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Original article

Effect of fish oil on performance and serum adipokine levels of dairy does during gestation period

K. Öztabak[1], U. Serbester[2], F. Esen Gürsel[1], I. Akış [1], A. Ateş[1], H. Yardibi[1], G. Atmaca[1], N. Koluman[2]

[1] Istanbul University-Cerrahpasa, Faculty of Veterinary Medicine, Department of Biochemistry, 34320 Avcilar Istanbul, Turkey
[2] Cukurova University, Agricultural Faculty, Department of Animal Science, 01330, Adana, Turkey

Abstract

Fatty acids are very important biological substances due to their metabolic, structural and signaling functions. Omega-3 has different beneficial, harmful and neutral effects on adipokines. Adipokines have autocrine, paracrine and endocrine effects on metabolism. In the study 54 German Fawn x Hair crossbred goats were synchronized using intravaginal sponges. During the first period (mating-75 days), all animals were fed a diet supplemented with protected fat and during the second period of pregnancy (76 days-kidding), one of the groups was fed a diet supplemented with fish oil and other was fed a diet supplemented with protected fat. Serum leptin, ghrelin, adiponektin and omentin levels were measured by ELISA system. Distributed fed (roughage and concentrate) were sampled and dry matter, crude protein, fat, and ash were determined by AOAC (1988) analysis methods. The Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) analysis were conducted using heat stable α-amylase and sodium sulphite. Fat source (fish oil or protected fat) affected feed consumption and the highest feed consumption was found in the group fed with protected oil first half of the pregnancy and with fish oil in the second half of the pregnancy and in the fish oil group during the pregnancy. It was determined that the use of fish oil during pregnancy did not affect ghrelin, leptin and omentin concentrations in serum. Adipokine levels of fish oil fed animals during any period of pregnancy were found to be high and it was also found that serum adiponectin levels in goats fed with diet containing fish oil in the first half of pregnancy and protected fat in the second half were statistically significantly high in adipokines.

Key words: fish oil, adipokine, does, gestation

Correspondence to: I. Akis, e-mail: iraz@istanbul.edu.tr

Introduction

Fat cells are a connective tissue cells whose main function is to store body fat. Fat tissue has physiological functions such as physical protection, water storing, and heat production, as well as storing energy and fat soluble vitamins. In recent years, in addition to these functions, it has been shown that some proteins called "adipokines" are secreted from connective tissue cells located between fat cells and adipose tissue cells, which have very important metabolic functions and that these proteins have autocrine, paracrine and endocrine effects. Leptin, adiponectin, resistin, apelin, adipsin, visfatin, vaspin, interleukin-6 (IL-6), TNF-alpha, angiotensinogen, omentin, retinal binding protein (RBP)-4 and plasminogen activator inhibitor (PAI)-1 are the adipokines, which are thought to be related to obesity and metabolic disorders secreted by fat tissue. Studies have shown that adipokines derived from fat tissue play a role in the pathogenesis of obesity complications such as hyperlipidemia, diabetes, hypertension, atherosclerosis and heart failure (Meier et al. 2004, Lau et al. 2005, Zhang et al. 2010).

Fatty acids are very important biological substances due to their metabolic, structural and signaling functions. Studies showed that diets containing n-3 fatty acids and n-3: n-6 ratio have positive effects on cardiovascular diseases, cancer, atypical eczema, quality of sperm in mice, brain, nervous system and retinal development in humans, follicle development in ruminants, progesterone concentration, increase in oocyte count and quality and placental and early embryo development in humans and animals (Robinson et al. 2004, Duvaux-Ponter et al. 2008, Simopoulos 2011, Akbarinejad et al. 2012, Ebrahimi et al. 2013). It is known that omega-3 has different beneficial, harmful and neutral effects on adipokines (Patel et al. 2007, Mostowik et al. 2013). It has been reported that there is a positive correlation between dietary intake of omega-3 and circulating adiponectin (Flachs et al. 2006). This can be interpreted as a low cardiovascular disease, metabolic syndrome, and diabetes risk for obese patients (Gray et al. 2013). High circulating adiponectin levels have anti-diabetic, anti-atherosclerotic, and anti-carcinogenic effects (Wanders et al. 2010). The intake of n-3 PUFAs during pregnancy increases the duration of pregnancy and fetal development and reduces the risk of pregnancy complications (Oken et al. 2007, Imhoff-Kunsch et al. 2012, Jones et al. 2013). It has been found that enzymes responsible for the long chain - polyunsaturated fatty acids (LC-PUFA) synthesis are either absent or very low in placenta (Hanebutt et al. 2008, Wadhwani et al. 2013). In this case, the fetus is entirely dependent on maternal resources, especially maternal diets, for fatty acids that are so important in terms of physiology (Jones et al. 2013). It is suggested that adipokines secreted during pregnancy originate from maternal adipose tissue, placenta and fetus (Zavalza-Gómez et al. 2008). The aim of this study was to determine the effects of dietary rations containing fish oil rich in omega-3 fatty acids or palm oil rich in saturated fatty acids during pregnancy on serum concentration of leptin, adiponectin, ghrelin and omentin adipokines secreted from visceral adipose tissue, and metabolic effects of diets containing unsaturated and saturated fatty acids on adipose tissue during pregnancy.

Materials and Methods

The study was carried out between August 2015 and March 2016 at Çukurova University, Faculty of Agriculture, Dairy Goat Research and Application Unite (Adana/Turkey). Animals were maintained under protocols approved by the University of Cukurova Animal Care and Use Committee (protocol number: 2015-2:4).

Animals

Fifty four German Fawn x Hair crossbred does varying in age between 2 and 5 years were used in this study. The body weights of does were 48.5 ± 8.11 kg (means±SD). Oestrus was induced and synchronized using the method by Özer and Doğruer (2011). Pregnancy tests were carried out on the 45th day of pregnancy using a real-time ultrasonography device (Pie Medical, Falco, The Netherlands) equipped with a 5-7,5 MHz linear rectal probe transrectally after completion of the matings.

Feeding regime

The feeding regimen applied to pregnant does in the present study was as follows. The gestation period was segmented to 2 periods, mating-75 days and 76 days-kidding day. During the first period, all animals were fed a diet supplemented with protected fat. During the second period of pregnancy (76 days – kidding), one of the groups fed a diet supplemented with fish oil and other fed a diet supplemented with protected fat.

During pregnancy period roughage concentrate feed in total mixed rations (TMR) was 60:40%. Alfalfa hay and wheat straw were used as roughage sources respectively, 75% and 25%. Concentrate feed manufactured with a special formulation contained both fats (protected and fish oil) at the rate of 7% for

Table 1. TMR content and nutrient composition used in the experiment.

Ingredients	%	
Barley	8.00	
Corn	2.00	
Wheat bran	10.00	
Corn bran	2.59	
Sunflower seeds (%25 Crude protein)	10.43	
Molasses	2.40	
Fat[1]	2.80	
Alfalfa hay	45.00	
Wheat straw	15.00	
Limestone	1.42	
Salt	0.32	
Vitamin-Mineral[2]	0.04	
Nutrient composition (%)	Protected Fat TMR	Fish Oil TMR
Dry Matter	92.3	92.1
Crude protein	16.7	17.3
Fat	4.6	4.6
ADF	28.9	29.7
NDF	45.1	45.5
Ash	6.6	7.2

[1] Fish Oil or Protected Fat.
[2] Vitamin-Mineral (kg): 15.000.000 IU vitamin A, 3.000.000 IU vitamin D3, 30.000 mg Vitamin E, 150.000 mg Niasin, 10.000 mg Cu, 800 mg I, 150 mg Co, 150 mg Se, 50.000 mg Mn, 50.000 mg Fe, 50.000 mg Zn, 6.800 mg organic Mn, 1.400 mg organic Cu, 6.800 mg organic Zn, 6.800 mg organic Fe, 50 mg organic Se.

each type of oil in the feed. Pregnancy TMR is given in Table 1.

Method

The study lasted 4.5 months in total (2.5 months of pregnancy and 2 months of lactation period). The study was carried out in a special designed open holster. During the experimentation, animal materials were housed in 6 sections of 6×12 m (width×length) dimensions. Three of groups encompassed does fed with TMR containing fish oil and the other three were fed with TMR containing protected fat.

Data collection during pregnancy and lactation period

During the experimentation, feed intake was weekly measured based on subgroup. Feeding was ad libitum, 5-10% of distributed feed should be left. Does body weight was measured every 2 weeks. The feed conversion efficiency was determined by dividing feed consumption by live weight gain.

Blood samples

Blood samples were taken monthly from the animals individually during pregnancy. Blood samples were taken into serum tubes from the vena jugularis before feeding in the morning. Samples were centrifuged (Universal 320R, Hettich, Germany) to obtain serum samples and stored at -20°C until hormone analysis.

Serum hormone analysis

Serum leptin (SUNRED Elisa kit, cat no. 201-07-3168), ghrelin (SUNRED Elisa kit, cat no. 201-07-3127), adiponektin (SUNRED Elisa kit, cat no. 201-07-3177) and omentin (SUNRED Elisa kit, cat 201-07-3124) levels were measured by ELISA system (µQuant, BioTek Instruments Inc.) in the Routine Research Laboratory of the Department of Biochemistry, Faculty of Veterinary Medicine, Istanbul University, using goat-specific commercial ELISA assay kits according to the manufacturer's directions.

Feed analysis

During the experiment distributed fed (roughage and concentrate) were sampled and dry matter, crude protein, fat, and ash were determined by AOAC (1988) analysis methods. The Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) analysis were conducted according to Van Soest et al. (1991) (Ankom200

Table 2. Effect of using protected fat or fish oil in the diet on live weight and feed intake during pregnancy period in goats.

	Mating to 75 d of gestation				SEM[1]	P value		
	Protected fat		Fish oil					
	76 d of gestation to kidding							
	Months	Protected fat	Fish oil	Protected fat	Fish oil		Fat source	Month	Fat source x Month

	Months	Protected fat	Fish oil	Protected fat	Fish oil	SEM[1]	Fat source	Month	Fat source x Month
Live weight (kg)	1	45.8	50.6	46.6	49.3	1.13	0.131	<0.01	0.996
	2	48.2	50.0	48.8	47.0				
	3	49.7	51.9	51.5	53.1				
	4	52.3	55.1	52.6	56.1				
	5	52.9	57.8	52.6	55.5				
	Average	49.8	53.1	50.4	52.2				
Feed intake (kg/d)	1	2.76	3.23	2.91	3.11	0.03	<0.01	<0.01	<0.01
	2	3.00	3.47	3.04	3.30				
	3	2.76	3.00	2.87	2.91				
	4	2.12	2.32	2.08	2.14				
	5	2.15	2.46	2.18	2.52				
	Average	2.56^b	2.90^a	2.62^b	2.80^a				

[1] SEM: Standart error of means

Fiber Analyzer, ANKOM Technology Corp., NY) using heat stable α-amylase and sodium sulphite.

Statistical Analysis

The collected data during the study period were compiled in Microsoft Excel (version 2013 of Microsoft Corp.). The analysis were performed by SAS software (version 8.0, SAS, 2000). P values ≤0.05 were considered significant, and 0.05< p≤0.10 were considered a tendency.

Results

In the current study, the effect of using fish oil rich in omega-3 fatty acids during pregnancy on adipokine concentrations secreted from adipose tissue in goats was investigated. Protected fat source was used in the control ration to make Total Mixed Ration (TMR) isonitrogenous and isoenergetic. The results obtained from our study are summarized below.

Effect of using protected fat or fish oil during pregnancy on performance

The use of protected fat or fish oil in the ration did not affect the live weight of pregnant goats (p>0.05, Table 2). It was determined that live weight was 51.5 kg in the group fed TMR with protected fat, and 51.3 kg in in the group fed TMR with fish oil during pregnancy. In the study, live weight was increased (p<0.01) during pregnancy whereas no significant interaction (p>0.05) between fat source and month was found.

Feed intake increased (p<0.01) for goats consuming fish oil diets comparing with goats receiving protected fat diets. The highest feed consumption (2.90 kg/day) was in the group fed with protected fat in the first half and was fed with TMRs containing fish oil in the second half of pregnancy. The lowest feed consumption (2.56 kg/day) was found to be in goats fed with TMRs containing protected fat during pregnancy. However, it was found that the fat source by month interaction effect was also statistically significant (p<0.01) on feed intake, and that feed consumption of TMRs containing fish oil or protected fat during pregnancy was lower in the gestation period than in the groups receiving different fat sources.

Effect of using protected fat or fish oil during pregnancy on serum adipokine levels

The effect of pregnancy period nutrition on serum ghrelin, leptin, omentin and adiponectin concentrations is given in Table 3. The use of fat in the diet had no effect on pregnancy serum ghrelin concentrations (p>0.05). The highest serum ghrelin concentration (221.7 pg/mL) was determined in goats fed with fish oil-containing TMR during pregnancy. The lowest serum ghrelin concentration (134.7 pg/mL) was found to be in goats fed with protected fat-containing TMRs during pregnancy. Serum leptin and omentin concentrations were also similar between the groups (p>0.05). The highest concentrations of leptin (7.1 ng/mL) and omentin (27.4 ng/mL) were found to be in the group fed with rations containing protected fat in the first half of pregnancy while the lowest concen-

Table 3. Effect of using protected fat or fish oil on pregnancy adipokine concentrations in goats.

Mating to 75 d of gestation	Months	Protected fat		Fish oil		SEM[1]	P value		
76 d of gestation to kidding		Protected fat	Fish oil	Protected fat	Fish oil		Fat source	Month	Fat source x Month
Ghrelin (pg/mL)	1	217.8	238.0	248.6	659.5	59.07	0.623	0.072	0.503
	2	189.6	345.7	185.1	96.9	56.37			
	3	98.5	121.8	251.8	57.5	71.34			
	4	91.4	186.2	242.0	149.8	58.63			
	5	76.4	102.5	174.6	144.9	55.62			
	Average	134.7	198.8	220.42	221.7	52.10			
Leptin (ng/mL)	1	5.3	5.2	7.9	16.1	1.86	0.394	0.280	0.454
	2	6.4	7.8	2.9	1.7	1.78			
	3	1.7	1.9	8.3	0.74	2.25			
	4	1.6	3.5	11.9	2.62	1.85			
	5	1.4	1.8	4.6	6.6	1.75			
	Months	3.3	4.0	7.1	5.5	1.62			
Omentin (ng/mL)	1	17.0	18.3	24.8	26.0	4.83	0.135	0.614	0.921
	2	12.0	20.5	18.5	12.0	4.62			
	3	13.6	14.1	29.7	5.4	5.82			
	4	12.1	27.8	39.0	21.5	4.75			
	5	9.3	10.7	24.9	30.0	4.50			
	Months	12.8	18.3	27.4	19.0	3.99			
Adiponektin (mg/L)	1	16.1	16.9	24.2	22.6	12.8	0.012	0.091	0.895
	2	13.1	18.2	50.3	26.0	12.3			
	3	19.1	33.7	99.0	36.2	15.6			
	4	25.1	74.1	114.9	62.6	12.8			
	5	14.2	21.5	69.1	27.86	12.1			
	Months	17.5^{b}	32.9^{ab}	71.5^{a}	35.0^{ab}	11.3			

[1] SEM: Standart error of means

trations were found in the group fed with protected fat during pregnancy.

Serum adiponectin concentrations of the group fed with rations containing fish oil in the first 75 days of gestation and serum adiponectin concentrations (71.5 mg/L) of the group fed with protected fat from the 76th day of gestation until birth were found to be statistically significant ($p<0.05$). The concentration of serum adiponectin (32.9 mg/L and 35.0 mg/L, respectively) was similar for goats receiving TMRs containing fish oil during the first half of the gestational period and those receiving TMRs containing fish oil during pregnancy while the goats fed with TMRs with protected oil during pregnancy had the lowest serum adiponectin concentration (17.5 mg/L)

Discussion

The use of fat in animal feeding is generally aimed at 2 main purposes. For these purposes, energy levels are increased by using fat sources which have much higher energy density compared to grains. The second purpose is to increase the concentration of long chain unsaturated fatty acids by reducing the medium chain saturated fatty acid concentration in animal products. In recent years, studies have also suggested that rich sources of omega-3 fatty acids have an effect on the release of adipokines with anti-diabetic, anti-atherogenic and anti-inflammatory roles (Wu et al. 2013, Al-Dawood 2017).

In the study, fat source (fish oil or protected fat) affected feed consumption and the highest feed consumption was found to be in the group fed with protected oil first half of the pregnancy and with fish oil in the second half of the pregnancy and in the fish oil group during the pregnancy. The use of oil in the ration causes a general decrease in dry matter consumption (Thanh and Suksombat 2015). The magnitude of this negative effect is related to the types and quantities of fat and forage used in the ration (Allen 2000). It has been suggested that the decline in feed consumption due to the presence of fat in the ration may be results of an increase in ruminating time due to negative effects on rumen digestion (Jenkins 2004) and the effect of cholecystokinin of the intestinal hormones on the brain satiety center (Choi et al. 2000). However, Doreau and Chilliard (1997) and Pirondini et al. (2015) reported that fish oil increased NDF digestibility in the digestive tract. Another important point is that microbial populations can adapt to unsaturated fatty acids if fat is given as frequent meals (Oldick and Firkins 2000). It was evaluated that the study had a positive effect on the fish oil consumption due to the fact that the goats were fed with fat-containing rations for 5 months, the fat level in the diet was 2.8% of the dry matter and the alfalfa hay and wheat hay were preferred as forage sources.

In our study, it was determined that the use of fish oil during pregnancy did not affect ghrelin, leptin and omentin concentrations in serum. Body condition score and feeding management can have an effect on leptin level (Vailati-Riboni et al. 2016). It has been reported that there is a positive correlation between body condition score and leptin concentration in cows and heifers (Reist et al. 2003, Leon et al. 2004). The difference in leptin concentrations between the groups can be attributed to the fact that the live weights are close to each other in the present study.

Adipokine levels of fish oil-fed animals during any period of pregnancy were found to be high in our study. It was also found that serum adiponectin levels in goats fed with diet containing fish oil in the first half of pregnancy and protected fat in the second half were statistically significantly higher. In a human study, omega-3 fatty acid consumption of 0.7 g/day was reported to increase adiponectin level by 0.37 μg/mL (Wu et al. 2013). Similarly, it was also found that fish oil affects the adipokine profile and significantly increases adiponectin concentration compared to the control group in male C57BL / 6 mice (Yan and Lie 2015). Increases in omega-3 fatty acid consumption and adiponectin concentration are associated with peroxisome proliferator-activated receptor-γ or calcium ion channels (Banga et al. 2009, Tishinsky et al. 2011, Sukumar et al. 2012). The results obtained in our study were in parallel with the above-mentioned studies.

Gestation in goats varies between 145 and 155 days on average (Jainudeen and Hafez, 2013). Embryonic development occurred in first 41-49 days of gestation and offspring are very small and their mouth, nose and brain development occurs in this period. Also, eye formation also occurs in the first 75 days of pregnancy (Özer 2010). The importance of omega-3 intake in the first half of pregnancy is highlighted by several studies (Innis et al. 2008). The fetal omega-3 concentration is determined by the maternal diet. In the period from the 75th day of the pregnancy to the end of the pregnancy, the energy needs of the pregnants' are increasing dramatically due to the rapidly accelerated fetal development. Studies have shown that feeding ruminants with protected fat reduces the negative energy balance in pregnancy (Duske et al. 2009). In our study, there was no statistically significant difference between the live weight, serum leptin, ghrelin and omentin concentrations between treatment groups during pregnancy. Serum adiponectin concentrations in groups 3 and 4 fed fish oil between 0 and 75 days of gestation were found to be statistically significantly higher than in the groups 1 and 2 fed with protected fat between 0 and 75 days of gestation. From day 76 until birth time protected fat fed group 3 had the highest serum concentrations of adiponectin. The reason for this is that, omega-3 fatty acids provide a healthy development in the embryonic period and in the early stages of the fetal period, when the brain nervous system and eye development take place, as mentioned above. Omega-3 fatty acids are very important for embryonal development especially in the first part of pregnancy (Innis et al. 2008). Adiponectin is produced in many organs and tissues that complete their development in the stage of fetal development following a healthy embryonic development and this leads to a high concentration of adiponectin (Kiess et al. 2008). Adiponectin level increases as the fetus grows throughout pregnancy and body weight increases (Lindsay et al. 2003, Kotani et al. 2004, Mazaki-Tovi et al. 2005). In our study, we can suggest that increasing adiponectin concentrations in the group 3 and 4 towards the 5th month of gestation may be due to the increased energy demand as a result of growth of the fetal surface and volume. Feeding with protected fat increases progesterone concentration through cholesterol biosynthesis, which positively affects fetal development in the last period of pregnancy (Tyagi et al. 2010, Shelke et al. 2011). In our study, we can suggest that the remarkable decrease in serum adiponectin concentrations in serum samples shown in the last month of gestation and just before birth may be due to the fact that fetal growth has reached its final point.

In conclusion, we can suggest that supplementing the diet with omega-3 fatty acid sources at the beginning of the pregnancy and support dietary supplementation with protected fat, especially considering the development of the fetus and its associated energy requirement after the second half of pregnancy is important for a healthy pregnancy process.

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