

## EFFECT OF DIETARY SOYBEAN OIL AND FISH OIL SUPPLEMENTATION ON BLOOD METABOLITES AND TESTIS DEVELOPMENT OF MALE GROWING KIDS

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### ABSTRACT

The aim of this study was to investigate the effects of different dietary lipid sources on blood metabolites and testis development of male kids. Twenty-four goat kids (BW = 19.43±1.2 kg) were assigned to three equal groups and received one of three dietary treatments as follows: control (CO), soybean oil (SO) or fish oil (FO). All three diets were isonitrogenous and isoenergetic, but contained different fat sources. Prilled palm-oil (high in C16:0), soybean oil (high in C18:2 n-6) and fish oil (high in EPA 20:5 n-3 and DHA 22:6 n-3) were supplemented at 2% DM to control, soybean oil and fish oil diets, respectively. Testicular circumference, width, length and volume were obtained on kids at the beginning and end of experiment while testicular growth measures were defined as differences between these two measurements. At the end of experiment, blood samples were collected and kids were slaughtered. Immediately after killing testes were dissected, weighed, and fixed in 10% formalin for histological studies. Plasma glucose, triglycerides, total cholesterol, HDL and LDL cholesterol, and testosterone concentrations did not significantly differ among diets. FO diet significantly elevated ( $p<0.05$ ) the testicular growth measurements (circumference, volume, width and length) and absolute fresh testis masses at slaughter compared to CO or SO diets. Also, FO diet showed a significant increase in the seminiferous tubule and lumen diameter, leydig cell, sertoli cell, spermatogonia cell, spermatocyte and spermatid cell counts ( $p<0.01$ ) compared to other diets. Results show that fish oil supplementation to male growing kids improved testes development.

**Key words:** Blood metabolites, goat, fat supplementation, testis development

### INTRODUCTION

Maturation of mammalian testes is dependent on the normal proliferation and differentiation of germinal epithelium and sertoli cells and the number of sertoli cell which established during the prepubertal period which determines the final testicular size and the number of sperm produced in sexually mature animals (Johnson *et al.*, 1994). The early 1930s, Burr and Burr observed that polyunsaturated fatty acids (PUFA) were essential for the maintenance of normal testicular function in rats (Burr and Burr, 1929, 1930). Since then, the role of lipids in the structure and function of the male reproductive system has been an interesting and important area of investigation. Since early 1990s, there have been increasing efforts to characterize the lipids of the testis and to describe their metabolism in relation to the development and functions of this organ (Connor *et al.*, 1997). Polyunsaturated fatty acid supplementation to diets produced significant changes in testicular lipid composition, modifying the metabolism of C20-22 fatty acids with a strong impact on the physiology of germinal and steroidogenic cells (Ayala *et al.*, 1977; Coniglio, 1994; Retterstol *et al.*, 2000). There is considerable evidence that docosahexaenoic acid (DHA; 22:6 n-3) performs an essential role in the functional maturation of tissues such as brain and retina (Innis, 1991; Neuringer *et al.*, 1988; Sanders, 1988). In most mammals, spermatozoa share with brain and retina the specificity of being very rich in

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n-3 long chain polyunsaturated fatty acids, mainly DHA, that playing an essential role in sperm development and function (Neuringer *et al.*, 1988). Docosahexaenoic acid also play an important role in development of testis and in spermatogenesis (Ayala *et al.*, 1977). However, despite the positive effects of DHA sources on male fertility (Estienne *et al.*, 2008; Surai *et al.*, 2000), there is no investigation regarding the DHA impact on the development and maturation of goat testicles. The objective of this study was to investigate the effects of dietary PUFA of the n-6 and n-3 series on blood metabolites and testis development of male growing kids.

## MATERIALS AND METHODS

### *Animal management and experimental procedure*

This study was done in Experimental Farm of Agriculture and Natural Resource Collage, University of Tehran, Karaj, Iran. Twenty-four male goat kids (initial age 5 months) were allocated by stratified randomization on the basis of body weight ( $19.43 \pm 1.2$  kg) into three equal groups. Kids were individually penned and measurements were made on each kid.

Gradual adjustment to the 70:30 grain: hay diet took place over the 14 d quarantine period, and then kids were separated into three groups of an eight animals each; control (CO) soybean oil (SO) or fish oil (FO). All diets contained the same ingredients (alfalfa 13.32%, corn silage 14.34%, wheat straw 2.05%, barley grain 49.18%, canola meal 4.10%, corn grain 5.12%, wheat bran 2.58%, rice bran 2.60%, soybean meal 2.56%, mineral-vitamin mix 0.31%, sodium bicarbonate 0.51%, salt 0.2%, limestone 1.13%) and were isonitrogenous (CP= 14%) and isoenergetic (ME= 2.76 Mcal/kgDM), but contained different fat sources. Prilled palm-oil (high in C16: 0), soybean oil (high in C18:2 n-6) and fish oil (high in EPA; 20:5 n-3 and DHA; 22:6 n-3) were supplemented at 2% DM to CO, SO and FO diets, respectively.

Kids were allowed ad libitum access to water and offered feed twice daily at approximately 0900 and 1700 for 12 weeks. In order to prevent lipid oxidation, fat sources were added and mixed completely with the concentrate before preparing TMR ration every day. Feed bunks were inspected before the 0800 feeding, and the quantity offered was adjusted daily to ensure *ad libitum* consumption. Feed refusals were removed from the bunks, weighed, and recorded daily.

Testicular measurements of circumference, width, length and volume were obtained on kids at the beginning and end of experimental period. Scrotal circumference (in cm) was measured with a flexible cloth measuring tape around the greatest diameter of the testes and scrotum, after pushing the testes firmly into the scrotum. The measurements of testis length and the combined width of the two testes was taken at the point of maximum dimensions with calipers simpler (Hahn *et al.*, 1969). Testicular volume (in ml) was measured by the amount of water displaced when the testicles were immersed in a cylinder of warm water (37°C) filled to the brim (Bailey *et al.*, 1998; Bongso *et al.*, 1984). Testicles were placed in a 2-L graduated cylinder with a known volume of water. The water displaced by testicles was then read to the nearest 5 ml to determine testicular volume. Testicular growth measures were defined as differences between these measurements. At the end of experiment blood samples were collected from the jugular vein by jugular vein puncture into evacuated collection tubes containing sodium heparin then stored immediately on ice. Plasma samples were harvested by centrifuging at  $3,000 \times g$  for 15 min and were stored at -20°C until analysis. Plasma samples were analyzed for glucose, triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), and low-density lipoprotein cholesterol (LDL-cholesterol) concentration using enzymatic method and appropriate kits (Pars-azmon Co., Tehran, Iran) and Clima Plus Analyzer (RAL, Madrid, Spain). Testosterone was assayed using ELISA kit produced by Germany IBL Company; with catalogs number RE52151 and the ELISA Plate Reader (Biotek ELX808, made in USA). At the end of experiment, kids were sacrificed at the Meat Processing Facility of the Animal Sciences Department, University of Tehran. Immediately after killing testes were dissected, weighed, and fixed in 10% formalin. Histomorphology characteristic of samples were evaluated in veterinary histology lab of Tehran University. Tissue fragments were dehydrated in ethanol, embedded in paraffin and sectioned at 5  $\mu$  thicknesses. For histological processing, the sectioned tissues were stained with hematoxylin-eosin and examined for morphological and histological parameters (including: seminiferous tubule and lumen diameter, leydig cell, sertoli cell, spermatogonia cell, spermatocyte and spermatid cell counts) by light microscopy.

### *Statistical analysis*

Data were analyzed as a completely randomized design using the General Linear Model (GLM) procedure of the Statistical Analysis Software package (SAS Institute, 2002). Least-square means were computed and tested for differences by the Tukey's test. Differences of least squared means were considered to be significant at  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

### *Growth performance and plasma metabolites*

The influence of diets on feeding parameters is listed in Table 1. There were no significant differences among diets in average daily gain, dry matter intake and feed conversion ratio. Since intakes were similar across treatments of similar energy-protein levels, no differences in animal performance were expected and these data confirmed results obtained by other authors (Choi *et al.*, 2000; Manso *et al.*, 2009) in beef and lambs. Also, in response to different fat inclusion, there were no differences among dietary treatments in feed intake, average daily gain and final live weight of pigs (Realini *et al.*, 2010). In this regard, Doreau and Chilliard (1997) mentioned that the effects of adding fat to diets of ruminants depend not only on the type of fat but also on the amount added.

Also, plasma glucose, triglycerides, total cholesterol, HDL and LDL cholesterol, and testosterone concentrations did not differ among treatments (Table 1). Although, evidence suggests that dietary polyunsaturated fatty acids lower the plasma cholesterol concentration and that lipid-lowering effect may be more pronounced when feeding EPA and DHA (Sebokova *et al.*, 1990). In this study different dietary oils had no significant effect on the plasma metabolites, meanwhile, our result is in agreement with Agazzi *et al.* (2010) who reported that different dietary fatty acids supplementation to dairy goats did not affect blood metabolites.

Although, previous studies (Gromadzka-Ostrowska *et al.*, 2002; McVey *et al.*, 2008) have shown that dietary fats can modulate steroidogenic function of mammalian testis, testosterone concentration in the present study was not significantly differed among treatments. Plasma testosterone concentration was positively correlated with HDL cholesterol and the change in testosterone concentration was also positively correlated with change in HDL cholesterol (Dai *et al.*, 1984). On the other hand,

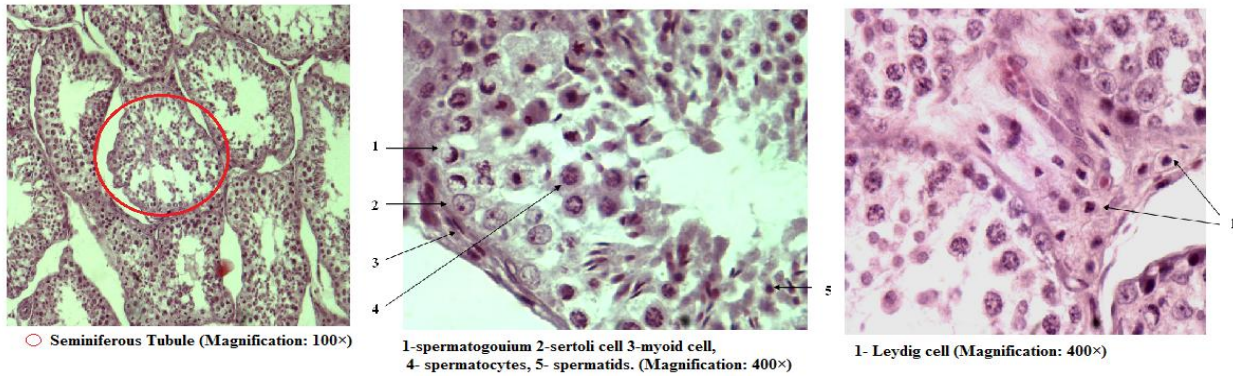
because different sources of fat supplementation could lead to the same plasma HDL cholesterol concentration, despite leydig cells activity, no difference in testosterone concentration was expected.

### *Testicular growth and histological parameters*

FO diet significantly elevated ( $p < 0.05$ ) the testicular growth measurements (circumference, volume, width and length) compared to CO or SO diets. Also, absolute fresh testis masses at slaughter were significantly higher ( $p < 0.05$ ) in FO diet (Table 2). Testicular measurements have been used to predict sperm production and semen quality (Bailey *et al.*, 1998). It is established that the testicular measurements, particularly scrotal circumference, critically affects male fertility (Toe *et al.*, 2000). Also, fish oil enhanced testis mass through improving testis development yet the higher testes masses with fish oil agree with study of Surai *et al.* (2000) in which testicular weight in broiler chicken at 60 weeks of age were 1.8 times greater as a result of dietary supplementation with tuna oil although the body weights of the birds were not significantly affected by the different dietary oils.

Micrographs of histological sections of testis are shown in Figure 1. The histological structure of testis differed among treatments at slaughtering. Results showed that FO diet had significantly elevated the seminiferous tubule and lumen diameter, leydig cell, sertoli cell, spermatogonia cell, and spermatocyte cell counts compared to CO or SO diets (Table 3;  $p < 0.01$ ). However, seminiferous tubule diameter and the number of leydig cells were significantly higher in SO diet compared to CO diet ( $p < 0.01$ ). Previous authors had demonstrated that during puberty the testes undergo profound physiological, biochemical, and structural changes, particularly in fatty acid profile (Connor *et al.*, 1997; Lin *et al.*, 2004). Increasing DHA and its effect on the maturation and function of cells was reported on brain and retina (Lin *et al.*, 2004). However, it is an idea that dietary supplementation of DHA or diet with n-3 PUFA source may increase activity and developments of such tissues (Lin *et al.*, 2004). Modern intensively farmed livestock have little, if any, access to fresh pasture. Also, in preserved forage n-3 PUFA concentrations are low (Wathes *et al.*, 2007), and diets fed to farm animals commonly contain large amounts of cereals. Thus, dietary fatty acids typically have no long chain n-3 polyunsaturated fatty acids. Therefore, fish oil as

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**Fig 1. Micrographs of histological sections prepared from goat testis.**

**Table 1: Growth performance and blood metabolites of growing kids fed diets differing in fatty acids sources**

	Diets			SEM <sup>1</sup>	P-value
	CO	SO	FO		
<b>Growth performance</b>					
Final body weight (kg)	34.47	33.21	34.10	1.2	ns
Average daily weight gain (g/d)	178.62	163.57	174.17	14.5	ns
Dry matter intake (g/d)	1156.96	1060.76	1009.85	47	ns
Feed conversion ratio	6.60	6.64	5.99	0.29	ns
<b>Plasma metabolites</b>					
Glucose (mg/dl)	77.09	75.44	73.71	2.33	ns
Triglycerides (mg/dl)	8.09	8.51	8.14	0.60	ns
Cholesterol (mg/dl)	113.7	110.6	107.5	3.55	ns
HDL (mg/dl)	69.6	71.3	70.7	6.07	ns
LDL (mg/dl)	42.6	44.1	38.1	3	ns
Testosterone (ng/ml)	6.47	5.44	5.58	0.69	ns

<sup>a,b</sup>Means within each row carrying no common letter are different at P<0.05 using Tukey's method.

<sup>1</sup>Standard error of the mean  
ns, not significant (P > 0.05)

**Table 2: Testicular growth measurements<sup>1</sup> (circumference, width, length and volume) and testis mass at slaughtering of kids**

	Diets			SEM <sup>2</sup>	p-value
	CO	SO	FO		
Circumference (cm)	7.51 <sup>b</sup>	7.09 <sup>b</sup>	9.38 <sup>a</sup>	0.46	*
Width (cm)	2.56 <sup>b</sup>	2.32 <sup>b</sup>	3.58 <sup>a</sup>	0.19	**
Length (cm)	2.80 <sup>b</sup>	2.64 <sup>b</sup>	3.70 <sup>a</sup>	0.24	*
Volume (ml)	114.46 <sup>b</sup>	121.68 <sup>b</sup>	161.96 <sup>a</sup>	10.57	*
Testis mass at slaughter (g)	275 <sup>b</sup>	265 <sup>b</sup>	304 <sup>a</sup>	8.0	*

<sup>a,b</sup>Means within each row carrying no common letter are different at P<0.05 using Tukey's method.

<sup>1</sup> End minus beginning measurements.

<sup>2</sup> Standard error of the mean

\* p < 0.05, \*\* p < 0.01

**Table 3: Histomorphology characteristic of testis tubules and cells of kids**

	Diets			SEM <sup>1</sup>	p-value
	CO	SO	FO		
Diameter of seminiferous tubule (mm)	0.149 <sup>c</sup>	0.178 <sup>b</sup>	0.257 <sup>a</sup>	0.005	**
Seminiferous tubule lumen diameter (mm)	0.09 <sup>b</sup>	0.08 <sup>b</sup>	0.12 <sup>a</sup>	0.006	**
Lydig cell count/mm <sup>2</sup>	1033 <sup>c</sup>	1247 <sup>b</sup>	1758 <sup>a</sup>	14.39	**
Sertoli cell count/mm <sup>2</sup>	1037 <sup>b</sup>	1020 <sup>b</sup>	1367 <sup>a</sup>	13.52	**
Spermatogonia cell count/mm <sup>2</sup>	1022 <sup>b</sup>	1005 <sup>b</sup>	1282 <sup>a</sup>	9.71	**
Spermatocyte count/mm <sup>2</sup>	1006 <sup>b</sup>	987 <sup>b</sup>	1286 <sup>a</sup>	6.96	**
Spermatid cell count/mm <sup>2</sup>	1007 <sup>b</sup>	998 <sup>b</sup>	1297 <sup>a</sup>	6.36	**

<sup>a,b,c</sup> Means within each row carrying no common letter are different at P<0.05 using Tukey's method.

<sup>1</sup> Standard error of the mean

\*\* p <0.01

n-3 PUFA supplementation could improve development and maturation of testes tissue.

The results showed that fish oil positively affected gonad developments (increased proliferation of many types of testis cells involved in spermatogenesis; Table 3) of the goats and similar results were reported in studies with rats by Ayala *et al.* (1977). It has been shown that polyunsaturated fatty acids (Jump and Clarke, 1999; Ojeda *et al.*, 1981) or their derived eicosanoids (Farr and Ellis, 1980; Free *et al.*, 1980; Saksena *et al.*, 1978) has a straight relation with the hypothalamo-pituitary-gonadal axis, which controls hormones involving in testicular development, and spermatogenesis. Post-natally, the sertoli and leydig cells development are reliant upon the pituitary gonadotrophins, so that Follicle Stimulating Hormone (FSH) (Almiron and Chemes, 1988; Orth, 1982, 1984) is considered as the main mitogenic factor responsible for sertoli cell divisions and luteinizing hormone (LH) considered as main factor affecting differentiation and maturation of the leydig cell population (Benton *et al.*, 1995; Huhtaniemi *et al.*, 1984). Chandolia *et al.* (1997) mentioned that GnRH treatment hastened the onset of puberty, increased sertoli cell counts, sperm output, and testicular weight. Also, Bagu *et al.* (2004) concluded that treatment of bull calves with bFSH increased testicular growth; hastened onset of puberty; and enhanced spermatogenesis. Although we did not measure FSH and LH, there is a possibility that supplementation with n-3 PUFA of fish oil might affect testis development through its impact on hypothalamo-pituitary-gonadal axis.

In addition, the significant increase in the number of spermatogonia, spermatocyte, and spermatid in the fish oil treatment showed a progress in spermatogenesis. The results of this trial is consistent with Surai *et al.* (2000) who indicated that number of spermatozoa at 60 weeks of age was significantly higher in birds given tuna oil.

The number of sertoli cell which is established during the prepubertal period determines the final testicular size and the number of sperm produced in sexually mature animals (Johnson *et al.*, 1994). Although we didn't measure sperm concentration in this trial, it is most likely has been increased. In agreement of this result, Estienne *et al.* (2008) showed an increase in the sperm concentration by supplementation with n-3 PUFA in Boars' diet. In human, reduction in the amount of 22:6n-3 in sperm lipids has been correlated with reduction in sperm concentration and in spermatozoa with progressive motility and normal morphology (Conquer *et al.*, 1999; Nissen and Kreysel, 1983; Zalata *et al.*, 1998). Also, the reduction in the number of spermatozoa and in sperm motility in ejaculates from ageing bulls is accompanied by a decrease in the proportion of 22:6 n-3 in sperm phospholipids (Kelso *et al.*, 1997).

### CONCLUSION

In this study source of dietary fat significantly affects testes developments and spermatogenesis in goat and the current results show that fish oil diet positively affect gonad development in the goat and that when dietary DHA level increased, better gonad development could observe.

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