Effects of Dietary Arsenical Inclusion on Lipid Metabolism and Liver Function in Mule Ducks

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ABSTRACT : This study evaluated the effectiveness of different arsenical sources on inducing fatty liver, on changes in lipid metabolism and on liver function in mule ducks. Sixty twelve-week-old mule ducks were selected and randomly divided into five treatments, including the control group and four different arsenical sources; Roxarsone (300 mg/kg), arsanilic acid, As_2O_5 or As_2O_3, containing 85.2 mg/kg arsenic were included in the basal diet. The ducks were fed the medicated basal diet for 3 weeks followed by a one-week drug withdrawal. The results showed Roxarsone treatment decreased body weight, feed intake, liver weight and abdominal fat weight ($p<0.05$), while it increased the relative liver weight ($p<0.05$) during medication period (3rd week). The As_2O_5 treatment decreased abdominal fat weight and relative abdominal fat weight when compared to the control ($p<0.05$). Only Roxarsone among the treatment groups increased feed intake, liver weight and relative liver weight, while the As_2O_3 group showed the lightest liver weight and relative liver weight among treatment groups during the withdrawal period (4th week). The Roxarsone group decreased ($p<0.05$) NADP-malic dehydrogenase (MDH) and acetyl-CoA carboxylase (ACC) activities and increased ($p<0.05$) cholesterol concentration during the medication period, and elevated the MDH and ACC activities during the withdrawal period. All four arsenical treatment groups showed lymphocytic infiltration in liver tissue, while the Roxarsone and As_2O_3 treatments showed an increase in aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities ($p<0.05$). During the withdrawal period, arsenical treatments resulted in liver vacuoles. However, the arsenicals differed in effectiveness and mechanisms of inducing fat vacuoles. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 412-417)***

Key Words : Arsenical, Fatty Liver, Lipid Metabolism, Mule Duck, Roxarsone

INTRODUCTION

Chronic arsenic toxicity has been discovered for more than a half century in some areas of the world. Arsenic in high dose 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 additive in poultry industries to improve production and control coccidiosis for decades (McDougald et al., 1992; Waldroup et al., 1995).

High dose (300 mg/kg) of Roxarsone inclusion in the diet induces fatty livers in laying hens, laying ducks and mule ducks (Chiou et al., 1997; Chen et al., 2000; Chen and Chiou, 2005; Wu et al., 2005). In mule ducks, the fatty liver probably is induced by arsenic or Roxarsone toxicity, which depresses feed intake during the treatment period. The large increase in feed intake after the arsenicals withdrawal causes an extra amount of lipid synthesis that cannot be metabolized efficiently (Chen and Chiou, 2001). Chen and Chiou (2005) indicated that the mechanism of fatty liver induced by Roxarsone is related to the structure of arsenic compound, and which is different from the restricted feeding. The degree of arsenic toxicity differs among the different arsenical structures. Inorganic arsenic is more toxic than the organic arsenic, while the trivalent arsenic is more toxic than the pentavalent arsenic (NRC, 1980). However, the mechanism on inducing fatty liver in different structural arsenic compounds is still not clear.

This study is therefore to evaluate the effectiveness of different arsenical sources on inducing fatty liver, changes in lipid metabolism and liver functions in mule ducks.

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MATERIALS AND METHODS

Mule ducks were a three way cross bred from male Peking ducks crossed with female domestic Tsaiya ducks as described in Chen and Chiou (2001). Eleven-week-old healthy mule ducks with similar live weights were selected and placed into individual 40×30 by 38 cm high cages for one-week adaptation. After adaptation, sixty healthy 12-week-old mule ducks (2,880±12 g) were selected and randomly allocated into five dietary treatments; a control without Arsenical inclusion and treatments with different arsenicals in the diet; Roxarsone, arsanilic acid, As_2O_3 or As_2O_5, respectively, at the same arsenic level of 85.2 mg/kg. Feed and water were provided *ad libitum*. Trial was conducted for 4 weeks with 3-weeks medication and 1-week withdrawal. The basal diet is presented in Table 1.

Feed intake and live weight were individually recorded weekly during the experimental period. Blood samples of the 3rd and 4th weeks were taken from the ulnar vein after 12-h feed withdrawal with water restriction. Ducks were

Table 1. Composition of the basal diet

Ingredients	g kg^{-1}
Yellow corn (grain)	661.8
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 (g/kg)	194
ME (kcal/kg)	3,000
Calcium (g/kg)	7.5
Available phosphorus (g/kg)	3.9

[1] Vitamin premix (per kg of diet): Vitamin A, 12,000 IU; vitamin D_3, 3,120 ICU; vitamin E, 37.5 IU; menadione sodium bisulphate, 6.25 mg; thiamin hydrochloride, 3.75 mg; riboflavin, 12.5 mg; pyridoxine hydrochloride, 10 mg; Ca-pantothenate, 18.8 mg; niacin, 50 mg; biotin, 0.06 mg; folic acid, 1.25 mg; cyanocobalamin, 0.05 mg.

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

sacrificed in the 3rd or 4th week in the trial, respectively.

Blood triacylglycerol (TG) and cholesterol (CHOL) concentrations, the serum enzyme activities included aspartate aminotransferase (AST, EC 2.6.1.2), lactate dehydrogenase (LDH, EC 1.1.1.27) and creatine kinase (CK, EC 2.7.3.2) and the hepatic lipogenic enzymes activities included ATP-citrate cleavage enzyme (CCE, EC 4.1.3.8), NADP-malic dehydrogenase (MDH, EC 1.1.40), fatty acid synthetase (FAS) and acetyl-CoA carboxylase (ACC, EC 6.4.1.2) were analyzed.

The abdominal fats included the fat surrounding the gizzard and fat pad in the abdominal cavity were stripped off immediately after livers removed. Approximately 0.5 cm of the liver tissue samples were taken from the livers 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 liver samples were sampled and homogenized at low temperature for 2 min (12,000 rpm). The supernatant was taken after centrifugation at 10,000×g for 10 min under 4°C. After repeating the centrifugation procedure, the supernatant was centrifuged again at 105,000×g 4°C for 60 min for cell microsomes precipitation. The supernatant cytoplasm was taken for hepatic enzyme and protein concentration analysis.

Serum TG and CHOL concentrations were analyzed referred to Lien et al. (2005) using an automatic blood chemical analyzer with Roche testing kits (Roche COBAS MIRA, Switzerland). The AST, LDH and CK activities

Table 2. Effects of different sources of arsenicals on growth performance and carcass traits in mule ducks

Period in trial	Control	Roxarsone	Arsanilic acid	As_2O_5	As_2O_3	SEM
Body weight (g)						
3 wk	2,853 ^{a}	2,219^{by}	2,785 ^{a}	2,842 ^{a}	2,850 ^{a}	31
4 wk	2,826 ^{a}	2,619^{bx}	2,832^{a}	2,926 ^{a}	2,840 ^{a}	30
SEM	12	24	15	16	19	
Feed intake (g/day)						
0-3 wk	160 ^{a}	83 ^{b}	160 ^{a}	158 ^{a}	157 ^{a}	1.7
3-4 wk	160 ^{b}	211 ^{a}	156 ^{b}	160 ^{b}	160 ^{b}	2.8
SEM	1.8	3.8	1.8	1.9	2.1	
Liver weight (g)						
3 wk	43.1 ^{a}	37.6 ^{by}	44.6 ^{a}	40.3 ^{ab}	41.1 ^{ab}	1.7
4 wk	42.0 ^{bc}	50.6 ^{ax}	43.1 ^{bc}	46.3 ^{ab}	39.8 ^{c}	1.7
SEM	2.2	2.9	0.9	2.9	2.2	
Abdominal fat weight (g)						
3 wk	29.5 ^{ax}	4.5 ^{cy}	19.6 ^{ab}	17.0^{b}	22.5 ^{ab}	3.7
4 wk	20.8 ^{y}	23.8 ^{x}	23.3	24.8	24.0	4.1
SEM	3.3	6.7	5.2	2.9	2.2	
Relative liver weight (g/100 g BW)						
3 wk	1.52^{bc}	1.68^{ay}	1.60^{ab}	1.41^{c}	1.44^{c}	0.05
4 wk	1.49 ^{bc}	1.93 ^{ax}	1.53 ^{bc}	1.59^{b}	1.40 ^{c}	0.06
SEM	0.05	0.06	0.04	0.08	0.04	
Relative abdominal fat weight (g/100 g BW)						
3 wk	1.036^{ax}	0.204^{c}	0.712^{ab}	0.596^{b}	0.771^{ab}	0.129
4 wk	0.737^{y}	0.889	0.823	0.844	0.841	0.144
SEM	0.08	0.17	0.13	0.09	0.18	

[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).

Table 3. Effects of different sources of arsenical on hepatic lipogenic enzymes and blood lipid in mule ducks

Period in trial	Control	Roxarsone	Arsanilic acid	As_2O_5	As_2O_3	SEM
NADP-malic dehydrogenase (unit)*						
3 wk	24.8 [a]	13.5 [by]	21.4 [a]	21.6 [a]	20.5 [a]	2.4
4 wk	23.8 [b]	37.3 [ax]	21.7 [b]	24.4 [b]	17.4 [b]	2.8
SEM	3.1	2.9	2.0	2.1	2.8	
Acetyl-CoA carboxylase (unit)*						
3 wk	4.9 [a]	2.2 [by]	3.9 [ab]	4.4 [a]	4.7 [a]	0.6
4 wk	4.1 [b]	8.0 [ax]	4.8 [b]	4.5 [b]	3.8	0.7
SEM	0.8	0.6	0.6	0.5	0.8	
Fatty acid synthetase (unit)*						
3 wk	9.6	5.8 [y]	9.5	8.9	8.6	1.6
4 wk	8.1 [ab]	11.7 [ax]	9.5 [ab]	9.4 [ab]	6.8 [b]	1.4
SEM	1.5	1.3	1.8	1.4	1.4	
ATP-citrate cleavage enzyme (unit)*						
3 wk	5.4	5.1	4.9	4.8	4.9	0.9
4 wk	7.3	6.2	5.1	6.6	5.8	0.9
SEM	1.0	1.2	0.9	0.7	0.8	
Triacylglycerol (mg/dL)						
3 wk	56	42 **	49	54	51	4.6
4 wk	49	52	55	54	62 **	2.9
SEM	3.4	4.9	5.1	7.9	4.0	
Cholesterol (mg/dL)						
3 wk	178 [b]	265 [ax]	176 [b]	170 [b]	158 [b]	12.0
4 wk	173	203 [y]	195	178	190	11.9
SEM	5	13	8	9	6	

[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 ($p<0.05$).
* The enzyme activities were measured NADH at 340 nm and expressed as amount of NADH consumed or produced per min.
1 unit = 1 n mole/mg protein.
** $p<0.1$ compared with control.

were measured using kinetic methods as recommended by the Germen Society for Clinical Chemistry (1972) using an automatic blood chemical analyzer with Roche testing kits (Roche COBAS MIRA, Switzerland). Serum enzyme activities were expressed in international units (U) per liter of serum (Bergmeyer, 1983).

The hepatic lipogenic enzyme activity of CCE, MDH, FAS and ACC were measured using the modified method of Takeda et al. (1963), Ochoa (1955), Kumar et al. (1970) and Numa (1969) respectively. The hepatic tissue protein content in the enzymatic activity calculation 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 arsenic chemical forms. Analyses of variance were calculated using the general linear model procedure 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

Production performance

Table 2 presents the effect of different arsenical sources on mule duck growth performance and carcass characteristics. Roxarsone inclusion decreased ($p<0.05$) feed intake, live weight and liver weight and abdominal fat weight, and As_2O_5 inclusion decreased abdominal fat weight ($p<0.05$). After a week of withdrawal, Roxarsone treatment increased ($p<0.05$) feed intake, liver and relative liver weight. However, these body weights were still lighter ($p<0.05$) as compared to the control.

Czarnecki and Baker (1984) demonstrated that the trivalent arsenic depressed more prominently chick growth performance on As_2O_3 versus As_2O_5 feeding, but this phenomenon did not occurred in this trial. Chen and Chiou (2001) indicated that after one-week of force-fed on the respective arsenical (11.36 mg/day arsenic), the feed intake and body weight of mule ducks were decreased by the Roxarsone treatment only, and did not show any significant effect by the arsanilic acid, As_2O_3 or As_2O_5 treatment. These results agreed to the observation in the present trial that only Roxarsone group ($p<0.05$), but not the other treatment group decreased ($p>0.05$) growth performance. Apparently, Roxarsone was the most effective among arsenicals on the growth performance effect in mule ducks.

Roxarsone inclusion decreased feed intake and caused a decrease in body weight, abdominal fat weight and liver weight ($p<0.05$) but an increase in relative liver weight ($p<0.05$), reflecting that the toxic influence of Roxarsone on

Table 4. Effects of different sources of arsenicals on blood chemistry and liver histology in mule ducks

Period in trial	Control	Roxarsone	Arsanilic acid	As_2O_5	As_2O_3	SEM
Creatine kinase (U/L)						
3 wk	778 ^{b}	1,099 ^{a}	896 ^{ab}	955 ^{aby}	771 ^{b}	98
4 wk	878 ^{b}	765 ^{b}	787 ^{b}	1420 ^{ax}	838 ^{b}	135
SEM	66	105	90	94	78	
Aspartate aminotransferase (U/L)						
3 wk	20.2 ^{b}	28.3 ^{a}	17.4 ^{b}	18.0 ^{b}	30.7 ^{a}	1.2
4 wk	22.2	21.3	17.8	21.8	31.5	5.0
SEM	1.4	2.4	1.5	1.3	4.3	
Lactate dehydrogenase (U/L)						
3 wk	795 ^{b}	906 ^{a}	837 ^{b}	855 ^{b}	1,163 ^{ax}	91
4 wk	818	751	677	892	860 ^{y}	87
SEM	51	64	54	68	81	
Lymphocytosis (No. of ducks)						
3 wk	2	6	6	6	6	
4 wk	1	6	6	6	6	
Fatty vacuoles (No. of ducks)						
3 wk	0	0	0	0	0	
4 wk	0	6	6	6	6	

$^{a, b}$ 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 ($p<0.05$).

the hepatic lipid metabolism is less prominent than on live weight. The As_2O_5 treatment did not affect ($p>0.05$) the feed intake but decreased ($p<0.05$) the abdominal fat weight and its relative weight, this implied that As_2O_5 directly affect the abdominal fat storage.

During the withdrawal period, Roxarsone treatment significantly increased ($p<0.05$) the feed intake, hence increased liver and relative liver weight compared to the control, and which were consistent with previous studies (Chen and Chiou, 2001; 2005). The highest liver weight and relative liver weight were found in Roxarsone group, followed by the order of As_2O_5, arsanilic acid and As_2O_3.

Lipid synthesis and metabolism

Table 3 presents the effect of different arsenical sources on the hepatic lipogenic enzymes and blood lipid in mule ducks. Roxarsone was the only treatment group that decreased MDH and ACC activities and increased cholesterol concentration ($p<0.05$), and these changes were reversed i.e., an increase in the MDH and ACC activities ($p<0.05$) during the withdrawal period.

Restricted feeding and fasting decrease lipid synthesis in animals (Donaldson, 1990). Increased feed intake followed by restricted feeding or fasting usually increases lipid synthesis. The increase in lipogenic enzyme activities is highly related to the source and amount of substrate (Tanaka et al., 1975). In this trial, Roxarsone decreased ($p<0.05$) feed intake and hence decreased ($p<0.05$) the MDH and ACC activities during treatment period and this trend was significantly reversed ($p<0.05$) after one week of drug withdrawal. Hence, the Roxarsone treatment group showed the higher MDH, ACC and FAS activities in withdrawal (4th week) than in medication period (3rd week). Hasegawa et al. (1994) also observed the phenomenon demonstrated in this trial. ACC is a limiting enzyme in lipid synthesis. This activity decreases in fasting and increases in re-feeding (Tanaka et al., 1984). Converting malate to pyruvate that catalyzed by NADP-MDH provides a major NADPH source required for lipid synthesis. The FAS activity in the Roxarsone group was higher than in the As_2O_3 group during the withdrawal period. This was implied that the mechanism of these two arsenicals were different, and resulted in higher liver and relative liver weight in the Roxarsone group than in the As_2O_3 group (Table 2).

Lipids are synthesized mainly in the liver and these newly synthesized lipids are transported through lipoproteins to the body tissues in poultry (Hermier et al., 1985). The TG concentration in the Roxarsone group showed a decreased trend ($p<0.1$) and about 25% lower than the control during the medication period reflected that lipid was minor released (Table 2) and mostly cumulated in the liver, and resulted in a higher relative liver weight in the Roxarsone group ($p<0.05$). The blood TG concentration did not show differences between the Roxarsone and the control group ($p<0.05$) during withdrawal period. This may be attributed to the dramatically increased feed intake in both treatment groups, and induced higher hepatic lipogenic enzymes activities. But these TG content did not release from liver and were accumulated in the liver ($p>0.05$), resulted in the increased liver and relative liver weight (Table 2). The blood TG concentration increased about 21% more in the As_2O_3 treatment than the control ($p<0.1$) reflected more lipids was released from liver, hence the lowest liver and relative liver weight in the As_2O_3 group. The cholesterol concentration was higher in the Roxarsone

group than control ($p<0.05$), and this agreed with Chen et al. (2000) who fed 300 mg Roxarsone to laying ducks; the cholesterol concentration was increased after 3-week Roxarsone treatment, but recovered to normal after withdrawal (Chen et al., 2000). Lien et al. (1999) also found that the cholesterol concentration in ducks increased after 3-day fasting, and was attributed to the body lipid lipolysis.

Liver function

Table 4 presents effects of different sources of arsenicals on blood chemistry and hepatic histology in mule ducks. During medication period, the CK and AST activities were higher in the Roxarsone group and the AST and LDH activities were higher in the As_2O_3 group. All arsenical treatment groups were demonstrated a liver lymphocytic infiltration from the histological observation. All hepatic enzyme activities returned ($p>0.05$) to the normal physiological range in all treatment groups after drug withdrawal, except the As_2O_5 treatment, which showed higher ($p<0.05$) CK activity compared to the other treatment groups. All the arsenical treatment groups showed the abnormal lymphocytosis and vacuoles in the liver cells from the microscope observation.

AST and LDH are widely distributed in the heart, liver, kidney and muscle cells in poultry and pigeons. CK is the specific enzyme for muscle cells. Increase in these enzyme activities without an increase in CK activity represents a damaged liver or kidney (Lumeij ad Wolfwinkel, 1988; Wang, 1992). The Roxarsone group showed an increase ($p<0.05$) in CK activity during medication and was agreed with our previous trials using 3- weeks Roxarsone inclusion (300 mg) in the diet in laying hens (Chiou et al., 1997) and laying Tsaiya ducks (Chen et al., 2000) and reflected that damaged muscle cells in poultry by Roxarsone treatment. The elevated CK activity in the As_2O_5 group means the damaged muscle tissue after withdrawn. From histological observation, all arsenical treatments caused abnormal lymphocytosis in the liver cell and Roxarsone treatment caused the bile duct proliferation in the liver cells in 50% ducks. These results proved that the over-dose arsenical had adverse effects on the liver function. These liver cell abnormalities were also observed in our previous trials using 300 mg Roxarsone inclusion in the diet on laying Tsaiya ducks (Chen et al., 2000) and mule ducks (Chen and Chiou, 2005). Arsanilic acid or As_2O_5 treatments did not increase AST and LDH activities. This reflected that liver was only slightly damaged and still had not reached the threshold to induce the increase in these enzyme activities.

CONCLUSION

During withdrawal period, the Roxarsone treatment but not the As_2O_3, As_2O_5 and arsanilic acid treatments showed the higher liver weight and relative liver weight than the control. All arsenical treatments would result in the liver vacuoles from histological observations, which actually defined as fatty liver, and would have adverse effects on animals. However, the arsenicals were different in effectiveness and in mechanisms in inducing the fat vacuoles.

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REFERANCES

Bergmeyer, H. U. 1983. Enzymes 1: Oxidoreductase, transferase. In Methods of Enzymatic Analysis III. Verlay Chemie, Deerfield Beach, Florida. Basel, pp. 126,416,510.

Chen, K. L. and C. P. Wu. 2000. Effect of roxarsone on growth performance, liver function and leg problem of broilers. Taiwan Lives. Res. 33(1):1-9.

Chen, K. L., C. P. Wu and P. W. S. Chiou. 2000. Effect of roxarsone inclusion in the diet on the performance and hepatic lipid metabolism of laying Tsaiya duck. Br. Poult. Sci. 41:363-369.

Chen, K. L. and P. W. S. Chiou. 2001. Oral treatment of mule ducks with arsenicals for inducing fatty liver. Poult. Sci. 80:295-301.

Chen, K. L. and P. W. S. Chiou. 2005. Effect of restrict feeding, roxarsone or its analogues in inducing fatty livers in mule ducks. Asian-Aust. J. Anim. Sci. 18(2):241-248.

Chiou, P. W. S., K. L. Chen and B. Yu. 1997. Effects of roxarsone on performance, toxicity, tissue accumulation and residue of eggs and excreta in laying hens. J. Sci. Food and Agric. 74:229-236.

Czarnecki, G. L. and D. H. Baker. 1984. Roxarsone toxicity in the chick as influenced by dietary cysteine and copper and by experimental infection with Eimeria acervulina. Poult. Sci. 61:516-520.

Donaldson, W. E. 1990. Lipid metabolism in liver of chicks: response to feeding. Poult. Sci. 69:1183-1187.

German Society for Clinic Chemistry. 1972. Empfehlungen der deutschen Gesellschaft fur Kinische Cheme. Standardisierung von Methoden zur Bestimmung von Enzymaktivitaten in biologischen Flussigketten. J. Clin. Chem. Clin. Biochem. 10:82-192.

Hasegawa, S., T. Kawakami, K. Honda and Y. Hilami. 1994. Effect of fasting on the activities of lipogenic enzymes in chick adipose tissue. Anim. Sci. Technol. (Jpn.) 65:656-660.

Hermier, D., P. Forgez and M. J. Chapman. 1985. A density gradient study of the lipoprotein and apolipoprotein distribution in the chicken, *Gallus* domesticus. Biochim. Biophys. Acta. 836:105-118.

Kumar, S., T. A. Dorsey, R. A. Muesing and J. W. Porter. 1970. Comparative studies of the pigeon liver fatty acid synthetase

complex and its subunit. J. Biol. Chem. 245(18):4732-4744.

Lien, T. F. and D. F. Jan. 1999. The effect on the lipid metabolism of Tsaiya ducks when high levels of choline or methionine are added to the ducks' diet. Asian-Aust. J. Anim. Sci. 12(7):1090-1095.

Lien, T. F., K. H. Yang and K. L. Lin. 2005. Effects of chromium propionate supplementation on growth performance, serum traits and immune response in weaned pigs. Asian-Aust. J. Anim. Sci. 18:403-408.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-270.

Lumeij, J. T. and J. Wolfswinkel. 1988. Blood chemistry reference values for use in columbine hepatology. Avian Pathol. 17:515-517.

McDougald, L. R., J. M. Gillbert, L. Fuller, A. Rotibi, M. Xie and G. Zhu. 1992. How much does roxarsone contribute to coccidiosis control in broilers when used in combination with ionphores. Appl. Poult. Res. 1:172-179.

McDowell, L. R. 1992. Minerals in Animal and Human Nutrition. Academic Press, Inc., New York. pp. 351-358.

National Reserch Council. 1980. Mineral Tolerance of Domestic Animals. National Academic Press, Washington, DC.

Numa, S. 1969. Acetyl-CoA carboxylase from chicken and rat liver. In: Methods in Enzymology (Vol. XIV, lipids). Academic Press, Inc., New York. pp. 9-17.

Ochoa, S. 1969. Malic enzyme. In: Method in Enzymeology. (Ed. S. P. Colowick and N. O. Kaplan). Academic Press, New York, Vol. 1 pp. 739-753.

SAS Institute Inc. 1984. SAS/STAT User's Guide: Version 6. 4th edn. SAS Institute Inc., Cary, North Carolina.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, New York, New York.

Takeda, Y., F. S. Zuki and H. Inoue. 1963. Citrate cleavage enzyme. In: Methods in Enzymology. Academic Press Inc. New York, Vol. 5:154.

Tanaka, K., T. Sen and K. Shigeno. 1975. The effect of fasting and refeeding on lipids of serum and liver in the meat-type chicken. Jpn. J. Zootech. Sci. 46:396-400.

Tanaka, K. J., K. Kenmochi, S. Ohtani, C. M. Collado and K. Shigeno. 1984. Effects of fasting and fasted-refeeding on activities of gluconeogenetic and glycolytic enzymes and acyl-CoA synthetase in growing chicks. Jpn. J. Zootech. Sci. 55(10):780-788.

Wang, C. H. 1992. Plasma and tissue enzyme activities in young chickens. J. Chin. Soc. Vet. Sci. 18(3):132-138.

Waldroup, P. W., S. E. Watkins and E. A. Saleh. 1995. Effect of dietary roxarsone level on leg condition of male broilers. Appl. Poult. Res. 4:186-192.

Wu, C. P., S. M. Tsay, P. W. S. Chiou and K. L. Chen. 2005. Recovery over time of egg production, organ weights and biological functions of laying hens after withdrawal of low to appetite-suppressing toxic levels of dietary roxarsone. Asian-Aust. J. Anim. Sci. (in press).