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EFFECT OF VARIOUS LEVELS OF DRIED TAMARIND PULP POWDER ON SERUM AND EGG YOLK BIOCHEMISTRY OF LAYER BIRDS

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ABSTRACT
The experimental study was conducted on 240 commercial layer birds of BV-300 strain to evaluate the effect of feeding various levels of dried tamarind pulp powder on serum biochemistry of birds. Four treatments are replicated in to 3 as 20 birds in each replicate. Group 1st (T_o) was treated as control fed with basal diet without dried tamarind pulp powder. Group 2nd (T_1) was treatment group with dried tamarind pulp powder 250g/100kg feed, Group 3rd (T_2) @ 500g/100kg and Group 4th (T_3) @ 1000g /100kg. Results shows that the significant ($P<0.01$) reduction in the serum cholesterol, LDL, triglyceride, significant ($P<0.05$) reduction in egg yolk cholesterol and significant ($P<0.01$) increase in the serum HDL level while no significant changes seen in the serum protein, albumin, globulin, egg yolk LDL, egg yolk HDL and egg yolk triglyceride.

KEY WORDS- Tamarind, Egg yolk cholesterol, layer.

INTRODUCTION

A modernistic challenge in poultry production is to exploit the use of specific dietary supplements to boost the intrinsic potential of poultry birds to perform better (Adil *et al.,* 2010). Probiotics, enzymes, amino acid supplements, available minerals and herbal plants are all relatively new additions to the harmony of poultry nutritionists and have a very positive effect on nutrient utilization when used with appropriate feed ingredients. However, this has compromised their immunity and immune response which cannot be improved by use of antibiotics in feed due to their inherent ill effects on public health. Hence, to fulfill these lacunae the use of herbal preparations and traditional remedies has become the need of the hour. A group of feed additives that have been generating interest in recent times as a replacement for banned antimicrobials in the poultry industry are the phytogenic feed additives (Jacela *et al.,* 2010). These phytogenic feed additives also called as phytobiotics or botanicals, are usually plant derived compounds that are used to improve productivity of livestock through improved feed intake, improved gut function, antimicrobial activity and anti-oxidative actions (Windisch *et al.,* 2008). Tamarind or *Tamarindus indica* L. of the Fabaceae, subfamily Caesalpinioideae, is an important food in the tropics. The tamarind fruit contains about 55% pulp, 34% seeds and 11% shell (pod). Tamarind has been reported to have anti-diabetic (Koyagura, 2013), anti-inflammatory (Landi librandi *et al*., 2007), cholesterol lowering (Chowdhury *et al*., 2005), anti-obesity (Ukwani *et al*., 2008; Khairunnuur *et al.,* 2011), antifungal (Abubakar *et al.,* 2010), antioxidant (Khairunnuur *et al.,* 2009; Bhutkar *et al.,* 2011; Atawodi *et al.,* 2014 ; Shridhar *et al.,* 2014), antipyretic (Izquierdo *et al., 2007)* and antimicrobial (Doughari 2006; Abukakar *et al.,* 2008; Daniyan *et al.,* 2008) properties. In addition, it has appetizing and stimulatory effect in the digestive process (Cabuk *et al.,* 2003). Aengwanich *et al*. (2009) found that polyphenolic compound in the extracts could reduce heat stress in broiler chickens.

MATERIALS & METHODS

Two hundred and forty healthy 20 weeks old layer birds of 'BV-300' strain were used. Layer birds were divided into four groups of 60 birds each. Each group was further divided into 3 replicates of 20 birds each. Group I (T_o) was treated as control fed with basal diet without dried tamarind pulp powder. Group II (T_1) was treatment group with dried tamarind pulp powder 250g/100kg feed Group III (T_2) @ 500g/100kg and Group IV (T_3) @1000g / 100kg. Percent Ingredient Composition of Layer Ration was shown in table-1. The fresh sample of tamarind fruit was procured from local market. The seed of tamarind fruit was separated with an intension to get pulp of fruit only. The clean pulp of tamarind fruit was kept for oven drying at 100^0 C for 24 hours. The oven dried sample was grounded for its further use. Proximate composition (% DMB) of dried pulp of Tamarind fruit were shown in the table- 2. The blood samples was collected at the beginning of experiment (20th week), mid (28th week) and end (36th week) of experimental period. The specific volume of blood was collected from wing vein of representative experimental birds. The blood samples were subjected for centrifugation for separation of serum. Further the serum samples were maintained at - 20^0C until analyzed.

Serum samples were analyzed for total cholesterol, LDL, HDL, total protein, albumin, globulin and triglyceride. The biochemical estimations were performed using Cromtech Vet-biochemistry Semi – Auto Analyzer PUS-2018. The methodology and the set of reagents used in respect of each parameter was as per the recommendations of the manufacturer.

Egg yolk from representative eggs were processed for estimation of total cholesterol, triglyceride, LDL and HDL at beginning (20th week), mid (28th week) and end (36th week) of experiment. Initially yolk was separated carefully from the albumin. One gram of yolk was placed into a centrifuge tube. Fifteen milliliters of chloroform: methanol (2:1 v/v) was added, blended on a vortex mixture, and allowed to extract for 12 h and extracted yolk samples were analyzed for cholesterol according to the colorimetric method. Sample of 0.5 ml extracted yolk was transferred into sterile tube containing 6 ml glacial acetic acid and mixed then; 4 ml ferric chloride reagent was added, shaken and let to cool. The estimations were done by using Cromtech Vet-biochemistry Semi – Auto Analyzer PUS-2018.

TABLE 1: Percent Ingredient Composition of Layer Ration

Sr. No.	Ingredient (%)	T_0	T_1	T_2	T_3
1	Maize (Ground)	42	42	42	42
2	Jowar (Ground)	14	14	14	14
3	DORB	06	06	06	06
4	Sunflower meal	04	04	04	04
5	Soybean Meal	22	22	22	22
6	LSP	2.6	2.6	2.6	2.6
7	Shell Grit	7.5	7.5	7.5	7.5
8	Di-Calcium Phosphate	1.5	1.5	1.5	1.5
9	Salt	0.4	0.4	0.4	0.4
	Total	100	100	100	100
10	Dried Tamarind Pulp Powder (over and above)	00	0.25	0.50	1.00

TABLE 2: Proximate composition (% DMB) of dried pulp of Tamarind fruit

Nutrients	Composition (% DMB)
Dry Matter	84.8
Crude Protein	4.67
Crude Fiber	4.4
Ether Extract	2.2
Total Ash	2.0
Calcium	0.2
Phosphorus	0.40
Nitrogen Free Extract	86.73

RESULTS &DISCUSSION

Serum biochemical

The mean values of serum cholesterol, HDL, LDL, Total protein, albumin, globulin and triglyceride (mg/dl) observed in different experimental groups at 20th, 28th and 36th week were shown in Table -3. After supplementation of DTPP in different treatment group at the levels of 0.25%, 0.5% and 1% for 16 weeks period the levels of serum total cholesterol was significantly($P<0.01$) reduced by 25 to 28%. Similar observations were reported by Chowdhury *et al.* (2005) with significantly reduced serum cholesterol levels in layer after supplementation of DTPP at 2% level, also reported by Iftekhar *et al*. (2006) in humans, Jindal *et al.* (2011) in rats, Khairunnuur *et al*., (2011) in rats and Shinde *et al.* (2015) in broilers with significantly reduced serum total cholesterol levels after supplementation of DTPP/ tamarind pulp extract at different levels. The possible mechanism of reducing serum total cholesterol might be due to production of cholic and deoxycholic bile acids from cholesterol. The excess of cholesterol is converted to their bile acids by hepatocytes and are conjugated with glycine and taurine. These acids enter in to the small intestine where they are absorbed and directed to the liver and decrease in bile acid recycling would ultimately results in lowering in serum cholesterol concentration. The supplementation of DTPP at various suggested levels in present study significantly ($P<0.01$) increased serum HDL with 42 to 45% in experimental group as compared to control. Martinello *et al*. (2006) reported similar observations with increase in serum HDL levels up to 61% in hamster after supplementation of tamarind fruit pulp extract (5%). Similar observations were also reported by Jindal *et al*. (2011) and Khairunnuur *et al*. (2011) with significantly increased serum HDL levels in rats after supplementation of ethanolic extract (100mg/kg) and aqueous extract (50mg/kg) of tamarind pulp respectively.

The supplementation of DTPP at various suggested levels in present study significantly ($P<0.01$) reduced serum LDL with 30 to 39% in experimental group as compared to control. Similar observations were also reported by Khairunnuur *et al.* (2011) and Chor Yin lin *et al.* (2013) with significantly reduced serum LDL levels after supplementation of tamarind pulp extract in rats and hamsters respectively. The reduction in serum LDL levels might be due to epicatechins contents in *Tamarindus indica* (Luengthanaphol *et al*., 2004). The long term feeding of epicatechins believed to be beneficial for the suppression of high fat diet induced hypercholesteremia by modulating lipid metabolism. The supplementation of DTPP at various suggested levels for 16 weeks in present study significantly ($P<0.01$) reduced serum triglyceride with 29-31% in experimental group as compared to control. Similar observation were reported by Martinello *et al*. (2006) in hamster with 60% reduction in serum triglyceride levels after feeding of 5% dried tamarind pulp. The average serum total protein (g/dl) (4.59 ±0.13, 4.77 ±0.18, 4.54 ±0.12, 4.81 ±0.13), serum albumin (g/dl) (2.21

±0.05, 2.19 ±0.05, 2.5 ±0.13 and 2.42 ±0.15) and serum globulin (g/dl) (2.38 ±0.15, 2.58 ±0.18, 2.04 ±0.11, 2.39 ±0.17) observed at the end of trial (36th week) was physiologically normal and statistically non-significant in all experimental groups.

TABLE 3: Mean values of serum biochemical parameters in different week

	20th week				28th week				36th week			
Parameter	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
Serum Total cholesterol (mg/dl)	164.3 ±5.64	159.13± 5.11	160.38± 3.61	161.71±4 .23	157.73± 2.94[a]	125.96 ±1.67[b]	128.23 ±2.38[b]	125.26± 1.97[b]	155.1 ±3.04[a]	120.8 ±2.18[b]	119.48 ±2.03[b]	117.08± 1.57[b]
HDL (mg/dl)	30.24 ±4.51	29.43 ±4.21	31.38 ±3.98	31.50 ±4.16	35.87±2. 20[b]	50.02 ±2.85[a]	51.68 ±2.38[a]	53.21 ±2.31[a]	38.99 ±2.95[b]	56.34 ±2.55[ab]	55.45 ±2.55[a]	55.65 ±1.93[a]
LDL (mg/dl)	60.54 ±2.57	61.98 ±3.38	58.68 ±3.33	58.78 ±2.75	59.80±2. 67[a]	39.48 ±2.90[b]	42.36 ±4.28[b]	37.21 ±3.43[b]	57.15 ±1.72[a]	34.99 ±4.63[b]	40.50 ±3.39[b]	34.85 ±4.11[b]
Total Protein (g/dl)	4.55 ±0.09	4.79 ±0.07	4.62 ±0.15	4.99 ±0.20	4.57 ±0.12	4.89 ±0.18	4.62 ±0.15	4.63 ±0.23	4.59 ±0.13	4.77 ±0.18	4.54 ±0.12	4.81 ±0.13
Albumin (g/dl)	2.23 ±0.08	2.31 ±0.08	2.25 ±0.06	2.27 ±0.06	2.24 ±0.23	2.31 ±0.11	2.26 ±0.06	2.29 ±0.08	2.21 ±0.05	2.19 ±0.05	2.50 ±0.13	2.42 ±0.15
Globulin (g/dl)	2.43 ±0.08	2.66 ±0.17	2.64 ±0.17	2.64 ±0.13	2.38 ±0.28	2.48 ±0.21	2.36 ±0.15	2.34 ±0.28	2.38 ±0.15	2.58 ±0.18	2.04 ±0.11	2.39 ±0.17
Triglyceride (mg/dl)	1605.11 ±63.62	1657.44 ±113.19	1581.33 ±89.03	1756.11 ±77.61	1707.33 ±87.91[a]	1342 ±55.33[b]	1299.44 ±23.65[b]	1246.67 ±21.06[b]	1712.11 ±70.22[a]	1227.89 ±29.79[b]	1222.33 ±39.49[b]	1200.67 ±25.91[b]

Note: Mean with different superscript in column differs significantly (P<0.01).

TABLE 4: Mean values of egg yolk biochemical parameters in different week

Note: Mean with different superscript in column differs significantly (P<0.05).

	20th week				28th week				36th week			
Parameter	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
Egg yolk cholesterol (mg/g)	17.29 ±1.14	18.15 ±1.75	17.73 ±1.29	18.89 ±1.13	17.87± 0.17[a]	15.53 ±0.68[b]	15.92 ±0.80[b]	15.71 ±0.66[b]	17.58 ±0.70[a]	15.19 ±0.72[b]	15.33 ±0.39[b]	15.54 ±0.56[b]
HDL (mg/g)	9.38 ±0.70	9.62 ±1.21	9.83 ±1.05	9.91± 0.77	9.93 ±0.42	11.25 ±0.57	10.56 ±0.46	10.43 ±0.60	9.16 ±0.51	11.4 ±0.73	10.49±1. 11	10.54 ±0.51
LDL (mg/g)	6.12 ±0.57	6.58 ±0.62	6.6 ±0.31	6.28 ±0.38	6.33 ±0.53	5.96 ±0.39	5.99 ±0.54	5.82 ±0.29	6.46 ±0.56	5.49 ±0.15	5.93 ±0.29	5.41 ±0.25
Triglyceride (mg/g)	129.67 ±3.93	130.26 ±4.12	133.82 ±6.85	126.91 ±5.30	129.17 ±3.87	120.37 ±2.48	121.99 ±1.46	122.44 ±4.26	128.87 ±5.56	115.07 ±2.68	117.71 ±2.66	116.34 ±2.99

Egg yolk biochemical

The mean values of egg yolk cholesterol, HDL, LDL and triglyceride (mg/g) observed in different experimental groups at 20th, 28th and 36th week were shown in Table -4 The average values of yolk cholesterol (mg/g) at 36th week were 17.58 ±0.70, 15.19 ±0.72, 15.33 ±0.39 and 15.54 ±0.56 in control group T_0 and treatment group T_1, T_2 and T_3 respectively. It was observed that significantly lower ($p<0.05$) cholesterol levels were recorded in all treatment group as compared to control T_0. After supplementation of DTPP in different treatment group at the levels of 0.25%, 0.5% and 1% for 16 weeks period the levels of yolk cholesterol was reduced by 12 to 14%.Chowdhury *et al*., (2005) reported that egg yolk cholesterol concentrations was not affected by dietary tamarind however, serum cholesterol concentration were decreased quadratically at the level of 2, 4, 6 and 8% dietary tamarind fed for six weeks in layer poultry birds. Yingyuen *et al.* (2011) reported significantly lower egg yolk cholesterol levels in layer poultry birds after supplementation of 0.4% *Tamarindus indica* for sixteen weeks period. The observation recorded in present study is in agreement with Yingyuen *et al*. (2011).

It was reported that the composition of egg yolk fatty acid is reflection of fatty acid synthesis by liver of laying hen considering amount of egg yolk fatty acid provided by adipose tissue is about 20% (Grimes *et al*., 1996). The reduction in egg yolk cholesterol could be due to suppression of lipogenic enzymes as indicated by pronounced decrease in m-RNA abundance and enzyme synthesis (Tomilson *et al*., 1988).

CONCLUSION

The present study concludes that serum cholesterol can be reduced by 25to 28% and egg yolk cholesterol can be reduced by 12 to 14% by supplementation of DTPP between 0.25 to 1.00 % for 16 weeks period in layer birds.

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