

Effect of Restrict Feeding, Roxarsone or Its Analogues in Inducing Fatty Livers in Mule Ducks

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ABSTRACT : This study is aimed at understanding the role of arsenic in Roxarsone in causing fatty livers in mule ducks. One hundred 10-week-old mule ducks were randomly divided into 5 groups. Ducks received 2 weeks of various treatments followed by 2 weeks of withdrawal. The treatments were non-treatment (control), 300 mg/kg Roxarsone inclusion for 2 weeks (1st and 2nd week), Roxarsone inclusion for one week (2nd week only), restrict feeding, or Roxarsone analogue (3-nitro-4-hydroxyphenyl acid) inclusion. Results showed that feed intake and body weight in the Roxarsone groups and the restrict feeding group decreased significantly during the treatment period. However only the liver and heart weights were significantly decreased ($p < 0.05$) in the restrict feeding group. Fatty acid synthetase (FAS) activity showed a significant decrease ($p < 0.05$) in the Roxarsone groups and the restrict feeding group, two-week-Roxarsone treatment significantly increased NADP-malic dehydrogenase (MDH) activity compared to the restrict ($p < 0.05$). After 2 weeks drug withdrawal, the 1-week-Roxarsone or restrict feeding group showed significantly increased ($p < 0.05$) glucose-6-phosphate dehydrogenase (G-6-PDH) activity ($p < 0.05$). Two-week-Roxarsone treatment significantly decreased ($p < 0.05$) the high density lipoprotein (HDL) and increased ($p < 0.05$) the low density lipoprotein (LDL) and very low density lipoprotein (VLDL) ratio. After drug withdrawal, the 1-week-Roxarsone or restrict feeding group showed significantly increased ($p < 0.05$) creatine kinase (CK) activity. The 2-week-Roxarsone treatment group showed significantly increased ($p < 0.05$) aspartate aminotransferase (AST) activity. The restrict feeding treatment group showed significantly decreased ($p < 0.05$) total protein (TP) concentration. After drug withdrawal, the related enzyme activities in the blood that reflected the liver function were restored to the normal physiological range, except for the total bilirubin concentration and CK activity in the 1-week-Roxarsone group. This group showed a significant increase ($p < 0.05$). Thus, the reasons for liver enlargement in the Roxarsone and restrict feeding groups were different. (*Asian-Aust. J. Anim. Sci.* 2005, Vol 18, No. 2: 241-248)

Key Words : Roxarsone, Restrict Feeding, Analogues, Fatty Livers, Mule Ducks

INTRODUCTION

Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) is a pentavalent organic arsenical that has been used as feed additive for a half century. The inclusion of Roxarsone 22.5 to 50 mg/kg showed inconsistent results in broiler diets. Inclusion however, does improve production and *Eimeria tenella* or *E. bruneti* infection resistance (McDougald et al., 1992). Roxarsone has been widely accepted as a feed additive in combination with antibiotics in broiler diets in the US (Waldroup et al., 1995) as well as in the other parts of the world. Roxarsone has been banned from use as feed additive in the UK and the European Union for more than a decade.

Dietary inclusion of 312 mg/kg Roxarsone for 3 weeks with one-week withdrawal induced fatty livers in laying hens (Chiou et al., 1997). After feeding a 300 mg/kg Roxarsone medicated diet for 3 weeks, the liver weight of laying Tsaiya ducks increased as the withdrawal period was prolonged (Chen et al., 2000). Oral medicated 40 mg/kg Roxarsone daily for one week induced fatty livers in mule

ducks after two weeks of withdrawal. Mule ducks were treated with the same arsenic level (11.36 mg/d) from different sources that included a control without As, Roxarsone, arsanilic acid, phenylarsonic acid, O-nitrophenylarsonic acid, As₂O₃ or As₂O₅. After one week on the treatment followed by 2-weeks withdrawal, only the Roxarsone, As₂O₃ or As₂O₅ treatments significantly induced fatty livers in mule ducks. Phenylarsonic acid or O-nitrophenylarsonic acid treatments did not produce fatty livers (Chen and Chiou, 2001). Roxarsone medication induces fatty livers through two possible paths: 1) The toxic effect of Roxarsone depresses feed intake during the medication period and causes a large amount of feed intake for compensation after re-feeding during the withdrawal period. This creates a hepatic lipid synthesis imbalance in poultry. 2) The toxic effect of Roxarsone induces abnormal hepatic lipid synthesis and/or depresses lipid catabolism. This decreases hepatic lipid removal, causing accumulated lipids and fatty livers. The abnormal lipid metabolic mechanism that causes fatty livers includes an increase in lipogenesis or decrease in lipid transport out of the livers, a decrease in lipid accumulation or decrease in lipid oxidation in fat tissue (Butler, 1976). However, the Roxarsone mechanism that induces fatty livers in mule ducks is still not clear.

To understand the role of arsenic in fatty liver formation

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Table 1. Composition of the basal diet

Ingredients	g kg ⁻¹
Yellow corn, grain	658.0
Soybean meal, 44%	309.0
Limestone, pulverized	8.0
Dicalcium phosphate	15.0
Vitamin premix ¹	0.1
Mineral premix ²	0.1
Salts	4.0
DL-methionine	0.5
L-lysine	0.6
Choline chloride, 50%	0.9
Calculated analysis	
Crude protein	194
ME (kcal/kg)	3,000
Calcium	7.5
Total phosphorus	6.6
Available phosphorus	3.9
Analyzed values	
Crude protein	191
Calcium	7.6

¹ Vitamin premix (supplied per kilogram of diet): Vitamin A, 25,000 IU; Vitamin D₃, 3,125 ICU; Vitamin E, 37.5 IU; Vitamin K₃, 6.25 mg; Vitamin B₁, 3.75 mg; Vitamin B₂, 12.5 mg; Vitamin B₆, 10.0 mg; Ca-pantothenate, 18.8 mg; Niacin, 50 mg; Biotin, 0.06 mg; Folic acid, 1.25 mg; Vitamin B₁₂, 0.05 mg.

² Mineral premix (supplied per kilogram of diet): Cu (CuSO₄·5H₂O, 25.45% Cu), 6 mg; Fe (FeSO₄·7H₂O, 20.09% Fe), 50 mg; Mn (MnSO₄·H₂O, 32.49% Mn), 40 mg; Zn (ZnO, 80.35% Zn), 60 mg; Se (NaSeO₃, 45.56% Se), 0.075 mg.

in mule ducks, the experimental treatments included Roxarsone, its analogues, the analogue (3-nitro-4-hydroxyphenyl acid) without arsenic content, and restrict feed intake to the same level during Roxarsone (300 mg/kg) treatment. This experiment was designed to clarify arsenic toxicity, the chemical structure of Roxarsone and its effect on the feed intake that causes fatty livers in mule ducks.

MATERIALS AND METHODS

The mule ducks used in this trial were from a three way cross with male Peking ducks crossed with female domestic Tsaiya ducks as described in Chen and Chiou (2001). Eight-week-old healthy mule ducks with similar live weights were placed into individual 40×30 cm, 38 cm high cages for a 2-week adaptation. Feed and water were provided *ad libitum*. The basal diet was referred to the NRC (1994) nutrient requirements for ducks and is presented in Table 1. After two weeks of adaptation, one hundred healthy mule ducks were selected and randomly allocated into five dietary treatment groups that included 1) A control group without As inclusion in the diet, 2) The 2-week Roxarsone group, dietary inclusion of 300 mg/kg Roxarsone for 2 weeks (the 1st and 2nd week), 3) The one-week Roxarsone group (only in the 2nd week), 4) The restrict feeding group, restrict feed intake to the level of the 2-week Roxarsone group, and 5)

The analogue group, inclusion of a Roxarsone analogue (3-nitro-4-hydroxyphenyl acid) without arsenic content for 2 weeks. Birds were managed according to the animal welfare regulations.

Feed intake and live weight were individually recorded every week during the experimental period. Blood samples were taken from the ulnar vein after a 12 h feed withdrawal and water restriction. Six, 7 and 7 Ducks were sacrificed at the 2nd, 3rd and 4th week, respectively, in the trial. Blood constituent analysis included concentrations of total protein (TP), albumin (ALB), total bilirubin, triacylglycerol (TG), and cholesterol (CHOL) and aspartate aminotransferase (AST, EC2.6.1.2), lactate dehydrogenase (LDH, EC 1.1.1.27), and creatine kinase activities (CK, 2.7.3.2), and percentages of high-density lipoprotein (HDL), very low density lipoprotein (VLDL), and low density lipoprotein (LDL). Hepatic lipogenic enzyme analysis included ATP-citrate cleavage enzyme (CCE, EC 4.1.3.8), NADP-malic dehydrogenase (MDH, EC 1.1.40), fatty acid synthetase (FAS), acetyl-CoA carboxylase (ACC, EC 6.4.1.2) and glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49).

The abdominal fats were stripped off immediately after the livers and hearts were removed. This included abdominal fat, the fat surrounding the gizzard and fat pad in the abdominal cavity. The intestinal mesenteric fat was not included. Approximately 0.5 cm of the liver tissue was taken from the liver and preserved in neutral-buffered 10% formalin for tissue section. The tissues were then embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin. These liver slides were prepared for pathological examination.

Approximately 5 g of the liver was sampled and homogenized at low temperature for 1 min. The supernatant was taken after centrifugation at 10,000×g for 10 min under 4°C. After repeating the centrifugation procedure, the supernatant was recentrifuged at 105,000×g 4°C for 60 min, to precipitate microsomes from the cells. The supernatant cytoplasm was taken for hepatic enzyme activities and protein concentration analysis.

Serum TP, ALB, total bilirubin, TG and CHOL concentrations were analyzed using an automatic blood chemical analyzer (Roche COBAS MIRA) with Roche testing kits. The AST, LDH and CK activities were measured using kinetic methods as recommended by the German Society for Clinical Chemistry (1972) by an automatic blood chemical analyzer (Roche COBAS MIRA) with Roche testing kits. Serum enzyme activities were expressed in international units (U) per liter of serum (Bergmeyer, 1983). Serum lipoproteins were analyzed according to Houstmuller (1969) using Helena titian gel electrophoresis. Electrophoresis data center scanning densitometry was used to estimate the area of each fraction.

The hepatic lipogenic enzyme activity of CCE, MDH,

Table 2. Effects of dietary inclusion of Roxarsone, its analogue or restrict-feeding on production performance in mule ducks

Period in trial	Control	2-week Roxarsone	1-week Roxarsone	Restrict feeding	Roxarsone analogue	SEM
Body weight (g)						
2wk	2,402 ^a	2,183 ^{cy}	2,289 ^{by}	2,129 ^{cy}	2,381 ^{ab}	35
3wk	2,457 ^{ab}	2,547 ^{ax}	2,487 ^{abx}	2,548 ^{ax}	2,445 ^b	45
4wk	2,447	2,610 ^x	2,561 ^x	2,624 ^x	2,446	65
SEM	24	41	62	41	36	
Feed intake (g/day)						
2wk	155.5 ^a	85.2 ^{cz}	118.7 ^{bz}	80 ^{cz}	156.5 ^a	2.14
3wk	155.2 ^d	194.5 ^{bx}	182.7 ^{cx}	209 ^{ax}	152.5 ^d	3.44
4wk	156.7	157.5 ^y	156.5 ^y	156.5 ^y	154.2	2.91
SEM	2.5	2.6	2.7			
Liver weight (g)						
2wk	36 ^a	34 ^{az}	36 ^a	30 ^{by}	35 ^a	1.20
3wk	35 ^b	47 ^{ax}	45 ^{ax}	45 ^{ax}	36 ^b	1.19
4wk	35 ^b	42 ^{ay}	40 ^{abxy}	41 ^{ax}	37 ^{ab}	1.83
SEM	0.95	1.46	2.22	1.90	1.00	
Abdominal fat weight (g)						
2wk	3.35 ^{ab}	1.60 ^{by}	2.20 ^{aby}	1.61 ^{by}	4.36 ^a	0.82
3wk	3.75 ^b	6.22 ^{abx}	9.20 ^{ax}	7.37 ^{abx}	4.52 ^b	1.34
4wk	3.32	7.07 ^x	7.18 ^{xy}	7.67 ^x	3.47	1.37
SEM	0.928	0.643	1.982	1.259	0.758	
Heart weight (g)						
2wk	21 ^a	20 ^{ay}	21 ^a	16 ^{by}	22 ^a	0.81
3wk	21 ^b	25 ^{ax}	22 ^{ab}	24 ^{abx}	22 ^{ab}	0.90
4wk	21	23 ^{xy}	22	24 ^x	21	0.80
SEM	0.73	0.83	0.97	0.83	0.88	
Relative liver weight (%)						
2wk	1.51	1.61 ^y	1.57	1.49 ^y	1.47	0.049
3wk	1.47 ^c	1.88 ^{ax}	1.78 ^a	1.79 ^{ax}	1.53 ^c	0.068
4wk	1.43	1.62 ^y	1.56	1.56 ^y	1.50	0.047
SEM	0.0604	0.048	0.066	0.059	0.049	
Relative abdominal fat weight (%)						
2wk	0.14	0.07 ^y	0.10 ^y	0.07 ^y	0.18 ^y	0.037
3wk	0.15 ^b	0.24 ^{abx}	0.35 ^{ax}	0.29 ^{abx}	0.90 ^{abx}	0.053
4wk	0.13 ^b	0.27 ^{abx}	0.26 ^{abxy}	0.29 ^{ax}	0.14 ^{bx}	0.048
SEM	0.039	0.025	0.069	0.050	0.033	
Relative heart weight (%)						
2wk	0.90 ^{ab}	0.97 ^a	0.92 ^a	0.81 ^{by}	0.92 ^a	0.032
3wk	0.90 ^{ab}	0.99 ^a	0.89 ^b	0.95 ^{abx}	0.95 ^{ab}	0.03
4wk	0.89	0.89	0.89	0.91 ^x	0.89	0.028
SEM	0.32	0.42	0.31	0.031	0.03	

^{a, b} Means in the same row with different superscripts are significantly different ($p < 0.05$).

^{x, y} Means in the same column with different superscripts are significantly different ($p < 0.05$).

FAS, ACC and G-6-PDH were measured using the modified method of Takeda et al. (1963), Ochoa (1955), Kumar et al. (1970), Numa (1969) and Lohr and Walker (1974), respectively. The protein content in the hepatic tissue was measured according to Lowry et al. (1951) using bovine serum albumin as the standard protein.

A completely randomized design was applied to examine the effects of the arsenic levels or chemical forms. Analyses of variance were calculated using the general linear model principles and procedures of the SAS (1984). Duncan's new multiple-range test was used to compare the means according to Steel and Torrie (1960).

RESULTS AND DISCUSSION

Effects on growth performance

Table 2 presents the effect of Roxarsone or its analogue in the diets versus restricting feeding on the production performance of mule ducks. The feed intake and live weight in the 2-week, 1-week Roxarsone treatment and the restrict feeding groups were significantly lower ($p < 0.05$) than the control group. The 1-week Roxarsone group exhibited significant higher ($p < 0.05$) feed intake than the 2-week Roxarsone or restrict feeding group. The liver weight, heart weight and relative heart weight were significantly lighter

Table 3. Effects of dietary added Roxarsone, its analogue or restrict-feeding on the hepatic lipogenesis enzyme activities in mule ducks

Period in trial	Control	2-week Roxarsone	1-week Roxarsone	Restrict feeding	Roxarsone analogue	SEM
----- unit -----						
ATP-citrate cleavage enzyme						
2wk	6.75	7.61 ^x	7.65 ^x	7.02 ^x	6.11	0.717
3wk	5.35	4.25 ^y	5.11 ^{xy}	3.65 ^y	5.07	0.592
4wk	5.90	5.87 ^{xy}	5.92 ^y	6.43 ^{xy}	5.90	0.692
SEM	0.56	0.785	0.726	0.896	0.610	
Fatty acid synthetase						
2wk	6.51 ^a	3.68 ^b	3.34 ^b	3.19 ^b	5.60 ^{ab}	0.779
3wk	4.51 ^{ab}	4.03 ^{ab}	3.30 ^b	3.79 ^{ab}	4.97 ^a	0.849
4wk	4.88	4.56	4.04	4.63	4.97	0.456
SEM	1.037	0.679	0.506	0.531	0.568	
Acetyl-CoA carboxylase						
2wk	1.59 ^{ab}	1.63 ^{ab}	1.51 ^{ab}	1.37 ^b	1.79 ^{ax}	0.087
3wk	1.57	1.59	1.31	1.35	1.25 ^y	0.195
4wk	1.37	1.26	1.19	1.17	1.27 ^y	0.120
SEM	0.119	0.123	0.128	0.132	0.108	
Glucose-6-phosphate dehydrogenase						
2wk	6.56	5.67	3.76 ^y	3.23 ^y	6.10	1.36
3wk	6.16	6.64	6.07 ^y	5.89 ^y	6.08	1.228
4wk	5.98 ^b	8.99 ^b	13.5 ^{ax}	12.95 ^{ax}	7.04 ^b	1.094
SEM	0.885	1.605	0.956	1.306	0.985	
NADP-malic dehydrogenase						
2wk	33.4 ^{ab}	41.5 ^{ax}	34.1 ^{abx}	27.0 ^{bxy}	34.6 ^{abx}	2.918
3wk	28.6	29.1 ^y	21.3 ^y	22.3 ^y	24.8 ^y	3.051
4wk	31.5	34.6 ^{xy}	35.7 ^x	32.0 ^x	31.6 ^x	1.933
SEM	2.26	3.398	2.47	2.94	1.953	

1 unit=1 n mole/mg protein.

^{a, b} Means in the same row with different superscripts are significantly different ($p < 0.05$).^{x, y} Means in the same column with different superscripts are significantly different ($p < 0.05$).

($p < 0.05$) in only the restrict feeding group compared to the other treatment groups. After returning to the basal diet for one week, both Roxarsone and restrict feeding groups showed significantly increased ($p < 0.05$) feed intake, liver and relative liver weight over the other groups. The live weight returned to the level of the control group without significant differences from each other ($p > 0.05$). The abdominal and relative abdominal fat weight were significantly increased ($p < 0.05$) in the 1-week Roxarsone group. After two weeks of feeding on the basal diet, all except the liver weight increased ($p < 0.05$) in the 2-week-Roxarsone group. The liver weight and relative abdominal fat increased ($p < 0.05$) in the restrict feeding group. The production performance was not significantly different ($p > 0.05$) among the treatment groups.

Roxarsone inclusion above 300mg/kg in the diet depressed feed intake and live-weight in both laying hens and laying Tsaiya ducks (Chiou et al., 1997; Chen et al., 2000). This agreed with the current study that 300 mg/kg Roxarsone inclusion in the diet for one or two weeks depressed feed intake and live-weight ($p < 0.05$). This depression effect was also observed in the restrict feeding group in this trial. Except for significantly decreased ($p < 0.05$) liver weight in the restrict feeding group, this

treatment did not significantly influence the liver weight in mule ducks. Both the 1-week and 2-week Roxarsone groups and the restrict feeding group showed significantly increased ($p < 0.05$) liver and relative liver weight after the withdrawal period with basal diet feeding. This indicated that the liver enlargement in the Roxarsone treated ducks could probably be attributed to the direct toxic effects of Roxarsone on liver enlargement in addition to the extra large energy intake from the large increase in feed intake after the depressed feed intake during the medication period. The Roxarsone analogue, 3-nitro-4-hydroxyphenyl acid, which did not contain arsenic, has no toxic effects on the mule ducks. Fifty mg per kg Roxarsone inclusion in the diet decreased the hepatic copper concentration 2 to 4 fold in swine, chickens and rats (Czarnecki and Baker, 1982; 1985; Czarneck et al., 1984). The substitute of Roxarsone analogues, O-nitro-phenol and 3-nitro-4-hydroxyphenyl acid did not induce the same effects (Czarnecki and Baker, 1985). Our previous study derived similar results with oral arsenicals, including Roxarsone, As_2O_3 or As_2O_5 causing fatty livers in mule ducks (Chen and Chiou, 2001). Our previous trial showed that with the exception of Roxarsone, dietary inclusion of arsenicals did not cause liver enlargement (Unpublished). Roxarsone induces fatty liver because of the arsenic content in Roxarsone.

Effects on hepatic lipogenesis

Table 3 presents the effects of Roxarsone, its analogue or restrict feeding on the hepatic lipogenic enzymes in mule ducks. Two-week Roxarsone or restrict feeding significantly decreased FAS activity ($p < 0.05$). This treatment did not show a significant difference ($p > 0.05$) from the control on hepatic lipogenic enzyme activities including CCE, ACC, G-6-PDH and MDH. The 1-week Roxarsone and restrict feeding groups showed significantly increased G-6-PDH activity. The activities of the other hepatic lipogenic enzymes were not significantly different ($p > 0.05$).

Lipogenic enzymes generally decrease with decreasing lipid synthesis at fasting in poultry (Goldman et al., 1985; Hasegawa et al., 1994). Lien (1999) indicated a significant decrease ($p < 0.05$) in lipogenic enzymes at fasting in 12-week-old Brown Tsaiya ducks, with the exception of G-6-PDH activity. The current trial did not agree with Lien (1999) and did not show a decrease in lipogenic enzyme activities with the exception of FAS in feed intake depressed by Roxarsone or restrict feeding treatment. The lipogenic enzyme activities generally increased after the depressed intake birds were allowed to feed normally (Leveille et al., 1975; Tanaka et al., 1975). Conversely, this trial obtained different results in that the lipogenic enzyme activities did not increase significantly after normal food intake was resumed with the exception of G-6-PDH activity. In fact, the ACC, G-6-PDH and MDH activities in the restrict feeding group showed a trend toward decrease ($p > 0.05$). This indicated that the decrease in feed intake was not severe enough for the deficient substrate required for normal lipid synthesis and therefore did not trigger a

reaction to depress lipogenic enzyme activities. Generally, MDH provides the NADPH required for lipid synthesis in the liver. NADPH comes mainly from NADP-MDH. The pentose-phosphate-shunt is an alternate but minor source of supply in chickens, geese and Tsaiya ducks (Bogin et al., 1984; Mourot et al., 2000). MDH activity was significantly higher in the 2-week Roxarsone treatment group compared to the restrict feeding group ($p < 0.05$) in this trial. The liver enlargement mechanism in the Roxarsone and restrict feeding treatments are different. Roxarsone may promote MDH synthesis and lipid synthesis and accumulation. Liver enlargement occurs because the liver weight ($p < 0.05$) in the Roxarsone and restrict feeding groups differed under similar amounts of feed intake.

Effects on lipid metabolism

Table 4 presents the effect of Roxarsone dietary inclusion, its analogue or restrict feeding on the serum lipid and lipoprotein in mule ducks. The 2-week Roxarsone treatment significantly decreased the serum HDL ratio ($p < 0.05$) and increased VLDL and LDL ratio ($p < 0.05$). However, no significant difference ($p > 0.05$) was found among the treatment groups in TG and CHOL compared to the control.

In general, the lipoprotein ratio in growing birds is relatively higher in HDL and LDL and lower in VLDL. The HDL ratio however is highest and reaches up to 70% in mule ducks. Chen and Chiou (2001) used 40 mg/d Roxarsone on mule ducks and observed a decrease in the HDL ratio. The same phenomenon was also observed in this trial. The liver is the major site for lipid synthesis in poultry.

Table 4. Effects of dietary added Roxarsone, its analogue or restrict-feeding on serum lipid and lipoproteins in mule ducks

	Control	2-week Roxarsone	1-week Roxarsone	Restrict feeding	Roxarsone analogue	SEM
High density lipoprotein, %						
2W	77 ^a	70 ^b	72 ^{ab}	74 ^{ab}	74 ^{ab}	1.549
3W	74	72	64	71	72	4.525
4W	76	68	70	71	69	2.387
SEM	2.219	2.704	2.489	2.859	2.121	
Low density lipoprotein and very low density lipoprotein (%)						
2W	22 ^b	29 ^a	27 ^{ab}	25 ^{ab}	25 ^b	1.549
3W	25	27	35	28	27	4.525
34W	23	31	29	28	30	2.387
SEM	2.219	2.704	2.489	2.859	2.121	
Triacylglycerol (mg/dL)						
2W	53 ^{ab}	51.2 ^{ab}	46 ^b	54 ^a	58 ^{ab}	5.478
3W	65 ^{ab}	65.5 ^{ab}	68 ^{ab}	54 ^b	73 ^a	5.476
4W	62	56.2	56	61	63	3.729
SEM	5.257	4.362	5.425	4.853	4.986	
Cholesterol (mg/dL)						
2W	142	174	168	172	145	16
3W	156	162	157	174	150	11
4W	161	136	145	144	156	10
SEM	8	17	19	12	8	

^{a, b} Means in the same row with different superscripts are significantly different ($p < 0.05$).

In laying Tsaiya ducks and chicken, serum TG decreases and CHOL increases at fasting (Lien and Jan, 1999) because a decrease in feed intake leads to a decrease in the lipogenesis rate with a speeding up in the lipolysis rate. In this study however, the decrease in feed intake due to Roxarsone or restrict feeding did not significantly influence ($p>0.05$) the TG and CHOL concentrations compared to the control. This reflected that the feed intake was still adequate enough to sustain normal lipid synthesis in the liver.

Effects on liver function

Table 5 shows the effect of Roxarsone dietary inclusion, its analogue or restrict feeding on the serum constituents of mule ducks. The 1-week-Roxarsone treatment significantly decreased ($p<0.05$) TP and increased ($p<0.05$) CK activity, while the 2-week-Roxarsone treatment significantly increased ($p<0.05$) AST activity. Restrict feeding decreased ($p<0.05$) the TP concentration. After drug withdrawal, most of the mule ducks recovered and returned to normal serum levels except the 1-week-Roxarsone group which exhibited significantly increased ($p<0.05$) total bilirubin concentration and CK activity.

Body protein comes mainly from liver synthesis and is distributed to various body tissues through the blood stream. CCl_4 , P, Pb or As, depress protein synthesis in the liver (Friedman et al., 1970; Mayes, 1973). This depression effect was observed in the 1-week-Roxarsone and restrict feeding group ($p<0.05$). The average feed intake in the 1-week-Roxarsone group was significantly lower compared to the 2-week-Roxarsone treatment group. Serum samples were taken during the medication period for the 1-week-Roxarsone group. As compared to the withdrawal period of 1-week-Roxarsone treatment group, the maximum drug toxic effect of Roxarsone on feed intake and liver was reflected in the 2-week group. Hence, the 1-week-Roxarsone group exhibited more prominent serum characteristics than the 2-week-Roxarsone treatment group.

CK is a specific enzyme for muscular cells in laying hens, broilers and pigeons. LDH and AST are widely distributed in the liver, heart, kidneys and muscle cells, with more in liver. When only CK activity is low among these enzymes, the liver and kidney tissues may be damaged (Lumeij and Wolfswinkel, 1988; Wang, 1992). In this trial, CK activity was significantly higher ($p<0.05$) in the 1-

Table 5. The effects of dietary inclusion of Roxarsone, its analogue or the restrict-feeding on the serum constituents in mule ducks

	Control	2-week-Roxarsone	1-week-Roxarsone	Restrict-feeding	Roxarsone analogue	SEM
Total protein (g/dL)						
2W	4.90 ^a	4.22 ^{ab}	3.61 ^{bz}	3.48 ^{by}	5.01 ^a	0.300
3W	5.00	5.45	5.32 ^x	6.35 ^x	5.02	0.564
4W	5.06	4.60	5.05 ^y	4.50 ^y	5.28	3.690
SEM	0.293	0.405	0.373	0.377	0.298	
Albumin (g/dL)						
2W	1.23	1.22	1.25	1.15	1.27	0.090
3W	1.22	1.22	1.27	1.20	1.25	0.050
4W	1.26 ^{ab}	1.23 ^{ab}	1.35 ^a	1.15 ^b	1.30 ^{ab}	0.048
SEM	0.050	0.067	0.080	0.084	0.059	
Total bilirubin (mg/dL)						
2W	0.91	1.00	0.64 ^y	0.93	1.06	0.156
3W	0.75	0.97	0.82 ^y	0.47	0.87	0.163
4W	0.92 ^b	1.16 ^{ab}	1.43 ^{ax}	0.925 ^b	1.05 ^b	0.162
SEM	0.159	0.163	0.170	0.153	0.187	
Aspartate aminotransferase (U/L)						
2W	34 ^{by}	63 ^a	50 ^{abxy}	51 ^{ab}	50 ^{ab}	7
3W	43 ^{aby}	60 ^{by}	26 ^{by}	51 ^{ab}	55 ^a	8
4W	66 ^{abx}	54 ^{by}	90 ^{ax}	69 ^{ab}	59 ^b	9
SEM	7	6	10	10	10	
Lactate dehydrogenase (U/L)						
2W	784	968 ^x	780	880	818	86
3W	742	635 ^y	706	696	799	67
4W	762	847 ^x	772	808	888	73
SEM	96	45	65	106	83	
Creatine kinase (U/L)						
2W	1,121 ^b	1,622 ^{ab}	1,919 ^{ax}	1,581 ^{ab}	1,415 ^{ab}	185
3W	1,014	1,532	878 ^y	2,205	1,122	443
4W	1,152 ^b	1,130 ^b	1,805 ^{ax}	1,164 ^b	1,259 ^b	186
SEM	110	118	279	365	118	

^{a, b} Means in the same row with different superscripts are significantly different ($p<0.05$).

^{x, y} Means in the same column with different superscripts are significantly different ($p<0.05$).

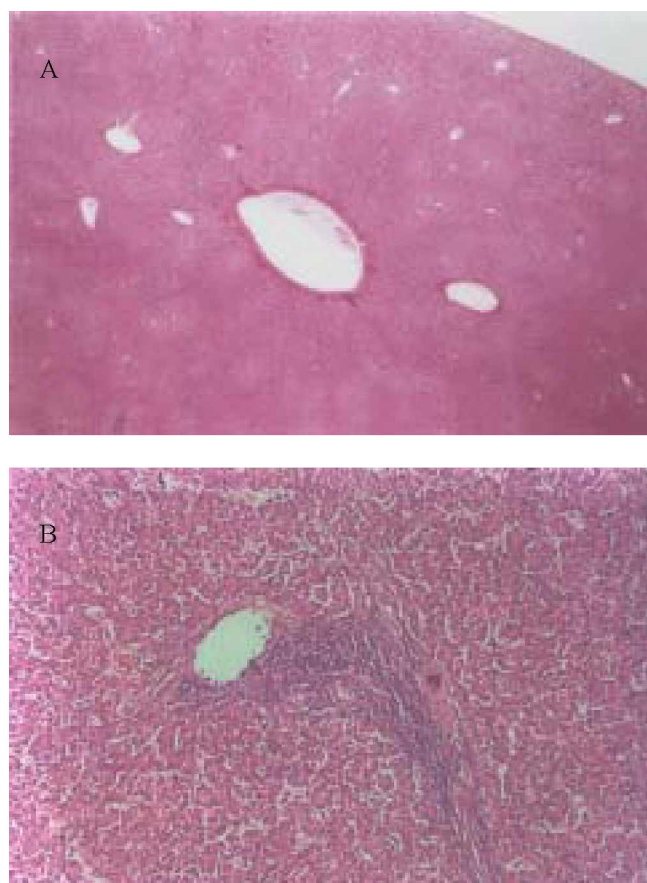


Figure 1. Microscopic photograph of the liver section from the mule ducks without (A) or with (B) 300 mg/kg Roxarsone treatment for 2 weeks.

week-Roxarsone group. Chiou et al. (1997) fed Roxarsone to laying hens for four weeks and observed a significant increase in all three enzymes, CK, AST and LDH, and a gradual decline afterward. This trend was also observed in this trial. The 300 mg/kg Roxarsone inclusion also increased CK activity in laying ducks. This implicated muscle cell damage by dietary Roxarsone inclusion. The AST activity increased only in the 2-week-Roxarsone group ($p < 0.05$). Chen and Chiou (2001) forced fed 40mg Roxarsone daily for one week to mule ducks without an increase in CK, LDH and CK activities during the treatment period. However, increased CK activity occurred one week after withdrawal. The abnormal liver histology with fatty vacuolated vesicles in the liver cells of Roxarsone treated groups included both one or two weeks treatments, as shown in the microscopic photograph in Figure 1.

Roxarsone treatment did not influence the LDH activity in mule ducks in this trial as observed both in laying ducks and mule ducks in our previous trials (Chen et al., 2000; Chen and Chiou, 2001). The LDH distribution in the body of mule ducks requires further investigation. From the microscopic liver histological slide photographs, liver damage is shown only in the Roxarsone treatment groups,

but not in the Roxarsone analogue treatment group.

CONCLUSION

It appears that Roxarsone caused liver enlargement is not only attributed to the large feed intake increase after intake depression, but also due to the toxic effects of Roxarsone in promoting the lipogenesis rate and depressed lipid transport out of the liver.

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