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DIETARY SUPPLEMENTATION OF COMBINATION OF INULIN AND SAPONIN ON EGG YOLK LIPID AND SERUM BIOCHEMICAL PROFILE IN LAYING HENS

Yenge G.D. [1], *Lonkar V.D.[2], Ranade A.S.[3], Patodkar V.R.[4], Mote C.S[5], Bhalerao S.M.[6] and Doiphode A.Y.[7]

[1]M.V.Sc Scholar, [2]Assistant Professor, [3]Professor & Head,
Department of Poultry Science,
[4]Associate Professor, Department of Veterinary Physiology,
[5]Assistant Professor, Department of Veterinary Pathology,
[6]Assistant Professor, Department of Animal Nutrition,
[7]Assistant Professor, Department of Animal Genetics & Breeding,
KNP College of Veterinary Science Shirwal Dist. Satara, MS., India
E-mail: vijulvet@rediffmail.com (*Corresponding author)

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Abstract: An effect of supplementation of combination of two feed additives, inulin from Chicory root powder (*Cichorium intybus L.*) and saponin from Sapindaceace plant powder on egg yolk lipid and serum biochemical profile of laying hens was studied. Sixteen weeks old "BV-300" pullets were housed in californian cages. Total 300 laying hens at 21 week of age were randomly assigned to three equal groups (A, B and C) of 100 hens each and further sub-divided into four replicates of 25 hens in each. The experiment was conducted up to 52 weeks age of laying hens. Group A received control basal diet. Group B received basal diet with combination of 0.1 g inulin/kg of feed through Chicory root powder @ 250 g/ton and 0.005 g saponin/kg of feed through Sapindaceae plant powder @ 50 g/ton of feed. Group C received basal diet with combination of 0.167 g inulin/kg of feed through Chicory root powder @ 417 g/ton and 0.0083 g saponin/kg of feed through Sapindaceae plant powder @ 83 g/ton of feed. The lipid profile with respect to the egg yolk Total Cholesterol (TC), Triglycerides (TG) and HDL Cholesterol (HDLC) were estimated at the end of 52 weeks of age. The serum biochemical parameters like serum Total Protein (TP), Albumin (Ab), Globulin (Gb) and ratio of Albumin to Globulin (A/G) were estimated at the end of 52 weeks of age. The both dose levels of combination of inulin and saponin employed in the present did not exert positive effect on egg yolk TC, TG and HDLC. Similarly, the levels used for combination of inulin and saponin in layers did not alter the serum biochemical parameters like serum TP, Ab, Gb and A/G ratio which indicated that the immunity of laying hens was maintained.

Keywords: Inulin, Saponin, egg yolk lipid profile, serum biochemical's.

INTRODUCTION

The current trend in poultry production is aimed at reducing the use of antibiotic growth promoters and increasing the use of non-antibiotic feed additives. The plant phytobiotics like inulin from Chicory root powder (*Cichorium intybus L.*) and saponin from *Sapindaceae* plant

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(soapnut) powder play important roles as feed additives in poultry. Inulin is one of the most commonly used and most effective prebiotics (Dankowiakowska *et al.,* 2013). Inulin is a mixture of linear polymers and oligomers of fructose linked by a β (2–1) glycosidic linkage often with a glucose terminal unit (Roberfroid *et al.,* 1998). The effect of inulin in poultry has been studied on gut microbiota, intestinal histomorphology, immune system, mineral absorption (mainly calcium and magnesium) and lipid metabolism (Samanta *et al.*, 2011). The application of prebiotics like inulin as feed additive in poultry is relatively a new commercial venture. Commercial forms of inulin are obtained from chicory root. Fresh chicory root contains 11-15 % inulin and it may increase upto 40 % in dried chicory root powder.

Saponins are steroidal glycosides which occur widely in plants that are consumed by animals and humans (Johnson *et al.,* 1986) and classically categorized as anti-nutritional factors present in many plant origin materials. The plant origin materials containing moderate amount of saponin could be useful for birds as they are capable of exhibiting similar protective effect in birds as in the plants. Thus, the responsible use of plant origin material containing lower level of saponin have proved to be beneficial than proving anti-nutritional. Plants like Yucca schidigera, Quillaja saponaria, Sapindus delavayi, Acacia concinna and Sapindaceae etc. have been used to extract saponin. Out of these, Sapindus mukorossi is a commonly available plant in India and saponin derived from its seeds commonly known as "Soapnut" has been found to be more useful at lower concentration. These saponins when used responsibly acts as cleansing agents in the intestines. Some of the positive effects of saponin as feed additives are reduction of environmental ammonia and odour, hypocholesterolemic activity. However, there are no studies on the use of dietary combination of inulin and saponin on the egg yolk lipid and serum biochemical profile in laying hens.

MATERIAL S AND METHODS

The 16 weeks old "BV-300" pullets were housed in high raised platform Californian cage system of housing under identical managemental conditions. The experiment was conducted for a period of 32 weeks (21 to 52 weeks of layer age). Total 300 numbers of laying hens at the age of 21 weeks were randomly divided into four equal groups of 100 layers each viz., A, B and C. Each group was further subdivided into four replicates of 25 birds each. Group A received control basal diet. Group B received control diet with 0.1 g of inulin/kg of feed (provided by mixing Chicory root powder (*Cichorium intybus L.*) @ 250 g/ton of feed) and

0.005 g saponin/kg of feed (provided by mixing *Sapindaceae* plant powder @ 50 g/ton of feed). Group C received control diet with (0.167 g of inulin/kg of feed (provided by mixing Chicory root powder (*Cichorium intybus L.*) @ 417 g/ton of feed) and 0.0083 g saponin/kg of feed (provided by mixing *Sapindaceae* plant powder @ 83 g/ton of feed). The lipid profile with respect to the egg yolk Total Cholesterol (TC), Triglycerides (TG) and HDL Cholesterol (HDLC) were estimated at the end of 52 weeks of age. The serum biochemical parameters like serum Total Protein (TP), Albumin (Ab), Globulin (Gb) and ratio of Albumin to Globulin (A/G) were estimated at the end of 52 weeks of age.

Egg yolk lipid profile:

At the end of 52 week of age, 12 eggs per group were randomly selected and used for analysis of egg yolk lipid profile. The egg lipid profile was carried out in Real World Nutrition Laboratory Foundation, Pune.

Preparation of egg yolk for analyses: The eggs used for analyses were prepared according to the procedure described by (Elkin *et al.,* 1999). The eggs were broken and egg yolk was separated from the eggs. A 1.0 g sample of each yolk was homogenized with 15 ml of chloroform-methanol 2:1 (v/v), thoroughly mixed and filtered. Egg homogenate filtrates were designated as egg yolk samples.

Egg Yolk Total cholesterol (TC): The TC was estimated as per quantitative determination of cholesterol concentration based upon enzymatic colorimetric method by using DELTA® Kit provided by Delta Lab, Mumbai-Goa Highway, Zarap, Dist. Sindhudurg. The various TC concentrations recorded as mg/g of egg yolk.

Egg Yolk HDL cholesterol (HDLC): The HDLC was estimated as per quantitative determination of cholesterol concentration based upon enzymatic colorimetric method by using DELTA® Kit provided by Delta Lab, Mumbai-Goa Highway, Zarap, Dist. Sindhudurg. The various HDLC concentrations recorded as mg/g of egg yolk.

Egg Yolk Total triglycerides (TG): The TG was estimated as per quantitative determination of TG concentration based upon enzymatic colorimetric method by using DELTA® Kit provided by Delta Lab, Mumbai-Goa Highway, Zarap, Dist. Sindhudurg. The various TG concentrations recorded as mg/g of egg yolk.

Serum biochemical parameters

At the end of 52 week of age, blood samples of two birds from each replicate (total eight blood samples per treatment) were collected when the birds were sacrificed. The blood samples were collected in dry labeled glass tube and were kept in a slanted position at room

temperature to facilitate the separation of serum. The serum total protein, serum total albumin was estimated calorimetrically by Biuret method utilizing the available kit in the market. The serum globulin level was calculated mathematically by subtracting serum albumin from total serum protein. The serum Albumin: Globulin ratio (A/G) was calculated.

Statistical Analysis

The data collected on various parameters were subjected to statistical analysis as per the methods suggested by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Egg yolk lipid profile:

The egg yolk total cholesterol (TC), Triglycerides (TG) and HDL Cholesterol (HDLC) in mg/g of yolk were estimated from the eggs laid by the laying hens from different dietary groups and its statistical analysis is depicted in Table 1.

The egg yolk TC level (mg/g of yolk) from the yolk of eggs laid by the laying hens at the end of 52 weeks age from group A, B and C was 12.48±1.11, 11.42±0.66 and 11.66±0.76, respectively. The TG (mg/g of yolk) was found to be comparable among the different dietary treatments. Feeding of chicory root powder (Aghazadeh and Nabiyar, 2015) and inulin (Praveen *et al.*, 2017) did not affect the serum and meat TC in broilers. Dietary saponin in layers failed to lower TC content in egg yolk (Sim *et al.*, 1984). These findings were in agreement with the present findings. However, in contrast to the present findings, supplementation of 1.5 %, and 2 % chicory (*Cichorium intybus L.*) powder (Mansoub, 2011), inulin (Chen *et al.,* 2005; Park and Park, 2012; Shang *et al.*, 2010) reduced egg yolk TC and serum TC. TC content in eggs decreased linearly with increasing levels (0.1, 0.5, 1.0, 1.5 or 2.0 %) of dietary inulin (Shang *et al.*, 2010). Addition of alfalfa meal containing saponin (Guclu *et al.*, 2004; Olgun and Yildiz, 2015, Fan *et al.,* 2018), dietary karaya saponin (Afrose *et al.,* 2010), saponin containing fenugreek seeds (Ali *et al.*, 2011) and *Yucca schedigera* supplementation (Wang and Kim, 2012; Kutlu *et al.*, 2001) in laying hens reduced egg yolk TC. These findings were not in agreement with present findings. The combination levels of inulin and saponin used in the present study did not reduce egg yolk TC significantly.

The corresponding values for egg yolk TG (mg/g of yolk) for group A, B and C were 114.31±9.48, 107.45±7.88 and 114.72±7.64, respectively. The total egg yolk TG values were comparable among different groups. Feeding various levels of inulin in broiler chickens did not have any significant effect serum TG (Praveen *et al.,* 2017) while the saponin containing alfalfa meal in layer quail diet had no significant effect on egg yolk TG (Olgun and Yildiz,

2015) which was in agreement with the present findings. But (Taraz *et al.*, 2015 and Mansoub, 2011) reported that the serum concentrations of TG significantly decreased in birds received *Cichorium intybus L.* root powder or extract. Karaya saponin (25, 50 or 75 mg/kg) in layers diet (Afrose *et al.,* 2010) reduced egg yolk TG, use of *S. rarak* fruit pericarp containing high saponin (Pasaribu *et al.*, 2014) reduced triglyceride in chicken blood plasma. The combination level of inulin and saponin used in the present study did not altered egg yolk TG significantly. The corresponding values for egg yolk HDLC for group A, B and C were 2.05±0.19, 1.23±0.06 and 1.63±0.11, respectively. It was seen that the egg yolk HDLC values were significantly ($p<0.05$) decreased in the eggs laid by the laying hens from group B and C than the group A. This indicated that there was no positive effect of combination of inulin and saponin on egg yolk HDLC. The reports of the present study corroborated with the findings of Aghazadeh and Nabiyar (2015), Mansoub (2011), Praveen *et al.* (2017), Fan *et al.* (2018) and Amirshekari *et al.* (2016)

Overall, it could be concluded that both the dose levels of combination of inulin and saponin employed in the present study might not be sufficient to exert positive effect on egg yolk TC, TG and HDLC.

Serum biochemical profile

The statistical analysis of data on serum Total Protein (TP), Albumin (Ab), Globulin (Gb) and ratio of Albumin to Globulin (A/G ratio) estimated at the end of 52 weeks of age in layers is given in Table 2. The corresponding values for serum TP (g/dL) were 7.11±0.22, 6.76±0.19 and 7.10±0.23, corresponding values for serum Ab (g/dL) were 2.38±0.15, 2.32±0.10 and 2.15±0.14, and corresponding values for serum Gb (g/dL) were 4.76±0.09, 4.44±0.11 and 4.94±0.16 for group A, B and C, respectively. The results of the study indicated that supplementation of both the levels of combination of inulin and saponin in layer diet of group B and C did not affect the serum TP and Ab in layers compared to control group A. While the serum Gb fraction was significantly ($p<0.05$) decreased in group B than group C but both the groups B and C were comparable with control group A. The corresponding values for serum A/G ratio for group A, B and C were 0.49±0.02, 0.52±0.01 and 0.44±0.02, respectively. It was observed that the A/G ratio was significantly ($p<0.05$) increased in group B than group C. but both the groups B and C were comparable with control group A.

This indicated that supplementation of lower dose level of inulin and saponin in the layer diet of group B and higher dose level of inulin and saponin in layer diet of group C maintained

immunity in layers. Many workers reported that use of different levels of inulin and saponin individually in different species like chicken, rat, fish and goat, did not alter the serum TP, Ab, Gb and A/G ratio than the control which was in agreement with the findings of present study. Khakzadihe *et al.* (2014) did not show any significant difference in serum TP and Ab by inclusion of 1.0 % inulin in quail. *Cichorium intybus L.* herb in chicken (Mousavinasab *et al.*, 2016 and Khodadadi *et al.,* 2016) and in rats under stress (Hanaa *et al.,* 2010) had no significant effect on serum TP and Ab. *Tribulus terrestris* plant powder containing saponin in laying hens (Amirshekari *et al.*, 2016), tea seed saponin in oat fodder based diet feeding in Gaddi goats (Jadhav *et al.*, 2017) and *Yucca schidigera* powder in rabbit diet (Ashour *et al.*, 2014) had no significant effect on levels of serum TP, Ab and Gb.

It was concluded that the levels used for combination of inulin and saponin in layers did not alter the serum biochemical parameters like serum TP, Ab, Gb and A/G ratio which indicated that the immunity was maintained in layers.

CONCLUSIONS

It was concluded that the dietary lower level of combination of 0.1 g inulin/kg of feed through Chicory root powder (*Cichorium intybus L.*) @ 250 g/ton and 0.005 g saponin/kg of feed through *Sapindaceae* plant powder @ 50 g/ton as well as higher level of combination of 0.167 g inulin/kg of feed through Chicory root powder @ 417 g/ton and 0.0083 g saponin/kg of feed through *Sapindaceae* plant powder @ 83 g/ton of feed did not exert positive effect on egg yolk TC, TG and HDLC. Similarly, the levels used for combination of inulin and saponin in layers did not alter the serum biochemical parameters like serum TP, Ab, Gb and A/G ratio which indicated that the immunity of laying hens was maintained.

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Table 1: Effect of supplementation of combination of inulin and saponin on lipid profile of egg at the end of 52 week of age, (Mean ± SE), (n = 16)

Parameter	Groups			SEM	P Value
	A	B	C		
TC (mg/g of yolk)	12.48±1.11	11.42±0.66	11.66±0.76	0.49	0.67
TG (mg/g of yolk)	114.31±9.48	107.45±7.88	114.72±7.64	4.75	0.79
HDLC (mg/g of yolk)	2.05 ± 0.19^{c}	1.23 ± 0.06^{a}	1.63 ± 0.11^{b}	0.09	0.001

a, b, c Means with different superscripts in a row differ significantly ($p<0.05$)

Table 2: Effect of supplementation of combination of inulin and saponin on serum profile at the end of 52 week age, (Mean ± SE), (n = 8)

Parameter	Groups			SEM	P Value
	A	B	C		
Serum TP (g/dL)	7.11±0.22	6.76±0.19	7.10±0.23	0.12	0.46

Serum Ab (g/dL)	2.38±0.15	2.32±0.10	2.15±0.14	0.07	0.49
Serum Gb (g/dL)	4.76 ± 0.09^{ab}	4.44 ± 0.11^{a}	4.94 ± 0.16^{b}	0.08	0.03
A/G Ratio	0.49 ± 0.02^{ab}	0.52 ± 0.01^{b}	0.44 ± 0.02^{a}	0.01	0.09

a, b, c Means with different superscripts in a row differ significantly ($p<0.05$)