

Effects of dietary dihydropyridine supplementation on growth performance and lipid metabolism of broiler chickens

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ABSTRACT: An experiment was conducted to investigate the effects of dihydropyridine supplementation on growth performance and lipid metabolism of broilers. A total of 480 one-day-old female Arbor Acres broiler chicks were randomly divided into four groups, each group had six replications of 20 birds. Each group was fed a maize-soybean meal diet supplemented with 0, 100, 200, 300 mg/kg dihydropyridine, respectively, for six weeks. At 42 days of age, body weight and feed intake were not affected by dihydropyridine, while feed efficiency was significantly increased by 8.4%, 15.0% and 12.0%, respectively ($P < 0.05$). The percentage of abdominal fat and the percentage of liver fat were reduced by 24.5%, 25.9%, 23.3%, and 23.6%, 26.7%, 26.0%, respectively ($P < 0.05$). The higher level of dietary dihydropyridine (200 or 300 mg/kg) increased the hormone-sensitive triglyceride lipase activity in liver and abdominal fat ($P < 0.05$). The lipoprotein lipase activity in abdominal fat was significantly decreased by dihydropyridine ($P < 0.05$). The activity of glucose-6-phosphate dehydrogenase and malic dehydrogenase in liver was significantly reduced, whereas the isocitrate dehydrogenase activity in liver was not affected by dietary dihydropyridine. The content of cAMP was significantly increased by dihydropyridine, but malondialdehyde content was decreased ($P < 0.05$). Dihydropyridine at doses of 100 and 200 mg/kg increased apolipoprotein B ($P < 0.05$), but 300 mg/kg dihydropyridine had no effect on apolipoprotein B compared with the control group. Triiodothyronine was significantly increased by dietary dihydropyridine ($P < 0.05$). There were no differences in apolipoprotein A, cholesterol, triglycerides, high density lipoprotein-cholesterol, very low density lipoprotein-cholesterol, thyroxine and insulin among dietary treatments. It is concluded that supplementing dihydropyridine has a beneficial effect on feed efficiency and lipid metabolism of broilers, and that 200 mg/kg dihydropyridine supplementation is the optimum dose.

Keywords: dihydropyridine; growth performance; lipid metabolism; lipogenesis, lipolysis; broilers

Genetic improvement in growth rate and feed efficiency has allowed modern broilers to reach market weight in a shorter period of time. However, selection for these economically important traits has been accompanied by an increase in the number of problems encountered during production, including obesity, ascites, sudden death syndrome and leg abnormalities (Siegel and Dunnington, 1985; Mench, 2002; Tolcamp et al., 2005). Broiler

chicks are prone to be overfatty, resulting from hyperpyagia when provided free access to feed. Although feed restriction could decrease the fat deposition in broilers (Jones and Farrell, 1992; Nielsen et al., 2003), it is accompanied by a decrease in growth rate.

Birds have the ability to store large quantities of excessive fat in liver, adipose tissue (Hermier, 1997). Lipogenesis (i.e. the conversion of glucose

to triglycerides) takes place primarily in the liver of birds and involves in a series of linked, enzyme-catalyzed reactions including glycolysis, the citric acid cycle and fatty acid synthesis. Hepatic lipogenesis is subjected to both the nutritional and hormonal control and this metabolic process is highly responsive to changes in the diet (Hillgartner et al., 1995; Kersten, 2001). The nutritional state of the bird, as determined by the amount and composition of feed consumed, dramatically affects the hepatic lipid metabolism (Hillgartner et al., 1995).

Dihydropyridine is a new multifunctional antioxidant in food (Kouřimská et al., 1993; Smagin, 1998; Tirzitis and Kirule, 1999; Pánek et al., 2000; Tirzitis et al., 2001). It has been shown that dihydropyridine has the antioxidant and immunological activity in ducks (Bakutis and Bukis, 1984) and chickens (Valdman et al., 1990). Dihydropyridine improved the health of cows, improved the quality of milk, and increased cow fertility (Sutkevicius et al., 1984). It also reduced the body weight loss in young fattened cattle during transport to the meat-packing plant (Spruzh and Igaune, 1991). Dihydropyridine also regulated the lipid metabolism in humans (Zhang et al., 2002). The study indicated that dihydropyridine could improve the laying performance and decrease hepatic fat and abdominal fat in laying hens (Zou et al., 2007). Furthermore, dihydropyridine, as a calcium channel blocker or calcium antagonist, is the effective drug in curing cardiovascular diseases in humans, including angina, hypertension, myocardial ischemia, cardiac failure and atherosclerosis (Luft and Haller, 1993; Zhang, 1996). However, there is no report on the regulation of lipid metabolism by dihydropyridine in broiler chickens. The objective of this study is to evaluate the effects of dietary dihydropyridine supplementation on growth performance and lipid metabolism of broilers to provide the clue for developing a new kind of feed additive which could effectively reduce the fat deposition in broilers.

MATERIAL AND METHODS

Experimental design and birds

This experiment was conducted according to protocols approved by the Northwest A and F University Animal Care and Use Committee. A total of 480 one-day-old female Arbor Acres broiler chicks were obtained from a local hatchery and

randomly divided into four groups, each group had six replications, and each replication 20 birds. The birds were fed maize-soybean meal basal diet supplemented with 0, 100, 200, or 300 mg/kg dihydropyridine, respectively. The dihydropyridine was provided by Sunpu Biochem. Tech. Co., Ltd, Beijing, China. Feed and water were provided *ad libitum*. The photoperiod was set at 22L:2D throughout the whole experimental period. The room temperature was at 35°C at the first day and gradually reduced to 25°C by the end of the third week.

At the end of the experiment, two birds from each replication were killed after 12 h fasting and *ad libitum* access to water. Abdominal fat pad and liver weight were determined.

Blood samples were obtained via the *vena cava* puncture. The serum was transferred into an Eppendorf centrifuge tube (10 ml), centrifuged for 10 min, and then kept at –30°C for analysis.

Experimental parameters measured

Hepatic fat percentage: Liver samples were homogenized and dried to constant weight at 105°C. A dried sample (2 g) was extracted with a chloroform: ether mixture (5:1) in a Soxhlet apparatus for 8 h, and the extracted sample was dried to constant weight at 105°C. Fat weight was calculated by subtracting the sample weight after extraction from the sample weight before extraction and hepatic fat percentage as: fat weight/dried liver weight × 100%.

SOD activity, apoA, apoB and MDA content in liver: The liver tissue was homogenized with 0.9% sodium chloride solution to make a 10% hepatic mixture, which was then centrifugated at 2 000 g for 10–15 min, the upper pellucid liquid was taken for parameter determination. The SOD activity was determined by colorimetry according to the assay kit provided by Nanjing Jiancheng Bioengineering Institute. ApoA, apoB and MDA contents were determined with a UV-2000 spectrophotometer (UNICO Instruments Co., Ltd., Shanghai, China) using analysis kits provided by Ningbo City Biochemical Reagent Factory and Nanjing Jiancheng Bioengineering Institute, China.

Biochemical parameters related to fat metabolism: Serum triglycerides (TG), cholesterol, high density lipoprotein-cholesterol (HDL-C), and very low density lipoprotein-cholesterol (VLDL-C) were measured with an ERBA CHEM-5 semi-automatic

Table 1. Ingredients and nutrient level of basal diets

Ingredients (g/kg)	1–21 days	22–42 days
Maize	610.0	640.0
Soybean meal	290.0	270.0
Rapeseed meal	30.0	40.0
Fish meal	20.0	–
Soy oil	10.0	10.0
Monocalcium phosphate	15.0	15.0
Limestone	12.0	12.0
Salt	3.0	3.0
Premix ¹	10.0	10.0
Nutrition level ²		
ME (MJ/kg)	12.46	12.65
CP (g/kg)	215.8	197.5
Ca (%)	1.0	1.0
Available phosphorus (%)	0.41	0.40
Lys (g/kg)	11.6	10.7
Met + Cys (g/kg)	9.3	7.9

¹provided per kilogram of diet: vitamin A 1 600 IU; vitamin E 10 mg; vitamin D₃ 220 IU; niacin 35.0 mg; D-pantothenic acid 12 mg; riboflavin 3.6 mg; pyridoxine 3.5 mg; thiamine 2.4 mg; menadione 1.3 mg; folic acid 1.4 mg; biotin 0.15 mg; vitamin B₁₂ 0.03 mg; Mn 60 mg; Zn 40 mg; Fe 80 mg; Cu 8 mg; I 0.3 mg; Se 0.2 mg

²ME value is calculated, the other nutrition levels are analyzed

biochemical analyzer using analysis kits provided by Ningbo City Bio-chemical Reagent Factory, China.

HSL activity was measured using the method reported by Shih et al. (1995). The fat was minced and homogenized in 10 volumes of pH 7.0 medium containing 0.25M sucrose, 1mM EDTA, 4 µg/ml leupeptin, 1 µg/ml pepstatin A, and 1mM dithiothreitol, then centrifuged at 105 000 g for 45 min at 4°C. The superficial fat cake was removed and the clear supernatant decanted and used for the assay of HSL activity based on the standard assay procedure of Nilsson-Ehle and Schotz. The assay substrate was prepared immediately before use to give a final concentration of each reagent in the 200 µl assay volume as follows: 100 mmol/l Tris HCl, pH 7.0; 5 g/l of BSA (fatty acid-free, fraction V), and 4.58 mmol/l triolein as substrate. A unit of HSL activity was defined as: one HSL activity unit is the concentration of enzyme that hydrolyzes 1 µmol olive oil in one minute at 37°C.

The activity of glucose-6-phosphate dehydrogenase (G-6-PDH), malic dehydrogenase (MDH), isocitrate dehydrogenase (ICD) in liver, and lipoprotein lipase (LPL) in abdominal fat were determined as reported before (Xu et al., 2003).

Hormone concentrations: Concentrations of triiodothyronine (T₃), thyroxine (T₄) and insulin in serum were measured using an RIA kit (Beijing North Immunological Institute, China) and a gamma-counter (Packard 8500, USA).

Statistical analysis: All the data were analyzed statistically using the general linear model procedure (SAS Institute, 1989) and the treatment means were separated by Duncan's multiple range test ($P = 0.05$).

RESULTS

Body weight at 42 days, feed intake, feed/gain, abdominal fat and liver fat are shown in Table 2.

Table 2. Effects of dihydropyridine on growth performance

Item	Dihydropyridine (mg/kg)				SEM
	0	100	200	300	
Body weight (g/bird)	2 123	2 133	2 198	2 138	24.80
Feed intake (g/bird)	4 085	3 768	3 687	3 633	3.65
Feed/gain	1.92 ^a	1.76 ^b	1.67 ^b	1.69 ^b	0.05
Liver fat (% in dry weight)	31.52 ^a	24.14 ^b	23.10 ^b	23.01 ^b	2.60
Abdominal fat (% BW)	4.21 ^a	3.18 ^b	3.12 ^b	3.23 ^b	0.32

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

Compared with the control group, there were significant differences ($P < 0.05$) in feed efficiency, abdominal fat percentage and liver fat percentage by supplementing dihydropyridine. Supplements of 100, 200 or 300 mg/kg dihydropyridine improved feed efficiency by 8.4%, 15.0%, and 12.0%, decreased liver fat by 23.6%, 26.7%, and 26.0%, and decreased abdominal fat by 24.5%, 25.9%, and 23.3% ($P < 0.05$) respectively. There were no significant differences in feed efficiency, abdominal fat percentage and liver fat percentage among 100, 200, and 300 mg/kg dietary dihydropyridine ($P < 0.05$). No significant differences were observed in body weight and feed intake ($P > 0.05$).

The activities of enzymes related to lipid metabolism are shown in Table 3. 100 mg/kg dihydropyridine had no effect on HSL activity in liver or abdominal fat, while a higher level of dietary dihydropyridine supplementation (200 or 300 mg/kg) increased the HSL activity ($P < 0.05$). The LPL activity in abdominal fat was significantly decreased by dihydropyridine ($P < 0.05$). The activity of G-6-PDH and MDH in liver was significantly decreased, whereas the ICD activity in liver was not significantly affected by dietary dihydropyridine.

There were significant effects of dietary dihydropyridine on cAMP, SOD, and MDA ($P < 0.05$, Table 3), content of cAMP was increased ($P < 0.05$),

Table 3. Effects of dihydropyridine on the activity of enzymes related to lipid metabolism and contents of cAMP and MDA¹

Item	Dihydropyridine (mg/kg)				SEM
	0	100	200	300	
HSL in liver (IU)	28.16 ^a	29.82 ^{a,b}	38.12 ^b	36.26 ^b	0.49
HSL in abdominal fat (IU)	15.21 ^a	17.34 ^{a,b}	22.25 ^b	21.67 ^b	0.70
LPL in abdominal fat (IU)	11.52 ^a	9.12 ^b	8.89 ^b	9.34 ^b	0.13
ICD in liver (IU)	19.83	17.45	17.67	17.87	1.23
MDH in liver (IU)	9.62 ^a	7.23 ^b	6.89 ^b	7.32 ^b	0.32
G-6-PDH in liver (IU)	6.51 ^a	5.15 ^b	4.98 ^b	4.45 ^b	0.13
cAMP (pm/g)	245.16 ^a	282.45 ^b	291.23 ^b	289.34 ^b	12.25
SOD (U/mg)	22.01 ^a	32.65 ^b	37.12 ^b	34.75 ^b	2.34
MDA (nmol/l)	1.43 ^a	1.13 ^b	1.01 ^b	1.06 ^b	0.15

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

¹HSL = hormone-sensitive triglyceride lipase; LPL = lipoprotein lipase; ICD = isocitrate dehydrogenase; MDH = malic dehydrogenase; G-6-PDH = glucose-6-phosphate dehydrogenase; SOD = superoxide dismutase; MDA = malondialdehyde

Table 4. Effects of dihydropyridine on apolipoprotein content in liver, and serum biochemical parameters¹

Item	Dihydropyridine (mg/kg)				SEM
	0	100	200	300	
ApoB (mg/100 mg protein)	32.15 ^a	41.62 ^b	44.75 ^b	38.23 ^b	3.02
ApoA (mg/100 mg protein)	21.12	21.25	23.05	20.88	1.20
Cholesterol (mg/dl)	114.58	111.24	114.15	116.35	23.34
TG (mmol/l)	12.21	11.58	10.15	12.67	2.68
VLDL-C (mmol/l)	1.45	1.31	1.15	1.27	0.78
HDL-C (mg/dl)	120.32	126.18	135.72	124.96	19.87
T ₃ (ng/ml)	1.92 ^a	2.25 ^b	2.33 ^b	2.30 ^b	0.12
T ₄ (ng/ml)	20.16	21.22	20.83	21.11	2.98
Insulin (ng/ml)	0.28	0.31	0.33	0.31	0.05

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

¹ApoA = apolipoprotein A; ApoB = apolipoprotein B; TG = triglycerides; VLDL-C = very low density lipoprotein-cholesterol; HDL-C = high density lipoprotein-cholesterol; T₃ = triiodothyronine; T₄ = thyroxine

SOD activity was increased ($P < 0.05$), and MDA content was decreased ($P < 0.05$) 100 and 200 mg per kg dihydropyridine increased ApoB ($P < 0.05$, Table 4), but 300 mg/kg dihydropyridine had no effect on ApoB compared with the control group. T₃ was significantly increased by dietary dihydropyridine ($P < 0.05$). There were no differences in ApoA, cholesterol, TG, HDL-C, VLDL-C, T₄, and insulin among dietary treatments.

DISCUSSION

The results of this study demonstrated that supplements of dihydropyridine could significantly improve feed efficiency and reduce fat deposition in broiler chickens. This is similar to the results reported by Chen et al. (1993) and Zou et al. (1999, 2007) in laying hens.

Lipogenic and lipolytic enzymes in liver and abdominal fat were determined to investigate the effect of dihydropyridine on lipid metabolism. MDH, ICD and G-6-PDH are responsible for generating NADPH for the support of lipogenesis while hormone-sensitive lipase has the opposite effect of mobilizing fatty acids from adipocytes into the blood stream in the process of lipolysis. Lipoprotein lipase in adipose tissue is involved in the movement of fatty acids from TG in blood chylomicrons and VLDL

to the adipocytes (Gondret and Leuret, 2002; Xu et al., 2003; Kokta and Dodson, 2004; Pashkov et al., 2005). At 42 days of age, the activities of NADPH-generating enzymes were significantly decreased in liver and abdominal fat by dietary dihydropyridine, whereas HSL was significantly increased. It is evident that changes in the deposition of liver and abdominal fat are closely associated with patterns of HSL and NADPH-generating enzymes. The enzymatic machinery of liver and adipose tissue has adapted in such a way as to increase or decrease the capacity for NADPH generating for fatty acid synthesis and mobilization of fatty acids from adipose tissue, thereby facilitating the use of energy toward the maintenance and growth process (Diaz et al., 1999; Lien and Jan, 1999).

The present study demonstrated that dihydropyridine significantly increased the SOD activity of serum and liver in broilers and significantly decreased MDA content in liver. This observation is similar to the results of Sniedze et al. (1977), who showed that dihydropyridine had an antioxidant function in animals and reduced the oxidation of lipid compounds. SOD protects important organs from the attack by free radicals and maintains their normal physiological function by disposing of excessive free radicals. Paradis et al. (1997) reported that dihydropyridine combined with terminal oxidase cytochrome p450 to form a complex which

significantly restrained the activity of NADPH-cytochrome reductase, restrained NADPH production and then reduced lipid superoxidation.

Results of this study also showed that dihydropyridine significantly improved the level of apolipoprotein B in liver. ApoB 100 is a major component of VLDL, which transports triglycerides out of liver. Yin et al. (2000) showed by immune electron microscopy that fat was joined to the apoB chain to form VLDL, which then transports endogenous fat to extrahepatic tissues.

The present results demonstrated that dihydropyridine increased the T_3 level in serum and simultaneously decreased the insulin level, which agrees with observations of Wu et al. (1999) in pigs. T_3 plays an important role in regulating the protein and lipid metabolism. An increase in T_3 concentration could increase fat mobilization and hydrolysis, and insulin has the reverse effects. Valcavi et al. (1997) and Tashi et al. (1998) found that there was a positive correlation between thyroxine and leptin levels and that thyroxine stimulated the secretion of leptin. Leptin is a protein secreted by adipocytes which maintains the relative stability of body fat by regulating the energy metabolism. Leptin absence will induce an increased insulin level in serum and stimulate hepatic fatty acid synthesis, as already shown in mice with leptin absence. In mice whose leptin receptor genes were knocked-out, symptoms of insulin resistance and fatty liver were still present even if the serum leptin level increased. So it is speculated that dihydropyridine can regulate the fat metabolism of laying hens through regulating the secretion of leptin in adipocytes.

In conclusion, the present research indicated that dietary dihydropyridine could improve feed efficiency and decrease hepatic fat percentage and abdominal fat percentage by affecting the fat metabolism in broilers. 200 mg/kg dihydropyridine is the optimum dose.

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