Recovery over Time of Production Performance and Biological Functions of Laying Hens after Withdrawal Toxic Levels of Dietary Roxarsone

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ABSTRACT : Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) has been used as feed additives in poultry industries to improve production and control coccidiosis. The effect of high dietary levels of Roxarsone (ROX) on the performance and function of internal organs and the kinetics of recovery as well as its after-effects were examined in laying hens. The inclusion rates of ROX were 0, 100, 200, 300, and 400 mg per kg feed. Inclusion up to 200 mg did not show any adverse effects ($p>0.05$), whereas in the 300 and 400 mg groups, significant effects, particularly in the latter, were observed for three weeks after ROX addition ($p<0.05$). Recovery of the physical appearance occurred soon after ROX addition was withdrawn. Recovery of performance and internal organs, however, appeared to be dependent on the amount of residual ROX in the body; as the amount of ROX decreased, the toxic effect of ROX also decreased. In the third week after the withdrawal of ROX, complete recovery was observed in the lower dosage groups (100 or 200 mg groups) ($p>0.05$), whereas in the higher dosage groups (300 or 400 mg groups), recovery took at least five weeks; when complete recovery was observed in egg production and in liver weight ($p>0.05$). On the other hand, ROX might have damaged the liver and other tissues. The recovery of liver weight was probably due to accumulation of fatty particles rather than repair. It appeared, therefore, there were little after-effects of ROX on the hen's physical appearance, but some internal organs were probably damaged. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 1 : 48-54)***

Key Words : Roxarsone, Laying Hen, Egg Production, Liver Function, Lipid Metabolism

INTRODUCTION

Chronic arsenic toxicity has been discovered for more than a half century in some areas of the world. Arsenic in high doses generally decreases animal performance and causes abnormalities in the skin and hair (McDowell, 1992). However, organic arsenicals i.e., Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) and arsanilic acid, has been used as feed additives in poultry industries to improve production and control coccidiosis for decades (McDougald et al., 1992; Waldroup et al., 1995).

Roxarsone is readily hydrated and coagulated by absorbing moisture, and therefore, it is difficult to prepare feedstuffs containing an even mix of ROX. As a result, chickens tend to overdose on ROX by taking in coagulated ROX (Chen and Wu, 2000). When a higher amount of ROX is used in the feedstuff, a number of physiological and other effects appear in the chicken. When the amount of ROX in laying hen's diet reaches 156 mg/kg, significant decreases in feed intake and egg production are observed; whereas with 312 mg/kg, egg production ceases in two weeks. Furthermore, formation of fatty liver is observed one week after ROX withdrawal (Chiou et al., 1997). When intake of nutrients is excessive, lipogenesis is promoted dwarfing the lipid catabolism in the liver, resulting in the fatty liver (Chen et al., 2000; Chen and Chiou, 2001; Chen et al., 2005).

It is therefore desirable to examine the recovery rate of the after-effects of ROX. In previous research, the effect of ROX withdrawal has been examined for one or two weeks after the withdrawal and on production performance (Chiou et al., 1997). Therefore, in the present trial, the time after the withdrawal was extended up to seven weeks to see the duration of ROX after-effect on a number of phenomena including the performance and functions of some internal organs.

MATERIALS AND METHODS

Chicken and feeding

One hundred healthy (force-moulted) 84-week-old Single Comb White Leghorn laying hens with comparable weight and egg producing rate was placed in individual wire floored cages (38×18.5×39 cm), and received a photoperiod of 14L:10D (Park et al., 2005). The feed and water was available *ad libitum*. The basal diet was formulated according to the National Reasearch Council (NRC, 1994) Nutrient Requirements of Poultry as listed in Table 1. The ROX premix was prepared with dextran as carrier, and was added to the mixer in the last stage of mixing.

Hens were randomly allocated to the five groups with 20 hens in each group. Twenty hens in each group were further divided into five smaller groups of four hens, and the hens in each group were fed with the same batch of feed

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Table 1. Composition of basal diet

Ingredients	g/kg
Maize	603.7
Soya bean meal	240.0
Fish meal	30.0
Soya bean oil	29.8
Dicalcium phosphate	13.0
Limestone (pulverised)	77.2
Iodized salt	3.0
dl-Methionine	0.8
Vitamin premixa	0.5
Mineral premix b	2.0
Total	1,000.0
Calculated value:	
Crude protein (g/kg)	178.0
Metabolizable energy (kcal /kg)	2,900
Calcium (g/kg)	34.0
Available phosphorus (g/kg)	4.7

[a] Vitamin premix supplied the following per kilogram of diet: Vitamin premix supplied per kilogram of diet: Vitamin A, 12,000 IU; Vitamin D3, 3,120 ICU; Vitamin E, 37.5 IU; menadione sodium bisulphite, 6.25 mg; thiamin hydrochloride, 3.75 mg; riboflavin, 12.5 mg; pyridoxine hydrochloride, 10.0 mg; Ca-pantothenate, 18.8 mg; niacin, 50 mg; biotin, 0.06 mg; folic acid, 1.25 mg; cyanocobalamin, 0.05 mg.

[b] Mineral premix supplied the following per kilogram of diet: Cu ($CuSO_4 \cdot 5H_2O$, 254.5 g Cu/kg), 6 mg; Fe ($FeSO_4 \cdot 7H_2O$, 200.9 g Fe/kg), 50 mg; Mn ($MnSO_4 \cdot H_2O$, 324.9 g Mn/kg), 40 mg; Zn (ZnO, 803.5 g Zn/kg), 60 mg; Se ($NaSeO_3$, 455.6 g Se/kg), 0.075 mg.

to avoid uneven intake of ROX. Five doses of ROX (RHÔNE-POULENC, France), 0, 100, 200, 300 and 400 mg per kg of diet, were respectively given to the hens. The feeding trial lasted for 10 weeks; the first three weeks with experimental diets containing ROX followed by 7 weeks of the basal diet (without ROX).

Tests and analyses

Feed intake, egg production and egg weight were recorded daily. All hens were individually weighed for their body weight after 12 h of water and feed restriction on every Tuesday of the 3rd, 6th, 8th and 10th week. Blood samples were taken from the brachial-vein at the same time. After centrifuging (3,000 rpm, 10 min), serum was stored at -40°C for further analysis. At each time point, five hens (one each from the five sub-groups) from each drug group were sacrificed and the follicle, oviduct, liver, heart and kidney were excised and weighed. Approximately 5 g of the liver tissue was taken from the left side of liver, and then was washed with saline for pathology determination.

The liver is the main organ where lipid and protein are synthesized, metabolized, and distributed to the body, e.g., lipoprotein is a pivotal medium for the transport of lipid from the liver to the whole body. Therefore, in order to examine the liver function, total protein (TP), albumin (ALB), triacylglycerol (TG), cholesterol (CHOL) and lipoprotein of the blood were measured, using an automatic blood chemical analyzer (Roche COBAS MIRA) with Roche test kits for the former four. The last, serum lipoprotein, was measured by Helena titian gel electrophoresis as described by Houstmuller (1969). An electrophoresis data center scanning densitometer (Helena Laboratories, USA) was used to estimate the area of each fraction, in which the proportion of lipoproteins was calculated (Lien et al., 2005).

High dose of ROX causes cell injury (Chiou et al., 1997), which releases cell enzymes including creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH), into the serum. These enzymes were therefore regularly measured clinically to examine the organ damage with the automatic blood chemical analyzer mentioned above (Roche testing kits) that measured NADH at a wavelength 340 nm (Bergmeyer, 1983).

Pathology

For pathological examination, the liver was preserved in buffered 10% formalin, pH 7, embedded in paraffin, sectioned (6 μm), stained with haematoxylin and eosin, then examined and photographed under a microscope (×100).

Statistics

A factorial arrangement was applied to examine the effects of ROX levels over time and interacions. Variance was calculated with the general linear model procedure of the SAS (1985). Dunnett's test was used for analysis of the data and for comparison of the differences between time periods as described by Steel and Torrie (1960).

RESULTS

Effect of ROX on performance

When laying hens were allowed to consume a ROX-containing diet for three weeks, 200 mg ROX per kg of diet did not significantly ($p<0.05$) influence the body weight, feed intake, and egg weight, as shown in Table 2. In the group with 300 or 400 mg ROX, however, significantly ($p<0.05$) lower body weight, feed intake and egg weight, compared to the control, were observed.

By the third week (Table 2) after drug withdrawal, all except the body weight and egg production in the 400 mg group returned to normal ($p>0.05$). In the higher dose (300 and 400 mg) groups, contrary to the other groups, the body weight increased ($p<0.05$). On the other hand, the recovery in egg production was slower and remained lower than the other groups. From the 5th week on after the drug withdrawal, even in the 400 mg group, egg production and body weight no longer showed significant ($p<0.05$) differences from the other groups including the control.

Effect of ROX on the internal organs

Next, the effect on the weight of the internal organs was

Table 2. Effect of ROX on the performance of laying hens*

T[1] in week (t)	Level of ROX (mg/kg)					Means	SED	Significance		
	0 (Control)	100	200	300	400			ROX	Time	R×T
Body weight (kg/bird)										
3 (0)	1.5	1.5	1.4	1.4[a]	1.3[a]	1.4	0.10	NS	0.001	0.001
6 (3)	1.5	1.6	1.6[x]	1.6[ax]	1.6[ax]	1.6	0.11			
8 (5)	1.5	1.5	1.6	1.5[x]	1.5[x]	1.5	0.16			
10 (7)	1.6	1.5	1.6[x]	1.6[x]	1.6[x]	1.6	0.21			
Means	1.5	1.6	1.5	1.5	1.5					
SED	0.13	0.13	0.14	0.13	0.12					
Feed intake (g)										
3 (0)	98	102	94	74[a]	54[a]	85	1.9	0.001	0.001	0.001
6 (3)	107	101	101	109[x]	102[x]	104	2.1			
8 (5)	108[x]	116[x]	111[x]	109[x]	113[x]	112	2.5			
10 (7)	104	103	107	107[x]	97[x]	104	2.5			
Means	104	105	103	99	90					
SED	2.4	1.9	2	1.9	2.8					
Egg production (%)										
3 (0)	71	53	52	27[a]	4.7[a]	42	2.9	0.001	0. 001	0.001
6 (3)	77	83	80[x]	79[x]	49[ax]	74	2.9			
8 (5)	71	74	77	76[x]	77[x]	75	2.6			
10 (7)	80	77	77	79[x]	81[x]	79	2			
Means	75	72	71	64	51					
SED	2.6	2.7	3.2	2.3	2.9					
Egg weight (g)										
3 (0)	61	61	62	61	58[a]	60	1	NS	NS	NS
6 (3)	61	61	61	61	61	61	0.7			
8 (5)	60	60	59	60	60[x]	60	0.9			
10 (7)	62	61	61	60	60	61	0.9			
Means	61	61	61	61	60					
SED	0.8	0.9	1	0.8	0.9					

* The values listed are the mean and standard deviation of each group. The superscript [a] indicates significant difference ($p<0.05$) versus control (ROX level = 0). The superscript [x] indicates significant difference ($p<0.05$) versus control (t = 0).
[1] T (time) indicates total time since the start of the test, and t in the parenthesis indicates total time after the drug withdrawal (withdrawal time only).
NS: $p>0.05$.

examined. As shown for body and egg weight (Table 3), the presence of ROX did not generally affect the weight of most organs except for a few at a higher dose.

As ROX dose increased, the follicle, oviduct and liver weights showed a significant ($p<0.05$) decrease in 300 and 400 mg groups. All organs recovered to normal ($p>0.05$) at the third week of the drug withdrawal; except the follicle weight of 400 mg group which was significantly ($p<0.05$) heavier than control.

Kinetics of recovery after ROX withdrawal

In all cases, by the third week, the body weight, feed intake, egg production, and the weight of eggs (Table 2), were similar to the control, indicating that recovery was complete. An astonishing degree of recovery was observed in feed intake and egg production in the groups given higher doses of ROX. In the 300 mg group, a 50% increase ($p<0.05$) in feed intake was observed, whereas there was a three fold increase in the egg production. The degree of recovery was even greater in the 400 mg group. The feed intake nearly doubled and the egg production increased about ten fold. ROX levels by time showed a significant interaction ($p<0.001$) for body weight, feed intake and egg production.

In the 300 mg group (Table 3), the weight of follicles increased nearly four fold (9 g to 40 g) in three weeks and in the tenth week, the weight was 20% more than the control. The gain was greater in the 400 mg group ($p<0.05$). By three weeks, the weight increased ten fold and by the tenth week, the weight was 32% higher than the control. A similar phenomenon was observed in the weight of oviduct, with a significant weight gain observed in the 300 and 400 mg groups. In the former, a threefold weight gain from 20 g to 59 g ($p<0.05$) was observed in three weeks, while in the latter, a sevenfold gain from 9 g to 49 g ($p<0.05$) in the same interval was observed. A similar trend was seen with the weight of the liver; however, the gain was modest. The change in liver weight was rather small except for the 400 mg group, even when a 40% gain was observed. ROX levels by time showed significant ($p<0.05$) interaction on the follicle, oviduct and liver weights.

Table 3. Effect of ROX on organ weight in laying hens*

T[1] in week (t)	Level of ROX (mg/kg)					Means	SED	Significance		
	0 (Control)	100	200	300	400			ROX	Time	R×T
Follicle weight (g)										
3 (0)	31	31	36	9 [a]	5 [a]	23	1.9	NS	0.001	0.001
6 (3)	30	35	41	40[x]	48 [ax]	39	1.8			
8 (5)	34	40	32	52[x]	40[x]	33	2.3			
10 (7)	39	41	37	47[x]	51[x]	44	2.0			
Means	34	37	37	37	36					
SED	1.8	2.0	2.2	2.2	1.8					
Oviduct weight (g)										
3 (0)	42	47	49	20 [a]	9 [a]	33	1.9	0.001	0.001	0.001
6 (3)	55	57	65	59[x]	49[x]	57	1.7			
8 (5)	56	61[x]	63	57[x]	54[x]	58	1.8			
10 (7)	60[x]	61[x]	56	60[x]	62[x]	60	2.1			
Means	53	56	58	49	43					
SED	1.9	1.7	2.2	1.9	1.6					
Liver weight (g)										
3 (0)	31	29	29	25 [a]	20 [a]	27	1.2	0.026	0.001	0.031
6 (3)	36	37[x]	36	41[x]	34[x]	37	1.4			
8 (5)	34	35	42[x]	38[x]	35[x]	37	1.5			
10 (7)	36	33	34	37[x]	34	35	1.3			
Means	35	34	33	35	31					
SED	1.4	1.4	1.5	1.4	1.3					
Heart weight (g)										
3 (0)	7.4	6.3	6.4	6.6	5.6[a]	6.5	0.64	NS	0.003	NS
6 (3)	6.6	7.3	7.1	7.8	7.8[x]	7.3	0.71			
8 (5)	6.1	7.1	7.6	7.2	6.8	6.9	0.71			
10 (7)	7.7	7.7	7.3	8.5	7.8[x]	7.9	0.79			
Means	6.9	7.1	7.1	7.5	7.0					
SED	0.67	0.77	0.65	0.71	0.71					

[a,] * The values listed are the mean and standard deviation of each group. The superscript [a] indicates significant difference ($p<0.05$) versus control (ROX level = 0). The superscript [x] indicates significant difference ($p<0.05$) versus control (t = 0).

[1] T (time) indicates total time since the start of the test, and t in the parenthesis indicates total time after the drug withdrawal (withdrawal time only).

NS: $p>0.05$.

Effect of ROX withdrawal on blood lipid and lipoprotein

During the treatment, the blood TG, VLDL and HDL showed significant ($p<0.05$) change in 300 or 400mg groups. After withdrawal, however, all these effects disappeared and the level returned normal within three weeks (Table 4).

Effect of ROX withdrawal on blood proteins

As ROX dose increased, AST, LDH and CK activities showed a significant ($p<0.05$) increase in the 300 and 400 mg group (Table 5). After ROX withdrawal, the 400 mg group returned to normal within 3 weeks (all except for creatine kinase). Recovery of the enzyme activity in the 400 mg group took 5 weeks. However, ROX levels by time showed a significant interaction for these three enzymes.

Effect on the liver

ROX affected the weight of the liver and liver-related molecules including enzymes such as AST and LDH (Tables 3 and 5). These were signs that the liver might be damaged. Therefore, a micrograph of the liver was prepared and examined as shown in Figure 1. During the three weeks of 300 mg ROX treatment, the liver showed lymphocytic infiltration (Figure 1A), and these persisted for at least three weeks after the removal of ROX from the feed (Figure 1B), indicating that the liver lipid containing vacuoles and have been damaged.

DISCUSSION

During the three weeks when ROX was provided in the feed, all body parts and biological functions including egg production were affected. The extent of the effect was greater as the dose increased. The effect of the lower doses (100 or 200 mg per kg) were marginal, but the higher doses of 300 mg/kg showed a marked effect. An even more drastic effect was observed with 400 mg/kg. The effect was such that the organs including the body, liver, and heart lost weight. Such effects were consistent with previous

Table 4. Effect of ROX on blood lipid and lipoprotein of laying hens*

T[1] in week (t)	Level of ROX (mg/kg)					Means	SED	Significance		
	0 (Control)	100	200	300	400			ROX	Time	R×T
Triacylglycerol (mg/dl)										
3 (0)	1,244	1,468	1,171	824 [a]	590 [a]	1,031	8.2	0.032	0.001	0.001
6 (3)	1,237	1,219	1,332	1,302[x]	1,334[x]	1,286	8.3			
8 (5)	1,190	1,335	1,164	1,360[x]	1,241[x]	1,285	8.6			
10 (7)	1,325	1,361	1,175	1,263[x]	1,205[x]	1,263	10			
Means	1,240	1,334	1,245	1,190	1,067					
SED	7.7	11	9.7	8.3	6.7					
VLDL[2] (%)										
3 (0)	87	88	88	62 [a]	56[a]	77	1.5	0.001	0.001	0.001
6 (3)	87	88	87	88[x]	88[x]	88	0.7			
8 (5)	87	88	88	88[x]	87[x]	88	0.7			
10 (7)	88	86	89	88[x]	87[x]	88	0.9			
Means	87	88	88	81	79					
SED	0.9	0.8	0.7	1.5	1.4					
HDL[2] (%)										
3 (0)	13	12	12	38[a]	44[a]	19	1.5	0.001	0.001	0.001
6 (3)	13	12	13	12[x]	12[x]	12	0.7			
8 (5)	13	12	12	12[x]	13[x]	12	0.7			
10 (7)	12	14	11	12[x]	13[x]	12	0.9			
Means	13	12	12	19	21					
SED	0.9	0.5	0.7	1.5	1.4					

* The values listed are the mean and standard deviation of each group. The superscript [a] indicates significant difference ($p<0.05$) versus control (ROX level = 0). The superscript [x] indicates significant difference ($p<0.05$) versus control (t = 0).

[1] T (time) indicates total time since the start of the test, and t in the parenthesis indicates total time after the drug withdrawal (withdrawal time only).

[2] % of total lipoprotein, HDL+VLDL. NS: $p>0.05$.

observations (Chiou et al., 1997) where similar effects were observed with a dose of 312 mg/kg. This result supported the notion (Chen et al., 2005) that the effect was due to the toxicity of ROX, because a comparable effect was seen here with 300 mg dose and an even greater effect was observed with a higher dose of 400 mg.

Such effects were apparently not due to the direct toxicity of ROX itself. Rather its effect was an indirect one by diminishing the appetite of the animal as evidenced by the reduced feed intake. Reduced amount of nutrients would consequently reduce the weights of follicle, oviduct and liver. ROX also affected egg production, which has also been observed earlier by Chiou et al. (1997). This apparently was a result of retardation development of egg producing organs, the follicle and oviduct, which showed deterioration as evidenced by the shriveling of the organs. This blocking of the development of egg producing organs was probably due to the toxicity of ROX. On the other hand, the oocyte requires a large energy supply for production of egg yolk (Schneider, 1992; 1995) and therefore, the reduction in feed (energy) intake was apparently also affecting the egg production.

The toxicity might be due to the altered biochemical activities such as the activity of several enzymes and the amount of blood molecules. Interestingly, the enzymatic activities of the three enzymes, AST, CK and LDH, increased as if to compensate for the toxic effect of ROX.

By week 3 after the withdrawal of ROX, except for a few organs and biological molecules of the group with a high dose like 400 mg, most organs and biological functions had already returned to normal. However, the body weight, follicles weight and CK in the 400 mg group remained high. Such effects were likely due to the increased feed intake and return of the appetite of the animal as ROX content in the body diminished. The increase in body weight after the withdrawal by overeating was compensation for the previous reduction. Similar overcompensation has been observed before with laying hens and broilers (Chiou 1997; 1998, Chen and Wu, 2000). Thus, there appeared to be no permanent or even short term after-effects on the phenotypic appearances such as feed intake and body weight. On the other hand, ROX might have a direct effect on lipid metabolism such as VLDL, HDL and triacylglycerol. Reduced amount of feed intake would decrease lipogenesis and increase lipid catabolism, resulting in the lower concentration of triacylglycerol (Chen et al., 2000). In the present observation, the amount of triacylglycerol and HDL ratio increased, consistent with earlier observations. Earlier we have shown that ROX inhibited egg production and removal of it restored the development of egg producing organs and production of the egg. The former effect apparently was due to blockage of lipogenesis by the drug is removal subsequently promoted lipogenesis, allowing the oocyte to accumulate a large

Table 5. Effect of ROX on blood biochemicals of laying hens*

T[1] in week (t)	Level of ROX (mg/kg)					Means	SED	Significance		
	0 (Control)	100	200	300	400			ROX	Time	R×T
Aspartate aminotransferase (U/L)										
3 (0)	230	224	238	284[a]	317[a]	260	3.4	NS	0.023	0.001
6 (3)	250	238	238	240	229[x]	239	3.2			
8 (5)	234	245	217	234[x]	236[x]	236	2.7			
10 (7)	253	198[a]	255	226[x]	227[x]	230	3.8			
Means	240	231	239	248	256					
SED	3.0	3.2	3.4	3.3	3.4					
Creatine kinase (U/L)										
3 (0)	1,551	1,454	1,635	2,114[a]	2,387[a]	1,864	10	0.0001	NS	0.010
6 (3)	1,483	1,577	1,538	1,601	2,234[a]	1,692	10			
8 (5)	1,758	1,769	1,452	1,780	1,677[x]	1,729	9			
10 (7)	1,524	1,400	1,602	1,539	1,616[x]	1,550	13			
Means	1,589	1,579	1,609	1,782	2,046					
SED	10	11	9	11	11					
Lactate dehydrogenase (U/L)										
3 (0)	1,132	1,311	1,325	2,048[a]	2,424[a]	1,609	11	0.005	0.001	0.001
6 (3)	1,223	1,442	1,494	1,300[x]	1,418[x]	1,374	10			
8 (5)	1,309	1,325	1,259	1,232[x]	1,099[x]	1,258	8			
10 (7)	1,281	1,208	1,137	1,365[x]	1,425[x]	1,309	13			
Means	1,230	1,334	1,351	1,509	1,563					
SED	9	11	10	11	10					

* The values listed are the mean and standard deviation of each group. The superscript [a] indicates significant difference ($p<0.05$) versus control (ROX level = 0). The superscript [x] indicates significant difference ($p<0.05$) versus control (t = 0).
[1] T (time) indicates total time since the start of the test, and t in the parenthesis indicates total time after the drug withdrawal (withdrawal time only).
NS: $p>0.05$.

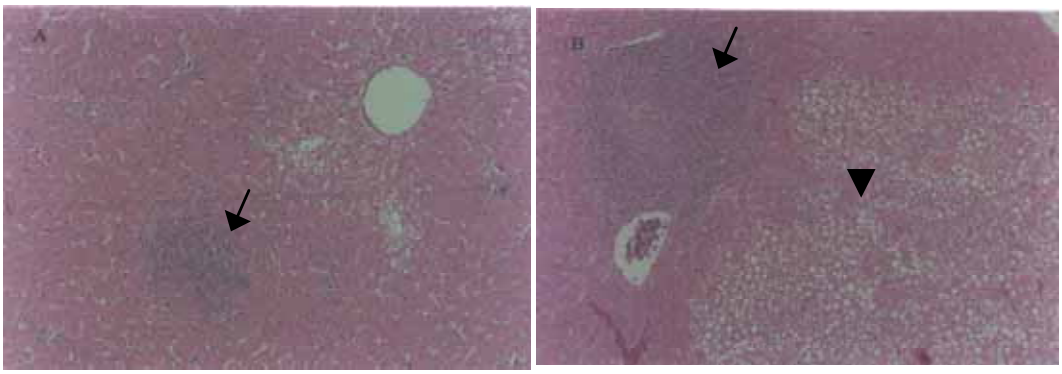

Figure 1. Microraphs of the liver section. Panel (A): the liver from the laying hens with 300 mg/kg of ROX, the liver showed lymphocytic infiltration (arrow). Panel (B): the liver obtained at t = 3 (3 weeks) after the drug withdrawal, and these persisted for at least three weeks after the removal of ROX from the feed, indicating that the liver lipid containing vacuoles (arrow head) and have been damaged. The liver was processed as described in Materials and methods. The section was stained with hematoxylin-eosin. ×100.

amount of energy (Chen et al., 2000).

ROX also enhanced the activity of certain enzymes such as AST, CK and LDH. It has been reported by Wang (1992) that CK is a specific enzyme in the muscle, while LDH and AST are widely distributed in the liver, kidney, heart, and muscle in broilers and laying hens. Here, we observed increased activity of all the above enzymes, whereas the weight of the internal organs, the liver and heart (muscle), was conspicuously decreased, indicating that these organs might have been damaged so that the enzymes were released from the cells. The microscope observation that showed lymphocytic infiltration and lipid-containing vacuoles supported this notion of liver damage. After the withdrawal of ROX, the damage persisted at least for three weeks as seen in the micrograph as well as in the conspicuously slow recovery observed with higher doses of 300 and 400 mg (Tables 2-5). The recovery of the liver was slow, only about 50% in the 400 mg group. The withdrawal restored the feed intake which promoted lipogenesis, and the recovery in liver weight might be due to accumulation of fat rather than due to recovery from the damage (or due to cell division), as evidenced by the abundant presence of

fat granules in the liver. Such effects have been observed before in laying hens (Chiou et al., 1997). The microscope observation, particularly the formation of lipid-containing vacuoles that was an indication of fatty liver, was consistent with the observation made with Tsaiya duck (Chen et al., 2000). Thus, while the effect of ROX on physical appearances such as feed intake and body weight was short term, its toxicity exerted a relatively long term effect on damage of internal organs.

CONCLUSION

The recovery of liver weight was probably due to accumulation of fatty particles rather than repair. It appeared, therefore, there were little after-effects of ROX on the hen's physical appearance, but some internal organs were probably damaged.

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