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Effect of Different Dietary Energy Sources on Induction of Fatty Liver-Hemorrhagic Syndrome in Laying Hens

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Abstract: This experiment was conducted to determine the effect of different dietary energy sources on hepatic lipid content and induction of Fatty Liver-Hemorrhagic Syndrome in laying hens. One hundred and fifty Lohmann commercial layers were divided into three groups of fifty birds each. According to a single factorial arrangement, birds were given one of three experimental diets with control(group 1, 2.65Mcal/kg),or high energy diet which was offered with 7% lard (group 2, 3.06Mcal/kg) and offered with 26% cornstarch (group 3, 3.00Mcal/kg). High energy diets decreased feed intake and egg production ($p<0.01$), average egg weight of group 3 (cornstarch) was decreased ($p<0.01$); In chicks fed the 7% lard diet or 26% cornstarch *ad libitum*, hepatic EE (ether extract) and triglyceride were increased significantly, cornstarch could increase hepatic EE, triglyceride and total cholesterol more significant than lard. The average activity of ALT and AST of high energy was increased to be more than 2 times than control treatment of the last three times blood collection. The LDH of high energy diet was increased with no significant difference of the first three times blood collection. In our experiment, the birds fed the 7% lard diets had the largest increase in plasma triglyceride, but there were no significant treatment effects on plasma triglyceride content by cornstarch all the experiment period. The average hemorrhage score of group 2(lard diet) was the highest in the three groups. In chicks fed the 7% lard diet or 26% cornstarch *ad libitum* had significantly higher MDA values. In the present study, plasma ALT, AST, LDH activities and TC concentration were positively correlated with hepatic TG concentration ($p<0.01$). The results showed that measurement of enzyme activities indicative of liver damage in birds, particularly AST, ALT, and plasma TC concentration, is a valuable tool in the diagnosis of fatty liver-hemorrhagic syndrome in a flock of layers. These results suggest that dietary carbohydrate can induce FLHS more efficiently that dietary fat, the hens overfeed high fat diets have significantly more liver hemorrhage than high carbohydrate diets.

Key words: Energy sources, fatty liver-hemorrhagic syndrome, carbohydrate, fat, laying hen

INTRODUCTION

Fatty Liver-Hemorrhagic Syndrom (FLHS) is a metabolic disorder that occurs widely in poultry flocks and has been reported to be the most frequently diagnosed condition in commercial laying hens (Squires and Leeson, 1988). The decrease in egg production and increased mortality associated with FLHS cause considerable economic loss to egg producers. Several factors have been implicated as having potential contributory elements towards the occurrence of FLHS, including nutrition, environment, genetics, endocrine, toxins etc (Squires and Leeson, 1988; Walzem *et al*., 1993). Despite extensive research in all these areas, the aetiology of FLHS remains idiopathic and no definitive prevention and cure methods for FLHS have been outlined for use in live birds.

Previous experiments have demonstrated high energy diet can effectively induce FLHS in the laying hen and some plasma enzyme activities have been used to diagnose FLHS (Diaz *et al*., 1999; Yousefi *et al*., 2005), but the researches about the effect of different dietary energy sources such as carbohydrate and fat on induction of fatty liver-hemorrhagic syndrome in commercial laying hens have not been described before.

It has been suggested that oxidative damage to the cellular and organelle membranes of the liver increases the susceptibility of the liver to haemorrhage (Squires and Leeson, 1988; Schumann *et al*., 2003). Some researches on the mammals find that there are two "hits" in the process of Steatohepatitis, the first "hit" is the homeostasis of lipid metabolism destroyed, hepatic net lipid can probably increase; the second "hit" is the inflammation (Day and James, 1998), so we think that the hepatic net lipid deposition is probably the first step in the process of FLHS of the laying hen, and the relationship between the selected plasma enzyme activities for the diagnosis of FLHS and hepatic lipid must be determined.

MATERIALS AND METHODS

Animals and diets: This experiment was conducted according to protocols approved by Sichuan Agricultural University Animal Care and Use Committee. One hundred and fifty Lohmann layers (30 weeks of age, individually caged) were used in this experiment. Hens

were divided into three groups of fifty birds each. All birds were given experimental diets *ad libitum* from battery cages equipped with separate nipple drinkers. Three diets were partially formulated in accordance with the nutrient requirements of poultry (National Research Councile, 1994). The groups were: 1) group 1 (control, 2.65Mcal/kg); 2) group 2 (high fat diet, offered by lard, 3.06Mcal/kg) ;3) group 3 (high carbohydrate diet, offered by cornstarch , 3.00Mcal/kg). The experiment continued for 85 days.

Sample collection and analysis: Egg production and feed intake was recorded daily and expressed by whole experiment period per group. The weight of the birds were measured at the start and the end of the 85-d experiment period. From d 30, blood samples were taken from wing vein at 14-d interval from 9 birds of each groups which were marked by symbol. The plasma was separated by centrifugation on 2000g for 5 min and subsequently stored at -20°C until analysis. The activities of plasma ALT, AST, LDH, GLU,TG,TC were determined with commercial kits by SHIMADZU CL8000 clinical chemistry analyzer, and MDA by spectrophotometer thiobarbituric acid method (TBA). All the marked birds were humanly killed at the end of the experiment and the livers were carefully removed, weighed, and scored for liver hemorrhage by assigning a score from 0 to 3 with 0 indicating no hemorrhages; 1, up to 10 subcapsular petechial or ecchymotic hemorrhages; 2, more than 10 subcapsular petechial or ecchymotic hemorrhages; and 3, massive liver hemorrhage (Diaz *et al*., 1999). Liver samples were taken for the determination of dry matter (Vacuum Freezing) and fat content.

Statistical analysis: Date are expressed as the mean ± SEM. Data were analyzed using the general linear model procedure of SPSS 11.0. Mean values were compared by multiple range test (Duncan). The level of significance was $p<0.05$.

RESULTS

Productive performance: The effect of different dietary energy sources on productive performance of laying hen were shown in Table 2, compared with group 1, body weight of group 2 and group 3 were increased significantly ($p<0.05$); high energy diets decreased Feed intake and Egg production significantly ($p<0.01$); compared with group 1 and group 2, average egg weight of group 3 (cornstarch) was decreased ($p<0.01$); feed-egg conversion rate of group 2 was decreased with no significant difference.

The composition of liver lipid: Composition of liver lipids were shown in table 3. For chicks fed the 7% lard diet or 26% cornstarch *ad libitum*, hepatic ether extract and triglyceride were increased significantly ($p<0.01$) comparing with basic diet treatment. Total cholesterol was increased with no significant difference ($p>0.05$). As can be seen from the Table 3, compared with group 1, phospholipid of group 2 was increased significantly ($p<0.05$), there was no significant difference between the group 2 and group 3. From table 3, cornstarch could increase hepatic EE (ether extract), triglyceride and total cholesterol more significant than lard.

Plasma biochemical indices: Plasma biochemical indices which were used to diagnosis FLHSH were shown in Table 3. In the present study, plasma ALT, AST, LDH , GLU activities and TG ,TC concentration were determined. In chicks fed the high energy diet ad libitum, The average activity of ALT and AST was increased to be more than 2 times than control treatment of the last three times blood collection . The LDH of high energy diet was increased with no significant difference of the first three times blood collection, but average activity of LDH was more higher than the other two groups when the chicks were fed the 7% lard diet. Besides these enzymes, plasma TG and TC were influenced by high energy diets (Table 4). In our experiment ,the birds fed the 7% lard diets had the largest increase in plasma triglyceride, but there were no significant treatment effects on plasma triglyceride content by cornstarch all the experiment period, and it was interesting that plasma triglyceride content of group 3 was even lower than that of the control treatment all the experiment period. In the experiment, plasma TC content of group 2 was increased significantly ($p<0.01$) comparing with diet treatment of the last two times blood collection.

Liver hemorrhage score: In the experiment, hens consuming lard and cornstarch ad libitum develop fatty liver (Table 5). In general, the hens overfeed high energy diets become obese, develop large fatty liver and have significantly more liver hemorrhage (walzem, 1993). But in our experiment, the average hemorrhage score of group 2 (lard diet) was the highest in the three groups. The fatty liver of group 3 have no significant hemorrhagic phenomenon. The concentration of MDA in plasma were influenced by high energy diets (Table 5). As can be seen from the table 5, hemorrhage score and the MDA concentration of plasma have the same trend. In chicks fed the 7% lard diet or 26% cornstarch *ad libitum* had significantly higher MDA values than the control treatment. Result showed that hens fed the lard had higher plasma MDA value and had significantly more severe liver hemorrhage scores than the hens fed on the cornstarch diet. Compared with group 1, the MDA concentration of group 2 and group 3 was increased significantly ($p<0.01$).

Correlation coefficients: In the present study, plasma ALT, AST, LDH activities and TC concentration were positively correlated with hepatic TG concentration ($p<0.01$), because the hepatic EE is most formed with

Table 1: Composition and nutrient levels of experimental diets

Ingredients	Group 1 (Control)	Group 2 (Lard)	Group 3 (Cornstarch)
Corn	65.30	56.60	39.50
Soybean meal	24.90	26.60	11.30
Zein Meal	-	-	13.00
Cornstarch	-	-	26.00
Lard	-	7.00	-
Calcium carbonate	7.52	7.52	7.49
Monocalcium phosphate	0.70	0.72	0.80
DL-methionine	0.10	0.10	0.12
Lysine HCL	0.02	-	0.33
Chloride	0.05	0.05	0.05
Salt	0.37	0.37	0.37
Vitamin premix[a]	0.04	0.04	0.04
Mineral premix[b]	1.00	1.00	1.00
Calculated analysis			
CP (%)	16.50	16.50	16.50
ME (Mcal/kg)	2.65	3.06	3.00
Met (sulphur amino acids)	0.37 (0.65)	0.36 (0.65)	0.40 (0.65)
Lys (%)	0.84	0.84	0.84
Ca (%)	3.50	3.50	3.50
AP (%)	0.32	0.32	0.32

[a]Supplied per kilogram of diets: Vitamin A, 5000 IU; Vitamin D_3,500 IU; Vitmin E, 5 IU; Vitmin K, 1 IU; Vitmin B_1, 1.5 mg, Vitmin B_2, 2.5mg, Ca-pantothenate, 2.5mg, niacin acid, 10 mg; pyridoxine,3mg; biotin, 0.1mg; folic acid, 0.25mg; Vitmin B_{12}, 0.005mg. [b]Supplied per kilogram of diets: $MnSO_4$. $7H_2O$,100mg,; $FeSO_4$. $7H_2O$, 220mg; $ZnSO_4$. $7H_2O$, 150mg; $CuSO_4$. $7H_2O$, 20mg; KI, 2mg; Na_2SeO_3,0.4 mg.

Table 2: Treatment effects on productive performance of laying hen

	Control	Lard	Cornstarch
Body weight gain (g)	113.33±23.71[a]	185.56±25.12[b]	186.67±39.41[b]
Feed intake (g/d)	121.72±0.00[B]	111.13±0.00[aA]	111.47±0.00[aA]
Average egg weight (g)	59.35±0.63[aA]	59.17±0.50[aA]	55.97±0.52[B]
Egg production (%)	94.06±0.01[B]	82.44±0.11[aA]	86.11±0.13[bA]
feed-egg conversion rate	2.04	1.89	2.00

Mean values in a row without the same superscript small letter are different($p<0.05$),those without the same superscript capital letter are significantly different($p<0.01$).

Table 3: Treatment effects on the composition of liver lipid

	Control	Lard	Cornstarch
EE% (EE/Fresh liver)	8.20±0.01[B]	14.10±0.01[aA]	15.00±0.01[aA]
TG% (TG/Fresh liver)	4.93±0.27[B]	8.72±0.67[aA]	9.65±0.45[aA]
TC (mg/100g Fresh liver)	389.34±17.05	418.37±24.88	420.52±12.84
PL% (PL/Fresh liver)	2.09±0.11[b]	2.61±0.16[a]	2.28±0.12[ab]

Mean values in a row without the same superscript small letter are different($p<0.05$),those without the same superscript capital letter are significantly different($p<0.01$).

triglyceride, hepatic EE was positively correlated with hepatic TG concentration ($p<0.01$); hepatic TG concentration also positively correlated with hepatic TG concentration ($p<0.01$). Plasma GLU and TG concentration were not significantly correlated with liver TG concentration ($p>0.05$)

DISCUSSION

This study was carried out to investigate the effects of different dietary energy sources on induction of Fatty Liver-Hemorrhagic Syndrome and liver lipid content in laying hens. Two energy sources diets were used in this experiment. Egg production and feed intake of hens fed high energy diets were considerably less than the hens of control group. This result was consistent with that of Harms *et al.* (2000) and Yousefi *et al.* (2005), who reported that hens fed a high energy diet consumed less feed than hens fed control diet, one of the major reasons is that energy content has a key role in the control of food intake (McNab and Boorman, 2002).

Changes in dietary energy concentration modulate feed efficiency through a dependent pathways. As dietary energy increases, energy needs are satisfied with decreasing feed intake. (Plavnik *et al.*, 1997), so, egg production was limited by decreased feed intake.

Hens consuming the diet ad libitum or intubated with the diet in quantities equivalent to usual daily energy intake maintained normal rates of laying, did not become obese, and did not develop liver hemorrhage. Hepatic lipidosis was often referred to as Fatty liver, the etiology of these syndrome has been proposed to be nutritional in nature, observed practically in response to force-feeding high energy diets (Van Elswyk *et al.*, 1994).

Only a few researchers have investigated the effect of different energy sources on hepatic lipids of laying hens. Our experiment has showed that not only carbohydrate but also fat could increase hepatic lipid content. And triglyceride is the main lipid which deposited in the liver (Walzem, 1996). The marked increasing in percentage of triglyceride and phospholipid is caused by high energy diets and there is a direct relationship between triglyceride and total fat content in fatty livers of laying hens.

In the present study, plasma ALT activity was found to be about 2 times higher in hens of group 3 than in hens of control group, plasma AST activity was increased with the same trend. As two important transaminases in hepatocytes, these transaminases could be released from the cytolymph when the hapatocytes had been destroyed. So, some transaminases and LDH were used to detecting the Hemorrhagic Syndrom, damnification of the hepatocytes and inflammation. In our study, from the second blood collection, plasma AST, ALT and LDH activity of hens in group 2 was higher than group 3. this result was supported by the liver hemorrhage score and MDA in plasma in this study. Lipid and glucose are main energy resource of animals, the first step in lipid metabolism is the hydrolysis of the lipid in the cytoplasm to produce glycerol and fatty acids. The liver has a central role in the storage and distribution within the body of all fuels, including glucose, but the metabolism of glucose and fatty acids is different. Glucose will be oxidized by all tissues to synthesize ATP. The most important pathway which begins the complete oxidation of glucose is called glycolysis. And Beta oxidation is the main pathway of fatty acids metabolism. So , when fatty acid deposited in liver, Beta oxidation stress would be strengthened, this is a

Table 4: Treatment effects on plasma biochemical indices

	ALT (U/L)	AST (U/L)	LDH (U/L)	GLU (mmol/L)	TG (mmol/L)	TC (mmol/L)
			The first blood collection			
Control	0.73±0.10	122.14±2.60	144.44±4.30	13.81±0.18	14.99±0.99^{bAB}	3.14±0.16
Cornstarch	0.98±0.10	128.58±3.76	140.44±13.86	14.08±0.19	18.23±1.07^{aA}	3.87±0.27
lard	0.81±0.09	129.43±4.30	147.11±7.17	14.18±0.17	13.87±0.67^{bB}	3.76±0.33
			The second blood collection			
Control	0.38±0.11^{bB}	145.01±5.44^{ab}	169.91±12.30	15.84±0.41^{bB}	15.14±1.77^{aB}	3.27±0.50^{b}
Cornstarch	1.84±0.61^{aA}	147.34±5.23^{a}	217.29±22.21	17.50±0.42^{aA}	23.81±1.36^{A}	4.52±0.09^{a}
lard	0.69±0.18^{bAB}	132.59±2.58^{b}	184.33±12.76	16.06±0.31^{bAB}	15.06±2.56^{aB}	3.46±0.43^{ab}
			The third blood collection			
Control	0.61±0.11^{b}	132.40±4.35^{b}	162.89±11.54	15.83±0.36	15.19±1.86^{b}	4.38±0.31
Cornstarch	1.50±0.34^{a}	152.89±22.97^{a}	190.78±18.76	16.71±0.26	20.50±1.61^{a}	5.27±0.36
lard	1.21±0.17^{ab}	135.61±3.37^{b}	193.33±13.50	16.13±0.34	14.49±1.12^{b}	4.20±0.38
			The fourth blood collection			
Control	0.67±0.13^{B}	135.20±5.56^{bB}	147.50±9.48^{b}	14.28±0.41^{C}	14.93±1.82^{b}	2.91±0.30^{aB}
Cornstarch	1.94±0.29^{aA}	210.68±21.23^{aA}	214.56±13.72^{a}	17.31±0.40^{A}	28.34±2.05^{a}	4.83±0.54^{A}
lard	1.58±0.19^{aA}	165.73±15.19^{ab}	190.11±22.38^{ab}	18.93±0.35^{B}	13.18±1.65^{b}	4.04±0.53^{aB}
			The fifth blood collection			
Control	0.84±0.17^{B}	136.08±5.04^{bB}	161.78±15.91^{b}	15.43±0.35^{b}	14.72±1.17^{aB}	2.28±0.28^{B}
Cornstarch	1.97±0.20^{aA}	211.16±16.55^{aA}	213.11±15.05^{a}	16.60±0.28^{a}	25.76±2.41^{A}	4.70±0.62^{aA}
lard	1.62±0.14^{aA}	169.87±11.03^{bAB}	162.44±7.91^{b}	16.10±0.46^{ab}	14.22±1.43^{aB}	4.22±0.45^{aA}

Mean values in a row without the same superscript small letter are different ($p<0.05$), those without the same superscript capital letter are significantly different ($p<0.01$).

Table 5: Treatment effects on the liver hemorrhage score and MDA in plasma

	Control	Lard	Cornstarch
hemorrhage score	0.89±0.35	1.78±0.32	1.22±0.32
MDA (nmol/L)	4.93±0.27^{B}	9.65±0.45^{aA}	8.72±0.67^{aA}

Mean values in a row without the same superscript small letter are different ($p<0.05$), those without the same superscript capital letter are significantly different ($p<0.01$).

Table 6: Correlation coefficients among hepatic TG and some biochemical indices

	Hepatic TG	Significant level
ALT (plasma)	0.611	**
AST (plasma)	0.580	**
LDH (plasma)	0.527	**
GLU (plasma)	0.363	NS
TG (plasma)	0.300	NS
TC (plasma)	0.508	**
EE (liver)	0.924	**
TC (liver)	0.624	**
PL (liver)	0.433	*

NS=not significant; * $p<0.05$; ** $p<0.01$

cause of oxidative damage from an increased production of free radicals in hepatocytes. MDA is a lipid per oxidative products, indirectly reflecting the degree of per oxidative damage of the body by the per oxidative degree of lipids (Squires and Wu, 1992; Bucala, 1996; Ahsan *et al*., 2003; Tao *et al*., 2005). So, our result showed that hens consumed the diet with lard, the liver hemorrhage score and MDA in plasma were higher than hens consumed the cornstarch diets. The same trend could be found within the AST, ALT and LDH activity.

The results showed that measurement of enzyme activities indicative of liver damage in birds, particularly AST, LDH, and GDH, is a valuable tool in the diagnosis of fatty liver-hemorrhagic syndrome in a flock of layers (Diaz *et al*., 1999). As can be seen from our result (Table 6), correlation coefficients among hepatic TG and some biochemical indices were first researched. In the present study, plasma ALT, AST, LDH activities and TC concentration were positively correlated with hepatic TG concentration ($p<0.01$), but the plasma LDH activities of hens in group 3 has no significant difference with control group. So ,we do not think that plasma LDH activities was a sensitive tool which could be used to diagnose the fatty liver-hemorrhagic syndrome in a flock of layers. These results suggest that dietary carbohydrate can induce FLHS more efficiently that dietary fat, the hens overfeed high fat diets have significantly more liver hemorrhage than high carbohydrate diets.

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REFERENCES

Ahsan, H., A. Ali and R. Ali, 2003. Oxygen free radicals and systemic autoimmunity. Clin. Exp. Immunol., 131: 398-404.

Bucala, R., 1996. Lipid and lipoprotein oxidation: basic mechanisms and unresolved questions in vivo. Redox Rep., 2: 291-307.

Day, C. and O. James, 1998. Steatohepatitis: a tale of two "hits". Gastroenterology, 114: 842-845.

Diaz, G.J., E.J. Squires, R.J. Julian, 1999. The Use of Selected Plasma Enzyme Activities for the Diagnosis of Fatty Liver-Hemorrhagic Syndrome in Laying Hens. Avian Dis., 43: 768-773.

Harms, R.H., G.B. Russell and D.K. Sloan, 2000. Performance of four strains of commercial layers with major changes in dietary energy. J. Appl. Poult. Res., 9: 535-541.

McNab, J.M. and K.N. Boorman, 2002. Poultry feedstuffs: supply, composition and nutrition value. CAB International, 81: 1681-1693.

Plavnik, I., E. Wax, D. Sklan and S. Hurwitz, 1997. The response of broiler chickens and turkey poults to dietary energy supplied either by fat or carbohydrates . Poult. Sci., 76: 1000-1005.

National Research Council, 1994. Nutrient Requirements of Poultry, National Research Council National Academy Press Washington, D.C. 1994.

Schumann, B.E., E.J. Squires, S. Leeson and B. Hunter, 2003. Effect of hens fed dietary flaxseed with and without a fatty liver supplement on hepatic, plasma and production characteristics relevant to fatty liver haemorrhagic syndrome in laying hens. Br. Poult. Sci., 44: 234-244.

Squires, E.J. and S. Leeson, 1988. Aetiology of fatty liver syndrome in laying hens. Br. Vet. J., 144: 602-609.

Squires, E.J. and J. Wu, 1992. Enhanced induction of hepatic lipid peroxidation by ferric nitrilotriacetate in chickens susceptible to fatty liver rupture. Br. Poult. Sci., 33: 329-337.

Tao, X., Z.R. Xu, X.Y. Han., Y.Z. Wang and L.H. Zhou, 2005. Effects of fluoride levels on lipid peroxidation and antioxidant systems of growing/finishing pigs. Asian-Aust. J. Anim. Sci., 18: 552-556.

Van Elswyk, M.E., B.M. Hargis, J.D. Williams and P.S. Hargis, 1994. Dietary menhaden oil contributes to hepatic lipidosis in laying hens. Poult. Sci., 73: 653-662.

Yousefi, M., M. Shivazad and I. Sohrabi-Haghdoost, 2005. Effect of Dietary Factors on Induction of Fatty Liver-Hemorrhagic Syndrome and its Diagnosis Methods with Use of Serum and Liver Parameters in Laying Hens Int. J. Poult. Sci., 4: 568-572.

Walzem, R.L., C. Simon, T. Morishita, L. Lowenstine and R.J. Hansen, 1993. Fatty liver hemorrhagic syndrome in hens overfed a purified diet. Selected enzyme activities and liver histology in relation to liver hemorrhage and reproductive performance. Poult. Sci., 72: 1479-1491.

Walzem, R.L., 1996. Lipoproteins and the laying hen: form follows function. Poult. Avian Biol. Rev.,7:31-64.