vereczkey A, Banhidy F. Folate deficiency and folic acid supplementation: the prevention of neural-tube defects and congenital heart defects. Nutrients. 2013;5(11):4760-4775.
4. Duncan DB. Multiple range and multiple F tests. Biometrics. 1955;11(1):1-42.
5. El-Din TT, Hassan RH, El-Samra AE, Hanaa AB. Effects of vitamin C and/or folic acid supplementations in alleviating the negative effects of heat stress in local laying hens. Egyptian Journal of Animal Production. 2008;45:333-346.
6. Hajela S, Srivastava R, Kumari N. Development and nutritional analysis of stevia chocolates fortified with flaxseeds (*Linum usitatissimum*). International Journal of Food Science and Nutrition. 2017;2(6):139-141.
7. Hebert K, House JD, Guenter W. Effect of dietary folic acid supplementation on egg folate content and the performance and folate status of two strains of laying hens. Poultry Science. 2005;84(10):1533-1538.
8. Husseiny OM, Soliman AZ, Omara II, El-Sherif HMR. Evaluation of dietary methionine, folic acid and cyanocobalamin (B) and their interactions in laying hen performance. International Journal of Poultry Science. 2008;7(5):461-469.
9. ICAR. Nutrient requirements of poultry. 3rd ed. New Delhi: Indian Council of Agricultural Research; 2013.
10. ICMR-NIN. Nutrient requirements for Indians: A report of the expert group. Hyderabad: National Institute of Nutrition; 2020.
11. Sahoo A, Jena B. Designer egg and meat through nutrient manipulation. Journal of Poultry Science and Technology. 2014;2(3):38-47.

12. Snedecor GW, Cochran WG. Statistical methods. 7th ed. Iowa: Iowa State University Press; 1989.
13. Valavan SE, Omprakash AV, Bharatidhasan A. Production performance of Kadaknath in an organised poultry farm. International Journal of Applied Agriculture Science. 2016;2(11):125-128.