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By Saravanakumar Marimuthu & Prashanth D'Souza

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Effect of Polyherbal Formulation on the Performance of White Leghorn Layers

Saravanakumar Marimuthu [α] & Prashanth D'Souza [σ]

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Keywords: layer poultry birds; choline chloride; polyherbal formulation; egg production.

I. Introduction

Choline is a constituent of the vitamin B complex and an essential nutrient in the poultry diet (Beheshti Moghadam et al., 2021). It is a vital component for building cell structure, metabolism and mobilization of fat. In laying birds, choline plays a crucial role in the removal of needless fat deposited in the hepatic tissue and prevents fatty liver disease (Chaudhari et al., 2017). It helps in synthesizing phosphatidylcholine (PC) that has a significant function in egg yolk formation (Khairani et al., 2016). Additionally, choline serves as a methyl group donor in methionine synthesis and actively influences egg production as well as the performance of the layers (Chaudhari et al., 2017). Choline is necessary for acetylcholine formation which helps in transmitting the nerve impulses (Chaudhari et al., 2017).

The layer birds often suffer from choline deficiency as they are unable to synthesize abundant choline. So layers necessitate choline intake through their diet (Parsons & Leeper, 1984). It was reported that the inclusion of choline in layers' diet enhances their reproductive performance, egg mass, egg weight, egg yield, albumin height, percentage of egg yolk weight, yolk colour and improves the Haugh unit (Chaudhari et al., 2017; Zhai et al., 2013). Thus, it is an indispensable nutrient in improving egg production and the quality of laying birds. The laying hens need an approximate amount of 1300, 900 and 500 mg/kg choline as a dietary supplement from 0 to 6th, 6th to 12th week and 12th week to age of laying eggs respectively (Chaudhari et al., 2017). Each egg comprises 12-13 mg of choline/g of dried whole egg mass (Chaudhari et al., 2017). According to Dänicke *et al.* (2006), the layers require choline at a concentration of 1500 mg/kg of feed for optimum performance. The dietary requisition of choline in layers as recommended by the US National Research Council (NRC) is 1050 mg/kg to get optimum yield (Zhai et al., 2013).

However, synthetic choline has several drawbacks. Choline is usually converted into trimethylamine (TMA) by gut microbiota which is corrosive in nature and causes toxicity (Selvam et al., 2018). The maximum permissible limit of TMA for birds is 200 ppm (Singh Rajesh Kumar, 2019). It triggers malabsorption and respiratory distress resulting in low

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reproductive performance and even death. Choline develops the lump of particles due to the hygroscopic property. Synthetic choline should have a uniform particle size for obtaining the optimum bio-availability (Singh Rajesh Kumar, 2019). Myriads of literatures have reported that the bioactive substances derived from plants may counter the demerits of synthetic choline (Selvam et al., 2018). Therefore, scientists are actively investigating the choline-like nutritional supplement of herbal origin to replace the synthetic choline from the layer's ration.

Kolin Plus™ is a proprietary polyherbal formulation (POH) comprised of *Acacia nilotica (A. nilotica)* and *Curcuma longa (C. longa)* plant parts developed by M/s Natural Remedies Pvt Ltd, Bengaluru, India. The phytoactive compounds present in POH are catechin, curcumin, gallic acid and polyphenols. They are proven to be antioxidant and lipotropic in nature. In the present study, the effect of POH supplementation in layers fed with a high energy diet (HED) has been explored for the rate of egg production and egg quality in comparison with synthetic choline chloride.

II. Materials and Method

a) Feed supplements

All protocols of the *in vivo* experiments were prepared and approved by Animal Ethics Committee of Natural Remedies Private Limited. The POH used as feed supplement in layers' diet, was comprised of powder of phytopharmaceuticals derived from *A. nilotica* and *C. longa* plant parts. HED was provided to the birds of all groups except normal control. HED contains basal diet with additional100 Kcal metabolic energy (ME). Whereas birds of normal control group were fed with basal diet only. The composition of basal diet and HED was mentioned in table 1. Choline chloride 60% was used as synthetic feed supplement in the experiment.

Table 1: Feed Composition of the layers

Nutrient	Basal Diet	HED
M.E (Kcal/kg)	2550	2650
CP (%)	13.7	13.7
dLysine, %	0.605	0.590
dMethionine+Cysteine, %	0.500	0.500
dTryptophan, %	0.139	0.137
dThreonine, %	0.512	0.498
dIso-leucine, %	0.512	0.500
dValine, %	0.637	0.603
Calcium, %	3.800	3.800
Sodium, %	0.170	0.170

b) Study design

The trial was conducted at Sri Ramadhoodha Poultry Farm, India. The experimental shed for housing the layer birds was maintained with standard room temperature and humidity as per the guidelines. The study was carried out for a duration of 12 weeks. The White Leghorn (WL) layers at the age of 41 to 55 weeks were distributed into 7 (G1 to G7) study groups as described in table 2. All birds except the normal control group were supplied with HED. The layers of G6 and G7 were fed with choline chloride 60% along with HED at a dose level of 500 and 1000 g/ton of feed, respectively. Whereas the birds of G3, G4 and G5 groups were fed with POH added diet at the dose range of 250, 500 and 750 g/ton of feed, respectively.

Table 2: Experimental design

Group	Dose (g/ton)	No. of birds / Replicate	No. of Replicates / Group	Duration
G1:Normal Control	-	20	6	12 weeks
G2:High Energy Diet Control (HED)	-	20	6	
G3:HED + POH	250	20	6	
G4:HED + POH	500	20	6	
G5:HED + POH	750	20	6	
G6:HED + Choline Chloride 60%	500	20	6	
G7:HED + Choline Chloride 60%	1000	20	6	

POH - Polyherbal formulation; HED - High Energy Diet; HED - Basal Diet + 100 Kcal ME. The extra energy should be provided by increasing the carbohydrate levels in feed *c) Assessment parameters*

The birds were monitored for the parameters like egg production percentage, feed intake and egg quality traits. The weekly experimental data of each group were recorded for a period of 12 weeks starting from 43 to 55 weeks of age of the birds and the mean value was calculated. The average value of 3 weeks *i.e.* week 41 to 43 of age of the birds was considered as baseline and used to compare with the recorded weekly values. The average feed consumption and feed intake per egg were estimated from week 1 to 12 of the entire study period. The weekly egg shell defect percentage for a period of 12 weeks study was evaluated. The egg quality parameters like egg density, Haugh unit (HU), egg shell weight and egg shell thickness were measured and compared between the groups.

d) Statistical analysis

All raw data of the trial were compiled and expressed as mean. The statistical analysis was performed using the one-way analysis of variance (ANOVA). The p value <0.05 was considered as statistically significant.

III. Results

The present study outcomes exhibited the effect POH as feed additive along with HED in the performance of the layer birds of 43 to 55 weeks of age. The baseline value of each parameter was calculated as per the average values from week 41 to 43 of the layers. Then the data were normalized using baseline value, and the POH results were compared with the data obtained from normal diet fed, HED fed, choline chloride 60%, and standard hen day production percentage (HDP).

a) Egg production %

As shown in table 3, the normalized egg production percentage (from week 1 to 6) of the layers fed with POH at a dose level of 500 g/ton of feed was similar as that of choline chloride 60% (1000 g/ton) supplementation. The results of normalized egg production rate of 7th to 12th week of POH supplementation at a dose range of 500 g/ton of feed revealed better performance as compared to normal, HED diet fed, and choline chloride 60% supplemented groups. Although, the baseline egg production was less in POH (500 g/ton) group as compared to standard HDP layers, the mean egg production rate (7-12 weeks) of POH was similar as that of standard value.

Table 3: Average percentage of egg production (EP) of layers

Group	Baseline	Average (1-6)	Diff	Average (7-12)	Diff	Average (1-12)	Diff
G1:Normal Control	94.67	90.74	-3.93	88.03	-6.64	89.38	-5.29
G2:High Energy Diet (HED)	93.83	90.10	-3.73	85.67	-8.16	87.88	-5.95
G3:HED + POH 250g	94.75	89.76	-4.99	85.76	-8.99	87.76	-6.99
G4:HED + POH 500g	93.50	90.52	-2.98	91.24	-2.26	90.88	-2.62
G5:HED + POH 750g	95.96	89.60	-6.36	83.08	-12.89	86.34	-9.62
G6:HED + CCL (60%) 500g	94.63	91.41	-3.22	88.53	-6.10	89.97	-4.66
G7:HED + CCL (60%) 1000g	96.54	93.51	-3.03	89.67	-6.87	91.59	-4.95
BV-300 Standard HDP (%)	94.97	93.65	-1.32	91.85	-3.12	92.75	-2.22

Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride; HDP – Henday egg production

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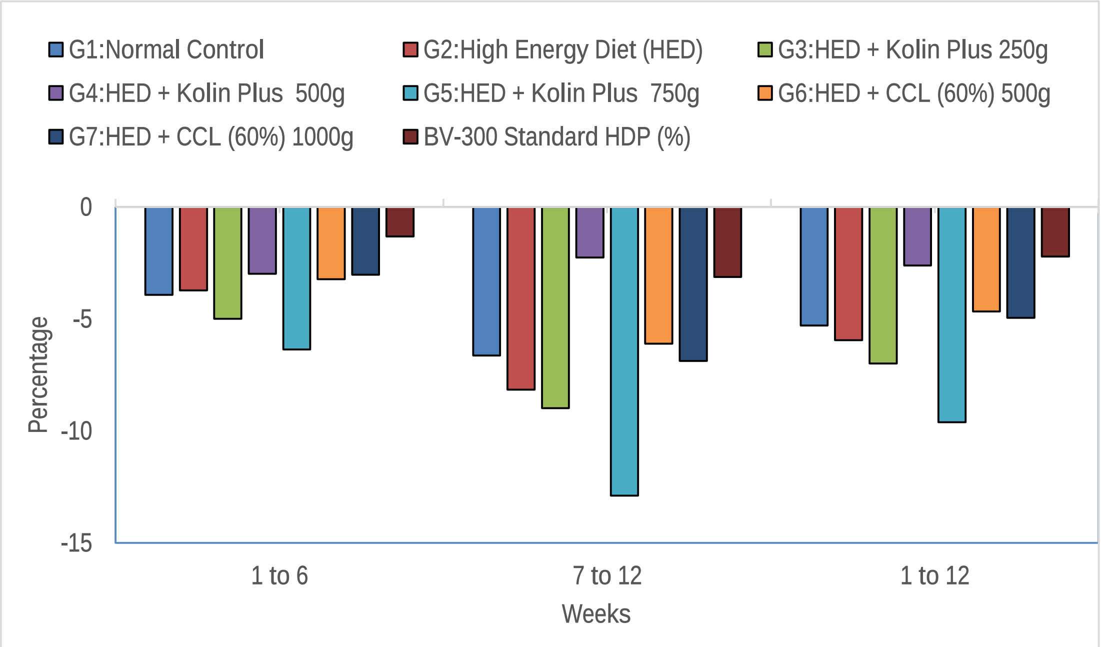

Figure 1: Difference in Egg Production (%) Compared to Baseline

POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride

The histogram (Figure 1) showed that, the dip in egg production of POH (500g/ton) group in the first 6 weeks was similar as that of choline chloride 60% (1000g/ton) fed birds. The average (7-12 weeks and 1-12 weeks) egg production loss was less in POH (500g/ton) supplemented birds as compared to all other groups.

b) Feed intake

The results presented in table 4, showed that, the normalized (1st to 6th week) total feed consumption of the layer birds of POH (500 g/ton) supplemented group was less when compared to the HED control group. However, the feed intake was higher than choline chloride 60% (1000 g/ton) added group. The data of the normalized feed intake from 7 to 12 weeks revealed that POH (500 g/ton) supplementation resulted in low feed intake as compared to HED fed layers, but higher than choline chloride 60% (1000 g/ton) fed group. Similar result was observed in case of normalized feed intake from 1st to 12th week duration.

Table 4: Average feed intake (g) of the birds

Group	Baseline	Average (1-6)	Diff	Average (7-12)	Diff	Average (1-12)	Diff
G1:Normal Control	108.9	112.8	3.93	112.7	3.75	112.74	3.84
G2:High Energy Diet (HED)	110.9	108.7	-2.20	110.3	-0.65	109.48	-1.43
G3:HED + POH 250g	113.1	109.0	-4.08	109.1	-4.03	109.04	-4.06
G4:HED + POH 500g	113.1	109.1	-3.12	111.0	-2.08	110.50	-2.6
G5:HED + POH 750g	109.7	109.8	0.15	109.4	-0.33	109.61	-0.09
G6:HED + CCL (60%) 500g	110.5	109.4	-1.10	109.0	-1.53	109.18	-1.32
G7:HED + CCL (60%) 1000g	113.6	109.0	-4.60	110.0	-3.65	109.48	-4.12
BV-300 Standard	112.0	112.0	0.00	113.5	1.50	112.75	0.75

Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride

The normalized feed intake per egg in first 6 weeks duration was less in POH (500 g/ton) added group as compared to normal control and HED fed layers. But the feed intake of POH fed layers was observed as higher than choline chloride 60% (1000 g/ton) supplemented group. The data obtained from the layers regarding the normalized feed intake per egg of week 7 to 12, exerted that POH (500 g/ton) supplemented birds consumed less feed as compared to choline chloride 60% (1000g/ton) group. Similar results were observed in case of normalized feed intake per egg from 1st to 12th week of the experiment.

Table 5: Average feed intake (g) per egg of the layers

Group	Baseline	Average (1-6)	Diff.	Average (7-12)	Diff.	Average (1-12)	Diff.
G1:Normal Control	115.1	124.6	9.47	128.3	13.20	126.43	11.33
G2:High Energy Diet (HED)	118.3	120.8	2.53	129.4	11.05	125.09	6.79
G3:HED + POH 250g	119.4	121.7	2.28	127.5	8.08	124.58	5.18
G4:HED + POH 500g	121.0	121.7	0.70	121.9	0.88	121.79	0.79
G5:HED + POH 750g	114.3	122.7	8.43	132.0	17.72	127.38	13.08
G6:HED + CCL (60%) 500g	116.8	119.9	3.10	123.3	6.53	121.62	4.82
G7:HED + CCL (60%) 1000g	117.7	116.7	-1.03	122.8	5.05	119.71	2.01
BV-300 Standard	118.0	119.7	1.67	123.5	5.5	121.58	3.58

Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride

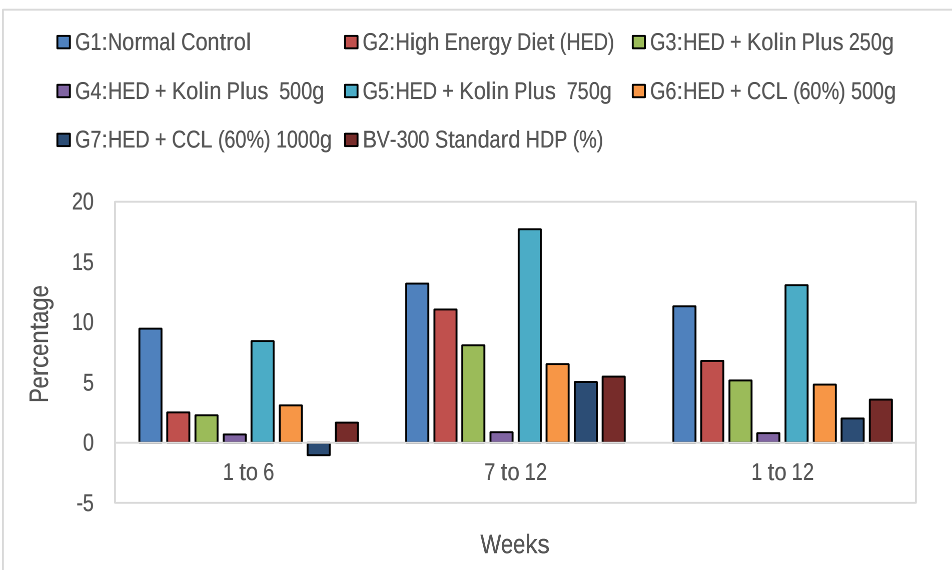

Figure 2: Difference in Feed Intake per Egg (%) compared to baseline

The normalized (1-12 weeks) feed intake per egg of POH (500g/ton) group was better than all the groups including choline chloride 60% (1000g/ton) added diet fed layers.

c) *Egg shell defects (ESD)* %

As shown in table 6, the average egg shell defects percentage of 1 to 6, 7 to 12 and 1 to 12 weeks of study duration showed values of within the range in all groups in case of before as well as after supplementation.

Table 6: Average percentage of egg shell defects of the layers

Group	Baseline	Average (1-6)	Average (7-12)	Average (1-12)
G1:Normal Control	0.840	0.570	0.350	0.490
G2:High Energy Diet (HED)	1.420	0.760	0.260	0.580
G3:HED + POH 250g	0.710	0.970	0.490	0.730
G4:HED + POH 500g	0.940	0.490	0.200	0.390
G5:HED + POH 750g	1.350	0.710	0.380	0.60
G6:HED + CCL (60%) 500g	1.670	0.580	0.50	0.630
G7:HED + CCL (60%) 1000g	0.390	1.020	0.840	0.890
Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride				

d) Egg quality

The egg quality parameters like average egg shell density, Haugh unit, egg shell weight and egg shell thickness were assessed for 6 weeks and 12 weeks duration of the study and compared between the groups. There was no significant difference noticed between the groups (table 7 and 8).

Table 7: Average density (g/cm^3) and Haugh Unit (HU) of the eggs

Group	Egg Density (g/cm^3)			HU		
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
G1:Normal Control	1.086	1.077	1.067	72.17	76.83	75.50
G2:High Energy Diet (HED)	1.083	1.072	1.065	73.83	75.83	72.50
G3:HED + POH 250g	1.082	1.077	1.060	73.83	75.50	72.83
G4:HED + POH 500g	1.082	1.078	1.062	76.83	77.67	75.83
G5:HED + POH 750g	1.086	1.076	1.060	75.83	75.67	70.17
G6:HED + CCL (60%) 500g	1.083	1.078	1.059	73.67	73.67	70.00
G7:HED + CCL (60%) 1000g	1.089	1.074	1.066	80.00	63.17	70.50
Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride						

Table 8: Average eggshell weight (g) and egg shell thickness (mm)

Group	Egg Shell Weight (g)			Egg Shell Thickness (mm)		
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
G1:Normal Control	6.169	5.890	5.084	0.413	0.372	0.378
G2:High Energy Diet (HED)	6.009	5.282	5.294	0.401	0.362	0.370
G3:HED + POH 250g	5.735	5.687	5.059	0.402	0.380	0.352
G4:HED + POH 500g	5.796	5.606	4.953	0.414	0.385	0.358
G5:HED + POH 750g	5.838	5.465	5.063	0.401	0.375	0.353
G6:HED + CCL (60%) 500g	5.778	5.589	4.965	0.404	0.380	0.357
G7:HED + CCL (60%) 1000g	5.992	5.431	5.219	0.404	0.370	0.365
Values were expressed as Mean; POH - Polyherbal formulation; HED - High Energy Diet; CCL – Choline chloride						

IV. Discussion

Choline is an essential nutrient for poultry and ubiquitously present in plant as well as animal cells. It is the fundamental component to build the structure of cells. Choline is required for synthesizing phospholipid and phosphatidylcholine that are obligatory for maintaining the integrity of cell membrane (Dänicke et al., 2006). Choline acts as methyl group donor to form methionine which has an important role in protein synthesis (Dänicke et al., 2006). It helps to metabolize fat and mobilize the excess lipid accumulated in the liver of the layers fed with HED (Beheshti Moghadam et al., 2021). In spite of numerous functions of choline, poultry birds cannot synthesize it in sufficient amount (Zhai et al., 2013). Therefore, choline supplementation is necessary in the diet of the birds. Synthetic choline chloride is routinely included as feed additive in layers' diet for a longer period. Although, it has lots of drawbacks like poor absorption rate, hygroscopicity, corrosiveness, non-uniform particle size, residual TMA formation (Selvam et al., 2018). Moreover, TMA concentration exceeding the permissible amount causes severe respiratory distress leading to fatality among the birds. To get rid of these demerits, scientists are in quest of choline like feed additives of natural origin.

POH is a polyherbal compound developed by M/s Natural Remedies Pvt Ltd, Bengaluru, India. The formulation is comprised of the bioactive constituents of *A. nilotica* and *C. longa* plant parts. Both the plants are well known for their multifarious ethnomedicinal properties. The key chemical constituents present in *A. nilotica* are polyphenols like gallic acid and catechin (Malviya et al., 2011). It is a promising antioxidant and proficient in reducing the oxidative stress (Adewale, 2016). It upsurged the oxidative enzyme activity like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-Stransferase (GST) in the liver of N-nitrosodiethylamine induced toxicity in rats (Malviya et al., 2011). Thus, it may act as hepatoprotectant and prevent lipid peroxidation. This statement was in agreement with the investigations done by Narayanan et al (Kannan et al., 2013). The treatment with the plant caused a significant reduction in cholesterol and triglyceride levels in the rodents (Kannan et al., 2013). *C. longa* is the rich source of phytopharmaceutical curcumin which is a natural antioxidant and hepatoprotectant. It scavenges the oxygen free radicals and enhances the activity of antioxidant enzymes. Previous literatures suggested that curcumin efficiently metabolized the dietary fat and accumulated lipid in liver (Labban, 2014; Tranchida et al., 2015). It may alter the fatty changes in liver, biliary hyperplasia and CCl4 induced hepatic injury in rats (Akram et al., 2010). In an experiment, hot water extract of *C. longa* showed a preventive effect against hepatic lipid deposition (Mun et al., 2019). Thus, the choline-like property of POH may exhibit a potential role in fat metabolism and lipotropic effect.

In the present study, POH supplementation in the diet of the layers of 43 to 55 weeks of age showed promising performance among the birds. HED in layer birds results in accumulation of fat in the abdomen and hepatic cells (Shini et al., 2019). However, previous studies also corroborated that herbal choline was a good alternative of synthetic choline chloride and might efficiently metabolize the hepatic fat of the birds (Gangane et al., 2010). Furthermore, it was reported that POH is efficient in fat metabolism and mobilization from hepatocytes to egg cells and improves the production rate. POH at a dose range of 500 g/ton in feed showed better egg production percentage (normalized %) as compared to choline chloride 60%. In addition, the results showed that normalized feed intake per egg percentage, in case of POH (500 g/ton) group was less than choline chloride fed birds. These results revealed that POH might efficiently enhance the performance of the layers. However, the egg quality based on egg density, HU, egg shell weight and egg shell thickness showed no significant differences between the groups. The bioactive compounds present in plant parts of *A. nilotica* and *C. longa* of POH helped in fat metabolism and prevented abdominal fat deposition leading to optimal egg production.

V. Conclusion

In brief, POH at a dose range of 500 g/ton as a natural feed supplement exhibited better performance among the layers of 43 to 55 weeks of age. The cumulative performance parameters viz. percentage of egg production, feed intake (g/egg) were improved following supplementation of POH (500g/ton) as compared to normal control and HED control groups and the performance of POH was similar to CCL 60% (1000g) group. However, no significant change in egg quality parameters was observed between the groups. POH (500 g/ton) exerted choline like characteristics and an ideal replacement of synthetic choline chloride 60% (1000 g/ton) in BV 300 layers fed with HED.

Abbreviations

Phosphatidylcholine (PC); Haugh unit (HU); Trimethylamine (TMA); Polyherbal formulation (POF); High-energy diet (HED); White Leg (WL); Metabolic energy (ME); Difference (diff); One-way analysis of variance (ANOVA); Egg shell defects (ESD); Catalase (CAT); Superoxide dismutase (SOD); Glutathione peroxidase (GPx); Glutathione-S transferase (GST)

Conflict of Interest

None

Acknowledgement

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References Références Referencias

1. Adewale, A. (2016). Evaluation of Root Extract of Acacia nilotica on Haematological and Lipid Profile in Rats. *European Journal of Medicinal Plants,* 17(4), 1–7. https://doi.org/10.9734/ejmp/2016/31262
2. Akram, M., Ahmed, A., Usmanghani, K., Hannan, A., Mohiuddin, E., & Asif, M. (2010). Curcuma Longa and Curcumin: a Review Article. *Romanian Journal of Biology,* 55(2), 65–70. http://ns.ibiol.ro/plant/volume 55/art201.pdf
3. Beheshti Moghadam, M. H., Aziza, A. E., & Cherian, G. (2021). Choline and methionine supplementation in layer hens fed flaxseed: effects on hen production performance, egg fatty acid composition, tocopherol content, and oxidative stability. *Poultry Science, 100*(9), 101299. https://doi.org/10.1016/j.psj.2021.101299
4. Chaudhari, Prajapati, K. I., Lunagariya, D. C., Sorathiya, P. M., Patel, & Nayak. (2017). an Importance of Choline Chloride for Poultry and

Cattle: an Overview. *International Journal of Science, Environment and Technology,* 6(5), 2804–2810. www.ijset.net

5. Dänicke, S., Ueberschär, K. H., Reese, K., & Weigend, S. (2006). Investigations on the effects of rape oil quality, choline and methionine concentration in diets for laying hens on the trimethylamine content of the eggs, on trimethylamine metabolism and on laying performance. *Archives of Animal Nutrition,* 60(1), 57–79. https://doi.org/10.1080/17450390500468404
6. Gangane, G. R., Gaikwad, N. Z., Ravikanth, K., & Maini, S. (2010). The comparative effects of synthetic choline and herbal choline on hepatic lipid metabolism in broilers. *Veterinary World,* 3(7), 318–320. https://doi.org/10.5455/vetworld.2010.318-320
7. Kannan, N., Sakthivel, K. M., & Guruvayoorappan, C. (2013). Protective effect of acacia nilotica (L.) against acetaminophen-induced Hepatocellular Damage in Wistar rats. *Advances in Pharmacological Sciences, 2013.* https://doi.org/10.1155/2013/987692
8. Khairani, Sumiati, & Wiryawan, K. G. (2016). Egg production and quality of quails fed diets with varying levels of methionine and choline chloride. *Media Peternakan,* 39(1), 34–39. https://doi.org/10.5398/medpet.2016.39.1.34
9. Labban, L. (2014). Medicinal and pharmacological properties of Turmeric (Curcuma longa): A review. *Int J Pharm Biomed Sci,* 5(1), 17–23. www.pharmainterscience.com
10. Malviya, S., Rawat, S., Kharia, A., & Verma, M. (2011). Medicinal attributes of Acacia nilotica Linn.-A comprehensive review on ethnopharmacological claims. *Int. J. of Pharm. & Life Sci. (IJPLS),* 2(6), 830–837.
11. Mun, J., Kim, S., Yoon, H. G., You, Y., Kim, O. K., Choi, K. C., Lee, Y. H., Lee, J., Park, J., & Jun, W. (2019). Water extract of Curcuma longa l. Ameliorates non-alcoholic fatty liver disease. *Nutrients, 11*(10). https://doi.org/10.3390/nu11102536
12. Parsons, C. M., & Leeper, R. W. (1984). Choline and methionine supplementation of layer diets varying in protein content. *Poultry Science,* 63(8), 1604–1609. https://doi.org/10.3382/ps.0631604
13. Selvam, R., Saravanakumar, M., Suresh, S., Chandrasekeran, C. V., & Prashanth, D. (2018). Evaluation of polyherbal formulation and synthetic choline chloride on choline deficiency model in broilers: Implications on zootechnical parameters, serum biochemistry and liver histopathology. *Asian-Australasian Journal of Animal Sciences, 31*(11), 1795–1806. https://doi.org/10.5713/ajas.18.0018
14. Shini, A., Shini, S., & Bryden, W. L. (2019). Fatty liver haemorrhagic syndrome occurrence in laying hens: impact of production system. *Avian Pathology, 48*(1), 25–34. https://doi.org/10.1080/03079457.2018.1538550
15. Singh Rajesh Kumar. (2019). *Role of Choline Chloride in Poultry (Layers & Broilers) Feed.* Www.Pashudhanpraharee.Com.
16. Tranchida, F., Shintu, L., Rakotoniaina, Z., Tchiakpe, L., Deyris, V., Hiol, A., & Caldarelli, S. (2015). Metabolomic and lipidomic analysis of serum samples following Curcuma longa extract supplementation in high-fructose and saturated fat fed rats. *PLoS ONE, 10*(8), 1–19. https://doi.org/10.1371/journal.pone.0135948
17. Zhai, Q. H., Dong, X. F., Tong, J. M., Guo, Y. M., & Bao, Y. E. (2013). Long-term effects of choline on productive performance and egg quality of brown-egg laying hens. *Poultry Science, 92*(7), 1824–1829. https://doi.org/10.3382/ps.2012-02854