# Changes in the milk fatty acid profile of Awassi sheep in response to supplementation with agro-industrial by-products

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## ABSTRACT

Awassi dairy sheep farmers in the Middle East are facing high feed costs, particularly during the milk production season, along with large fluctuation in grain and straw prices.

Incorporating agro-industrial byproducts into the diet of Awassi dairy sheep can help to balance diets and decrease costs. But it may affect milk quality through changes in the fatty acid profile. Six experimental diets were compared to a control diet in the research station of the International Center for Agricultural Research in the Dry Areas (ICARDA) in Tal Hadya, Syria.

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Milking Awassi ewes (n = 56) were used to test six experimental diets, in which supplements comprised molasses, sugar beet pulp, or cotton seed cake to a traditional control diet contained barley, wheat bran, and barley straw. Milk samples were collected on a weekly basis from April to June.

The daily milk production and fat content were enhanced in diets containing urea-treated wheat straw ($P < 0.01$). The vetch-based diet resulted in a remarkable increase in C6:0, C8:0, C10:0, C12:0, C14:0, and decrease in C18:0 and C20:0 ($P < 0.01$). An increase in C6:0, C8:0, and C10:0 was also observed in diets containing molasses and sugar beet pulp ($P < 0.01$). Monounsaturated fatty acids (MUFA) increased by 10% with cotton seed cake-based diets, whereas it decreased by 25% in the vetch-based diet ($P < 0.01$). Polyunsaturated fatty acids (PUFA) increased in five diets by 10-19%, compared to that of the control group ($P < 0.01$). This study provides evidence that supplementation using agro-industrial by-product feed stuff influences milk fatty acids profile and therefore enhances potential health benefits.

**(Key words:** fatty acids; agro-industrial by-products; Awassi sheep milk; Middle East)

## 1. Introduction

In the Middle East, sheep milk production plays an important role in supporting the livelihoods of resource-poor farmers. Dairy processing is done at a small scale in many sheep-keeping households and the products are important in the diets of families (Hilali et al., 2011a; Titi and Al-Fataftah, 2013). However, sheep producers are often constrained by high feed costs and feed shortages due to severe degradation of rangeland and limited opportunities to cultivate forages. To overcome these constraints, farmers have to rely on crop residues and the purchase

of costly supplements to reduce feed gaps, particularly during the milk production season (Hilali et al., 2011b). The use of available agro-industrial by-products and urea-treated straw can reduce feed costs (Bencini, 2001; Abbeddou et al., 2011a; Hilali et al., 2011b).

It is well known that milk fat quality is affected by the composition of feed diets (Vasta et al., 2008), and that feed ingredients influence the composition of milk fatty acids (FA) (Buccioni et al., 2012). The inclusion of sugar beet pulp could increase the proportion of vaccenic acid and reduce the total polyunsaturated fatty acids (PUFA) (Cabiddu et al., 2006). The source of carbohydrate in concentrates also influences the FAs profile in cheese (Cabiddu et al., 2006). The proportion of most of the C18 FAs, including CLA, increased in the milk and cheese when the cattle fed on cottonseeds (Dhiman et al., 1999). Moreover, starchy feed increased the level of short chain fatty acids (SCFA) (Toral et al., 2014). However, studies reporting the effect of agro-industrial by-products like sugar beet pulp and cotton seed cake on milk fatty acid profiles are scarce.

The quality of dietary fat is recognized as an important determinant of cardiovascular disease, cancer and diabetes (Wilkins et al., 2017). Recent studies *in vitro* and *in vivo* with laboratory animals showed that CLA had a positive effect against cancer and obesity (Wahle et al., 2004; Prieto-Hontoria et al., 2011). Specific attention has been given to maximizing the level of FAs, which have an anti-atherogenic and anti-obesity properties, such as butyric acid; C4:0, oleic acid C18:1, PUFA, especially omega 3 FA and conjugated linoleic acids (CLA). Lowering FA, such as C12:0 (lauric), C14:0 (myristic), and C16:0 (palmitic) could reduce atherogenicity index, which is a health benefit (Ulbricht and Southgate, 1991).

Nutritional quality is becoming a major issue in food choices because of rising consumer awareness of the link between diet and health. This awareness will lead to an increased market demand for functional foods. Over the last years, many studies have demonstrated that milk fat

may offer health benefits compared to other sources of dietary fats (MacRae et al., 2005). Milk and dairy products can be used as functional food due to their bioactive components like PUFA, CLA, vaccenic acid (VA), (Sanders, 1998; Gill and Cross, 2000; Tsiplakou and Zervas, 2013a). The beneficial effect of these bioactive components on human health has been shown in a number of studies (Wahle et al., 2004; Recio et al., 2009). For example, an increase of unsaturated fatty acids leads to a reduction of the ratio between saturated fatty acids (SFA) and PUFAs, thus improving milk's nutritional quality (Bernardini et al., 2010) and products like ghee, widely consumed in the Middle East (Hilali et al., 2011a). Several studies (Tsiplakou and Zervas, 2013b; Toral et al., 2014) have addressed the effects of lipid supplementation in the diets (i.e., seeds, oils) on milk FA synthesis in ruminants. The objective of this study is to assess the level of the fatty acids profile in the milk when agro-industrial by-products are incorporated into the diets of Awassi sheep.

## 2. Material and methods

### *2.1. Animals and diets*

The experiment was carried out in the main research station of ICARDA in Tal Hadya, Syria, which has semi-arid conditions with an average annual rainfall of 330 mm. Seven groups of lactating Awassi ewes, aged between 3 and 6 years and in their second parity or higher were used to test dietary composition on milk FA. Each group consisted of eight ewes that were weaned at 56 ± 3 d after parturition, and were 67 ± 15 days in milk at the start of the experiment. Prior to the trial, all ewes were managed alike under a standard diet consisting of range grazing supplemented with concentrates. The experimental groups were formed so that means and variances for ewe BW, initial milk yield and initial DIM, recorded prior to the outset of the experiment, were similar among the groups. Homogeneity of means and variances was

confirmed by using Bartlett's test for homogeneity. Likewise, animals were distributed so that the distribution of litter size and age of ewes were similar in all the groups.

Seven experimental diets, including a control diet, were formulated (Table 1) as reported by Hilali et al. (2011b) and were assigned randomly to the respective animal groups. The control diet was based on conventional feeds used by farmers: barley, wheat bran, and barley straw. The 6 experimental diets involved RG or vetch (*Vicia sativa*) pasture (VP) and supplements based on different non-conventional feeds: cotton seed cake (CSC), molasses (M), sugar beet pulp (SBP) and urea-treated straw (UTS). The experimental diets (Table ):

1. CSC: RG and a supplement, which has similar ingredients like the control diet, except that it contained CSC to reduce the use of barley grain.
2. M-SBP-UTS: RG and a supplement, including M, SBP, UTS, and CSC to reduce the use of barley grain and replace barley straw.
3. CSC-UTS: RG and a supplement with similar ingredients like those of the control diet, except that it contained CSC and UTS to reduce barley grain and replace barley straw.
4. M-SBP: RG and a supplement similar to that of the second experimental M-SBP-UTS diet, except that it uses only barley straw as in the control diet.
5. M-SBP-CSC: RG and a supplement in which the total use of barley grain was replaced by M, SBP, CSC, wheat bran, and barley straw.
6. M-SBP-VP: VP and a supplement based on M, SBP, wheat bran, and barley straw in which the entire barley grain was also replaced.

The diets were formulated and fed to the animals from April to June. All the animals had a weekly strip grazing (at a stocking rate of 54 ewes/ha/week) as a basal diet component with unrestricted access to water, and were given the experimental supplements detailed in Table1. The ewes grazed on natural rangeland with the exception of those fed on the M-SBP-VP diet,

where the ewes grazed on vetch (*Vicia sativa*). The nutrients available in the rangeland and VP, from April to June, are presented in Table 2. All the animals were sent for grazing after milking, from 7 a.m. to 2 p.m.

It is estimated that the grazing on rangeland covered 40% of the animal requirements (Hilali et al., 2011b). On this basis, and according to their maintenance and production requirements (MAFF, 1987), each animal in the experimental diets received on average 229 g of CP and 18 MJ of ME per day from both the supplement and pasture. Under the traditional feeding regime (control) that was set to resemble the 'average' diet used by farmers, with ewes receiving less protein (180 g/d) and similar energy levels as those fed on the experimental diets. In the M-SBP-VP diet, involving vetch pasture, the supplement contributed on average only 72 g of CP and 9.8 MJ of ME per day, as vetch was covering the remaining crude protein and energy demands of ewes (Hilali et al., 2011b). The experimental diets were not balanced for the content of fat, as diets were formulated to be energy-balanced feed options on milk production (Hilali et al., 2011b). Fifty percent of the supplement was offered in the morning before grazing, and the remaining 50% in the evening after milking. The animals' weights were recorded at the beginning and at the end of the experiment.

### *2.2. Pasture and feed composition*

Pasture and range samples were taken and processed as described in Hilali et al. (2011b). All the pasture samples and feed diets used in the experiment were analyzed for DM, CP, and fat content according to AOAC (2000); ADF and NDF were analyzed according to Van Soest et al. (1991). *In vitro* digestibility was determined according to Tilley and Terry (1963).

*2.3. Milk measurements and analysis*

Ewes were machine milked twice daily at 6:30 a.m. and 4:30 p.m., using a double 12 milking parlor (Bonsaglia & C. SRL, Brasica, Italy), except once a week when they were hand milked to record bulk milk production and collect milk samples. Samples of bulk milk were collected every two weeks for analysis without preservatives. The main milk components (%) of fat, protein, and total solids were determined using an infrared spectroscopy device (MilkoScan 133B, Foss Electric, Hillerød, Denmark). One ml of each milk sample was frozen at -18°C until fatty acid profile analysis could be done.

*2.4. Fatty acid profile*

Milk fatty acids extraction and methylation were performed according to Bligh and Dyer (1959) adapted to milk samples and Molkentin and Precht (2000). One ml of thawed milk was vortexed then mixed with 2 ml methanol and 1 ml chloroform and vortexed for 1 min. Another 1 ml of chloroform and 1 ml of water were added to the tube and vortexed again for phase separation. The lower phase was carefully extracted through the upper phase using Pasteur pipettes, and evaporated under $N_2$. The extracted lipids were dissolved in 1 ml Hexane and mixed with 20 µl sodium methylate solution (2 *N* in methanol) and vortexed for 3 min. The tubes were centrifuged for 1 min at 493 × *g* (Kubota 3110, Kubota Corp., Tokyo, Japan). The reaction was stopped by adding 100 mg of sodium hydrogen sulphate-monohydrate and vortexed for 2 min. The tubes were centrifuged again for 1 min at 493 × *g*. One µl of the clear supernatant was manually injected into the gas chromatograph (GC 2010, Shimadzu, Kyoto, Japan). The GC was equipped with highly polar (90% biscyanopropyl / 10% phenylcyanopropyl polysiloxane) capillary column (105m × 0.25 mm ID, 0.20 µm film, Supelco, Rtx®-2330, USA).

The analysis was performed using split-injection and flame ionization detector (FID). The following conditions were applied: Helium as a carrier gas at a column flow rate of 1.30 ml/min (linear velocity control); split ratio 1/100; injector and detector temperatures 250°C; oven program 50°C, 3 min isothermal, then with 5°C/min to 140°C, 2 min isothermal, then with 2°C/min to 170°C, 5 min isothermal, then by 10°C/min to 220°C, 5 min isothermal, then 5°/min to 225°C, 20 min isotherm. Supelco 37 mix (Sigma Aldrich, Vienna, Austria) and Mixture Me 62 (Larodan, Malmö, Sweden) were used as standards for fatty acid identification (Collomb and Bühler, 2000). The FAME proportions were corrected by applying theoretical FID response factors (Schreiner and Hulan, 2004).

*2.5. Indexes of lipid quality*

The ratio of SFA to unsaturated fatty acids (UFA) was calculated including *trans* FA in the UFA. The Index of atherogenicity (AI) and thrombogenicity (TI) was calculated from FA profiles (Ulbricht and Southgate, 1991; Stockdale et al., 2003) as follows:

1- Atherogenicity Index (AI) indicates the relationship between the main SFA and the main UFA, the first being considered pro-atherogenic (lipids adhesion ability to cells of the immunological and circulatory system), and the second anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids by preventing the appearance of micro and macro coronary diseases).

   $AI = [C12 + (4 \times C14:0) + C16:0] / [\Sigma MUFA + \Sigma PUFA\text{-}n6 + \Sigma PUFA\text{-}n3]$.

2- Thrombogenicity Index (TI) indicates the tendency to form clots in the blood vessels. This is the relationship between the pro-thrombogenetic SFA and the anti-thrombogenetic fatty acids namely MUFAs, PUFAs – *n*6 and PUFAs – *n*3.

$$TI = [C14:0 + C16:0 + C18:0] / [(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma \text{ PUFA-}n6) + (3 \times \Sigma \text{ PUFA-}n3) + (\Sigma \text{ PUFA-}n3 / \Sigma \text{ PUFA-}n6)].$$

*2.6. Statistical analysis*

Animal weights at the beginning and at the end of the experiment in the different dietary groups were performed using SAS MIXED procedure, software v. 9.2 (SAS Institute Inc., Cary, NC, USA), considering the diet groups as a fixed effect.

Comparisons of milk components and FA among dietary groups were performed, using SAS GLM procedure. The fat and FA were considered as dependent variables; the effects of the diets, bulk milk collection days, and their interactions were considered as a fixed effect, and the LS means were provided. Contrast statements were included in the model to compare treatment effects. Correlations among the measured variables were performed, using SAS-CORR procedure.

## 3. Results

*3.1. Animal weights*

Animal weights at the start of the experiment was 58.7±4.9 kg and the variation among the different groups were not significant P = 0.825. The average weight at the end of the experiment was 59.5±4.6 kg and the variation among the groups were not significant P = 0.663.

*3.2. Milk yield and composition*

Milk yield and milk components were significantly influenced by the experimental diets (Table 3). Diets M-SBP-UTS and CSC-UTS promoted the largest increase in daily milk production (26%) and milk fat content (9%) over the control diet (P < 0.01). In addition, diets

M-SBP and M-SBP-CSC promoted higher protein content compared to the control diet, with all other diets resulting in lower protein content. However, the decrease or increase was not larger than 3-4% in relation to the control diet (Table 3). Milk total solids were 1-4% higher compared to the control diet. In contrast, the use of urea- treated wheat straw enhanced the milk total solids up to 2% ($P < 0.01$). As expected, milk production declined and components increased with the advance of lactation period ($P < 0.01$).

*3.3. Fatty acid profile*

The inclusion of agro-industrial by-products in the feed diet caused changes in the milk FA profile (Table 4). The major FAs were palmitic (C16:0; 30%), oleic (C18:1c9; 17%), and myristic (C14:0; 11%). From the nutritional point of view, Palmitic acid (C16:0) increased in milk fat from diet groups containing SBP (4 to 9%; $P < 0.01$). In addition, the concentration of C8:0, C10:0, C12:0, C14:0, C16:0 in milk fat from CSC and CSC-UTS diet groups was clearly reduced compared to the control ($P < 0.01$; Table 4) and less pronounced for caproic acid (C6:0). Stearic acid (C18:0) was increased 16-20% in milk fat from the same diet groups, whereas the molasses based diet (M-SBP-VP) reduced it by 52% ($P < 0.01$).

The control and M-SBP-VP diets increased C13:0 in milk fat by 72% and 100%, respectively ($P < 0.01$). Also, M-SBP-VP diet increased C15:0 FA was by 24% and reduced the C17:0 FA. Likewise, the C18:0, the same effect was observed for C18:1c9 and MUFA ($P < 0.01$). The C18:2c9t11 (CLA) were highest in diets containing CSC. However, this was more pronounced for CSC, CSC-UTS, and M-SBP-CSC with an increase of at least 35% over the control diet ($P < 0.01$). This effect was much clearer in the content of vaccenic acid (VA; C18:1t11) which increased from 60 to 90% over the control ($P < 0.01$).

The linoleic acid (C18:2c9,12) was 19-28% higher in five experimental diets compared to the control, while it was reduced by 12% in milk fat from M-SBP-VP ($P < 0.01$). Compared to the control, molasses-based diet promoted C20:5c5,8,11,14,17 and depressed C18:1c9 ($P < 0.01$).

Fat content of SFA (Table 5), short chain FA (SCFA, C4:0 to C11:0), medium chain FA (MCFA), C12:0 to C16:0 and the ratio SFA to UFA were highest in the M-SBP-VP and lowest in CSC and CSC-UTS diets ($P < 0.01$). This finding was observed for AI ($P < 0.01$) and TI, indicating that milk fat from CSC and CSC-UTS is much healthier as it has lower risk of coronary and blood circulation disease. In fact, the unfavorable elevated values of TI (Table 5.) was observed in starch and sugar rich diets like the control and the M-SBP-VP diets ($P < 0.01$).

Generally, the C4:0, C6:0, C8:0, and C18:0 were steadily decreasing, while C14:0, C16:0, and SFA were increasing with the advance of the lactation stage ($P < 0.01$). This was the case of some MUFA like C12:1, C14:1, C16:1c9, and *n*-6 FA ($P < 0.01$). The fat content of VA, CLA, C18:1c9, C18:1t12, C18:3c9,12,15, and *n*-3 FA was steadily decreasing ($P < 0.01$).

### *3.4. Correlation among fat and fatty acids and its clusters*

A negative correlation was found between the dietary unsaturated fatty acids and C8:0, C10:0, C12:0, and C14:0 ($r > 0.87$; $P < 0.01$). The CLA was correlated with C18:1t11 and PUFA ($r = 0.81$; $P < 0.01$) and C18:2n6t ($r = 0.70$; $P < 0.01$). It was negatively correlated with MCFA ($r = -0.68$; $P < 0.01$) and C16:0 ($r = -0.69$; $P < 0.01$). The AI was significantly correlated with C8:0 ($r= 0.72$) and with C10:0 ($r = 0.89$). It was negatively correlated with C18:1c9 ($r = -0.87$).

## 4. DISCUSSION

Generally, the experimental diets promote milk production. Daily milk production of ewes fed on diets CSC, M-SBP-UTS, CSC-UTS, and M-SBP were 15% to 27% higher compared to ewes fed the control diet ($P < 0.01$). This increase appears to reflect the use of balanced diets with a balanced CP:ME ratio, as opposed to the control diet with an unbalanced CP:ME ratio of less than 10 (Table 1). This shows a deficit in protein, which is known to lead to sub-optimal milk production (Cowan et al., 1981; Gabler and Heinrichs, 2003). The unbalanced starchy diet and high temperatures from mid-May could divert the energy to body fat deposition and lead to a reduction in milk yield, because gluconeogenesis stimulation is followed by an increase in insulin and adipose tissue uptake capacity (Molle et al., 2008). Diets M-SBP and M-SBP-CSC were promoting a higher milk protein content c compared with the control diet. Other diets have resulted in decreases in milk protein content. However, the decrease or increase was ≤ 3% compared to the control diet. Milk protein content was higher than the range values reported by Galal et al. (2008).

Regardless fat rich diets, it is possible that the increased milk fat yield was related to the inclusion of urea- treated wheat straw ($P < 0.01$) that improves the digestible fiber content (Lu et al., 2005; Nurfeta et al., 2009). Such observation was also reported by Cabiddu et al. (2006) in Sarda sheep fed on corn and sugar beet pulp concentrates. Diet M-SBP-VP completely replaced high-cost barley grain and had the highest concentration of molasses (34%), rich in soluble sugars. With this diet, rumen fermentation would increase, reducing rumen pH and fiber digestibility due to the reduction of the acetate:propionate ratio, known to depress milk fat content (Van Soest, 1994; Lu et al., 2005).

The average fat percentage of the milk produced by ewes under balanced experimental diets and with access to natural grazing (Animal groups on diet CSC, M-SBP-UTS, CSC-UTS,

M-SBP, and M-SBP-CSC) was slightly higher than that of Awassi ewes, reported by Nudda et al. (2002).

Despite the ample data on milk FA, there is paucity of studies investigating the effect of CSC, SBP, wheat bran (WB), and other concentrate ingredients on milk FA. Thus, the present study aimed to evaluate the effect of some agro-industrial by-products on milk FA. The changes observed in FA C6:0 to C16:0 can be seen as a positive effect. There is evidence of strong link between intake of C12:0 and C14:0 and the incidents of cardiovascular diseases (Mensink et al., 2003). The changes in FA profiles could be explained by the fact that FA with chain lengths from C4:0 to C16:0 are mainly produced by *de novo* synthesis from β-hydroxybutyrate in the mammary gland (Chilliard et al., 2000), whereas FA with C16:0 and higher are normally incorporated via blood from dietary sources and storage tissues (Schreiner and Windisch, 2006).

Diets containing beet pulp increased C16:0 ($P < 0.01$). This is consistent with the findings reported by Cabiddu et al. (2006). Interestingly, the CSC and CSC-UTS diets that did not contain SBS and molasses provided the highest feed C16:0 and MUFA intake differed from other experimental diets and the control diet. Moreover, these two diets promoted MUFA and *n*-9 FA and depressed the C12:0, C14:0, and C16:0, which affect AI negatively. These two diets had a higher lipid content with elevated concentration of PUFA and MUFA compared to other diets. Such observation was reported by Tsiplakou and Zervas (2013a), who used fish and soybean oil in dairy sheep diets. In addition, the low values of VA could be due to the low daily intake of linoleic acid from the supplement compared to other experimental diets (Tsiplakou and Zervas, 2013a), which could be due to a combination effect of WB, barley, and CSC that enhanced the diet with C18:2c9,12. Such results were reported by Toral et al. (2014). It is well known that the ruminal linoleic acid biohdrogination promote the formation of VA as an intermediate metabolite (Griinari et al., 1998; Tsiplakou et al., 2006). The VA is *trans* C18:1, which is present

mainly in milk and dairy products whose role in preventing cardiovascular disease has been proven (Tyburczy et al., 2009). Based on contrasts for diets, it seems that CSC and urea-treated straw has an effect on the fat content of C18:2c9,12. However, the starchy and sugar-rich diets (control and M-SBP-VP) had the lowest content due to the extensive FA biohydrogenation in the rumen (Buccioni et al., 2012).

In general, *trans* FA are derived from incomplete biohydrogenation of PUFA in the rumen. The VA is considered as a major *trans* C18:1 isomer and is an intermediate that promotes the synthesis of C18:2c9t11 (CLA) (Lock and Garnsworthy 2002). This fact was observed in our study on milk content of CLA and VA. It was also confirmed by the high correlation ($r = 0.81$; $P < 0.01$). Diets containing CSC, WB, and Barley grain could contribute to this finding. Additionally, starchy or sugar-rich diets, observed under the control and M-SBP-VP diets, could depress CLA in milk fat (Colman et al., 2013). However, the unclear feed effect on the content of CLA could be due to the fact that the CLA is primarily synthesized in the mammary gland during lactation (Bauman et al., 1999) and the factors that affect the content in milk fat is due to individual variation between animals (Kelsey et al., 2003; Lock and Garnsworthy, 2003). The differences in total *trans* C18:1 FA could be associated with certain inhibition of milk fat synthesis (Bauman and Griinari, 2003; Abbeddou et al., 2011b). The variation in C18:1 *trans* could be due to the effect of rumen pH, particularly based on the assumption of lower rumen pH resulting from M-SBP-VP and control diets. This study's findings suggest that reduced rumen pH resulting from starchy and soluble-rich diets will reduce the CLA in a way that is similar to silage *vs* green hay (Bernardini et al., 2010).

The negative relation between the increased activity of $\Delta^9$-desaturase calculated in the control and M-SBP-VP diets could inhibit the synthesis of fat and protein. Such calculation was reported for cattle (Soyeurt et al., 2008). The activity of $\Delta^9$-desaturase is measured indirectly by

using the ratio of C14:1 to C14:0 (Lock and Garnsworthy, 2003). This ratio was higher in M-SBP-VP and the control diets ($P < 0.01$). Also, this ratio fluctuated with the advance of lactation. Such seasonal variation was due to changes in $\Delta^9$-desaturase activity that could be related to individual genetic variation (Lock and Garnsworthy, 2003). Generally, the values obtained are consistent with the findings reported by Addis et al. (2005). The highest value for C18:3c9,12,15 was obtained in M-SBP-VP and control diet in agreement with Cabiddu et al. (2006) who obtained similar results in cheese from ewes fed on corn-based diet. The low fiber and soluble sugar-rich diet like diet M-SBP-VP could lead to subacute ruminal acidosis that could be responsible for the low values of the bacterial fatty acids; C15i (Colman et al., 2013; Li et al., 2014) and high levels of C15 (Colman et al., 2013). Moreover, the quantity of FA produced under the different experimental diet groups is even more pronounced by the combined diet effect on milk production and the content of fat in milk.

The advance in lactation season affected FA composition, which could be explained by the effect of availability of green fodder and the advance of lactation period. In general, the observed behavior of the advance in lactation on fatty acids are reported for cattle (Lock and Garnsworthy, 2003; Colman et al., 2013). Additionally, there were some similarities between FA obtained in this study and other experiments reported. However, the content of C18:2c9t11 (CLA) were less than what was reported by Abbeddou et al. (2015) in Awassi ewes fed on olive cake or tomato pulp, and what was reported by Tsiplakou and Zervas (2013a). This was also the case for total *n*-3 FAs, C10:0, C12:0, and C14:0. However the content of was similar. The C16:0 and MUFA reported in this study are similar to the results reported by Abbeddou et al. (2015).

## 5. CONCLUSION

This study demonstrates that feed could affect the fatty acid composition in sheep milk. Diets containing CSC could be used as a nutritional strategy to enhance both milk yields and milk FAs with a clear benefit for human health. In contrast, excess use of molasses reduced milk yield and negatively affected the quality of milk fat. Moderate use of molasses could have a positive effect on FA profile for better health. In addition, balanced diets will enhance the nutritional quality of milk fat.

## Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper entitled “Changes in the milk fatty acid profile of Awassi sheep in response to supplementation with agro-industrial by-products.”

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Table 1. Ingredients of experimental diets, and its nutritional value.

| Item | Control diet | Experimental diets[1] | | | | | |
|---|---|---|---|---|---|---|---|
| | | CSC | M-SBP-UTS | CSC-UTS | M-SBP | M-SBP-CSC | M-SBP-VP |
| Ingredients, % of DM[*] | | | | | | | |
| Barley | 40 | 20 | 20 | 30 | 26 | | |
| Sugar beet pulp | | | 14 | | 8 | 18 | 17 |
| Molasses | | | 17 | | 19 | 22 | 34 |
| Cotton seed cake | | 15 | 25 | 17 | 26 | 20 | |
| Wheat bran | 15 | 26 | | 17 | | 20 | 8 |
| Urea treated wheat straw | | | 24 | 36 | | | |
| Barley straw | 45 | 39 | | | 21 | 20 | 41 |
| Intake and nutritional value of diets[*2] | | | | | | | |
| DMI, kg/d | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.3 |
| CPI, g/d | 101.3 | 147.3 | 151.1 | 156.1 | 143.7 | 147.8 | 71.9 |
| MEI, MJ/d | 10.9 | 10.4 | 11 | 11.7 | 10.8 | 10.1 | 9.8 |
| CP:ME ratio | 9.3 | 14.1 | 13.8 | 13.4 | 13.4 | 14. 7 | 7.3 |
| ADF, % | 25.7 | 25.6 | 23.4 | 26 | 20.2 | 21.2 | 24.8 |
| NDF, % | 57.9 | 52.5 | 42.3 | 52.2 | 39.5 | 38.7 | 44.4 |
| Lipid, % | 1.7 | 2.2 | 1.2 | 1.8 | 1.4 | 1.6 | 0.8 |
| Fatty acids, % of lipid | | | | | | | |
| C14:0 | 1.1 | 0.9 | 0.8 | 0.5 | 1.1 | 0.7 | 2.1 |
| C16:0 | 18.2 | 19.8 | 25.5 | 20.1 | 25.2 | 21.3 | 19.7 |
| C18:0 | 1.8 | 1.9 | 2.2 | 1.7 | 2.2 | 1.9 | 2.5 |
| C18:1c9 | 13.5 | 14.6 | 15.2 | 15.6 | 14.4 | 15.3 | 10.1 |
| C18:1c11 | 0.7 | 0.8 | 0.7 | 0.7 | 0.6 | 0.8 | 0.6 |
| C18:2c9,12 | 32.2 | 34.59 | 35.8 | 37.4 | 34.0 | 36.0 | 22.5 |
| C18:3c9,12,15 | 3.5 | 2.7 | 1.8 | 2.8 | 2.0 | 2.0 | 2.5 |
| SFA[3] | 41.9 | 39.4 | 42.3 | 36.1 | 44.5 | 38.2 | 54.8 |
| MUFA[4] | 16.6 | 18.3 | 17.4 | 19.3 | 16.5 | 19.3 | 13.0 |
| PUFA[5] | 39.9 | 40.9 | 39.0 | 43.2 | 37.9 | 41.2 | 29.6 |

[*] Source: Hilali et al, 2011b.

[1]CSC = cotton seed cake based diet; M = molasses, SBP = sugar beet pulp; UTS = urea wheat treated straw; VP = vetch pasture.

[2]DMI = dry matter intake; CPI = crude protein intake; MEI = metabolized energy intake; ADF; acid detergent fiber; NDF = natural detergent fiber.

[3]SFA = saturated fatty acids: C6:0; C8:0; C10:0; C14:0; C16:0; C18:0; C20:0; C21:0; C22:0; C23:0; C24:0.

[4]MUFA = mono unsaturated fatty acids C16:1c7; C16:1c9; C18:1c9; C20:1c11.

[5]PUFA = poly unsaturated fatty acids: C18:2t9,12; C18:2c9,12; C18:2 minor isomers (C18:2c9t12-13); C18:3c6,9,12, C20:3c8,11,14.

Table 2. Nutritional value of pasture during the experiment.

| | Months | | |
|---|---|---|---|
| Pasture*[1] | April | May | June |
| Range | | | |
| DMI, kg/d | 1.8 | 1.0 | 0.8 |
| Grasses, % of DM | 42 | 49 | 57 |
| Legumes, % of DM | 15 | 3 | 2 |
| CP, g/d | 159.0 | 70.8 | 47.1 |
| ME, MJ/d | 13.8 | 7.7 | 6.0 |
| ADF, % | 31.3 | 39.9 | 43.3 |
| NDF, % | 49.7 | 59.5 | 65.3 |
| Lipid, % | 2.1 | 2.3 | 1.6 |
| Vetch | 1 | 1 | 1 |
| DMI, kg/d | 1.3 | 1.4 | 1.0 |
| CP, g/d | 213.2 | 211.1 | 150.3 |
| ME, MJ/d | 12.7 | 11.9 | 8.1 |
| ADF, % | 18.7 | 23.3 | 26.4 |
| NDF, % | 32.2 | 37.8 | 43.4 |
| Lipid, % | 1.3 | 0.7 | 0.9 |

*Source: Hilali et al, 2011b.

[1]values were average of weekly measurements during the experimental period.

Table 3. Daily milk production (g/day) and milk composition (%) produced under the different experimental diet groups.

| | Control diet | Treatments[1] | | | | | | SEM | P Values[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CSC | M-SBP-UTS | CSC-UTS | M-SBP | M-SBP-CSC | M-SBP-VP | | D | T | D × T |
| Milk yield (g/day) | 937$^{b}$ | 1100$^{c}$ | 1184$^{d}$ | 1188$^{d}$ | 1080$^{c}$ | 961$^{b}$ | 756$^{a}$ | 22.15 | ** | ** | NS |
| Milk composition (%) | | | | | | | | | | | |
| Fat | 6.42$^{a}$ | 6.57$^{ab}$ | 7.00$^{c}$ | 7.00$^{c}$ | 6.63$^{ab}$ | 6.46$^{ab}$ | 6.70$^{b}$ | 0.1 | ** | ** | NS |
| Protein | 5.68$^{bc}$ | 5.51$^{a}$ | 5.64$^{b}$ | 5.48$^{a}$ | 5.86$^{d}$ | 5.78$^{cd}$ | 5.65$^{b}$ | 0.04 | ** | ** | NS |
| Total Solids | 17.66$^{a}$ | 17.76$^{a}$ | 18.37$^{c}$ | 18.13$^{bc}$ | 18.22$^{c}$ | 17.85$^{ab}$ | 18.08$^{abc}$ | 0.13 | ** | ** | NS |

$^{a-d}$ Least square means within a raw, having different superscripts differ ($P < 0.05$).

[1]CSC = cotton seed cake based diet; M = molasses, SBP = sugar beet pulp; UTS = urea wheat treated straw; VP = vetch pasture;

[2]D = Diet; T = Time.

* $P < 0.05$, ** $P < 0.01$, ns = not significant

Table 4. Effect of the experimental diets on fatty acid composition of milk fat (% of total fatty acids).

| Fatty Acids | Control diet | Experimental Diets[1] | | | | | | SEM | P value[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CSC | M-SBP-UTS | CSC-UTS | M-SBP | M-SBP-CSC | M-SBP-VP | | D | T | D × T |
| C4:0 | 3.62$^{d}$ | 3.997$^{a}$ | 4.48$^{ab}$ | 3.82$^{bc}$ | 3.98$^{bc}$ | 3.87$^{c}$ | 3.50$^{c}$ | 0.089 | ** | ** | NS |
| C6:0 | 2.50$^{bc}$ | 2.415$^{ab}$ | 2.81$^{de}$ | 2.27$^{a}$ | 2.66$^{cde}$ | 2.6$^{bcd}$ | 2.83$^{e}$ | 0.077 | ** | ** | NS |
| C8:0 | 2.17$^{b}$ | 1.93$^{a}$ | 2.31$^{c}$ | 2.02$^{a}$ | 2.25$^{bc}$ | 2.2$^{bc}$ | 2.72$^{d}$ | 0.048 | ** | ** | NS |
| C10:0 | 6.87$^{b}$ | 5.51$^{a}$ | 6.64$^{b}$ | 5.57$^{a}$ | 6.76$^{b}$ | 6.65$^{b}$ | 9.54$^{c}$ | 0.170 | ** | ** | * |
| C12:0 | 4.11$^{c}$ | 3.118$^{ab}$ | 3.39$^{b}$ | 2.97$^{a}$ | 3.73$^{c}$ | 3.81$^{c}$ | 6.15$^{d}$ | 0.143 | ** | ** | NS |
| C13:0 | 0.089$^{c}$ | 0.041$^{a}$ | 0.049$^{ab}$ | 0.047$^{ab}$ | 0.064$^{b}$ | 0.056$^{ab}$ | 0.105$^{c}$ | 0.007 | ** | * | NS |
| C14:0 | 11.39$^{d}$ | 10.36$^{b}$ | 11.62$^{d}$ | 9.90$^{a}$ | 10.88$^{c}$ | 11.34$^{d}$ | 14.14$^{e}$ | 0.125 | ** | ** | NS |
| C14i | 0.150$^{de}$ | 0.113$^{a}$ | 0.14$^{cd}$ | 0.12$^{ab}$ | 0.13$^{bc}$ | 0.16$^{f}$ | 0.15$^{ef}$ | 0.004 | ** | ** | NS |
| C15:0 | 0.924$^{ab}$ | 0.846$^{a}$ | 0.802$^{a}$ | 0.836$^{a}$ | 0.822$^{a}$ | 0.901$^{a}$ | 1.043$^{b}$ | 0.043 | ** | * | NS |
| C15i | 0.308$^{e}$ | 0.269$^{cd}$ | 0.256$^{bcd}$ | 0.287$^{de}$ | 0.227$^{ab}$ | 0.238$^{bc}$ | 0.209$^{a}$ | 0.013 | ** | ** | NS |
| C16:0 | 28.5$^{a}$ | 27.92$^{a}$ | 30.67$^{cd}$ | 28.34$^{a}$ | 29.68$^{b}$ | 30.45$^{c}$ | 31.03$^{d}$ | 0.234 | ** | ** | NS |
| C17:0 | 0.547$^{a}$ | 0.597$^{cd}$ | 0.587$^{cd}$ | 0.615$^{d}$ | 0.606$^{d}$ | 0.606$^{cd}$ | 0.558$^{bc}$ | 0.017 | * | ** | NS |
| C17ai | 0.497$^{c}$ | 0.511$^{c}$ | 0.439$^{b}$ | 0.536$^{c}$ | 0.388$^{ab}$ | 0.394$^{ab}$ | 0.373$^{a}$ | 0.020 | ** | * | NS |
| C18:0 | 9.26$^{c}$ | 10.78$^{d}$ | 8.95$^{bc}$ | 11.08$^{d}$ | 9.13$^{bc}$ | 8.37$^{b}$ | 4.41$^{a}$ | 0.270 | ** | ** | NS |
| C20:0 | 0.338$^{f}$ | 0.348$^{f}$ | 0.254$^{b}$ | 0.284$^{cd}$ | 0.259$^{bc}$ | 0.288$^{de}$ | 0.213$^{a}$ | 0.010 | ** | ** | NS |
| C22:0 | 0.125$^{cd}$ | 0.148$^{e}$ | 0.116$^{bc}$ | 0.115$^{bc}$ | 0.11$^{ab}$ | 0.135$^{de}$ | 0.099$^{a}$ | 0.005 | ** | ** | NS |
| C24:0 | 0.011 | 0.013 | 0.008 | 0.012 | 0.007 | 0.010 | 0.013 | 0.004 | NS | NS | NS |
| C10:1 | 0.266 | 0.187 | 0.189 | 0.567 | 0.228 | 0.256 | 0.376 | 0.144 | NS | NS | NS |
| C12:1 | 0.055$^{b}$ | 0.005$^{a}$ | 0.012$^{a}$ | 0.013$^{a}$ | 0.021$^{a}$ | 0.04$^{b}$ | 0.129$^{c}$ | 0.006 | ** | ** | NS |
| C14:1 | 0.214$^{b}$ | 0.123$^{a}$ | 0.146$^{ab}$ | 0.146$^{ab}$ | 0.159$^{ab}$ | 0.183$^{ab}$ | 0.324$^{c}$ | 0.025 | ** | ** | NS |
| C16:1c9 | 0.958$^{cd}$ | 0.846$^{abc}$ | 0.753$^{a}$ | 0.789$^{ab}$ | 0.885$^{bcd}$ | 0.997$^{d}$ | 1.232$^{e}$ | 0.040 | ** | ** | NS |
| C16:1c7 | 0.359 | 0.380 | 0.423 | 0.522 | 0.423 | 0.330 | 0.338 | 0.047 | NS | ** | NS |
| C18:1t11 | 0.675$^{a}$ | 1.288$^{b}$ | 1.221$^{b}$ | 1.169$^{b}$ | 1.157$^{b}$ | 1.079$^{b}$ | 0.738$^{a}$ | 0.094 | ** | ** | NS |
| C18:1c9 | 18.32$^{d}$ | 20.10$^{e}$ | 15.94$^{b}$ | 19.82$^{e}$ | 17.51$^{cd}$ | 16.82$^{c}$ | 12.17$^{a}$ | 0.293 | ** | ** | ** |
| C18:2t9,12 | 0.209$^{b}$ | 0.243$^{c}$ | 0.185$^{a}$ | 0.223$^{bc}$ | 0.208$^{b}$ | 0.229$^{bc}$ | 0.24$^{c}$ | 0.007 | ** | ** | ** |
| C18:2c9,12 | 2.336$^{b}$ | 2.886$^{de}$ | 2.769$^{c}$ | 2.79$^{cd}$ | 2.987$^{e}$ | 2.823$^{cd}$ | 2.055$^{a}$ | 0.041 | ** | ** | ** |
| C18:2c9t11(CLA) | 0.335$^{a}$ | 0.468$^{e}$ | 0.39$^{b}$ | 0.452$^{cd}$ | 0.410$^{bc}$ | 0.457$^{de}$ | 0.371$^{ab}$ | 0.015 | ** | ** | NS |
| C18:3c6,9,12 | 0.045$^{cd}$ | 0.014$^{a}$ | 0.029$^{abc}$ | 0.033$^{bcd}$ | 0.039$^{cd}$ | 0.018$^{ab}$ | 0.047$^{d}$ | 0.006 | ** | ** | NS |
| C18:3c9,12,15 | 0.285$^{c}$ | 0.251$^{bc}$ | 0.214$^{ab}$ | 0.21$^{a}$ | 0.218$^{ab}$ | 0.244$^{ab}$ | 0.413$^{d}$ | 0.013 | ** | ** | ** |
| C20:1c11 | 0.053$^{cd}$ | 0.058$^{de}$ | 0.042$^{ab}$ | 0.057$^{cde}$ | 0.04$^{a}$ | 0.064$^{e}$ | 0.049$^{bc}$ | 0.003 | ** | ** | NS |
| C20:2c11,14 | 0.019 | 0.012 | 0.016 | 0.014 | 0.017 | 0.012 | 0.024 | 0.006 | NS | * | NS |
| C20:3c8,11,14 | 0.004 | 0.005 | 0.006 | 0.008 | 0.005 | 0.009 | 0.004 | 0.005 | NS | * | NS |
| C20:3c11,14,17 | 0.062 | 0.045 | 0.029 | 0.043 | 0.057 | 0.062 | 0.039 | 0.016 | NS | ** | NS |
| C20:4c5,8,11,14 | 0.126 | 0.151 | 0.145 | 0.141 | 0.137 | 0.138 | 0.165 | 0.017 | NS | ** | NS |
| C20:5c5,8,11,14,17 | 0.001$^{a}$ | 0.001$^{a}$ | 0.002$^{a}$ | 0.001$^{a}$ | 0.002$^{a}$ | 0.002$^{a}$ | 0.017$^{b}$ | 0.002 | ** | ** | ** |

[a-e] Least square means within a raw, having different superscripts differ (P < 0.05).

[1]CSC = cotton seed cake based diet; M = molasses, SBP = sugar beet pulp; UTS = urea wheat treated straw; VP = vetch pasture.

[2]D = Diet; T = Time.

* P < 0.05, ** P < 0.01, ns = not significant

Table 5. Grouped fatty acids (%) and indexes of lipid quality by experimental diet groups.

| Item | Control diet | Experimental Diets[1] | | | | | | SEM | P value[13] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CSC | M-SBP-UTS | CSC-UTS | M-SBP | M-SBP-CSC | M-SBP-VP | | D | T | D × T |
| SFA[2] | 72.00$^{b}$ | 69.47$^{a}$ | 74.37$^{c}$ | 69.61$^{a}$ | 72.25$^{b}$ | 72.72$^{b}$ | 77.75$^{d}$ | 0.361 | ** | ** | ** |
| MUFA[3] | 22.76$^{d}$ | 24.99$^{e}$ | 20.43$^{b}$ | 24.98$^{e}$ | 22.35$^{cd}$ | 21.75$^{c}$ | 17.11$^{a}$ | 0.342 | ** | ** | ** |
| PUFA[4] | 3.77$^{a}$ | 4.47$^{d}$ | 4.15$^{b}$ | 4.29$^{bc}$ | 4.46$^{cd}$ | 4.38$^{cd}$ | 3.77$^{a}$ | 0.061 | ** | ** | ** |
| Omega 3[5] | 0.35$^{bc}$ | 0.30$^{ab}$ | 0.24$^{a}$ | 0.25$^{ab}$ | 0.28$^{ab}$ | 0.31$^{ab}$ | 0.48$^{c}$ | 0.248 | ** | ** | * |
| Omega 6[6] | 2.72$^{b}$ | 3.30$^{de}$ | 3.13$^{c}$ | 3.20$^{cd}$ | 3.38$^{e}$ | 3.22$^{cd}$ | 2.51$^{a}$ | 0.059 | ** | ** | NS |
| Omega 9[7] | 18.68$^{d}$ | 20.48$^{e}$ | 16.36$^{b}$ | 20.34$^{e}$ | 17.93$^{cd}$ | 17.15$^{bc}$ | 12.50$^{a}$ | 0.306 | ** | ** | * |
| SCFA[8] | 11.60$^{b}$ | 9.88$^{a}$ | 12.05$^{b}$ | 10.12$^{a}$ | 11.68$^{b}$ | 11.48$^{b}$ | 15.19$^{c}$ | 0.324 | ** | ** | NS |
| MCFA[9] | 45.82$^{b}$ | 43.00$^{a}$ | 47.23$^{c}$ | 42.84$^{a}$ | 45.89$^{b}$ | 47.34$^{c}$ | 53.17$^{d}$ | 0.361 | ** | ** | NS |
| SFA/USFA | 2.75$^{b}$ | 2.37$^{a}$ | 3.07$^{c}$ | 2.44$^{a}$ | 2.75$^{b}$ | 2.85$^{b}$ | 3.75$^{d}$ | 0.056 | ** | ** | * |
| Desaturase index[10] | 0.021$^{c}$ | 0.017$^{ab}$ | 0.014$^{a}$ | 0.016$^{ab}$ | 0.018$^{bc}$ | 0.018$^{bc}$ | 0.025$^{d}$ | 0.001 | ** | ** | NS |
| AI[11] | 2.97$^{b}$ | 2.48$^{a}$ | 3.34$^{c}$ | 2.50$^{a}$ | 2.93$^{b}$ | 3.10$^{b}$ | 4.42$^{d}$ | 0.071 | ** | ** | NS |
| TI[12] | 3.19$^{bc}$ | 2.99$^{a}$ | 3.79$^{e}$ | 3.16$^{ab}$ | 3.38$^{cd}$ | 3.41$^{d}$ | 3.68$^{e}$ | 0.070 | ** | ** | NS |

$^{a-d}$ Least square means within a raw, having different superscripts differ (P < 0.05).

[1]CSC = cotton seed cake based diet; M = molasses, SBP = sugar beet pulp; UTS = urea wheat treated straw; VP = vetch pasture.

[2]SFA = saturated fatty acids: C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14i, C15:0, C15i, C15ai, C16:0, C16i, C17:0, C17i, C17ai, C18:0, C18i, C20:0, C21:0, C22:0, C23:0, C24:0.

[3]MUFA = mono unsaturated fatty acids: C10:1; C12:1; C14:1; C16:1c7; C16:1c9; C18:1t11; C18:1c9; C18:1 minor isomers (C18:1t 8-13; C18:1c9-12); C20:1; C22:1c13.

[4]PUFA = polyunsaturated fatty acids: C18:2t9,12; C18:2c9,12; C18:2c9t11; C18:3c6,9,12; C18:3c9,12,15; C20:2c11,14; C20:3c8,11,14; C20:3c11,14,17; C20:4c5,8,11,14; C20:5c5,8,11,14,17; C22:2c13,16.

[5]Omega 3: C18:3c9,12,15; C20:3c11,14,17; C20:5c5,8,11,14,17.

[6]Omega 6: C18:2c9,12; C18:2t9,12; C18:3c6,9,12; C20:3c8,11,14; C20:4c5,8,11,14.

[7]Omega 9: C18:1c9; C22: 1c13.

[8]SCFA = short chain fatty acids: C4:0 to C11:0.

[9]MCFA = Medium chain fatty acids: C12:0 to C16:0.

[10]Desaturase index = C14:1/(C14:1 + C14:0);

[11]AI = Atherogenicity Index.

[12]TI = Thrombogenicity Index.

[13]D = Diet; T = Time.

* P < 0.05, ** P < 0.01, ns = not significant