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EFFECT OF DIETARY LIPIDS AND ALFALFA LEAF MEAL ON SERUM LIPIDS PROFILE OF RATS

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المستخلص

تم استخدام سبعة وعشرون فارا من سلالة وستر البينو متوسط أعمارها ما بين شهرين إلى ثلاثة أشهر لتحديد اثر إضافة صفار البيض المغلي مع البيرقر البقري المطبوخ و مركز أوراق البرسيم على دهون الدم في الفئران. المعاملات هي **ت1** العليقة الشاهدة (63% ردة القمح، 31% الذرة و3% زيت الذرة، بالإضافة إلى البريمكس، كالسيوم بكربونات و ملح الطعام)، **ت2** يضاف يوميا 15جرام من صفار البيض المغلي و45 جرام من البيرقر البقري المطبوخ إلى العليقة الضابطة، **ت3** يضاف يوميا 15جرام من صفار البيض المغلي و45 جرام من البيرقر البقري المطبوخ و5 جرام من مركز أوراق البرسيم إلى العليقة الشاهدة. التجربة مصممه على أساس التوزيع العشوائي الكامل يتضمن ثلاثة معاملات لكل منها ثلاثة تكرارات وبكل تكراره 3 فئران، استمرت التجربة لمدة ستة أسابيع ثم جمعت عينات من مصل الدم للحصول على تركيز مستويات الدهون المختلفة (الكولسترول، الجليسريدات الثلاثية، البروتينات الداهنة ذات الكثافة المنخفضة و البروتينات الداهنة ذات الكثافة العالية). اظهرت النتائج الإحصائية إن إضافة مصدر الدهون (صفار البيض + البيرقر البقري) له أثرا معنويا كبيرا على زيادة مستوى الكولسترول، الجليسريدات الثلاثية، البروتينات الداهنة ذات الكثافة المنخفضة و البروتينات الداهنة ذات الكثافة العالية على مصل دم الفئران. بينما تناول مركز أوراق البرسيم (5 جرام) اظهر أثرا معنويا كبيرا في خفض مستوى الكولسترول، الجليسريدات الثلاثية و البروتينات الداهنة ذات الكثافة المنخفضة علاوة على ذلك فان مركز أوراق البرسيم له أثر معنوي كبيرعلى زيادة مستوى البروتينات الداهنة ذات الكثافة المرتفعة

Abstract

Twenty seven 2 to 3 month old Wister albino male rats were used to determine the serum lipids of rats fed ration containing boiled egg yolk (BEY), cooked beef burger (CBB) and alfalfa leaf meal (ALM). The treatments were T_1 basal diet as control (mixture of wheat bran, corn, maize oil, premix, calcium bicarbonate and salt), T_2 basal diet supplemented with 15g BEY + 45g CBB daily and T_3 basal diet supplemented with 15g BEY + 45g CBB + 5g alfalfa leaf meal daily. The experiment was arranged in a completely randomized design (CRD) with 3 treatments each replicated three times with 3 rats per replicate, and the experiment lasted for six weeks. Blood samples were collected for determined serum cholesterol, triglyceride, low density lipoprotein (LDL) and high density lipoprotein (HDL). The result showed that feeding lipids source (T_2 = egg yolk and beef burger) caused a significant increased ($P < 0.01$) serum cholesterol, triglyceride, low density lipoprotein and high density lipoprotein of rats than control diet (T_1). Feeding alfalfa leaf meal (T_3) significantly ($P < 0.01$) decreased serum cholesterol, triglyceride and low density lipoprotein. Moreover, alfalfa leaf meal (T_3) significantly ($P < 0.01$) increased serum high density lipoprotein of rats.

Keywords: Cholesterol, triglyceride, low and high density lipoprotein, Rat

Introduction

Dehydrated alfalfa leaf is commercially available as dietary supplement in several forms such as tablets, powder and tea bag (Long, 2005). Alfalfa meal is a good source of hypocholesterolemic compounds such as saponin and fiber (Roa and Girfinkel, 2000; Francis *et al*., 2002).

Cholesterol in the blood is mainly present in the lipoprotein fractions including low density lipoprotein (LDL) and high density lipoprotein (HDL). Cholesterol is of biochemical significant because it is a precursor of large number of important sterol as bile acids, adrenocortical hormones, sex hormone, vitamin D and cardiac glycosides (Murray *et al*., 1999). Egg yolk, milk products and red meat are rich sources of dietary cholesterol (Martin, 2006). In addition of being metabolic precursors, cholesterol has been implicated in atherosclerosis, this disease result when cholesterol that comes from LDL is deposited in the wall of blood vessels (Ganong, 1997). However, a high blood cholesterol level is one of the factors causing heart disease (Thorpe *et al*., 1970). High blood levels of triglyceride are found in many cases, like in fat metabolism disorders, diabetes mellitus and obesity. It was reported that high levels of triglyceride increase the chance of having heart attack and atherosclerosis (John, 1995). The objective of this study was to assess the effect of dietary alfalfa leaf meal on the level of serum cholesterol and triglyceride of rats fed lipids source.

Materials and Methods

Experimental animals and design: Twenty seven healthy male Wister albino rats two to three month old were used for this study. The average weight of rats was 135±3g. The rats were randomly assigned to three experimental diets. Nine rats per treatment with three replicate (3 rats/replicate) in a completely randomized design.

Formulation of experimental diets: Three experimental diets were formulated as follows: Diet **T1** (basal diet) contained 63% wheat bran, 31% corn and 3% maize oil, provided with premixes, calcium bicarbonate and salt,

diet **T2** contained same ingredients of diet **T1** (basal diet) supplemented with 15g boiled egg yolk + 45g cooked beef burger daily and diet **T3** contained same ingredients of diet **T1** (basal diet) supplemented with 15g boiled egg yolk + 45g cooked beef burger and 5g alfalfa leaf meal daily. All the diets were iso-nitrogenous and iso-energetic. (Table 1).

Table 1. Ingredient composition and proximate analysis of basal diet, beef burger and alfalfa leaf meal

I**ngredients**	%		
Wheat bran	63		
Corn (maize)	31		
Maize oil	3		
Premix*	0.5		
Calcium bicarbonate	1.5		
Salt	1		
Proximate analysis	Basal diet %	Beef burger %	Alfalfa leaf meal %
Dry matter	93.05	31.88	94.85
Crude protein	20.00	19.25	25.95
Crude fiber	21.64	—	21.93
Ether extract	8.56	7.96	3.97
Ash	6.96	1.05	14.38
NFE	35.89	—	28.62

*Multiple vitamins each g contain: Vit A 15000IU. Vit D_3 1500IU. Vit E 2mg. Vit K_3 2mg. Riboflavin 2.5mg. Calcium-D-pentothenate 5.5mg. Niacin amid 10mg. Pyridoxine HCl 3mg. Thiamine HCl 2mg. Vit B_{12} 5mg. Folic acid 2mg**.**

Management and Data collection: The diet were randomly allotted to the three experimental dietary groups. Feed and water were offered *ad libitum*. Normal management practices were observed. At the end of the experiment, blood samples were taken from the orbital plexus of rats by the means of capillary glass tubes under inhalation anesthesia according to the methods of Khanna *et al* (1993) and allowed to clot at room temperature for 20 minutes then serum was separated by centrifugation at 500 r.p.m for 10 minutes. Clear

serum was stored at 20˚C pending analysis for cholesterol, triglyceride, low density lipoprotein and high density lipoprotein.

Chemical analysis: Blood samples were collected into clean tubes for estimation of serum parameters using Allain *et al* (1974) methods.

Statistical analysis: The data obtained were entered into Excel (2003) and retrieved for analysis using Statistical Package for Social Sciences (SPSS) Version 16. Analysis were done by descriptive statistics to calculate the variance and the means were separated using Duncan's Multiple Range Test as described by Steel and Torrie (1980)

Results and Discussion

Serum constituents of rats are presented in Table 2. The effect of dietary lipids and alfalfa leaf meal on cholesterol levels and triglyceride were significantly ($P < 0.01$) different between the treatments. Feeding lipids source (T_2 = egg yolk and beef burger) caused a significant increase ($P < 0.01$) in serum cholesterol and triglyceride levels. Increase of dietary lipids intake has been linked to increase risk of obesity, coronary heart disease and certain types of cancer (Ciscuolo, 1994). However, feeding alfalfa leaf meal (T_3) significantly ($P < 0.01$) decreased serum cholesterol levels of rats. The reduction in cholesterol levels might be due to the presences of saponins in alfalfa formed insoluble complexes with cholesterol in digesta and increased faecal ability of excretion of cholesterol and inhibited intestinal absorption of cholesterol (Matsuula, 2001). Moreover, the diet supplemented with 5g alfalfa leaf meal caused a significant reduction in serum triglyceride levels. Dietary triglycerides induced hyperlipidemia in rabbits (Yanaura and Sakamoto, 1975), which was lowered by additional alfalfa leaf meal. Serum low density lipoprotein (LDL) and high density lipoprotein (HDL) were significantly ($P < 0.01$) affected by the dietary lipids and alfalfa leaf meal. Additional lipids to the basal diet has a significant increase on serum LDL levels, while feeding alfalfa leaf meal significantly ($P < 0.01$) lowered serum LDL level. Adding alfalfa meal with hyperlipidemic diet to humans decreased serum LDL-cholesterol (Molgaard, 1987). On the other hand,

feeding alfalfa leaf meal to an induced hyperlipidemic rats caused a significant increase in HDL levels (Hassan, 2001). High level of HDL-cholesterol has protective effects against the risk of coronary heart disease (Murray *et al.*, 2003).

Table 2. Effect of dietary lipids and alfalfa leaf meal on serum constituents of Wister rats

Parameters	Treatments			±SEM
	T_1	T_2	T_3	
Cholesterol (mg/dl)	90.67^c	154.67^a	119.44^b	2.11
Triglyceride (mg/dl)	173.00^c	318.56^a	212.78^b	5.40
Low density lipoprotein (mg/dl)	58.14^c	88.47^a	69.00^b	0.35
High density lipoprotein (mg/dl)	36.88^c	46.86^b	51.20^a	0.51

a.b.c means with different superscript in the same row were significantly different ($P < 0.01$)
T_1= basal diet (control)
T_2 = basal diet supplemented with 15g boiled egg yolk + 45g cooked beef burger daily
T_3= basal diet supplemented with 15g boiled egg yolk + 45g cooked beef burger with 5g alfalfa leaf meal daily

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