



Study of combination of *curcuma longa*, lutein and *cissus quadrangularis* on production performance and egg quality in layers

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ABSTRACT

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The study was undertaken to evaluate the effect of supplementation of *Curcuma longa*, Lutein and *Cissus quadrangularis* on production performance and egg quality in layers. The birds from control group A and group C received diets with normal and 25% reduced levels of Calcium (Ca) and Phosphorus (P), respectively. The birds from groups B and D received diets similar to groups A and C supplemented with combination product of *Curcuma longa*, Lutein and *Cissus quadrangularis* @ 500 g/T, respectively. The trial was conducted on 208 White Leghorn (BV 300) layers for 12 weeks at age of fifty five weeks old layers randomly divided into four groups, viz A, B, C and D with two replicates per group. The birds from group B also recorded numerically lower number of broken eggs and feed consumption per bird per day. The birds from group B showed reduction in Total Viable Count (TVC) than the birds from all groups. The birds from group B recorded better profit margins than the birds from all groups. It is concluded that, the supplementation of combination product containing *Curcuma longa*, Lutein and *Cissus quadrangularis* @ 500 g/T to the diet with normal levels of Ca (4%) and P (0.05%) is beneficial in improving production performance, egg quality and profit margins in layers.

Keywords: Combination product, White Leghorn layer, Egg quality, Production performance

INTRODUCTION

There is tremendous potential for poultry industry to grow especially in India due to its growing population, migration of the youth to cities and changing food habits (Kolluri *et al.*, 2021). Egg breakage is one of the most sensitive issues in poultry industry. The egg shell quality is important for table eggs as well as for embryonic chamber and gas exchange medium (Roland and David, 1988; Keshavarz and Nakajima, 1993; Hunton, 2005). In addition to this, better gut health of layers is also responsible for improvement in egg production and egg quality. Thus, to maintain optimal production performance, new antibiotic alternative strategies including gut health enhancers and phytogetic compounds have been incorporated into poultry diets (Sharma *et al.*, 2020). A phytogetic product improves calcium and phosphorus, vitamins and minerals absorption and also improves the egg yolk colouration and skin pigmentation in broilers as well as in layers (Gopi *et al.*, 2020). Phytogetic feed additives have the potential to promote production performance and productivity (Muthusamy *et al.*, 2015) and thus add to the set of non-antibiotic growth promoters such as organic acids and probiotics.

Hence, in the present study *Curcuma longa*, Lutein and *Cissus quadrangularis* (Asthisamharaka) were selected to be used in layers for their various beneficial

effects on production performance and egg quality. It was thought that *Curcuma longa* will help to reduce microbial or bacterial load from the intestinal content of birds, Lutein will help to increase the colour of the egg yolk and *Cissus quadrangularis* will help layers for improving egg shell thickness by improving their calcium metabolism. The use of this combination in layers has been studied individually for their beneficial effects.

MATERIALS AND METHODS

Experimental design

A trial was conducted on 208 White Leghorn layers of BV-300 strain for a period of 12 weeks. The trial was conducted in the Department of Poultry Science, Mumbai Veterinary College, Parel, Mumbai- 400 012. The trial started at 55th week of age of birds and lasted till 67th week of age. The layers were randomly divided into four equal groups of 54 birds each and each group was further divided into two replicates of 27 birds each. Groups received one of the following treatments.

- Group A - Control-diet with normal levels of Calcium and Phosphorus
- Group B - Diet similar to group A + Combination product* @ 500 g/T
- Group C - Diet with 25% reduction in levels of Calcium and Phosphorus
- Group D- Diet similar to group C + Combination product* @ 500 g/T

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*Combination product is Protegg under a name marketed by Nurture aqua technology pvt. Ltd., Mumbai contains powder form of *Curcuma longa* 6%, Lutein 3% and *Cissus quadrangularis* (Asthisamharaka) 55%.

Feeding: Diets were formulated according to the standards prescribed in manual of BV-300 strain. Feed prepared was in the mash form and weighed quantity was offered daily throughout the trial period. The feed refusal was measured from each replicate on daily basis and based on this daily feed consumption of birds was calculated. Fresh water was offered *ad libitum* every day.

Parameters studied during experiment

We are going to study these parameters those are Total number of eggs produced, Egg production percentage, Total egg mass, Hen house and hen day egg production, Number of broken eggs, Feed consumption per bird per day, Feed conversion ratio on egg number basis, Feed conversion ratio on egg mass basis, Mortality, Egg weight, Shape index, Albumen index, Yolk index, Yolk colour index, Shell thickness, Haugh unit, Calcium and Phosphorus levels of egg shell, Total viable count and Coliform count of the intestinal contents, Economics of egg production

Egg quality parameters studied

Internal and external egg quality estimations were carried out every four weeks by randomly selecting six eggs per group. Selected eggs were weighed on digital scale (0.5 g precision). The measurements of length and width of egg were recorded by using digital vernier calliper (Mitutoyo®). Then the eggs were cracked on the flat glass surface to measure width and height of albumen and yolk. Height was measured using manual circular spherometer. Shell thickness without shell membrane was measured with a digital micrometer screw gauge (Mitutoyo®). Yolk colour index was judged using standard yolk colour fan (RocheR). Shape index (%), Albumen index (%), Yolk index (%) and Haugh unit were calculated as follows:

$$\text{Shape index (\%)} = \frac{\text{Width of egg in mm}}{\text{Length of egg in mm}} \times 100$$

$$\text{Albumen index (\%)} = \frac{\text{Average height of albumen in mm}}{\text{Average width of albumen in mm}} \times 100$$

$$\text{Yolk index (\%)} = \frac{\text{Average height of yolk in mm}}{\text{Average width of yolk in mm}} \times 100$$

$$\text{Haugh unit} = 100 \times \log (H - 1.7W^{0.37} + 7.6)$$

Where, H = Average height of albumen in mm

W = Weight of egg in grams.

Economics of egg production

The economics of egg production was calculated by considering cost of feed as the only variable. The

economics was worked out by considering cost of raw materials used for the feed. Average selling price of egg was considered as Rs. 6/- and the cost of combination product is considered as Rs. 600/- per kg.

Calcium and Phosphorus levels of egg shell

At the end of trial, Calcium and Phosphorus levels of egg shell with shell membrane were estimated as per AOAC (2009).

Total viable count and Coliform count of the intestinal contents

At the end of trial, six birds from each group were randomly selected and sacrificed. Ileum content sample of each sacrificed bird was collected by dissecting 2 cm long portion of the ileum. Then samples were placed in 50 ml sample container in phosphate buffered saline (PBS) and then kept at 4°C until used for sampling. For estimation of the total viable count and coliform count, 0.1 ml of inoculum was taken from 10⁻³ dilution of intestinal sample content and cultured by pour plate technique on plate count agar and eosin methylene blue agar. The agar plates were then incubated at 37°C for 24 hours. Colonies developed were counted by colony counter to arrive at final readings of the total viable count and total coliform count. (Andrew, 1992)

Statistical analysis: The data were subjected to statistical analysis for all the parameters as per Snedecor and Cochran (1994), considering treatments as the only variable and using Completely Randomized Design for all parameters.

RESULTS AND DISCUSSION

Total number of egg produced

The statistical analysis revealed that the differences in the total number of eggs produced by birds from different groups were statistically significant (P≤0.05). The findings of the present study are in accordance with the work of Park *et al.* (2012), Rahardja *et al.* (2015) and Marchiori *et al.* (2019), who reported that egg production was significantly (P≤0.05) higher in layer and quail diet fed with turmeric powder supplementation. However, Hadj Ayed *et al.* (2018), Gumus *et al.* (2018), Rahardja *et al.* (2015) and Attia (2018) who observed that the egg production was marginally higher in hens fed diet with turmeric. Similarly, Rezaei *et al.* (2019) and Lokaewmanee *et al.* (2010) observed that egg production was marginally higher in diet with marigold flower extract. These results corroborate with the findings of the present study.

Percent egg production

The statistical analysis revealed that the differences in the percent egg production (hen-day) of birds from different groups were statistically significant (P≤0.05). The findings of the present study are in accordance with the work of Rahardja *et al.* (2015) and Park *et al.* (2012) who reported that the percent egg production was

significantly ($P \leq 0.05$) higher in diet fed with supplementation of turmeric. Further, Zacaria and Ampode (2021) who observed that the percent egg production was marginal increase in diet with turmeric rhizome powder in quail. Similarly, Silva *et al.* (2018) observed that the percent egg production was marginally increased by adding turmeric powder in diet of layer (sorghum based). These observations are also in accordance with present study. However, Lokaewmanee *et al.* (2010) reported that the percent egg production was marginally reduced with the diets containing marigold flower meal and marigold flower extract. These findings do not corroborate with the findings of the present study.

Total egg mass

The statistical analysis revealed that the differences in the average total egg mass produced by birds from different groups were statistically non-significant. The findings of the present study are in accordance with research conducted by Rezaei *et al.* (2019), who observed that the total egg mass was marginally better with marigold flower in the diet. Similarly, Gumus *et al.* (2018) and Attia (2018), reported that the total egg mass was marginally improved with turmeric powder. Moreover, Skrivan *et al.* (2015), who observed that the total egg mass significantly ($P = 0.01$) increased with 150 mg/kg marigold flower extract and Park *et al.* (2012) and Marchiori *et al.* (2019), who reported that the total egg mass was significantly ($P \leq 0.05$) increased with turmeric powder in layer as well as in quail diet. These findings corroborate with the results of the present finding. However, Lokaewmanee *et al.* (2010) who observed that the total egg mass was not affected significantly with dietary marigold flower meal and marigold flower extract supplementation. These studies do not corroborate with the present findings.

Number of broken eggs

From the study it is revealed that the differences in the number of broken eggs obtained from different groups were statistically significant ($P \leq 0.01$). The statistical analysis revealed that group C recorded significantly ($P \leq 0.01$) higher number of broken eggs as compared to control group receiving diet with normal levels of Ca and P. Hadj Ayed *et al.* (2018) reported that the number of broken eggs was numerically decreased with diet supplemented with dry turmeric. These results corroborate with the findings of the present study. However, the findings of the present study are not in accordance with the work done by Moorthy *et al.* (2009), who found that the percent broken eggs was numerically higher in group fed with turmeric.

Feed consumption per bird per day

From the study it is revealed that the differences in the average feed consumption per bird per day of the birds from different groups were statistically non-significant. The findings of the present study are in

accordance with Marchiori *et al.* (2019) and Rahardja *et al.* (2015), who found that, the feed consumption per bird per day was marginally reduced with turmeric in the diet. Similarly, Chowdhury *et al.* (2008) observed that the feed consumption per bird per day was numerically decreased by dietary treatments of marigold flower meal and marigold flower extract. Furthermore, Rahardja *et al.* (2015) and Zacaria and Ampode (2021) reported that feed consumption per bird per day was significantly ($P \leq 0.05$) reduced with turmeric powder in diets of layers as well as in quails. However, Attia (2018) and Gumus *et al.* (2018) observed that, the feed consumption per bird per day was numerically improved with addition of turmeric in diet. These results do not corroborate with present study. Similarly, Skrivan *et al.* (2015) and Rezaei *et al.* (2019) reported that feed consumption was marginally higher with marigold flower extract and marigold flower meal in the diet.

FCR on egg number

The statistical analysis of the data revealed that the differences in the average FCR expressed on egg number basis for the birds from different groups were statistically non-significant. The findings of the present study are in accordance with the observations recorded by Gumus *et al.* (2018), Hadj Ayed *et al.* (2018) and Moorthy *et al.* (2009) who observed that the FCR on egg number basis was numerically improved in birds fed with turmeric. Further, Marchiori *et al.* (2019) and Zacaria and Ampode (2021) observed that, FCR on egg number was significantly ($P \leq 0.01$) better by adding turmeric in quail diet. However, Rezaei *et al.* (2019) noted that the FCR on egg number was numerically poor with marigold extract. This finding do not corroborate with present study.

FCR on egg mass

The statistical analysis of the data revealed that the differences in the average FCR expressed on egg mass basis for the birds from different groups were statistically non-significant. The findings of the present study corroborate with the work done by Gumus *et al.* (2018) and Attia (2018), who recorded that, the feed conversion ratio on egg mass basis was marginally improved after feeding birds with turmeric. Similarly, Skrivan *et al.* (2015) recorded marginally better FCR on egg mass basis with marigold flower extract. Further, Marchiori *et al.* (2019) recorded significantly better feed conversion ratio on egg mass basis with turmeric powder @ 30 mg/kg to control diet. These results are also in accordance with the findings of present study.

Mortality

There was no mortality in any groups during trial period of 12 weeks. This suggested that the supplementation of combination product @ 500 g/T had no ill effect on the health of the birds.

Egg weight

The statistical analysis of the data revealed that the differences in the average egg weight of the birds from different groups were statistically non-significant. The findings of the present study corroborate with the work done by Rahardja *et al.* (2015), who found that the egg weight was marginally poor with turmeric supplementation. However, Gumus *et al.* (2018), Hadj Aayed *et al.* (2018) and Attia (2018) reported that the egg weights were numerically improved with supplementation of turmeric. Similarly, Rezaei *et al.* (2019), Grcevic *et al.* (2019) and Chowdhury *et al.* (2008) recorded that the egg weight was numerically improved with marigold extract. Furthermore, Skrivan *et al.* (2019) reported that egg weight was significantly ($P \leq 0.05$) improved with marigold extract in layers and Marchiori *et al.* (2019) who reported that significantly ($P \leq 0.05$) improved with turmeric in quails. These results do not corroborate with the findings of present study.

Shape index

The statistical analysis of the data revealed that the differences in shape index of eggs produced by birds from different groups were statistically non-significant. The findings of the present study are in accordance with the work done by Zacaria and Ampode (2021) and Gumus *et al.* (2018) reported that the shape index was marginally reduced with turmeric in the diet. However, Chowdhury *et al.* (2008) and Attia (2018) observed that, the shape index was numerically improved with marigold and turmeric in the diet. These observations do not corroborate with the findings of the present study.

Albumen index

The statistical analysis of the data revealed that the differences in the albumen index of eggs produced by birds from different groups were statistically non-significant. The findings of the present study are in accordance with the work done by Kostic (2017) who observed that, during the last period of the trial the albumen index was significantly ($P \leq 0.05$) higher when turmeric powder was fed to birds. These findings are in accordance with the findings of the present study. However, Gumus *et al.* (2018) found that the albumen index was marginally reduced by the dietary treatment of turmeric. Similarly, Chowdhury *et al.* (2008) recorded that the albumen index was numerically reduced with marigold flower supplementation in the diet. These findings do not corroborate with the observations of the present study.

Yolk index

The statistical analysis of the data revealed that the differences in the yolk index of eggs produced by the birds from different groups were statistically non-significant. Attia (2018) observed that, the yolk index was numerically lower with curcumin supplementation to the diets. This finding is in accordance with the

observations of the present study. Similarly, Chowdhury *et al.* (2008), recorded that, the yolk index was not affected with marigold extract fed in the diet and Marchiori *et al.* (2019), observed that the yolk index was not affected with curcumin supplementation in quails diet. However, Gumus *et al.* (2018) and Galli *et al.* (2018) found that the yolk index was numerically higher with turmeric supplementation. These observations do not corroborate with the findings of the present study.

Yolk colour index

The statistical analysis of data revealed that the differences in yolk colour index of eggs produced by the birds from different groups were statistically non-significant. Park *et al.* (2012), Galli *et al.* (2018), Silva *et al.* (2018) and Attia (2018), recorded that, the yolk colour index was significantly higher with supplementation of Curcumin ($P \geq 0.05$). Further, Rezaei *et al.* (2019), Lokaewmanee *et al.* (2010) and Chowdhury *et al.* (2008) reported that the yolk colour index was significantly higher with marigold flower supplementation. These findings are in accordance with the findings of present study. However, Gumus *et al.* (2018) found that, the yolk colour index was not affected with 0.50% turmeric in the diet. These findings do not corroborate with the observations of present study.

Shell thickness

The statistical analysis of the data revealed that the differences in shell thickness of eggs produced by the birds from different groups were statistically non-significant. The findings of the present study are in accordance with the work done by Skrivan *et al.* (2015) and Grcevic *et al.* (2019), who reported that the shell thickness was marginally better with dietary addition of marigold flower extract. Similarly, Lokaewmanee *et al.* (2010) recorded that, the shell thickness was not affected with supplementation of marigold extract. Furthermore, Skrivan *et al.* (2015) found that the shell thickness was significantly ($P \leq 0.05$) higher at the sharp end of the egg with supplementation of marigold flower extract in the diet. However, Chowdhury *et al.* (2008), who reported that the shell thickness was numerically reduced with marigold flower extract ($P \geq 0.05$). These results do not corroborate with the findings of the present study.

Haugh unit

The statistical analysis of the data revealed that the differences in Haugh unit of the eggs produced by the birds from different groups were statistically non-significant. The findings of the present study corroborate with the work done by Marchiori *et al.* (2019) and Lokaewmanee *et al.* (2010) who found that, Haugh units were marginally improved with curcumin supplementation in quails and with marigold flower meal supplementation in layers. Similarly, Park *et al.* (2012) observed that the Haugh unit was significantly ($P \leq 0.05$) higher with turmeric in the diet after 14 days storage of eggs.

Moreover, Galli et al. (2018) and Silva et al. (2018) recorded that the Haugh unit was not affected with curcumin in the diet. However, Gumus et al. (2018), Skrivan et al. (2015) and Chowdhury et al. (2008) reported that the Haugh unit was numerically decreased with turmeric and marigold flower extract supplementation in the diets. These results do not corroborate with the findings of the present study.

Calcium and phosphorus levels of egg shell

From the data, it is observed that, the levels of Calcium in egg shells of the birds from groups A, B, C and D were 37.31, 37.29, 37.26 and 37.19 %, respectively. Similarly, Phosphorus levels in egg shells of the birds from groups A, B, C and D were 0.22, 0.21, 0.23 and 0.20%, respectively. It is noted that, there was no apparent effect on the Calcium and Phosphorus levels in egg shells of the eggs produced by birds from different treatment groups.

Total viable count and coliform count of intestinal content

The statistical analysis of data revealed that the differences in TVC of intestinal content of birds from different groups were statistically significant (P≤0.01).

It is revealed that the birds from only group C receiving diet with 25% reduced levels of Ca and P recorded the coliform bacteria. However, the birds from other treatment groups A, B and D did not record any coliform bacteria.

Overall performance

Hence, it can be concluded that supplementation of combination product containing *Curcuma longa*, Lutein and *Cissus quadrangularis* @ 500 g/T to layer diets with normal levels of Ca (4%) and P (0.05%) is beneficial for improving production performance, egg quality and profits.

Table 1: Overall performance with statistical analysis

Parameters	Gr. A	Gr. B	Gr. C	Gr. D
Total number of egg produced	4019 ^a	4163 ^b	4081 ^{ab}	4076 ^{ab}
Percent egg production (%)	92.01 ^a	95.46 ^b	93.43 ^{ab}	93.32 ^{ab}
Total mass produced (kg)	222.69	231.31	224.94	225.39
Hen day egg production for 12 weeks	77.29	80.06	78.48	78.38
Hen house egg production for 12 weeks	77.29	80.06	78.48	78.38
Total number of broken eggs	122 ^{ab}	71 ^a	216 ^c	185 ^{bc}
Feed consumption per bird per day (g)	137.41	137.03	136.89	136.19
Feed conversion ratio on egg number basis	1.80	1.72	1.76	1.75
Feed conversion ratio on egg mass basis	2.70	2.58	2.66	2.64
Mortality %	0	0	0	0
Egg weight (g)	56.48	55.65	56.26	54.77
Shape index	75.56	74.80	75.53	75.50
Albumen index	10.94	11.07	10.40	10.93
Yolk index	41.30	40.42	40.53	40.89
Yolk colour index	6.46	6.93	6.53	6.92
Shell thickness (mm)	0.40	0.42	0.38	0.42
Haugh unit	87.82	90.09	87.06	90.10
Ca level of egg shell (%)	37.31	37.29	37.26	37.19
P level of egg shell (%)	0.22	0.21	0.23	0.20
Total viable count of the intestinal content (x 10 ³ CFU/ml)	196.17 ^a	70.50 ^c	146.33 ^{ab}	101.83 ^{bc}
Coliform count of the intestinal content (x 10 ³ CFU/ml)	0	0	2	0
Total profit (Rs.)	454.53	1519.41	343.10	422.15
Net profit per egg (Rs.)	0.12	0.37	0.09	0.11

CONCLUSION

Thus, the overall results of the experiment indicated that the birds receiving diet with normal Ca and P levels supplemented with combination product @ 500 g/T recorded significantly (P≤0.05) better egg production, percent egg production, hen house and hen day egg

production and marginally better egg mass and feed conversion ratio on egg number and egg mass basis. The birds from group B also recorded numerically lower feed consumption per bird per day and lesser number of broken eggs and significantly (P≤0.01) lower total viable count of the intestinal contents of birds than the birds

from its non-supplemented counterparts. Similarly, significant ($P \leq 0.01$) reduction in total viable count and marginal reduction in coliform count from their intestinal contents was observed in the birds from group A and D as compared to their respective non-supplemented counterparts. Moreover, the birds receiving diets with normal as well as 25% reduced levels of Ca and P recorded better profit margins when supplemented with combination product @ 500 g/T as compared to their respective non-supplemented counterparts.

Hence, it is concluded that, the supplementation of combination product containing *Curcuma longa*, Lutein and *Cissus quadrangularis* @ 500 g/T to the diets with normal levels of Calcium (4%) and Phosphorus (0.5%) is beneficial in improving production performance, egg quality and profit margins in layers.

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