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Full Length Research paper

Effects of polysorbate-80 on liver and kidney function in broiler chicken during juvenile growth period

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An experiment was conducted to examine the effects of polysorbate-80 (PS-80) on liver and kidney function in broiler chicken using 120 one-day-old Cobb 500 chicks. The birds were randomly divided into 6 groups, of 20 birds each (as one replicate) and then allocated to 1 of 6 floor pens (90×180 cm) at the stocking density of 0.08 m^2/ bird in a concrete floor, cross ventilated house. All the birds received the same corn and soybean meal based starter (1 to 21 days) and grower (15 to 28 days) diets for *ad libitum* consumption. The birds in 3 pens continually received water supplemented with either 0 (control) or 3500 ppm PS-80 throughout the experiment. Live weight gain as well as, feed conversion ratio were significantly suppressed in response to supplementation of drinking water with 3500 ppm PS-80 ($P<0.05$). Mean liver weight and liver pH did not significantly change, for the birds received treated water compared to those grown on normal water ($P>0.05$). No alteration in concentration of GPT, GOT, Urea, uric acid and creatinine in blood serum were observed in the chicks receiving PS-80-supplemented water as compared to control group ($P>0.05$). The PS-80 treated water resulted in increased levels of serum ALP ($P<0.05$). Based on these findings, persistent exposure of broiler chicks to 3500 ppm PS-80 through drinking water has a negative impact on juvenile growth performance in chicks. No indication found that such discouraging impact is caused by liver or kidney dysfunction.

Key words: Polysorbate-80, GPT, GOT, ALP, liver, kidney.

INTRODUCTION

Emerging use of poorly soluble or permeable compounds such as vaccines, herbal extracts and essential oils in poultry production, represents a reason to introduce inexpensive and non toxic vehicle formulations and emulsifiers (Gad et al., 2006). Polysorbate-80 (PS-80), also known as Tween-80, is a commercially available non-ionic surfactant (Chou et al., 2005) which originates from polyethoxylated sorbitan and oleic acid and is mainly used in food industry (Goff, 1997). PS-80 is also used as an excipient as well as, emulsifier in the manufacture of anti arrhythmis amiodarone (Path et al., 1991), microbial media culture (Jacques et al., 1980), cosmetics and oral, parentral and topical pharmaceutical formulations (Rowe, 2009).

PS-80 is shown to be safe for few spices of laboratory animals and is well-tolerated by human (Steele et al., 2005; Roberts, 2010). Rats fed on diets supplemented with 5% PS-80 (v/v) showed no toxic effects (Oser and Oser, 1956). In the same study, the adverse effects of PS-80 on reproduction performance were pointed out where the dietary dose increased to 20%. Early reports on safety study of PS-80 in dogs and other canine species reveal that IV administration has been associated with an idiosyncratic reaction characterized by a prolonged depressor response (Krantz et al., 1951). Elder, (1984) and Masini et al. (1985) showed that this hypotensive response was caused by a marked release

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Table 1. Mean water and tween-80 intake for experimental flock in different periods from 1 to 28 days of age.

Age (day)	Water consumption (ml/bird)			ADG[1]	Tween intake (µg)	
	Control birds	Treated birds	Difference (%)		Per day	µg/g WG/day
1 - 7	990	1232	+24.00	13.25	616	46.50
8-14	2650	2686	+1.30	28.35	1434	50.62
15 - 21	4540	4750	+4.60	70.04	2375	33.91
22 - 28	13500	13100	-2.96	66.64	5614	82.24
1-28	21680	21760	-2.45	45.57	2510	55.01

Average daily weight gain.

of histamine after IV intake of PS-80. Thackaberry et al. (2010) confirmed that PS-80 at the rate of 10 mg/kg was safe and well tolerated when administered to mice, rats, dogs and cynomolgus monkeys through oral gavage over the course of 3 months administration. However, experimental results on effects of PS-80 on chicken as well as, other avian species are scarce.

Wide application of vaccines and increasing use of herbal extracts and essential oils in avian drinking water, warrant further investigation on the effects of continual administration of PS-80 in avian species. This study was undertaken to study the response of juvenile broiler chicks to intake of PS-80 through drinking water with respect to indicators of liver and kidney function

MATERIALS AND METHODS

Experimental flock

A total of 120 one-day-old Cobb 500 broiler chicks of mixed sex were provided from Zarbal hatchery, Borujerd, Iran. Up on arrival, the birds were randomly divided in 6 groups of 20 birds each and then randomly allocated to 1 of the 6 floor pens (90×180 cm). The pens were located in a cross-ventilated negative-pressure house equipped with infra red brooders. The space allowance for each bird was 0.08 m^2. Corn and soybean meal based starter (230 g/kg CP, 3100 Kcal ME/kg 1 to 21 days) and grower (191 g/kg CP, 3220 Kcal ME/kg 15 to 28 days) diets and water were provided for *ad libitum* consumption under a round the clock lighting regime.

Data collection

Live weight gain, feed intake, feed conversion ratio and mortality per cent were weekly determined from 1 to 28 days. At 28 days of age, two male and two female chicks per pen (those with the minimum difference from the mean pen weight for each sex) were killed and manually processed to obtain liver-to-body weight ratio and liver pH data. Blood samples (4 ml) were collected from all the killed birds to assess the serum enzymes including serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (SALP) and certain serum biochemical parameters. Concentration of SGPT and SGOT enzymes were assayed based on IFCC method without pyridoxal phosphate (P-5-P) as described by Bermeyer et al. (1986). SALP were measured based on DGKC method (Anonymous, 1972) with slight modification as adopted by SCE (Anonymous, 1974). The blood serum samples were evaluated for concentration of urea, uric acid and creatinine using an automatic analyzer (Selects E Autoanlyzer, Sr. No. 8-7140, Vital Company, Netherlands), following the indications for commercial kits at 25ºC.

Experimental design

A supplemented water (with 0 or 3500 ppm PS-80) × gender (male or female) factorial design was used to evaluate the effects of two main fixed factors (PS-80 level and sex) and their interaction on liver function of broiler chicks during early growth period, up to 28 d. The birds in each replicate (a pen) were of mixed sex and received drinking water supplemented with either 0 (control) or 3500 ppm PS-80 (PS-80). However, the PS-80 intake was associated with water consumption which by itself is a function of feed intake. The ultimate PS-80 intake based on water consumption and average daily gain is presented in Table 1.

Statistical analysis

Collected data were subjected to analysis of variance utilizing the General Linear Model Procedure of Statistical Analysis System (SAS Institute, 2002). Means for each fixed effect as well as, interactions were separated based on Duncan's Multiple Range Test (DMRT). Means were considered significantly different at P<0.05.

RESULTS

A summary of the results achieved for the response of the chicks in conventional productive performance parameters to continual receiving polysorbate-80 (PS-80) through drinking water is presented in Table 2. The birds with access to treated water demonstrated significantly lower average daily gain (P<0.05). Weight gain suppression for treated birds initiated from neonatal days improved by age such that it reached 117 g lower body weight at 28 days as compared to control group (Figure 1). Mean feed intake did not significantly differ for treated birds as compared to the control group (P>0.05), while feed conversion ratio was significantly increased for the birds receiving PS-80 supplemented water compared to the birds fed on normal water (P<0.05). No mortality was confirmed for the birds receiving either normal or treated water.

Supplementation of 3500 ppm PS-80 into drinking water showed no significant effect on liver weight and liver significant differences were pointed out in SGPT and SGOT enzymes between the birds receiving either normal or treated water (P>0.05). However, administration

Table 2. Effect (mean ± S.E.) of Polysorbate 80 on average daily weight gain (AVG), average daily fed intake (ADFI), feed conversion ratio (FCR) and mortality in chicks.

Variable	Tween-80 (ppm)		P-Value
	0	3500	
ADG (g; 1-28 d)	47.54±0.17[a]	45.57±0.66[b]	0.0582
ADFI (g; 1-28 d)	67.89±0.20[a]	68.42±0.36[a]	0.3609
FCR (g:g; 1-28 d)	1.43±0.01[b]	1.50±0.02[a]	0.0212
Mortality (%, 1-28 d)	0.00±0.00[a]	0.00±0.00[a]	0.3295

[a-b] Means within a raw without a common superscript differ significantly (P<0.05).

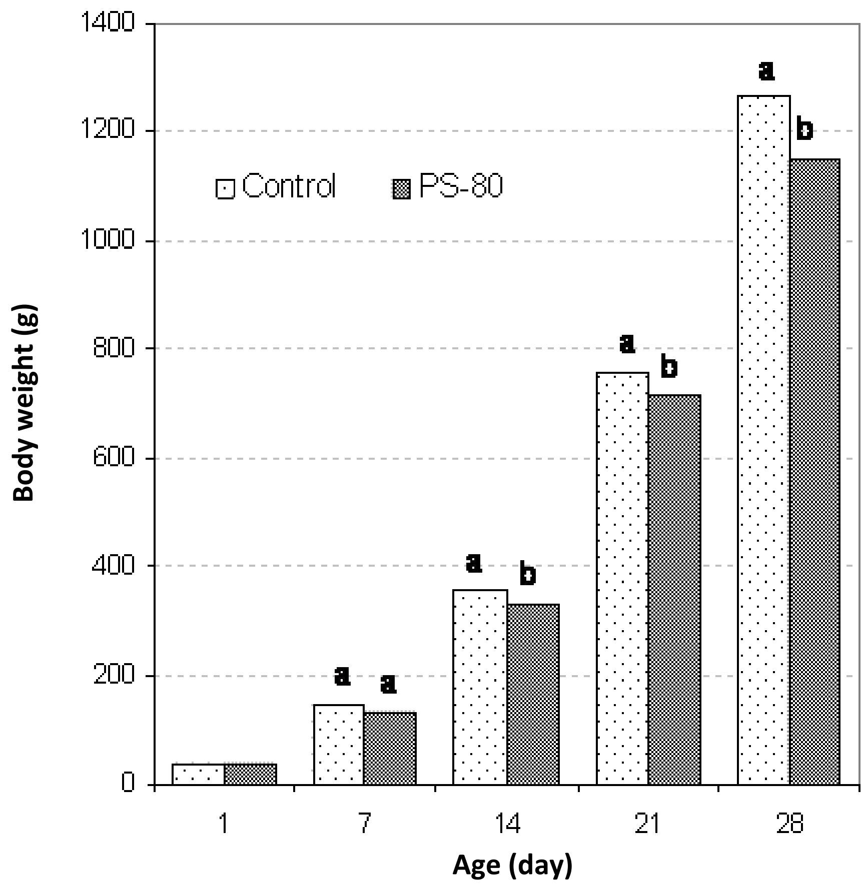

Figure 1. Change in live body weight (g) of the birds received normal water (control) and supplemented water with 3500 ppm polysorbate-80 at 1 to 28 days.

of PS-80 caused a considerable increase in ALP enzyme as compared to the normal chicks (62 vs. 45 IU/l; P=0.0984). The mean liver weight, liver pH, SGPT and SGOT were not significantly differ for the treated birds compared to control group for gender as well as gender × PS-80 interaction. The female birds generally showed lower ALP concentration in serum and their response in enhanced levels of the same enzyme with PS-80 was greater than the male chicks.

No significant differences in average uric acid, urea and creatinine concentrations (mg/dl) in serum were seen at 28 days of age between the birds received either normal or PS-80 supplemented water (P>0.05; Table 4). The mean values for all three variables with no significant differences were greater in male chicks as compared to females. There was a significant difference on uric acid level in serum for interaction between PS-80 and gender. However, it does not seem that administration of PS-80 through drinking water affects male and female birds in contrary manners. The PS-80 supplanted water resulted in lowered serum uric acid in males and females by 0.74 and 0.71 mg/dl, respectively.

Table 3. Effects (Mean ± SE) of normal and PS-80 treated water on liver percentage and pH, blood serum level for glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (SALP) in broiler chicks at 28 days of age.

Factor\ level		Liver (%)	Liver pH	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
Polysorbate-80 (ppm)						
0		2.42 ± 0.13^{a}	6.24 ± 0.03^{a}	36.67 ± 11.24^{a}	26.18 ± 1.74^{a}	45.87 ± 6.29^{b}
3500		2.44 ± 0.12^{a}	6.27 ± 0.04^{a}	29.27 ± 6.57^{a}	25.98 ± 1.01^{a}	62.33 ± 6.98^{a}
Sex						
Female		2.42 ± 0.13^{a}	6.24 ± 0.03^{a}	36.67 ± 11.24^{a}	26.18 ± 1.74^{a}	45.87 ± 6.29^{b}
Male		2.44 ± 0.12^{a}	6.27 ± 0.04^{a}	29.27 ± 6.57^{a}	25.98 ± 1.01^{a}	62.33 ± 6.98^{a}
Sex						
Female		2.45 ± 0.12^{a}	6.24 ± 0.03^{a}	32.31 ± 7.35^{a}	27.32 ± 1.08^{a}	45.62 ± 5.07^{b}
Male		2.42 ± 0.12^{a}	6.27 ± 0.04^{a}	33.64 ± 11.38^{a}	24.60 ± 1.67^{a}	66.78 ± 8.03^{a}
PS-80 × Sex						
0	Female	2.50 ± 0.17^{a}	6.23 ± 0.03^{a}	31.11 ± 7.22^{a}	27.41 ± 1.44^{a}	46.82 ± 6.23^{b}
0	Male	2.22 ± 0.15^{a}	6.27 ± 0.05^{a}	53.33 ± 43.33^{a}	22.47 ± 5.73^{a}	67.28 ± 9.56^{a}
3500	Female	2.35 ± 0.16^{a}	6.27 ± 0.09^{a}	35.00 ± 18.48^{a}	27.13 ± 1.67^{a}	53.11 ± 8.43^{a}
3500	Male	2.49 ± 0.16^{a}	6.28 ± 0.05^{a}	26.25 ± 4.98^{a}	25.40 ± 1.28^{a}	66.28 ± 9.26^{a}
SEM		0.08	0.03	6.41	0.98	4.99
			P > F			
PS-80		0.8889	0.5275	0.5774	0.9206	0.0984
Sex		0.8547	0.5524	0.9214	0.1859	0.0407
PS-80 × Sex		0.2909	1.0000	0.2787	0.3827	1.0000

[a-b] Means within a column for each main effect and interaction without a common superscript differ significantly (P<0.05).

DISCUSSION

The present study intended to evaluate the effect of high levels of polysorbate-80 (PS-80) on liver and kidney functions in broiler chicks when it may be consumed through oily medications or additives as in the case of phytogenic preparations. A noticeable indication of hepatic damage is the leaking of cellular enzymes into the plasma (Schmidt and Schmidt, 1983), due to instability reasoned in the transport functions on hepatocytes (Pratt and Kaplan, 2000). The estimation of serum marker enzymes was reported as a useful quantitative marker for determining the extent and type of hepatocellular damage in chicken as well as, many other species exposed to toxic substances through feed (Celyk et al., 2003), water (Wu, 1997) and air (Amakiri et al., 2008). When liver cell plasma is injured, a variety of enzymes situated in the cytosol is released into the circulation, thus, causing increased enzyme levels in the serum. Constant exposure of the chicks to 3500 ppm PS-80 through drinking water resulted in no increase in liver weight (hypertrophy), no change in liver pH and no elevation in GPT and GOT marker enzymes in serum. These results considered as pathological evidences to confirm the non toxicity of PS-80 with respect to liver function. However, the increased ALP is interesting, but may be misleading, since it was minimal and not associated with an increase in any other liver-specific enzymes.

In avian species the kidney function is well evaluated based on concentration of uric acid, urea and creatinine (Selvaraj et al., 1998). The concentration of these three biochemical components in peripheral blood have shown to be reliable indicators for nitrogen metabolism and kidney function (Zantop, 1997). Huff et al. (1988) also confirmed that significant increase in serum uric acid and creatinine levels are indicative of nephrotoxicity in broiler chicken. Among them, uric acid is of prime importance. Broiler chickens like other birds are urecotelic and eliminates 60 to 80 percent of nitrogen in the form of uric acid. Uric acid serum levels changes with protein content in feed, quantity of feed ingested (Costa et al., 1993), and water consumption and kidney health for normal filtration rate, among many other factors. In our study, no noticeable changes were pointed out in concentration of uric acid, urea and creatinine in the serum of the treated birds. It reveals that PS-80 is safe for kidney function at the high dose of 3500 ppm.

The results evidently demonstrated that exposure of a

Table 4. Effects (Mean ± SE) of normal and PS-80 treated water on blood serum level (mg/dl) of uric acid, urea and creatinine in male (M) and female (F) broiler chicks at 28 days of age.

Factor\ level		Acid uric (mg/dl)	Urea (mg/dl)	Ceratinine (mg/dl)
PS-80 (ppm)				
0		5.11 ± 0.67^{a}	2.92 ± 0.19^{a}	0.28 ± 0.02^{a}
3500		4.82 ± 0.70^{a}	3.00 ± 0.21^{a}	0.28 ± 0.01^{a}
Sex				
Female		4.12 ± 0.49^{a}	2.92 ± 0.18^{a}	0.27 ± 0.02^{a}
Male		5.96 ± 0.79^{a}	3.00 ± 0.23^{a}	0.28 ± 0.01^{a}
PS-80 × Sex				
0	F	4.64 ± 0.60^{ab}	2.89 ± 0.20^{a}	0.27 ± 0.02^{a}
0	M	6.50 ± 2.08^{a}	3.00 ± 0.58^{a}	0.30 ± 0.00^{a}
3500	F	3.93 ± 0.47^{b}	3.00 ± 0.41^{a}	0.28 ± 0.03^{a}
3500	M	5.76 ± 0.85^{ab}	3.00 ± 0.27^{a}	0.28 ± 0.02^{a}
SEM		0.48	0.01	0.14
			P > F	
PS-80		0.7471	0.7849	0.9988
Sex		0.0521	0.8017	0.5887
PS-80× Sex		0.1881	0.9987	0.4886

$^{a-b}$ Means within a column for each main effect and interaction without a common superscript differ significantly (P<0.05).

juvenile chick to PS-80 supplemented water leads to no negative consequence with respect to liver as well as, kidney metabolic pathways. However, the observations on growth and feed utilization parameters are not in favor of inclusion PS-80 to drinking water for chicks at the level of 3500 ppm. Such adverse impacts might rise in commercial broiler practices whereas PS-80 contained fat soluble substances such as phytogenic additives are given to birds via water or feed as medication or growth promoters. These evidences give an idea about presumable occurrence of an antagonistic impact of PS-80 on digestion or absorption process of one or more nutrient in gastrointestinal tract of chicks. Interpretation of the data gathered in this study offer no evidences to sustain the idea mentioned. Evaluation of digestibility for different nutrients with the diets containing PS-80 may approve the idea. From the economic standpoint, these results are rather significant as the minor differences multiply by huge number of birds in a commercial chicken house.

In conclusion, results of the present study demonstrate that persistent exposure of broiler chicks to 3500 ppm PS-80 through drinking water suppress gain weight and feed conversion ratio in chicks during the early growth periods. However, no evidences were found that such negative impacts are arising from either liver or kidney dysfunction.

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