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Effect of *in ovo* injection of vitamin A on serum lipid profile and liver function in broiler

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Abstract

The aim of this study was to investigate the effects of *in ovo* injection of vitamin A on some blood parameters in broiler chickens at 7 and 35 day of age. Six hundred fertile eggs of Hubbard broiler breeder were divided into 4 groups. One group served as a control (T0) while the other groups were injected with 100 (T1), 150 (T2) and 200 I.U (T3) vitamin A at day zero before placing in the hatching machine. After hatching, serum lipid profile and liver function enzymes were determined. Results showed no significant differences between control and treated groups at 7 day of age in the form of GOT, GPT, ALP, cholesterol, triglyceride, HDL and VLDL whereas LDL was significantly higher in treated groups compared to control. At day 35, GOT, GPT and ALP were significantly lower in treated groups compared to control. Furthermore, serum cholesterol and LDL was significantly lower in T3 compared to control. No significant difference was found in triglycerides and VLDL between treated and control groups. Data suggested that, *in ovo* injection of vitamin A can significantly reduce serum GOT, GPT, ALP, cholesterol, LDL and increased HDL at day 35 in broiler chickens.

Keywords: In ovo injection; blood parameters; broiler; vitamin A

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Introduction

Vitamin A is one of the important vitamins which are necessary during the life for proper functioning of the body (Olson, 2003). It is required for multiple functions in the body such as vision, immune function, growth (Azais-Braesco and Pascal, 2000) and embryonic development (Maden, 2001). This vitamin is easily oxidized and affected by light, temperature, humidity and storage conditions (Al-Husainy and Abu-Alella1990) which may result in early embryo death and reduced hatchability (Wilson, 1997). Recent advances in poultry production have opened a new erea of injecting various biologically important compounds *in ovo* to support the embryo formation and subsequent growth and development of chicks after hatching (Ellen, 2004; Tangara et al., 2010).

The site of absorption of vitamin A in the chicks is parallel to the site of absorption of fatty acids and cholesterol (Sklan et al., 1975). Orlov et al. (1987) found that the percentage of feed additive that pass from hens body to egg is about 25-30% while the rest is going to the hens body, therefore, the researchers recognized *in ovo* injection as one of the quick route to transfer nutrient compounds like amino acids and glucose directly to the developing embryo (Al-Asady, 2006) or vitamins (Robel, 2002; Mahmood, 2010).

It was found that there is a positive relationship between the levels of *in ovo* injected with vitamin A and its concentration in liver chicks post hatch (March et al., 1972; Sauri et al., 1998). In an experiment, the effect of *in ovo* injection of vitamins on embryonic and post-hatch growth performance, Bhanja et al. (2007) found that vitamin A and C may influence the embryonic development and improved hatchability and increased chicks weight to egg weight ratio. The objective of the present study was to determine the effect of different concentrations of vitamin A injected into eggs during the embryonic stage on some blood parameters of broilers at 7 and 35 days old.

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Materials and Methods

Six hundred fertile eggs from the broiler breeder (64 week old) were divided equally into 4 groups. Eggs of group 1 served as control (T0; without injection), while groups 1, 2 and 3 were injected with 100 I.U (T1), 150 I.U (T2) and 200 I.U (T3) of vitamin A respectively at day zero before placing in the hatching machine. The *in ovo* injection was done through a pinhole made at the broad end of the egg. The egg was thoroughly disinfected using 75% ethyl alcohol. The pinhole was mad using 25 mm needle as per the method standardized by Bhanja et al. (2004). After puncturing the egg shell, 0.1 ml of freshly prepared solution of vitamin A was gently injected into air cell with the assistance of a micropipette. Then the hole was sealed with liquid paraffin and eggs were placed in the incubator. Incubation of eggs was carried out in a hatching machine (Zeddan, Holland). The incubation process was conducted in accordance with the commonly accepted procedures applying optimal hatching parameters. On 19th day, the eggs were shifted to the Hatcher and kept in the respective pedigree hatching boxes on the day of hatch, chicks were weighed, wing banded and transferred to the rearing house for growth.

Chicks hatched from the respective treatments were distributed in pens with three replicates per treatment. All pens were bedded with wood shavings litter and equipped with feeders and waters. The birds were fed a starter diet until 21 day of age followed by a finishing diet from day 22 until day 35. The basal diet was formulated using NRC (1994) guideline and contained 21.5% crude protein, 2928 kcal/kg ME in starter, and 19.7% crude protein and 2988 kcal/kg ME in finisher diet (Table 1).

The diet and fresh water were offered *ad libitum*. Light was provided continuously (24 h). At the end of day 7 and day 35, 3 birds randomly chosen from each treatment were slaughtered and blood was collected. Blood samples were centrifuged at 3000 rpm/min for 15 min, and then serum was collected and stored at -20ºC for later analysis. Serum samples were thawed at room temperature to determine glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were determined using biochemical analyzer kit with the help of spectrophotometer (Biosystems, Espain).

Statistical analysis

All data obtained were analyzed using ANOVA (SAS, 2010). Significant difference among treatment was determined by Duncan's multiple rang tests (Duncan, 1955). P value less than 0.05 was considered as statistically significant.

Table 1: Composition and calculated analysis of experimental diet

Ingredient%	Starter (0-4wks)	Finisher (4-7wks)
Yellow corn	58.6	63.6
Soybean meal (44%)	31	26
Protein concentrate*	10	10
Food salt	0.4	0.4
Total	100	100
Calculated composition**		
Crude protein%	21.5	19.7
ME (kcal/kg)	2928	2988
Methionine%	0.79	0.74
Lysine%	1.19	1.05
Fat%	2.7	2.9
Fibre%	3.8	3.6
Calcium%	0.77	0.76
Available phosphorus%	0.73	0.53
Vitamin A (I.U/kg)***	13406.4	13526.4

*Provides per kg of diet: oil 3.64%, crude fibre 4.3%, lysine 1.7%, methionin 1.5%, methionine+systine 1.94%, calcium 6.9%, available phosphorus 3.3%, lenolic acid 0.26%, vit A 12000 IU/kg, vit D 350000 IU/kg, vit K 450 mg, vit B 130 mg, vit B 260 mg, vit B6 25mg, vit B12 0.015 mg, biotin 500µg, folic acid 10mg, nicotinamide 10 mg, Mn 1300 mg, Iodide 17 mg, Co 1 mg, Zn 800 mg, Fe 1500 mg, Se 2 mg, Cu 200 mg; **NRC (1994); ***Lesson and summer (1991 & 1997)

Results and Discussion

Table 2 presents the effects of *in ovo* injection of different concentrations of vitamin A on serum total cholesterol, triglycerides, HDL, LDL and VLDL at 7 and 35 day of age. At 7 days, results showed no significant statistical difference between the control and experimental groups except LDL. LDL was found to be significantly ($P<0.05$) higher in T1 and T2 in comparison with control group. Chicks treated with 200 IU vitamin A did not differ significantly with control group. No significant difference was found in other parameters. At day 35, cholesterol and LDL concentration decreased significantly in T3 whereas HDL increased significantly in the same group.

Reports on the effect of *in ovo* injection of vitamin A are not available. Kaya et al. (2001a) found that feeding vitamin A and zinc at the rate of 10 IU/kg and 200 mg/kg respectively reduced plasma triglyceride in laying hens. March and Biely (1962) concluded that administration of large amount of vitamin A reduced serum cholesterol in 6-7 weeks old chickens. Similar to our results, Salmanzadeh and Shahryar (2012) did not find any significant effect of *in ovo* injection of L-carnitine on blood cholesterol level in turkey poults. Similar to our results, Kaya et al. (2001b) found no significant difference on plasma cholesterol, triglyceride and HDL in laying hens in response to vitamin A supplementation.

Table 2: Effect of *in ovo* injection of vitamin A on serum lipid profile at 7 and 35 day old broiler

Age (day)	Treatments	Serum lipid profile				
		Triglycerides (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Cholesterol (mg/dl)
7	T_0	186.00±35.8	37.21±7.16	61.22±9.55^{b}	46.32±0.00	151.83±18.15
	T_1	121.09±37.70	24.22±7.54	122.46±19.9^{a}	43.74±3.40	190.43±24.84
	T_2	169.19±30.76	27.17±10.74	128.51±22.8^{a}	36.02±4.63	191.71±27.68
	T_3	126.99 ±44.69	25.40±8.93	110.99±16.1^{ab}	46.32±2.22	182.71±21.64
35	T_0	168.34±61.38	42.42±15.07	65.66±3.72^{a}	36.49±1.96^{a}	138.96±11.14^{a}
	T_1	115.18±31.11	16.83±0.51	60.72±4.14^{a}	36.67±1.11^{a}	108.08±3.36^{ab}
	T_2	128.39±17.90	25.69±3.57	61.16±22.04^{a}	30.88±0.00^{b}	117.73±25.62^{a}
	T_3	106.32±0.00	21.26±000	45.76±0.01^{b}	38.60±0.00^{a}	65.62±0.00^{b}

Values within different superscript differ significantly (P≤0.05).

Table 3: Effect of in ovo injection of vitamin A on broilers serum GOT, GPT and ALP at 7 and 35 days old

Age (day)	Treatments	ALP (U/l)	GPT (U/l)	GOT (U/l)
7	T_0	8.59±0.53	6.66±0.33	6.000±0.55
	T_1	8.26±0.24	6.333±0.66	5.00±0.57
	T_2	8.21±0.46	6.330±0.66	5.00±0.57
	T_3	7.97±0.28	6.333±0.66	5.00±0.57
35	T_0	6.80±1.36^{a}	7.16±1.16^{a}	3.50±0.86^{a}
	T_1	3.38±0.42^{b}	2.50±2.50^{b}	1.66±0.33^{b}
	T_2	3.14±0.18^{b}	4.50±0.86^{b}	1.50±0.28^{b}
	T_3	2.67±0.00^{b}	2.00±0.00^{b}	1.00±0.00^{b}

Values with different superscripts within a row differ significantly (P≤0.05)

Statistical analysis of serum GOT, GPT and ALP are shown in Table 3. It is clear from these results that there were no significant difference between control and experimental groups at 7 day of age. At day 35, we found that *in ovo* vitamin A injection resulted in significant reduction in these traits compared to un-injected group (T0) and the lowest values was in group that was treated with 200 IU vitamin A.

Lipid profile usually includes total cholesterol, triglycerides, HDL, LDL and VLDL. Lipoproteins are proteins in blood whose main purpose is to transport cholesterol, triglycerides and other insoluble fats. HDL (alpha lipoproteins) is predominantly protein with small amount of cholesterol. In the current study, the significant increase in serum HDL at 35 day in 200 IU vitamin A treated eggs is a good indicator of protective cardiovascular effect associated with HDL (good cholesterol). LDL cholesterol (bad cholesterol) is deposited in peripheral tissues and is associated with increased risk of heart and vascular diseases. Therefore, in current study, reducing levels of serum LDL at 35 day in 200 IU vitamin A *in ovo* injected group would have a beneficial effect on broiler.

Vitamin A is a potent biological antioxidant (Denli et al., 2003). Reports concluded that liver plays a major role in vitamin A homeostasis (Sklan, 1983) and it is the main organ for storage of vitamin A. From liver, it is transferred to other tissues of the body as needed (Scotter et al., 1992). The first few days after hatching are a critical period for survival and development of neonates in poultry because of considerable energy catabolism (Ebrahimnezhad et al., 2011). This could be the reason for the absence of significant difference in serum liver function enzymes and lipid profile between control and treated groups at 7 day of age. Vitamin A is one of antioxidant vitamins that have been found to be useful in preventing tissue injury caused by toxic agents (Al-Fartosi et al., 2011). Decreased levels of GOT, GPT and ALP in birds at day 35 of age in treated groups offered protection by preserving the structural integrity of the hepatocellular membrane against oxidative factors by inhibiting the free radicals on liver cells, thereby maintaining the structural and functional integrity of cells (Chew, 1995; McDowell, 2000).

Conclusion

Based on the results of present study, it can be concluded that *in ovo* injection of vitamin A at zero day could offer hepatoprotective effect in broilers at market age.

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