International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print) 2222-5234 (Online)
Vol. 2, No. 10(2), p. 11-18, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

# Effect of extruded and roasted soybeans on lactation performance and milk fatty acid profile of early lactating dairy cows

**Amanlou H[1], Maheri-Sis N[2], Bassiri Sh[2*], Mirza-Aghazadeh A[3], Salamatdust R[2], Ghorbani A[2]**

[1]*Department of Animal Science., Faculty of Agriculture., Zanjan University, Zanjan, Iran, P.O.Box 45195-313, Zanjan, Iran*

[2]*Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, East Azerbaijan, Iran*

[3]*Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran*

**Received:** 14 September 2012
**Revised:** 04 October 2012
**Accepted:** 05 October 2012

**Key words:** Full fat soybean, milk fatty acid, dairy cow.

## Abstract

Thirty multiparous Holstein cows (29.80 ± 4.01day in milk; 671.6 ± 31.47 kg of body weight) randomly designed to one of the control (no fat) or 4 diets with either raw soybean, extruded soybeans, roasted soybeans, or tallow, added to provide 1.93% supplemental fat for 8 weeks. Cows fed fat diets had significantly ($P$<0.01) high NEL intakes and FCM production ($P$<0.01) when compared to control with no fat. Milk fat yield and percentage of cows fed fat-supplemented diets were significantly ($P$<0.01 and $P$<0.02 respectively) higher than control. There was no significant effect of supplemental fat on the milk protein and lactose content and yield. Total saturated fatty acids percentage in milk fat was significantly ($P$ = 0.01) decreased by fat supplementation. Full fat soybeans significantly ($P$<0.01) decreased concentration of short- to medium- chain (C6:0 to C16:0) saturated fatty acids compared with control, with an outstanding value for extruded soybean. A tendency for lower C6:0 and C16:0 and higher C18:0 contents in the milk of cows fed the supplemental fat was detected ($P$ = 0.14, $P$ = 0.16 and $P$ = 0.11 respectively). Mono unsaturated fatty acid, total C18:1 and trans-11 C18:1 proportion was significantly ($P$<0.01) increased by supplemental fat. The concentration of milk fat cis-9 trans-11 C18:2 were also increased by all fat sources, but it was only significant for extruded soybeans. There were no significant differences between extruded and roasted soybeans oil in lactation performance and energy balance of dairy cows, as well as milk fatty acid profile however, cows fed roasted soybean had numerically greater FCM production and fat content and yield than cows fed extruded soybeans, and milk fat of cows fed extruded had numerically higher cis-9 trans-11 C18:2 content when compared milk fat of cows fed roasted soybeans (respectively $P$=0.19, $P$=0.054).

*Corresponding Author: Bassiri Sh ✉ sh_Bassiri@yahoo.com

## Introduction

Soybeans are excellent source of energy and protein for dairy cows and depending on how they have been processed can provide degradable, undegradable and soluble protein, fat and fiber (Ishler and Varga, 2000)). Full-fat soybeans have been investigated mainly as a source of protein; but recently a little attention has been given to the fat portion of seeds (Chouinard et al., 1997a, b). Inclusion of fat increases the energy content of the diet and enhances the absorption of fat-soluble nutrients in support of high milk production without the negative effects associated with increased dietary starch (Miller et al., 2009). Moreover, feeding lipid sources rich in polyunsaturated fatty acids as oilseeds to dairy cows decrease the saturated fatty acids content of milk fat. The ratio of saturated fatty acids to polyunsaturated fatty acids in dairy fat has generally been regarded as undesirable from a human nutrition perspective because of a probable link between dietary medium-chain saturated fatty acids (C12:0, C14:0, and C16:0), serum cholesterol, and heart disease (Gómez et al., 2009). Including oilseed to dairy cows diets also leads to an increase in milk *cis*- 9, *trans*-11-octadecadienoic acid content, which is attributed to several health benefits in human as *anti*-carcinogenic, *anti*-atherogeni and *anti*-diabetagenic.

Soybeans may be successfully fed to dairy cows whole, rolled or ground and raw or heated (Reddy et al., 1994). Extrusion by rupturing the seeds and liberation oil from the soybeans cells influenced the metabolism of fatty acids in the rumen and the fatty acid profile of milk fat (Chouinard et al., 1997b). There are some investigations examining the effect of heated soybeans on performance and milk fatty acid of dairy cows (Chouinard et al, 1997a, Chouinard et al, 1997b, Faldet and Satter, 1991, Scott et al., 1991), however, all of them have been primarily designed to examine the effect of heating process on digestibility or protein degradability of seeds; consequently, equality in degradability of protein was ignored, thus the net effect of fat wasn't observed. In current experiment soybeans has been evaluated merely as oil source, the objective of study was to determine the effect of adding 1.93% fat originated from extruded or roasted soybeans on performance and milk fatty acids of dairy cows, in compare with raw soybeans or tallow (as a reference fat). In contrast to previous experiments, we tried to provide the same levels of rumen degradable protein in Raw and heated soybean treatments, prohibition the confounding effect of differences in RUP.

## Materials and methods

Experimental cows, housing management, diets, laboratory analyses, milking and experimental design for performance have been given in bassiri et al. (2012) paper. Sampling of milk for fatty acid determination was conducted on 27 June. Samples were stored in ice and immediately were sent to Chemistry Faculty of Tabriz University for fatty acid analysis. Milk fat was extracted by the method of Bligh and Dyer (1959). The derivation of fatty acids was done by the method of Shanta and Decker (1993). Milk fatty acid was analyzed in a gas chromatograph. The fatty acid composition of milk fat is expressed as amount of each individual fatty acid per total fatty acid present.

### *Extraction of lipids*

Milk fat was extracted using a modification of the Bligh and Dyer method (1959). In brief, a sample of milk (10 g) was mixed (vortexed for 2 min) with methanol (10 ml) and dichloromethane (5 ml) in a polyethylene centrifuge tube (50 ml). Dichloromethane (5 ml) and NaCl (0.1 g) was then added and mixed (vortexed for 30 s). The mixture was centrifuged (1780 × *g*, 20 min, 0°C) to partition into two distinct solvent layers separated by a white gelatinous layer. The bottom layer (dichloromethane) was collected. A dichloromethane wash (5 ml) was added to the gelatinous layer, mixed (vortexed for 1 min) and centrifuged (1780 × *g*, 10 min, 0°C). Dichloromethane wash was combined with the original extract, and then the lipid samples were stored in amber-coloured glass vials with screw-top lids fitted with PTFE/silicone inserts at −20° until analysed.

**Table 1.** Ingredient and nutrient composition of experimental diets.

| Item, % of DM | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Ingredient | | | | | |
| Alfalfa hay | 27.38 | 27.38 | 27.37 | 27.42 | 27.45 |
| Corn silage | 22.56 | 22.56 | 22.55 | 22.01 | 22.04 |
| Corn, ground | 24.29 | 22.36 | 22.36 | 23.56 | 23.58 |
| Tallow | - | 1.93 | - | - | - |
| Corn gluten feed | 6.94 | 6.94 | 6.94 | 8.11 | 8.10 |
| Soybean meal | 8.10 | 8.10 | - | - | - |
| Raw soybean | - | - | 10.02 | - | - |
| Extruded soybean | - | - | - | 10.03 | - |
| Roasted soybean | - | - | - | - | 10.03 |
| Fishmeal | 4.78 | 4.78 | 4.96 | 1.85 | 1.74 |
| Meat meal | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 |
| Calcium PhosDi | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 |
| Molasses | 3.86 | 3.86 | 3.86 | 2.70 | 2.70 |
| Fish oil | - | - | - | 0.186 | 0.186 |
| Urea | 0.15 | 0.15 | - | 0.62 | 0.64 |
| Mineral-vitamin mix | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| Wheat straw | - | - | - | 1.55 | 1.55 |
| Nutrient | | | | | |
| DM, % | 55.4 | 56.33 | 56.27 | 57.04 | 57.00 |
| CP, % of DM | 18.6 | 18.5 | 18.5 | 18.5 | 18.5 |
| RDP*, % of CP | 58.9 | 59 | 58.7 | 58.4 | 58.4 |
| RUP*,% of CP | 41.1 | 41 | 41.3 | 41.6 | 41.6 |
| NDF,% of DM | 28.9 | 28.8 | 28.9 | 29.6 | 29.6 |
| NFC,[1]% of DM | 44.3 | 42.8 | 42.8 | 43.2 | 43.3 |
| Ether extract,% of DM | 3.5 | 5.4 | 5.1 | 5.3 | 5.3 |
| Calcium,% of DM | 0.9 | 0.96 | 0.9 | 0.73 | 0.72 |
| Phosphorus.% of DM | 0.61 | 0.55 | 0.63 | 0.54 | 0.52 |
| $NE_L$*, Mcal/kg DM | 1.69 | 1.75 | 1.74 | 1.72 | 1.72 |

1, 2, 3, 4 and 5 are respectively: control and diet containing 1.93% tallow, 10% raw soybeans, 10% extruded soybeans and 10% roasted soybeans. *Calculated using the CPM-DairyV3computer program from composition of feedstuffs. [1]NFC = 100 – (NDF + CP + ether extract + ash)

*Derivation of fatty acids*

The fatty acids were derived by Shanta and Decker (1993) method. Frozen samples were melted at 40°C water bath and were dried using nitrogen injection, and then 2 ml of hexane were added to dried samples. Next, 100 µL of samples were transferred to new tubes and 25 µL of internal standard (heneicosanoic acid) was added to them. This was followed by an addition of 400 µL of methanol and 100 µL methyl guanidine. Samples were dried using nitrogen and boiled for 10 min, after which the tubes were recapped. After cooling the mixture to room temperature, 5 cc of saturated saline were added to the tubes, and then 2 cc petroleum ether were added to the mixture and dried using nitrogen. In the next stage, the mixture was shaken for 3 min and was centrifuged for 5 min to partition into two distinct layers. Afterward, the bottom layer was omitted and the top layer was dried by nitrogen. Finally, 5cc hexane was added to the mixture and samples were dried again by nitrogen and transferred to freezer.

*Experimental design*

Fatty acid data were analyzed using a general linear model (SAS Institute Inc., Cary, NC), according to:

$$y_{ij} = \mu + \beta_j + \alpha_i + \varepsilon_{ij}$$

Where, $y_{ij}$ is the jth observation in ith group; $\beta_j$ is the fixed effect of treatment; $\alpha_i$ the random effect of cow and $\varepsilon_{ij}$ is the residual error. Tukey's test was used to test treatment means ($P < 0.05$) of the experiment (Table 2).

**Table 2.** Least squares means and significance of differences in dry matter intake; milk production and composition from cows fed diets containing 4 fat sources and control diet.

| | traatment | | | | | | *P*-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Item** | **1** | **2** | **3** | **4** | **5** | **SEM** | **metreatnt** | **fat vs control** | **week** | **treatment*week** |
| DMI, kg/d | 24.27 | 23.91 | 24.29 | 24.41 | 24.91 | 0.25 | 0.1916 | 0.7148 | <.0001 | 0.5434 |
| Milk yield, Kg/d | 43.66 | 45.55 | 45.55 | 45.77 | 46.11 | 0.57 | 0.0850 | 0.0087 | 0.0005 | 0.0337 |
| 3.5% FCM[1] | 41.46b | 43.57a | 43.87a | 43.28a | 44.25a | 0.48 | 0.0208 | 0.0022 | 0.0006 | 0.0442 |
| Milk fat, % | 3.10 | 3.23 | 3.30 | 3.17 | 3.31 | 0.05 | 0.1089 | 0.0304 | 0.1429 | 0.3316 |
| Milk fat, Kg/d | 1.35b | 1.46a | 1.49a | 1.45a | 1.51a | 0.022 | 0.0039 | 0.0004 | 0.0038 | 0.5276 |
| Milk protein, % | 3.04 | 3.03 | 3.06 | 2.86 | 2.83 | 0.08 | 0.3364 | 0.3584 | 0.4013 | 0.5576 |
| Milk protein, Kg/d | 1.32 | 1.38 | 1.39 | 1.31 | 1.30 | 0.04 | 0.5728 | 0.6889 | 0.0691 | 0.0612 |
| Milk lactose, % | 5.07 | 4.73 | 5.05 | 4.88 | 4.70 | 0.13 | 0.2324 | 0.1485 | 0.0074 | 0.0010 |
| Milk lactose, Kg/d | 2.20 | 2.14 | 2.30 | 2.23 | 2.17 | 0.004 | 0.3150 | 0.3824 | 0.0894 | 0.0021 |

1, 2, 3, 4 and 5 are respectively: control and diet containing 1.93% tallow, 10% raw soybeans, 10% extruded soybeans and 10% roasted soybeans. 13.5% FCM = 0.432 (kilograms of milk) + 16.2 (kilograms of fat).

**Table 3.** Fatty acid profile (g/100 g of total fatty acid) in milk fat from cows fed diets containing 4 fat sources and control diet.

| | treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Fatty acid | 1 | 2 | 3 | 4 | 5 | SEM | P-value | fat vs control | Soy vs Tallow | Ex vs Ro |
| C4:0 | 3.1 | 3.16 | 3.09 | 2.99 | 2.97 | 0.12 | 0.8000 | 0.7116 | 0.2994 | 0.9205 |
| C6:0 | 2.50 | 2.30 | 2.29 | 2.18 | 2.32 | 0.08 | 0.1819 | 0.0289 | 0.7440 | 0.2872 |
| C8:0 | 1.51$^{a}$ | 1.29$^{ab}$ | 1.23$^{ab}$ | 1.18$^{b}$ | 1.32$^{ab}$ | 0.07 | 0.0482 | 0.0057 | 0.6037 | 0.2044 |
| C10:0 | 3.59$^{a}$ | 3.11$^{b}$ | 3.23$^{a}$ | 3.10$^{b}$ | 3.30$^{a}$ | 0.09 | 0.004 | 0.0004 | 0.3209 | 0.1281 |
| C12:0 | 4.12$^{a}$ | 3.24$^{b}$ | 3.29$^{b}$ | 3.18$^{b}$ | 3.41$^{b}$ | 0.15 | 0.002 | 0.0001 | 0.7748 | 0.3218 |
| C14:0 | 11.00$^{a}$ | 10.44$^{ab}$ | 10.19$^{b}$ | 10.21$^{b}$ | 10.52$^{ab}$ | 0.16 | 0.0105 | 0.0012 | 0.4840 | 0.1887 |
| C16:0 | 30.08 | 29.81 | 28.74 | 28.23 | 28.77 | 0.57 | 0.1523 | 0.0759 | 0.0777 | 0.5152 |
| C18:0 | 10.52 | 11.52 | 12.34 | 12.05 | 11.99 | 0.62 | 0.2943 | 0.0472 | 0.4132 | 0.9449 |
| C14:1 | 1.06 | 0.92 | 0.95 | 0.89 | 0.92 | 0.05 | 0.2209 | 0.0248 | 1.0000 | 0.7896 |
| C16:1 | 1.88 | 1.91 | 1.75 | 1.77 | 1.79 | 0.05 | 0.2350 | 0.2489 | 0.0526 | 0.6356 |
| C18:1 | 21.38$^{c}$ | 23.22$^{ab}$ | 22.40$^{bc}$ | 24.03$^{a}$ | 22.69$^{b}$ | 0.27 | 0.0001 | 0.0001 | 0.5635 | 0.0020 |
| C18:1 trans-11 | 1.38$^{b}$ | 1.86$^{a}$ | 1.99$^{a}$ | 2.26$^{a}$ | 2.02$^{a}$ | 0.11 | 0.0002 | 0.0001 | 0.0856 | 0.1445 |
| cis-9, trans-11 C18:2 | 0.52$^{b}$ | 0.72$^{ab}$ | 0.77$^{ab}$ | 0.98$^{a}$ | 0.82$^{a}$ | 0.06 | 0.0003 | 0.0001 | 0.0722 | 0.0547 |
| SFA* | 66.54$^{a}$ | 64.89$^{ab}$ | 64.44$^{ab}$ | 63.15$^{b}$ | 64.77$^{ab}$ | 0.67 | 0.0274 | 0.0064 | 0.3298 | 0.0994 |
| MUFA** | 24.23 | 26.09 | 25.12 | 26.50 | 25.41 | 0.64 | 0.1439 | 0.0417 | 0.5845 | 0.2441 |
| Short-medium *** | 55.68$^{a}$ | 53.41$^{ab}$ | 52.12$^{b}$ | 51.05$^{b}$ | 52.66$^{b}$ | 0.65 | 0.0006 | 0.0001 | 0.0631 | 0.0918 |

*Saturated fatty acids **Mono unsaturated fatty acids: $C_{14:1}$, $C_{16:1}$, $C_{18:1}$ *** $C_{4:0}$–$C_{16:0}$

**Results**

Dry matter intake, milk yield and composition, and FCM were shown in Table 2. Dry matter intake was similar among diets, but milk production and FCM was greatest ($P < 0.01$) for cows consuming fat supplemented diets. Milk fat yield and content from cows fed the fat supplements was higher ($P < 0.01$ and $P < 0.05$ respectively) than that of cows fed the control (Table 2), but milk protein yield and lactose contents were not affected by fat supplementation.

Milk fatty acid composition (g/100g fatty acid) from cows fed different diets is presented in Table 2. As shown, the concentration of total saturated fatty acid in milk fat was significantly ($P$=0.01) decreased by extruded soybeans. Full fat soybeans significantly ($P$<0.01) decreased proportion of short- to medium- chain (C6:0 to C16:0) saturated fatty acids in milk fat. A tendency for higher C18:0 and lower C14:1 in milk fat of cows fed supplemental fat observed ($P$=0.19 and $P$=0.11 respectively). The concentration of milk fat of vaccenic acid (trans-11 C18:1) and conjugated linoleic acid (cis-9, trans-11C18:2) were increased by all fat sources, but only extruded soybeans caused significant increase in milk fat content of conjugated linoleic acid(cis-9, trans-11C18:2).

**Discussion**

The lack of negative effect of feeding fat sources on DMI has been previously reported in previous research (Rong Yan et al., 2010, Miller et al., 2009, AbuGhazaleh et al., 2004, Grummer et al., 1993, Bernard et al., 1990) although a decrease in DMI was reported with others (Chilliard et al., 2009, Mercedes et al., 1997). Tree factors may explain this lack of effect in current experiment: 1) low level of fat was used in experiment (Petit et al., 2010), 2) encapsulation of anti microbial fatty acids within hard outer soybeans coat (Jenkins and Lundy, 2001), 3) the negative effect of lipid supplementation on dry matter intake would be more important in mid and late stage of lactation than first stage (Petit *et al.*, 2010).

In certain cases, added fat may elevate milk yield and milk fat, but different types and sources of fat may have divergent effects on these parameters (Chouinard et al., 1997a). The improved milk production and FCM by cows fed fat supplement diets in current study may be resulted from a numerical increase in DMI and consequently NEl intakes. Moreover, the direct incorporation of fatty acids into milk cause a decrease in de novo synthesis of fatty acids and a greater proportion of available glucose being used for lactose synthesis (Hammon et al., 2008). Milk fat depression has been observed when cows have been fed high-concentrate diets, fat-supplemented diets, or combinations of both (Miller et al., 2009). Increased milk fat content for cows fed fat supplemented diets may be due to increased dietary fatty acids being taken up by the mammary gland for milk fat synthesis (Knapp et al., 1991).

Previous researchers (Choinard et al., 2001, Kim et al., 1993) pointed decrease in proportion of short- and medium- chain saturated fatty acid of milk from cows fed dietary fat supplements. Fatty acids from C6:0 to C14:0 are synthesized de novo in the mammary gland, whereas C16:0 is derived from both diet and de novo synthesis (Gómez et al., 2009). The decrease in short- and medium-chain fatty acids can be attributed to the ruminal production of trans isomer inhibitors of de novo synthesis (trans-10 C18:1 and trans-10, *cis*-12 CLA). Linoleic acid (C18:2 *cis*-9, *cis*-12) and oleic acid(C18:1 *cis*-9), the major fatty acid present in soybeans and tallow respectively, both are precursors for synthesis mentioned isomers (AbuGhazaleh et al., 2004). Decrease in milk fat of C16:0 were minimal for tallow diets, as substantial amounts of C16:0 fatty acids are provided by tallow. The C4:0 concentration of milk fat was not affected substantially by supplemental fat, this may originate in C4:0 synthesis from beta hydroxyl butyrate, which is less susceptible to inhibition by long-chain fatty acid (Glasser et al., 2008).

Decrease in concentration of 14:1 and 16:1 by fat supplements were previously reported (Glasser et al., 2008). Glasser et al. (2008) stated trans-16:1 percentage was not significantly affected, possibly because of its ruminal origin, whereas cis-16:1 and cis-14:1 probably arising from mammary Δ9-desaturation of 16:0 and14:0 respectively, was inhibited, by most of the fat supplements.

The concentration of 18:0 and all of C18 fatty acids was increased by fat supplementation in experiments (Glasser et al., 2008, Dhiman et al., 1999, Chouinard et al., 1997a). Increase in milk fat of 18:0 was lower for extruded soybean than raw soybean in current experiment. This was unexpected, as the extrusion by rupturing the seeds makes more oil available for microbial hydrogenation (Chouinard et al., 1997a), which would causes more 18:0 production. Chouinard et al.(1997b) proposed free oil at the level of each extruded soybean particle might inhibit the activity of microorganisms that were responsible for the last step of biohydrogenation. The trans intermediates, thus accumulated, left the rumen without further modification, which could explain the lower proportion of 18:0 and the higher proportion of vaccenic acid found in milk of cows fed extruded soybean (Chouinard et al., 1997b).

Oleic, linoleic, and linolenic acids are all precursors for synthesis of vaccinic acid and conjugated linoleic acid during the ruminal biohydrogenation process (AbuGhazaleh et al., 2004). Soybean contain approximately 53% linoleic acid and tallow contain 43% oleic acid, which may explain the increase in conjugated linoleic acid and vaccinic acid in milk fat from cows fed full fat and tallow diets. Milk fat of cows fed extruded soybeans had higher concentration of conjugated linoleic acid and vaccinic acid than raw, possibly because of low release of oil from raw seeds in the rumen compared with heat-treated seeds, which may lead to complete biohydrogenation of linoleic acid to stearic acid in the rumen (*Khanal* and Olson, 2004).

## Conclusion

In conclusion, the addition of 1.93% oil from extruded or roasted soybeans added to the to diet of early lactation dairy cows, beside supporting equal or higher milk and milk composition, compared to raw soybean or tallow and help to minimizing the dietary level of imported bypass protein sources such as fish meal increased milk fat functional and healthy properties when compared the tallow and raw soybeans. Decrease in short- to medium- chain (C6:0 to C16:0) fatty acids was significant both for raw and extruded soybeans, but decrease in total saturated fatty acids was only significant for extruded soybeans. All fat sources significantly increased milk fat content of vaccenic acid compared with control. Proportions of conjugated linoleic acid in milk fat of cows fed extruded soybeans were significantly higher than control. The decrease of baneful fatty acids proportion and increasing functional fatty acids by the result of fat supplementation, specially extruded soybean, in current experiment has high value in standpoint of health concerns.

## Acknowledgements

The current paper is a part of Ph.D. thesis in animal nutrition, Islamic Azad University, Shabestar Branch, Iran, Under supervision of Dr. H. Amanlou and Dr. N.Maheri-Sis. We gratefully acknowledge the support by the Tehran Daneh Cooperation (Tehran, Iran) for providing us with required roasted soybean and Azar Negin Coorporation, specially, Mr. Gasemi the head of Coorporation and Dr. Musavi and Eg. Karimi for their friendly contribution to the study.

## References

**AbuGhazaleh A A, Schingoethe D J, Hippen A R, and Kalscheur K F.** Conjugated Linoleic Acid Increases in Milk When Cows Fed Fish Meal and Extruded Soybeans for an Extended Period of Time. 2004. Dairy Sciences **87**, 1758–1766.

**AOAC**. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

**Bassiri Sh, Taghizadeh A, Angadji L, Dusti-Fard M and Alizadeh-Tofigi A**. The comparison of lactation performance and milk fatty acid composition of Sarabi indigenous and Holstein cows. Cell and Animal Biology **6(12)**, 182-187.

**Bligh EG and Dyer WJ .1959. A rapid method of total lipid extraction and purification**. Canadian Journalo f Biochemistrya nd Physiology 37, 911- 917.

**Bobe G, Zimmerman S, Hammond EG, Freeman AE, Porter PA, Luhman CM and Beitz DC**. 2007. Butter Composition and Texture from Cows with Different Milk Fatty Acid Compositions Fed Fish Oil or Roasted Soybeans. Dairy Sciences. **90**, 2596–2603.

**Chilliard Y, Martin C, Rouel J and Doreau M**. 2009. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. Dairy Sciences **92**, 5199–5211.

**Chouinard PY, Corneau L, Butler WR, Chilliard Y, Drackley JK and Bauman DE**. 2001. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. Dairy Sciences **84**, 680–690.

**Chouinard PY, Levesque J, Girard V, and Brisson GJ**. 1997b. Dietary soybeans extruded at different temperatures: milk composition and in situ fatty acid reactions. Dairy Sciences **80**, 2913–2924.

**Chouinard PY, Girard V and Brisson G J**. 1997a. Performance and profiles of milk fatty acids of cows fed full fat, heat-treated soybeans using various processing methods. Dairy Sciences **80**, 334–342.

**Dhiman TR, Helmink ED, Mcmahon DJ, Fife RL and Pariza MW**. 1999. Conjugated Linoleic Acid Content of Milk and Cheese from Cows Fed Extruded Oilseeds. Dairy Sciences **82**, 412–419.

**Faldet MA, and Satter LD**. 1991. Feeding heat-treated full fat soybeans to cows in early lactation. Dairy Sciences **74**, 3047.

**Glasser FA, and Chilliard Y**. 2008.Oilseed Lipid Supplements and Fatty Acid Composition of Cow Milk: A Meta-Analysis. Dairy Sciences **91**, 4687–4703.

**Gómez-Cortés P, Bach A, Luna P, Juárez M, and de la Fuente M**. 2009. Effects of extruded linseed supplementation on n-3 fatty acids and conjugated linoleic acid in milk and cheee from ewes. Dairy Sciences **92**, 4122–4134.

**Ishler V and Varga G**. 2000. Soybeans and soybean byproducts for dairy cattle. Department of Dairy and Animal Science The Pennsylvania State University.www.das.psu.edu/teamdairy.

***Khanal* RC and Olson KC**. *2004. Factors Affecting Conjugated* Linoleic Acid (CLA) Content in Milk, Meat, and Egg: A Review . Pakistan Journal of Nutrition **3(2)**, 82-98.

**Kim YK, Schingoethe DJ, Casper DP and Ludens FC**. 1993. Supplemental Dietary Fat from Extruded Soybeans and Calcium Soaps of Fatty Acids for Lactating Dairy Cows. Dairy Sciences. **76**,197-204.

**Mercedes VA, Bertics J, and Grummer R**. 1997. The effect of dietary energy source during mid to late lactation on liver triglyceride and lactation performance of dairy cows. Dairy Sciences **80**, 2504–2512.

**Miller WF, Shirley JE, Titgemeyer and EC, and Brouk MJ**. 2009. Comparison of full-fat corn germ, whole cottonseed, and tallow as fat sources for lactating dairy cattle. Dairy Sciences **92**, 3386–3391.

**Reddy PV, Morrill JL, and Nagaraja TG**. 1994. Release of free fatty acids from raw or processed soybeans and subsequent effects on fiber digestibilities. Dairy Sciences **77**, 3410–3416.

**Scott TA, Combs DK, and Grummer RR**. 1991. Effects of roasting, extrusion, and particle size on the feeding value of soybeans for dairy cows. Dairy Sciences. **74**, 2555-2563.

**Shanta NC, Deker EA** .1993. Conjugated linoleic acid concentaration in processed cheese containing hydrogen donors, iron and dair-based additives.Food Chemistry **49**, 257-261.