Turk. J. Vet. Anim. Sci.
30 (2006) 315-318

Research Article

Alterations to Lipid Parameters in Response to Fig Tree (*Ficus carica*) Leaf Extract in Chicken Liver Slices

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Received: 15.07.2005

Abstract: Lipid storage, particularly in the abdominal cavity, is a major concern in poultry breeding because it affects the net meat yield. The major contributors to lipid storage in the abdominal cavity are hepatic fatty acid synthesis and VLDL secretion. These factors are strongly correlated with the body fatness in the chicken. The aim of our study was to determine if a traditional food supplement, the extract from fig tree leaves (fig tree leaf extract (FTE)), can be used to decrease hepatic triglyceride (TG) content and secretion of TG and cholesterol (TC) from the liver. Livers from 8-week-old roosters (n = 24) with high abdominal fat pad ratios were extracted, sliced, and cultured with increasing concentrations of FTE, insulin and both of them. While insulin significantly increased TG secretion (0.190 ± 0.013 mmol/l), TG content (0.523 ± 0.093 mmol/l) and TC secretion (1.727 ± 0.412 mmol/l) above the basal levels ($P < 0.001$), when FTE was added these effects were drastically reduced to the basal levels in a concentration-dependent manner ($P < 0.001$). Furthermore, we showed that in response to 1.7, 2.5 and 3.3 µl of FTE/ml of media hepatic TG content and TG secretion values from the liver significantly decreased in a concentration-dependent manner when compared to the control ($P < 0.001$). Our results suggest that *Ficus carica* leaf extract could be a beneficial supplement to modulate TG and TC secretion from the poultry liver.

Key Words: Fig tree leaf, chicken, lipid, liver

Introduction

Decreased meat yield from excessive abdominal fat tissue is a problem for modern breeders. The liver is the main target in birds because de novo lipogenesis is very limited in adipose tissue. Lipid storage in adipose tissue depends on availability of fatty acids originating from the diet or liver. Crespo and Esteve-Garcia (1) have proposed that abdominal fat deposition is more dependent on VLDL secretion than high dietary fat content. However, the problem cannot be corrected by simply decreasing secretion of TG (mostly in the form of VLDL) from the liver without addressing storage of TG because this leads to steatosis (a major concern for breeders with laying hens) (2). For a physiologically viable solution, both the storage and secretion of TG must be decreased in the liver.

Nutritional interventions have been previously investigated. Part of the problem is that common commercial diets provide low lipids (less than 10%) and lead to high levels of de novo fatty acid synthesis in the liver. This is then packaged and secreted into the plasma in the form of VLDL, which readily provide TG to adipose tissue. This ultimately leads to fattening of the birds. Partial replacement of dietary energy by protein has proven to be effective in reducing lipogenesis and subsequent fattening, but this method is not cost effective (2). Other methods, such as hormonal treatments, have been proposed but are not allowed (2).

Recently, some beneficial effects of fig tree (*Ficus carica*) leaf extract (FTE), a traditional supplement, have been argued (3,4). FTE has therapeutic benefits in cases of hyperglycemia (5), cancer (6), helminth infection (7) hypercholesterolemia (8), hypertriglyceridemia (9) and bovine papillomatosis (10). Moreover, previous studies suggested that FTE contains some substances capable of stimulating the lipolytic activity of the plasma LPL system

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(9). In this regard, hypolipidemic activity of an aqueous extract of *Ficus carica* has been shown in diabetic rats (11). Experiments are currently being conducted to show a novel activity of FTE on TG secretion from chicken hepatocytes. In this regard, we propose that FTE may also be used to reduce extrahepatic fattening in avian species by decreasing hepatic lipogenesis and VLDL secretion.

Materials and Methods

Fig tree leaf extract was collected from the south of Iran (Ahwas, June 2004) and freshly used in this experiment. For this purpose, fig tree leaves were cut and running extract was collected from the petiole. The livers from 24 healthy 8-week-old roosters were extracted after a 12-h fast (water *ad libitum*) at the School of Veterinary Medicine, University of Tehran. All of roosters had the same diet prior to the experiment. Roosters with high abdominal fat pad ratios (obese roosters) were decapitated and their livers were sliced and put into culture as previously described by Pullen et al. (12). Briefly, liver was cut into 20 × 10 × 10 mm pieces by a metal block and immersed into ice cold MEM (minimum essential medium eagle, Sigma, M-4642). Strips of approximately 0.5 mm thick were then cut (specific cutter designed at the Sharif Industrial University, Tehran, Iran). Liver slices were blotted on tissue paper and 1 g was placed in a 25-ml flask containing 3 ml of MEM. Treatments were performed 4 times and each time on 6 chickens. Flasks were gassed with O_2:CO_2 (95%:5%) for 20 s, sealed with parafilm and placed in a 37 °C shaking water bath (Memmert, WB/OB7-45, WBU 45) that was oscillating at 60 cycles/min for 4 h. After 4 h, media were removed from the slices using a 0.8 µm filter. Lipids in the slices were extracted according to the Hara and Radin method (13). Briefly, in a test tube, 0.5 g of liver tissue was weighed and 9 ml of hexane:isopropanol (3:2) was added. Glass beads were then added to each test tube, followed by shaking overnight. This mixture was then centrifuged at 1000 × g for 15 min and the supernatant was removed, mixed with 12 ml of aqueous natrium sulfate (6.6%) and decanted for 1 min. The supernatant was then removed, dried and reconstituted in 1.5 ml of isopropanol for lipid analysis. Moreover, media were mixed with chloroform:methanol (80:20), decanted, and after 4 h supernatant was removed, dried and reconstituted in 1.5 ml of isopropanol.

In both extracts, TG was measured as described by Neri and Fring (14) and total cholesterol of the media was measured according to Loeffler and McDougald (15). Total protein was measured by the method described by Bradford (16). TG and cholesterol levels are expressed per milligram of cell protein (as corrected values).

Nine experimental conditions were tested. After finding the toxic doses of FTE through a dose-response study (data not shown) incubations were carried out with just MEM (basal or 0), with increasing concentrations of FTE (1.7, 2.5 and 3.3 µl of FTE/ml of media), 10^{-6} M insulin, 1.7 µl of FTE/ml of media plus insulin and 3.3 µl of FTE/ml of media plus insulin.

Parametric (means, standard deviations) values were determined by the univariate procedure of statistical analysis system software (Sigma stat2 package). The mean values of TG secretion, TG content and TC secretion were tested among the treatment groups by ANOVA.

Results

Roosters had high abdominal fat pads, i.e. the liver weight of chickens was 57 ± 10.33 g and the weight of the abdominal fat pad was 57.5 ± 8.66 g. TG content, TG secretion and TC secretion in chicken liver slice cultures are shown in Table 1. FTE showed a significant decrease in cell TG content compared to the basal level ($P < 0.001$). Furthermore, there was an apparent trend towards a concentration-dependent decrease ($P < 0.001$). Our preliminary dose-dependent study showed that 10^{-6} M insulin had the highest efficacy. However, the induced cell TG content stimulated by 10^{-6} M insulin (0.523 ± 0.093 mmol/g liver) was significantly decreased in coincubation with either 1.7 or 3.3 µl of FTE/ml of media ($P < 0.001$). Moreover, the relative percentages of TG content values showed a significant decrease when compared to the control (Table 2).

As shown in Table 2, incubation of slices with FTE (1.7, 2.5 and 3.3 µl of FTE/ml of media) in the presence or absence of insulin caused a significant decrease in TG secretion ($P < 0.001$). The relative percentages of TG secretion values showed a significant decrease when compared to the control (Table 2). In this respect, induced TG secretion showed a significant decrease in coincubation with 1.7 and 3.3 µl of FTE/ml of media (Table 1).

Table 1. Lipid parameters in treatment groups I (0 or control), II (1.7 µl of FTE/ml of media), III (2.5 µl of FTE/ml of media), IV (3.3 µl of FTE/ml of media), V (10^{-6} M insulin or INS), VI (1.7 µl of FTE/ml of media plus INS) and VII (3.3 µl of FTE/ml of media plus INS). Values are expressed as mean ± SD.

Lipid Parameters	Treatment groups							P-values among different groups
	I	II	III	IV	V	VI	VII	
TG content (mmol/g of liver)	0.202 ± 0.038	0.090±0.016	0.062 ± 0.012	0.048 ± 0.007	0.523 ± 0.093	0.115 ± 0.025	0.040 ± 0.006	II,VI/II,III/III,VII/III,IV/IV,VII ($P > 0.05$) All other groups ($P < 0.001$)
TG secretion (mmol/l)	0.105 ± 0.015	0.090 ± 0.019	0.071 ± 0.010	0.065 ± 0.007	0.190 ± 0.013	0.080 ± 0.009	0.075 ± 0.022	II,VI/ VI,III/ VI,VII/ VII,IV/ VII,III/ III,IV ($P > 0.05$) All other groups ($P < 0.001$)
TC secretion (mmol/l)	0.347 ± 0.058	0.337 ± 0.098	0.260 ± 0.095	0.375 ± 0.181	1.727 ± 0.412	1.143 ± 0.212	0.674 ± 0.141	IV,III/ IV,II/ IV,I/ I,III/ I,II/ II,III ($P > 0.05$) All other groups ($P < 0.001$)

Table 2. Percentages of lipid parameters changes in different treatment groups (1.7, 2.5, 3.3 µl of FTE/ml of media, 10^{-6} M insulin or INS, 1.7 µl of FTE/ml of media plus INS, 3.3 µl of FTE/ml of media plus INS) when compared to the control. Values are expressed as percent of changes ± SD.

Lipid parameters	Treatment groups					
	1.7	2.5	3.3 (µl of FTE/ml of media)	INS	1.7 + INS	3.3 + INS
TG content (% ± SD)	44.6 ± 7.9	30.7 ± 5.9	23.8 ± 3.5	258.9 ± 46	56.9 ± 12.4	19.8 ± 2.9
TG secretion (% ± SD)	85.7 ± 12.2	67.6 ± 14.3	61.9 ± 6.7	180.9 ± 12.4	76.2 ± 8.6	71.4 ± 11.4
TC secretion (% ± SD)	97.1 ± 16.2	74.9 ± 12.9	108.1 ± 5.2	497.7 ± 57.9	329.4 ± 61.1	194.2 ± 40.6

Based on the change in percentages at the 4-h point (Tables 1 and 2), FTE had no significant effect on TC secretion of slices while FTE plus insulin significantly increased TC secretion ($P < 0.001$). In this regard, the insulin effect of increased TC secretion was diminished by FTE.

Discussion

The ease with which adipose tissue takes up VLDL-TG is the major contributor to fattening of the rooster (17). Hermier (2) showed that plasma VLDL-TG of obese roosters was twice that of lean roosters. The VLDL-TG in plasma comes from hepatic lipogenesis. Despite knowing the deleterious effects of hepatic lipogenesis, very little is known about the physiological and pharmacological agents mediating the synthesis and secretion of lipids, apolipoproteins, and VLDL-assembly and secretion in avian species.

Interestingly, the decrease in TG secretion observed in the FTE treatment was concurrent with decreases in cell TG content and TC secretion. In agreement with our findings, Canal et al. (8) showed that chloroform extract of *Ficus carica* leaves led to a decline in the levels of total cholesterol and total cholesterol/HDL cholesterol ratio in hypercholesterolemic streptozotocin induced diabetic rats. In this respect, Perez et al. (9) showed while a *Ficus carica* leaf decoction decreased serum TG values of hypertriglyceridemic rats, it had no effect on TC levels. The mechanism of the effect of FTE on the hepatic lipid is presently unclear.

It is well known that insulin acts on TG storage and secretion via the acetyl coenzyme A carboxylase, a key enzyme in the fatty acid synthase complex (2,18,19). In this study, we showed that FTE modulates the action of insulin, in terms of both TG storage and secretion, when compared to the control and insulin stimulated TG content and secretion. It remains to be seen if these effects are via the acetyl coenzyme A carboxylase. Previous studies suggested that fig leaves contain some substances capable of stimulating the lipolytic activity of the plasma LPL system (8,9). Experiments are currently

being conducted to show novel activities of the *Ficus carica* leaf extract on the lipid parameters of hepatocytes. However, we have shown for the first time that FTE can have a novel effect on both the insulin induced TG hepatic storage and insulin induced VLDL-TG secretion in vitro. As shown in Table 2, there is a significant alteration to basal TG storage and this suggests that FTE therapy may be a beneficial method of altering fat storage in vivo. Furthermore, we showed that in the 4-h incubation FTE can significantly reduce the insulin induced increase in cholesterol secretion (although not as completely as it abolished TG secretion). It is possible that with longer incubation we would see a complete reduction of the insulin induced cholesterol secretion.

Future studies will need to examine the mechanism of FTE effects on the basal and insulin induced lipid changes to deduce if the effect is due to decreased de novo fatty acid synthesis or increased lipolysis of TG (and hence increased secretion of fatty acids or oxidative products) before administration to roosters in vivo. However, these preliminary data suggest that FTE administration may be an alternative method to reduce fat mass, particularly in the abdominal cavity, providing increased meat yield and healthy roosters. Additional verification of these results in a long-term clinical trial is necessary to understand the long-term effects of FTE therapy in reducing the fat mass of roosters.

Acknowledgment

We thank Mr. Ali Naderloo for his technical support.

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