IJAS Iranian Journal of Applied Animal Science

Effect of Roasted Soybean and Canola Seeds on Peroxisome Proliferator-Activated Receptors Gamma (*PPARG*) Gene Expression and Cattle Milk Characteristics

Research Article

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Received on: 5 Dec 2018
Revised on: 26 Feb 2019
Accepted on: 2 Mar 2019
Online Published on: Dec 2019

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Online version is available on: www.ijas.ir

ABSTRACT

The aim of this research was to investigate the changes in milk yield and its composition, fatty acid profile and peroxisome proliferator-activated receptors gamma (*PPARG*) gene expression in adipose tissue of Iranian Holstein cattle in response to feeding isonitrogenous and isocaloric diets but formulated based on different oilseeds (soybean or canola seeds). Twenty cows were tested randomly with experimental diets. Sampling from subcutaneous adipose tissue was performed. Moreover, fatty acid composition was analyzed, total RNA was isolated, cDNA synthesized and samples were amplified. SPSS 16.0, LinRegPCR and REST software were used to analyze real-time polymerase chain reaction results of gene expression. The amount of *PPARG* gene expression in the adipose tissue for canola seed treatment, in comparison with the soybean seed was higher. The milk production, 4% fat corrected milk, fat percentage, milk urea nitrogen and body condition score between two treatments was significant ($P<0.05$). The results showed that the amount of some fatty acids extracted from adipose tissue was changed by altering the diet. As, for C16:1, C18:0 and C18:3t fatty acids, there was a significant effect between the two treatments ($P<0.05$). In general, the *PPARG* gene expression was not significant in canola that can be due to the similarity of most of the fatty acids between canola and soybean, and the similarity and nutrient balance of the diet. Therefore, canola seed can be a good option for dairy cattle diet due to high levels of fat and protein and lower prices than soybean seed. It can also be used to improve milk, with the effect of feeding on the fatty acid composition of the milk.

KEY WORDS adipose tissue, gene expression, Iranian Holstein cattle, *PPARG*.

INTRODUCTION

In Iran, cattle population including 7.9 million heads. From these animals, 45.9, 43.6 and 10.51% are indigenous, crossbred and registered (mainly Holstein), respectively. The contribution of livestock to the national economy is 4% of total gross domestic product (GDP). Based on report of Chupin and Schuh (1993), Iranian dairy production has gone through important and remarkable constructional alterations within the last two decades because of producing greater herds. Artificial insemination (AI) is used in many regions of the world, especially in Iran developed considerably over the 1980s (Ebrahimi *et al*. 2015a). Iranian dairy producers generally use four groups of sires; American,

Canadian, European and Iranian sires based on their source. On the other hand, these four groups can be also classified to two groups: summarized or sampling sires. The first group progeny tests and estimation of their daughter's producing ability is possible.

The second group selects to pass on high production characteristics or type-related traits according to their pedigree. Whereas, it is necessary to define more exactly which sires can transfer their high production and type-related traits to their offspring.

Unwillingness of many Iranian dairy producers for applying sampling sires is this fact that their daughters' efficiency is considered somewhat unpredictable. But, some dairy producers prefer to employ Iranian sampling sires on the repeat breeder cows as well as on the moderate to low foreign cows (Ebrahimi *et al*. 2015b). In addition, based on the latest official statistics of the Iranian Ministry of Agriculture, there are 18830 industrial cattle breeding unit (farm) in Iran that breed 2048563 dairy cattle (Kharrati Koopaei *et al*. 2012). There was an increasing tendency on Iranian milk production within 2004-2008 years (Kharrati Koopaei *et al*. 2012).

Regardless of gain in milk production in Iran, capita consumption for milk is under the international standard. This index in the world is 169 kg, in the Europe is 350 kg per person, but in Iran this index is 95 kg per person. Based on existing information, it can be understood that breeding goals must be to increase milk production (Kharrati Koopaei *et al*. 2012).

Moreover, regulation of the gene transcription rate by food components illustrates an interesting site for regulating phenotype (Ghormade *et al*. 2011). A group of the most important regulators of gene expression patterns is essential nutrients and other bioactive food components. Macronutrients, vitamins, minerals, and various phytochemicals can affect biological responses such as metabolism, cell growth and differentiation by means of gene transcription and translation. Transcriptomics determines level of gene expression based on the quantity of existent RNA in the tissue samples (Ghormade *et al*. 2011).

Tachibana *et al*. (2005) showed that soybean protein, in comparison with casein changed the expression of 120 genes involved in lipid metabolism, antioxidant activity and energy metabolism. Endo *et al*. (2002) also reported that various dietary protein sources resulted in a difference in expression of about 281 genes in rat liver, suggesting a nutritional function for protein components. Nutrition of oilseeds is performed to providing energy and protein for dairy cattle. This is particularly important in feeding livestock with high production, such as dairy cows that have a defined feed intake capacity.

Soybean seed is considered as an excellent protein (33-40%) and fat supplement (16-22%) in the diet of lactating cows due to its amino acid balance and fat content. But, the price of this seed is more expensive than others, such as canola seed. Canola seed oil can be a good choice for dairy cow diet due to its high oil content (40%) and a significant level of protein (30%). Also, its beneficial effect on milk fatty acids composition can be used to improve the milk quality (Beauchemin *et al*. 2009).

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor hormone superfamily and play basic and important role in cellular differentiation and lipid accumulation during adipogenesis (Lapsys *et al*. 2000). The PPAR has three isoforms, PPARα, PPARγ, and PPARδ that have shown similar regulation mechanisms (Escher and Wahli, 2000). One of the most important of these three isoforms is PPARγ or peroxisome proliferator-activated receptors gamma (PPARG) that is essential in the metabolism of lipids and carbohydrates in various tissues (Ji *et al*. 2014). The position of PPARγ gene was mapped on chromosomes 22 of bovine (Zimin *et al*. 2009). Sundvold *et al*. (1997) showed that this gene is expressed in spleen, ovary, fat, lung, mammary gland, small intestine, kidney, muscle and heart tissues. PPARG is expressed in adipose tissue more than other tissues in cattle (Huff *et al*. 2004). Several CCAAT/enhancer-binding proteins (CEBP); CEBPA, CEBPB, CEBPC and CEBPD that act as the important adipogenic and lipogenic transcription regulators. They work as parts of a cascade of events, with early induction of CEBPB and CEBPD leading to induction of CEBPA and CEBPA acts as a transactivator of PPARG, and these two transcription regulators work together to increase adipogenesis. *In vitro* PPARG activation studies in the adipose tissue of cattle (Lengi and Corl, 2010) demonstrated that it plays an important role to upregulate gene targets that are responsible for differentiating pre-adipocytes into mature adipocytes in the body. These mature cells are able to act as reservoir of triacylglycerol (TAG). It is obvious that study and understanding basic and essential processes controlling adipogenesis in dairy cattle plays an important role to increase our knowledge about dairy production. Although many studies have been performed on Iranian native and Holstein cattle (Alinaghizadeh *et al*. 2007; Ghasemi *et al*. 2010; Mohammadabadi *et al*. 2011; Pasandideh *et al*. 2015; Barazandeh *et al*. 2016), but correlation between PPARG gene expression and milk production was not investigated, hence the aim of this research was to study the effect of feeding isonitrogenous and isocaloric diets differ in oilseeds source (soybean or canola seeds) on milk yield and its composition and PPARG gene expression in adipose tissue of Iranian Holstein cattle.

MATERIALS AND METHODS

This research was carried out in dairy cattle breeding farm of Shahriar (Tehran province, Iran) in format of completely random design with two treatments and 10 repeats. Twenty cows were examined on the 20th day of lactation with the same gestational age (second pregnancy) and mean body weight of 680 ± 80 kg. Animals were tested randomly for two months after two weeks of habitual habitat with experimental diets consisting of whole processed soybean seed ratio (roasted soybean seed=treatment 1) and whole processed canola seed (roasted canola seed =treatment 2).

Experimental diets were similar in terms of energy, protein, starch, fiber, vitamins and minerals, which were formulated according to livestock needs with a pure protein and carbohydrate system of the Cornel University for dairy cattle using cornell net carbohydrate and protein system (CNCPS) with a production of 42 kg per day and 3.2% fat. Diets were formulated using the CNCPS v6.1 (Tylutki *et al.* 2008; Van Amburgh *et al.* 2010) to supply animal requirements. The components of the diets are listed in Table 1.

The diets were fed *ad libitum* as group feeding and total mixed ration (TMR) at two times in the morning and evening and animals had free access to water. Cows were milked three times a day. Milk yield was recorded at each two weeks in all experimental periods. Milk samples were analyzed for fat, protein, solids and solids without fat by milkoscan (EKOMILK, MilkanaKam 98-2A, Foss Electric, Denmark). Urea nitrogen levels, calculation of corrected milk for fat and changes in fat body stores were done.

Sampling from subcutaneous adipose tissue in tail region was performed to study the effect of soybean and canola seeds on fatty acid profiles and PPARG gene expression changes. For sampling, after washing and disinfecting the skin, using a surgical knife, a small gap was created on the skin surface, and after removing two small samples from the subcutaneous fat tissue, stitching was done. The tissue sample was placed in liquid nitrogen after being washed in a physiologic serum solution and wrapped in an aluminum foil, and was transferred to the laboratory. Moreover, fatty acid composition was analyzed using gas chromatography (GC).

GC analysis was performed using CP-3800 gas chromatograph (Varian, Palo Alto, CA, USA). All Institutional and National Guidelines for the care and use of animals (fisheries) were followed.

Total RNA extraction from tissues performed applying a One Step RNA Reagent Kit (Biobasic Co. Ltd., Iran). Spectrophotometry at 260 nm was performed to determine RNA concentration and the absorbance 260nm:280nm ratio and electrophoresis on 2% agarose gel stained with ethidium bromide were employed to define RNA quality.

RerertAid™ H Minus First Strand cDNA Synthesis Kit (#K1631, Fermentase Co., Iran) was employed to synthesis cDNA from total RNA and an oligo d(T) primer was used according to manufacturer's protocol. For each reaction was consumed 1 µg total RNA.

Primers 5'-TTGACTTCTCCAGCATTTCCAC-3' and 5'-ATACAGGCTCCACTTTGATTGC-3' for PPARG gene (GenBank accession number; NM_181024.2) and 5'-GTTCAACGGCACAGTCAAGG-3' and 5'-5'-GTTGATGTTGGCAGGATCTCG-3' for GAPDH gene (GenBank accession number; NM_001034034.2) used for RT-PCR were prepared by Bioneer Co. (Iran). Power SYBR Green PCR Master Mix (Iran) was employed to amplify samples and optical 96-well skirted microplates was used to carry out reactions in a volume of 15 µL.

Each microtube consisted of 7.5 µL from 2X SYBR Green PCR Master Mix, 1.5 µL template cDNA, 1 µL from 10 µM forward and reverse primers, 0.3 µL ROX and 4.7 µL ddH_2O. PCR protocol was done at 3 steps: step 1; 3 min at 94 ˚C, step 2 had 35 cycles consisted of 3 stages; 60 s at 94 ˚C, 60 s at 57 ˚C for, 60 s at 72 ˚C and step 3 for final extension 5 min at 72 ˚C. For deleting of the contamination of unspecific PCR products such as primer dimers, melting curve analysis was generated to all final PCR products. SPSS 16.0 (SPSS, 2015), LinRegPCR (11.0) and REST (2009) softwares were used to analyze Real-Time PCR results of gene expression.

RESULTS AND DISCUSSION

The extracted total RNA had a good quality and not contaminated, as the 260 nm:280 nm ratio ranged from 1.77 to 1.90. Moreover, all RNA extracted from adipose tissue of the Iranian Holstein dairy cattle used in the present study revealed two 18S and 28S bands (Figure 1). The melting and amplification curves of PPARG and GAPDHPCR products showed sharp single peaks indicating that dimers of primers were not produced and that the primers were specific and amplification product was not generated in the negative control sample. The proper annealing temperature for the specific primers was determined at 57 ˚C.

The amount of PPARG gene expression in the adipose tissue for treatment 2 (whole processed canola seed), in comparison with the treatment 1 (whole processed soybean seed) was higher, but this increase was not significant ($P>0.05$).

As it has shown in the Table 2, the milk production, 4% fat corrected milk, fat percentage, Milk urea nitrogen and Body condition score between two treatments was significant ($P<0.05$), however, no significant effect was observed on the other milk composition and dry matter between two treatments ($P>0.05$).

Table 1 The ingredient components of experimental diets (% of DM basis)

Ingredients	Soybean seed diet	Canola seed diet
Alfalfa hay	17.5	17.5
Corn silage	22.0	22.0
Barley grain, ground	17.5	17.5
Corn grain, ground	15.7	15.7
Soybean meal	8.7	6.0
Roasted soybean seed	9.0	0
Corn gluten meal	1.5	4.6
Roasted canola seed	0	9.0
Fish meal	1.3	1.3
Fat supplement (prilled)	2.0	1.6
Calcium carbonate	0.8	0.8
Di calcium phosphate	0.2	0.2
NaCl	0.6	0.6
Sodium bicarbonate	1.5	1.5
Vitamins and minerals[1]	1.3	1.3
Magnesium oxide	0.4	0.4
Chemical compositions		
Dry matter (DM, %)	50	51
Crude protein (CP, %)	16.5	16.1
Ether extract (EE, %)	5.15	5.78
Ash (%)	8.21	8.1
Neutral detergent fiber (NDF, %)	31.3	29.3
Acid detergent fiber (ADF, %)	18.4	17.5
NE_L (net energy for lactation, Mcal/kg of DM)	1.71	1.72

[1] Provided (per kilogram of DM): Zn: 56 mg; vitamin A: 6440 IU; vitamin D: 2000 IU; vitamin E: 16 IU; Mn: 46 mg; Fe: 22 mg; Cu: 12 mg; I: 0.9 mg; Co: 0.4 mg; Se: 0.3 mg and Monensin: 12 mg.

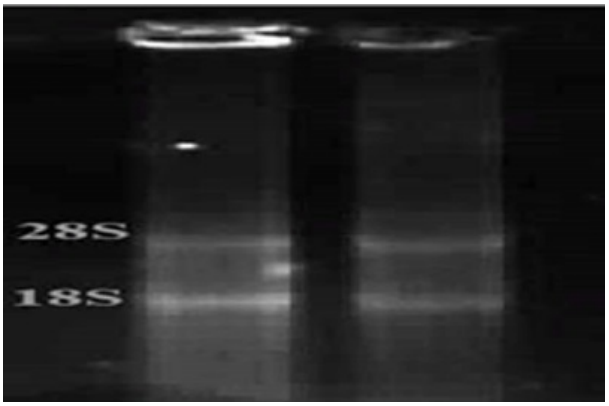

Figure 1 Some samples of the extracted RNA

The results showed that the amount of some fatty acids extracted from adipose tissue was changed by altering the diet (Table 3). As, for C16:1, C18:0 and C18:3t fatty acids, there was a significant effect between the two treatments ($P<0.05$).

The main difference between roasted canola and soybean seed was their protein and fat percentage. Soybean oilseed containing 37.6% protein and 21.7% fat and canola oilseed had 22.9% protein and 37.7% fat. Protein, Rumen-undegradable protein (% of total protein), Metabolisable protein (g/day), fat (ether extract), net energy lactation (NE_L) (mega calories per kilogram of dry matter=Macl/kg of DM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ash for treatment 1 were 16.5, 33, 2750, 5.15, 1.71, 31.3, 18.4 and 8.21, respectively and these values for the treatment 2 were 17, 33, 2750, 5.78, 1.72, 29.3, 17.5 and 8.10, respectively. In term of fatty acids composition, the main difference between soybean seed and canola seed was in the amount of cis oleic acid (C18: 1c) (25 versus 65 mol /100 mol fatty acid) and cis linoleic acid (C18: 2c) (49 vs. 17 mol/100 mol fatty acid). Palmitic and stearic fatty acids were also lower in canola seed (Table 4).

Table 2 Milk production and composition, body condition score of dairy cows fed with roasted canola and soybean seed

Parameter	Soybean seed diet	Canola seed diet	SEM	P-value
Dry matter intake (kg/d)	22.1	22.0	-	-
Initial body condition score	3.17	3.18	0.03	0.100
Final body condition score	2.92	3.01	0.02	0.010
Milk yield (kg/d)	51	53.5	0.61	0.003
4 % fat corrected milk yield (kg/d)	38.4	41.9	0.94	0.005
Milk composition (%)				
Fat (%)	2.36	2.56	0.06	0.010
Protein (%)	3.03	3.04	0.02	0.107
Solid non-fat (%)	9.34	9.35	0.01	0.502
Total solids (%)	11.92	12.05	0.06	0.064
Milk urea nitrogen (mg/dL)	13.25	14.61	0.69	0.060

SEM: standard error of the means.

Table 3 The compositions of fatty acids profiles in adipose tissue of dairy cows fed with roasted soybean and canola seed

Fatty acid (carbon number)	Treatment 1	Treatment 2	SEM	P-value
C4:0	0.14	0.02	0.12	0.50
C6:0	1.55	0.02	1.54	0.50
C8:0	0.21	0.04	0.20	0.50
C10:0	0.55	0.05	0.39	0.29
C12:0	0.10	0.12	0.01	0.35
C14:0	2.66	2.95	0.87	0.67
C14:1	1.39	0.64	0.29	0.83
C15:0	0.38	0.58	0.21	0.38
C15:1	0.22	0.23	0.05	0.71
C16:0	22.01	24.40	3.13	0.39
C16:1	8.11	5.90	0.33	0.01*
C17:0	0.78	0.91	0.16	0.65
C17:1	0.83	0.70	0.67	0.14
C18:0	8.44	14.16	1.77	0.05*
C18:1t	1.09	1.35	1.80	0.63
C18:1c	42.04	40.27	0.82	0.94
C18:2t	0.10	0.88	0.32	0.92
C18:2c	1.45	1.80	0.34	0.27
C18:3t	0.01	0.03	0.0001	0.0001*
C18:3c	0.23	0.31	0.07	0.28
C20:0	0.20	0.25	0.02	0.59
C20:1	0.04	0.03	0.001	0.64
CLA c9t11	0.26	0.27	0.08	0.82
CLA t10c12	0.09	0.08	0.005	0.22
C22:0	0.16	0.11	0.03	0.20
C20:4w6	0.16	0.10	0.07	0.23
C20:4w3	0.17	0.14	0.06	0.53
C20:5 EPA	0.02	0.07	0.01	0.30
C22:1	0.06	0.04	0.03	0.32
C24:0	0.23	0.05	0.21	0.46
C24:1	0.30	0.02	0.30	0.45
C22:5 W3	0.07	0.12	0.02	0.90
C22:6 DHA	0.09	0.05	0.07	0.67
C22:2	0.03	0.03	0.04	0.74

SEM: standard error of the means.

Conjugated fatty acids are one of the most important foods in the health of organisms. Replacement effects of the roasted soybean seed with roasted canola seed in dairy cattle by Esmaeili *et al.* (2016) has shown that the amount of linoleic acid and linolenic acid in the milk fat of cows fed with canola seed decreased but did not affect the level of conjugate fatty acids that was in confirmation with our results.

Huff *et al.* (2004) studied mRNA PPARG expression in adipose and muscle tissues of Holstein and Charolais cattle and did not observe tissue-specific differences in mRNA expression of PPARG between two studied breeds and concluded that expression levels of PPARG are similarly regulated in the two breeds.

Table 4 Fatty acids composition of roasted soybean and canola seeds (mole per 100 mole of fatty acid)

Fatty acid (carbon number)	Roasted canola seed	Roasted soybean seed	Fatty acid (carbon number)	Roasted canola seed	Roasted soybean seed
C12:0	0.03	No data	C18:2c	17.03	48.95
C14:0	0.08	0.12	C18:3t	0.11	0.03
C16:0	4.91	12.63	C18:3c	7.88	6.75
C16:1	0.33	0.17	C20:0	0.72	0.41
C17:0	0.06	0.15	C20:1	1.43	0.27
C17:1	0.07	0.07	C22:0	0.32	0.42
C18:0	2.67	4.76	C22:1	0.59	0.03
C18:1t	0.35	0.01	C22:2	0.01	ND
C18:1c	62.82	24.75	C24:0	0.14	0.14
C18:2t	0.04	0.06	C24:1	ND	0.03

In other investigations (Dimopoulos *et al*. 2007), it has been demonstrated that PPARG has a dominant task in moving metabolism in the direction of applying lipid as a fuel source. Rosen *et al*. (1999) also showed that PPARG gene is one of the most important regulators for gene expression of necessary encoding proteins for adipogenesis. Kelly *et al*. (2011) studied mRNA expression of genes regulating oxidative phosphorylation in the muscle of beef cattle divergently ranked on residual feed intake and showed that PPARG has negative association with feed conversion ratio, residual feed intake and dry matter intake.

McCabe *et al*. (2012) studied RNA-seq analysis of differential gene expression in liver from lactating dairy cows divergent in negative energy balance and reported that PPAR agonists acts as up regulator of angiopoietin-like 4 gene (ANGPTL4) and then inhibits function of lipoprotein lipase in adipose, decreases very low-density lipoproteins triacylglycerides (VLDL-TAG) consumption and increases lipolysis. Bionaz *et al.* (2013) also concluded that PPARγ plays an important role in controlling adipogenesis and lipogenesis in adipose tissue and in controlling fatty acid oxidation in ruminants. This gene also controls at least partially milk fat synthesis in dairy cows (Bionaz and Loor, 2008), because between pregnancy and lactation in dairy cattle its expression is increased. Based on reports of Kadegowda *et al*. (2009) PPARγ plays an essential role to control expression of key genes involved in milk fat synthesis, including sterol regulatory element binding transcription factor 1 (SREBF1). In studying Central Role of the PPARγ Gene Network in Coordinating Beef Cattle Intramuscular Adipogenesis in Response to Weaning Age and Nutrition, Moisa *et al*. (2014) concluded that precocious and sustained activation of the PPARG and its target genes is one factor leading to greater intramuscular fat deposition and consequently more carcasses grading greater than or equal to "High Choice". Ji *et al*. (2014) investigated the expression levels of PPARG, FASN, and ACADM genes in different adipose tissues, longissimus dorsi muscle (LD), heart, liver, kidney, colon and lung in Chinese Yanbian yellow cattle and Yan yellow cattle breeds.

They reported that PPARG and FASN mRNA expression levels were significantly higher in adipose tissues than in LD, which indicated that both genes play key roles in fat deposition.

PPARG gene showed higher expression in abdominal fat and in perirenal fat than in the subcutaneous fat in Yanbian yellow cattle, suggesting that the gene expression variation partly contributed to the differences of fatty acid composition in different adipose tissues.

Hence, in future studies, it is needed to consider expression patterns of other genes that have interaction with PPARG in adipose tissue to achieve more accurate results and conclusions.

Joseph *et al*. (2010) studied lipogenic gene expression in bovine subcutaneous adipose tissue using 3 treatments consisted of 3 corn oil (57% linoleic acid, 28% oleic acid; 11% palmitic acid) supplementation levels: 0 (NONE), 0.31 kg/d (MED) and 0.62 kg/d (HI) and showed that the MED level of oil supplementation (4.94% total fatty acids in diet) up-regulates gene expression of key lipogenic enzymes, but that as oil supplementation reaches HI level (7.99% total fatty acids in diet) genes encoding lipogenic enzymes responsible for de novo fatty acid synthesis and monounsaturated fatty acid (MUFA) synthesis are down-regulated. Their results were agreeing with changes in tissue fatty acid composition in which palmitic (C16:0) acid concentration, a product of de novo fatty acid synthesis, and CLA cis-9 trans-11 isomer, a product of desaturation, were reduced with the highest level of oil supplementation.

CONCLUSION

In general, the PPARG gene expression was not significant in canola that can be due to the similarity of most of the fatty acids between canola and soybean oil, and the similarity and nutrient balance of the diet. Therefore, canola oil can be a good option for dairy cattle diet due to high levels of oil and protein and lower prices than soybean oil. It can also be used to improve milk, with the effect of feeding on the fatty acid composition of the milk.

ACKNOWLEDGEMENT

Authors would like to thank Vice Chancellor for research and technology, Shahid Bahonar University of Kerman for their support and assistance for this project.

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