



# Influence of Chia on Total Fat, Cholesterol, and Fatty Acid Profile of Holstein Cow's Milk

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## Introduction

Coronary heart disease (CHD) is the leading cause of death in the world. In recent years clinical and epidemiological studies have indicated that dietary T-3 fatty acids may reduce the risk of coronary heart disease. Western diets, however, are typically low in T-3 fatty acids, and high in saturated and T-6 fatty acids (Wright et al., 1998).

Although T-6 and T-3 fatty acids have been known to be necessary for normal growth and dermal function since 1930, T-3 fatty acids have not received much attention until recently (Holman, 1998). Following Bang et al.'s (1976.) report of low mortality from CHD among Greenland Eskimos, which was attributed to antiatherosclerotic effects of a diet rich in fish oil, many studies have documented the effects of T-3 fatty acids on the biochemical and physiologic factors believed to influence the risk of CHD (Leaf and Kang, 1998).

There is considerable interest in providing natural sources of T-3 fatty acids in the human diet. Today marine products are the main food source of T-3 fatty acids; however availability, consumer preference, and other factors limit fish and marine product consumption (Marshall et al., 1994). Many attempts have been made to produce foods high in T-3 fatty acids, without imparting an unpleasant "fishy flavor" to them. Enriched T-3 eggs is one success (Ayerza and Coates, 1999).

Chia (*Salvia hispanica L.*) is an emerging oil seed crop that is rich in T-3, "-linolenic fatty acid (Ayerza, 1995). The seed is now being used to produce T-3 enriched eggs in Argentina. These eggs lack the off-flavors found in eggs produced by hens fed either flaxseed (*Linum usitatissimum L.*) or marine products (Ayerza and Coates, 1999).

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Milk enriched with higher levels of T-3 fatty acid is a product that because of its wide consumption, could bring important health benefits to people of all social classes. Changing milk composition by changing a lactating cows' diet would seem to be a logical process, following the example of producing T-3 enriched eggs. However because lipid metabolism in hens is very different from cows, dietary T-3 incorporation efficiencies cannot be directly compared between them. For example, even if dairy cattle consume relatively large amounts of polyunsaturated fatty acids (PUFAs) through eating green pastures, the amounts passed on to the milk is low. The reason is that PUFA's are hydrogenated by microorganisms in the rumen (Jensen, 1992). Techniques to reduce this degradation process, and hence increase T-3 deposition, have been developed (Ashes et al., 1992; Scott et al., 1971).

The US dairy industry, as is the case in other western countries, has been affected by the declining per capita consumption of milk and milk products in recent years. In the US, per capita consumption of fluid milk and cream has dropped from 241 pounds in 1985, to 224 pounds in 1996 (United States Department of Agriculture, 1996; 1998). Enriched milk, containing higher levels of T-3 fatty acids, would help to change the negative perception consumers have of milk, attributed to its less than ideal fatty acid composition.

The objective of the study reported herein was to determine the effects that adding whole chia seed to the diet of lactating cows would bring about in terms of milk yield, cholesterol content, total fat content, and fatty acid composition.

## Materials and methods

### Cows and Diets

The trial was undertaken at a commercial dairy located at Salazar, Province of Buenos Aires, Argentina. Eight multiparous lactating Holstein cows (average production of 18.9 liters/head/day, ranging from 13.8 to 25 liters/head/day) were selected for the trial. The cows were pastured for 18 hours/day in orchard grass (*Dactylis glomerata L.*) and alfalfa (*Medicago sativa L.*), both in the active growth stage, and 2 hours/day in a corn field which was in the milk stage.

The cows were monitored for 94 days, during which time they were fed a supplement. At day 64 of the trial, the cows were randomly assigned to one of two treatments, these being defined by the supplements each received. The supplements, their ingredients and their composition are shown in Table 1. The cows were milked twice daily, at 5:00 AM and at 5:00 PM, with 3 kg of the supplements fed at each milking. Milk weights were recorded four times (day 0, 4, 14 and 30) during the experimental period, as well as 64 and 60 days before the start of the experimental period. Milk samples were collected and combined from four consecutive milkings on day 29 and 30 of the experimental feeding period. Sub-samples were used for the laboratory analysis.

### **Laboratory Analyses**

Each milk sub-sample was analyzed for total fat content, cholesterol content and fatty acid composition. Total fat content was determined gravimetrically, according to the method of the International Dairy Federation (1992). Total lipids were converted to fatty acid methyl esters using the AOAC method (Association of Official Analytical Chemists, 1990a), Fatty acid methyl esters were separated and quantified according to the method of the AOAC (Association of Official Analytical Chemists, 1990b), using an automated gas chromatograph (Model 6890 GC, Hewlett Packard Co, Wilmington, DE<sup>1</sup>) equipped with flame ionization detectors and a 30 m x 530  $\mu$ m i.d. capillary column (Model HP-FFAP, Hewlett Packard Co, Wilmington, DE). HPChem Station (Hewlett Packard Co, Wilmington, DE) was used to integrate peak areas.

Cholesterol was extracted according to the method of the International Dairy Federation (Holman, 1998). The same equipment and procedures that were used for the fatty acids were used to quantify cholesterol levels, except for the use of a different column (Model HP-1 Methyl Siloxane, made by Hewlett Packard, Wilmington, DE).

### **Statistical Analysis**

The feeding trial was set up as a randomized block design, with the experimental unit being one cow. Each variable was compared using the Generalized Linear Model analysis of variance technique to assess treatment differences. When the F-value was significant ( $P < 0.05$ ) differences in means were analyzed for significance using Duncan's Multiple Range Test (SAS Institute, Inc., 1988).

### **Results**

#### **Milk Production**

Milk production was not significantly different ( $P < 0.05$ ) between the two groups of cows either before, or during the test period (Table 2). Nevertheless, milk yield was numerically lower for the cows fed chia, than for the cows fed the control diet. It should be noted, however, similar differences between groups of cows were also observed prior to the start of the trial, except at day 0 that is the day when feeding of the test ration began to be fed.

#### **Cholesterol and Total Fat Content**

Cholesterol and total fat content were numerically lower in the milk obtained from the cows fed the chia diet, than in the milk produced by the cows fed the control diet. However, no significant differences ( $P > 0.05$ ) were detected between treatments (Table 3).

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<sup>1</sup> Use of brand names is for informational purposes and does not imply an endorsement by the authors or The University of Arizona.

### **Fatty Acid Composition**

Table 3 presents the results of the chromatography analysis, including only those fatty acids found in amounts greater than a trace. Oleic, a monounsaturated fatty acid (MUFA), and linoleic (T-6) and linolenic (T-3) polyunsaturated fatty acids (PUFAs), were significantly higher with the chia diet, than with the control diet. No significant differences ( $P < 0.05$ ) in myristic, palmitic and stearic saturated fatty acids, or in palmitoleic, a MUFA, were detected between treatments.

Total PUFAs, calculated as the sum of linoleic and  $\alpha$ -linolenic fatty acids, were significantly ( $P < 0.05$ ) higher in the milk produced by the cows fed the chia diet, than in the milk produced by the cows fed the control diet (Table 4). No significant ( $P > 0.05$ ) differences between treatments were detected in total saturated fatty acids (SFAs), calculated as the sum of myristic, palmitic and stearic fatty acids, and in MUFA's, calculated as the sum of oleic and palmitoleic fatty acids (Table 4). Addition of chia to the cows' diet resulted in significantly ( $P < 0.05$ ) lower SFA:PUFA and SFA:T-3 ratios in the milk, compared to that produced by feeding the control diet (Table 4).

### **Discussion**

Altering milk properties by changing the diet of lactating cows has been demonstrated by others. The main concerns when feeding PUFA to ruminants are their deleterious effects on dry matter intake, depression of fiber digestibility, and change in milk fat percentage (Wright et al., 1998).

### **Milk Production**

No significant difference ( $P < 0.05$ ) in milk production between treatments was observed. This is consistent with the results for cows fed a 2% fish oil diet (Cant et al., 1997), cows fed 2.5, 5.0, 7.3, and 9.65% Jet-Sploding® canola fat (Khorasani et al., 1991), and cows fed 5% protected canola seeds (Ashes et al., 1992). The finding of no significant difference in milk production between treatments indicates that the chia supplement was not inappropriate for lactating cows.

### **Cholesterol and Total Fat**

The absence of a significant difference ( $P < 0.05$ ) in milk fat content between treatments was similar to that reported when Holstein cows were fed supplements containing 2.5, 5.0, 7.3, and 9.6% fat added as Jet-Sploding® whole canola seed (Khorasani et al., 1991). In contrast, Ashes et al. (1992), observed a 10% increase in milk fat content when Holstein cows were fed a diet supplemented with 6.5% protected canola seeds. Cant et al. (Cant et al., 1997) reported a reduction in milk fat content when Holstein cows were fed 4% red fish oil, compared to cows fed a control diet. Casper et al. (1988), found reduced milk fat content when Holstein cows were fed sunflower seeds, but increased fat content with a high oleic sunflower seed diet.

The variation in results reported when adding dietary fat to lactating cow's rations is expected. Khorasani et al. (1991), suggest that variations in milk fat content arise

because of changes in the balance of short and medium chain fatty acid synthesis in the inter-mammary tissues, and hence the extent of incorporation of dietary long chain fatty acids into the milk fat.

### **Fatty Acid Composition**

The most significant finding in the chia trial was the effect it had on the omega-3 fatty acid content of the milk. The 20% increase in “-linolenic fatty acid percentage is similar to that found by Cant et al. (Cant et al., 1997) for dairy cows fed fish oil. They reported that a diet supplemented with 2% fish oil produced a significant increase ( $P < 0.10$ ) in docosahexanoic (DHA) and eicosapentanoic (EPA) (24% and 33.8%, respectively) in the milk, compared to cows fed a control diet. This represented deposition efficiencies of the omega-3 fatty acids DHA and EPA of 9.3 and 16.2%, respectively. In their trial, however, “-linolenic fatty acid content was not significantly different between treatments. In the chia experiment, omega-3 “-linolenic fatty acid was deposited with an efficiency of 3.34%. It is worthy of note that both the chia seed and fish oil were fed in unprotected forms, yet transfer of the fatty acids from the feed to the milk took place.

Adding chia seed to the diet of lactating cows produced milk having over 48% of the advertised T-3 fatty acid content of T-3 enriched milk sold in stores in Argentina and Brazil. This commercially available T-3 enriched milk, however, is obtained by adding fish oil to partly skimmed milk (Parmalat.™, 1999). As such this milk cannot meet the requirements of the International Dairy Federation as being natural dairy milk (Chambon, 1996).

Isomeric fatty acids in ruminants are formed by biohydrogenation, as a result of bacterial fermentation in the rumen, and are similar to those formed in the commercial hydrogenation of vegetable oils (Craig-Schmidt, 1992). Although the capillary column used in the laboratory for the current trial could not resolve trans-isomers, other reports have shown that 91% of the 18:3 fatty acid found in cows milk is the T-3 isomer (Jensen, 1992). Because concerns have been raised about the effects trans-isomeric fatty acids have in promoting the incidence of CHD and cancer in humans, a determination of their presence in the milk must be included in any future chia tests.

### **Fatty Acid Ratios**

Addition of chia to the cows' diet lowered the total SFA content of the milk and improved its SFA:PUFA ratio, bringing it more into line with that recommend by the American Heart Association (American Heart Association, 1990). The chia also brought the SFA:T-3 ratio of the milk closer to that recommended by the British Nutrition Foundation (1992), for human consumption. This could reduce consumer risk of suffering CHD as has been described. Hence adding chia seed to a lactating cow's diet can improve the nutritional quality of the milk produced, from the standpoint of the fatty acid profile.

### Ruminal Degradation of PUFA's

PUFAs are normally hydrogenated by microorganisms in the rumen, and because of this process, ruminant triglycerides contain very low proportions of polyunsaturated fatty acids (Jensen, 1992). Scott et al. (1971), reported on a process wherein polyunsaturated oil droplets were protected from ruminal hydrogenation by encapsulation in a formaldehyde treated protein. The protein resists break-down in the rumen, thereby protecting the fatty acid against microbial hydrogenation. Ashes et al. (1992), fed dairy cows canola seed (10% "-linolenic content) which was protected from ruminal metabolism by emulsification and encapsulation in a matrix of aldehyde treated protein. When fed at 6.5% of the ration, significant reductions in milk palmitic, myristic and lauric fatty acids were found, and corresponding increases in the proportions of stearic, oleic, linoleic, and linolenic fatty acids were detected. In their trial, oleic plus polyunsaturated fatty acids in the milk increased by 54%.

Other techniques have been reported as reducing the detrimental effect of fat on ruminal fermentation. Feeding calcium salts of long-chain fatty acids (at 4 to 5% of the total ration) appears to overcome the adverse effect that feeding fat has on nutrient digestibility (Ashes et al., 1992; Grummer et al., 1984; Jenkins and Palmquist, 1984). Thus it is possible both to enhance milk fat composition, and overcome the adverse effect that feeding fat has on nutrient digestibility, however the protection efficiency is not the same with all of the technologies (Atwal et al., 1991; Khorasani et al., 1991; Wright et al., 1998). A trial feeding protected chia seeds must be undertaken to determine if the incorporation efficiency of the "-linolenic fatty acid found herein can be improved.

### Conclusions

Changes in the fatty acid composition of milk fat were obtained by feeding chia to lactating Holstein cows. Linoleic and "-linolenic fatty acid contents increased, and SFA:PUFA and SFA:T:3 ratios improved. These factors would make milk more acceptable to health conscious consumers, and could reverse the declining per capita consumption of milk that has occurred in recent years. While this study does show promising results, the effect of using protected chia at different levels over extended periods of time must be determined to fully assess the potential chia has in improving the fatty acid profile of dairy milk.

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**Tables**

**TABLE 1. Ingredients and composition of the supplements fed.**

|   | Control | Chia  |
|---|---------|-------|
| Corn (%)                                | 45,83   | 66,87 |
| Soybean meal (%)                        | 10,42   | -     |
| Wheat flour by-product (%) <sup>1</sup> | 43,75   | 15,63 |
| Chía (%)                                | -       | 17,5  |
| ME (Kcal/kgG-)                          | 2930    | 3100  |
| Protein (%)                             | 18,06   | 14    |
| Neutral Detergent Fiber (%)             | 22,4    | 17,9  |
| Acid Detergent Fiber (%)                | 1,37    | 5,7   |
| Ether Extract (%)                       | 5,31    | 11,1  |
| Calcium                                 | 0,09    | 0,2   |
| Phosphorous (%)                         | 0,7     | 0,4   |
| Fatty Acids (%)                         |         |       |
| Palmitic                                | 0,31    | 1,19  |
| Palmitoleic                             | 0       | 0,02  |
| Stearic                                 | 0,05    | 0,52  |
| Oleic                                   | 0,6     | 1,34  |
| Linoleic                                | 0,88    | 2,4   |
| Linolenic                               | 0,05    | 4,1   |

<sup>1</sup> Neutral Detergent Fiber (37%)

**TABLE 2. Influence of dietary chia on milk production.**

|                  | Treatment                |        |       | Production (liters/cow/day) |        |        |
|------------------|--------------------------|--------|-------|-----------------------------|--------|--------|
|                  | Before test period began |        |       | After test period began     |        |        |
|                  | Day 64                   | Day 60 | Day 0 | Day 4                       | Day 14 | Day 30 |
| Control          | 26,13                    | 28,13  | 18,4  | 20,3                        | 25,3   | 20,8   |
| Chia             | 24,47                    | 25,7   | 19,6  | 15,27                       | 17,93  | 15,13  |
| cr: <sup>1</sup> | 5,16                     | 5,06   | 8,56  | 10,59                       | 10,39  | 10,67  |

Means within each column did not differ (P<0.05) according to Duncan's multiple range test

<sup>1</sup>cr: critical range for means separation.

**TABLE 3. Influence of dietary chia on total fat content, cholesterol content, and fatty acid composition of milk.**

| Day       | Treatment | Myristic<br>Cholesterol | Palmitic<br>Total Fat | Palmitoleic | Stearic | Oleic   | Linoleic |           |          |        |
|-----------|-----------|-------------------------|-----------------------|-------------|---------|---------|----------|-----------|----------|--------|
| Linolenic |           | %                       | %                     | %           | %       | %       | %        | mg/100 ml | g/100 ml |        |
| 30        | Control   | 12.85a                  | 29.98 a               | 2.28 a      | 12.55 a | 28.08 b | 3.10 b   | 1.10 b    | 27.75 a  | 3.12 a |
| 30        | Chia      | 11.27 a                 | 25.13 a               | 2.47 a      | 13.90 a | 32.47 a | 3.96 a   | 1.32 a    | 24.33 a  | 2.92 a |
|           | cr        | 2,42                    | 5,99                  | 0,83        | 2,41    | 4,31    | 0,74     | 0,19      | 6,65     | 0,91   |

<sup>ab</sup> Means within a column lacking a common letter differ (P<0.05) according to Duncan's multiple range test;

<sup>1</sup>cr: critical range for means separation

**TABLE 4. Influence of dietary chia on saturated fatty acids (SFA), monounsaturated fatty**

| Day | Treatment | SFA<br>% | MUFA<br>% | PUFA<br>% | T-6:T-3 | SFA:PUFA | SAT:T-3 |
|-----|-----------|----------|-----------|-----------|---------|----------|---------|
| 30  | Control   | 55.38 a  | 30.35 a   | 4.20 b    | 2.84 a  | 13.29 a  | 51.04 a |
| 30  | Chia      | 50.30 a  | 34.93 a   | 5.28 a    | 3.00 a  | 9.61 b   | 38.09 b |
|     | cr        | 6,26     | 4,63      | 0,77      | 0,73    | 3,24     | 12,71   |

<sup>ab</sup> Means within a column lacking a common letter differ (P<0.05) according to Duncan's multiple range test;

cr: critical range for means separation