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The addition of *Citrus aurantium* leaves and tomato pomace in the diet of Awassi ewes and the impact on milk composition and matured cheese

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Abstract

The purpose of this study was to use concentrated feed alternatives with other less expensive feed alternatives in feeding the Awassi ewes and the impact on milk composition and matured cheese. A total of twenty Awassi ewes were used, they were selected in the middle of lactation and divided into two homogeneous groups with weights (47±1.7 kg) and aged 3-4 years, given two different servings of feed (1.5 kg/sheep/day) Milk composition was measured weekly, to make cheese, raw milk control (CON) and *Citrus aurantium* leaves and tomato pomace (CALTP) is kept separately and chilled. Results indicated that fat and total solid content were high in milk and cheese manufactured in the diet that contained CALTP; the proportion of medium-chain fatty acids (c10-c16) and free fatty acids was lower compared to the diet (CON). While the proportion of unsaturated fatty acids (UFA) in the diet increased CALTP. Therefore, the inclusion of CALTP in the diet is helpful because it has a positive impact on the composition of milk and mature cheese, reduces the cost of the diet and helps recycle by-products.

1. Introduction

The increase in population has led to increased demand for cereals and other food sources, leading to higher prices, resulting in the inability of most livestock breeders to feed their animals to meet their requirements. This situation has led to increased demand for cheaper non-traditional resources that could replace grain (Sharif *et al.,* 2018). The increasing preparation of sheep, adverse climatic conditions and the limited income of farmers have led to a lack of feed and the difficulty of providing them, all of which have led to the use of tree residues and residues of industrial products in diets as alternatives to straw and cereals. (Vasta *et al.,* 2008) however, these feed alternatives often contain anti-nutrient compounds, which may reduce their utilization and digestion (Makkar, 2003) and feed contributes about 75% of the total cost of livestock production (Omar, 2002). Many high-value by-products have increased interest in their use to feed ruminants (Mohsen *et al.,* 2021). Recently, considerable social and environmental pressure has been exerted to efficiently reuse the agricultural industry's waste (Pfaltzgraff *et al.,* 2013). Inclusion of this industrial and agricultural waste reduces the cost of feeding animals because it is important to assess the impact of these products on health and physiological status as well as on milk production and quality (Mizael *et al.,* 2020), where the use of crops and products has led Secondary in the diet of the ruminants to an increase in the common in recent years, this reduces feed costs and environmental problems resulting from these substances (Huanca *et al.,* 2021). Citrus trees create a lot of garbage, which is burnt, resulting in resource loss and the need for expensive waste management initiatives (Gutiérrez *et al.,* 2017). However, studies have shown that these by-products can be used to feed ruminants (Romero *et al.,* 2020), while a study has revealed that the use of some leaves and fresh fruit pulp can be a good source of protein, which in turn gives noticeable levels of essential amino acids in milk (Amata and Iwelu, 2012) use of portions of olive tree trim (twigs and leaves) in feeding the Awassi ewes improved the quantity of nutrients eaten and enhance coefficient (Al-Ghuraibawi and Sundos, 2020). Tomato pomace is an industrial by-product consisting of seeds, peel, and a small part of pulp (Domínguez *et al.,* 2020); produced from tomato paste, large quantities of it can become a serious environmental problem and waste of

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resources. Hence, they can be used as animal feed (Al-Harahsheh *et al.,* 2009) due to the chemical composition of tomato pomace and its good acceptance by animals, it has been used in the diet of the (Álvarez *et al.,* 2015) where it contains about 57% fiber, 16% raw protein, 8% ether extract, and 24% of lignin (Marcos *et al.,* 2019). The use of a mixture of tomato and apple pomace in feeding breast cows has increased milk production, dry materials (DM), and nutrition efficiency (Abdollahzadeh *et al*., 2010), where the nutrition and the season are among the factors that affect milk composition (Saadi and Hasan, 2019), nutrition significantly affects sheep's milk production and improve milk's suitability as food for humans, through the improve the quality of fat in the milk (Nudda *et al.,* 2020). Cheese is of great importance and a good source of nutrients essential to the human body (El-Sayed and Youssef, 2019); it is a nutritious fermented milk product containing 30-40% protein and fat and other minerals fat-soluble vitamins (Khanal *et al.,* 2019). This study aimed to assess the inclusion of *Citrus aurantium* leaves and tomato pomace in feeding Awassi ewes and affecting the quality of raw milk and ripe cheese.

2. Materials and methods

2.1 Materials

The materials (alfalfa, barley, bean soy, wheat bran, yellow corn grains, sodium bicarbonate, salt, limestone) were obtained from the local market; collected *Citrus aurantium* leaves from citrus groves, While the tomato pomace was obtained from one of the tomato paste factory farms in Erbil.

2.2 Experimental procedure

The study was conducted at a sheep breeder's farm in Mosul/ Iraq. Using twenty Awassi ewes selected in the middle of lactation and divided into two homogeneous groups of 10 each and with weights (47±1.7 kg) and an age of 3–4 years, they were fed twice daily with a restricted amount of feed (1.5 kg /ewe/day) through the experimental period which lasted 37 days (7 days for adaptation and 30 days for milk collection). The first group was fed with the control ratio which consisted mainly of alfalfa, barley grain, yellow corn, soybean meal, and wheat bran, while *Citrus aurantium* leaves and tomato pomace were included in the ratio of the second group (Table 1). The tomato pomace was dried at air temperature with continuous flipping before being used. Milk was collected from both groups (CON and CALTP) and stored until the cheese was produced. Where the cheese was manufactured according to (Quintanilla *et al*., 2019).

Table 1. Composition of the experimental diets of the diet used in feeding Awassi ewes.

Ingredients (g/kg)	CON	CALTP
Alfalfa	385	385
Barley	276	65
Citrus aurantium leave	0	145
Tomato pomace	0	193
Soybean meal	136	55
Wheat bran	198	130
Soya oil	0	22
Sodium bicarbonate	0.5	0.5
Salt	1	1
Limestone	3.5	3.5
Gross energy (kcal/kg)	2580	2435

CON: control, CALTP: *Citrus aurantium* leaves and tomato pomace.

2.3 Milk and cheese analysis

Milk components were measured using Lactoscac (brand name: Lohand) (Model: SP60); after segregating the lambs from the ewes for 12 hrs, milk yield was measured weekly. Somatic cell count (SCC) was measured using a Viscometric Milk Analyzer (Brand Name: HiYi) (Model: Somatos Mini); cheese composition of acidity, protein, fat, and total solid was evaluated after 60 days of maturation according to Association of Official Analytical Chemists (AOAC) (2010). Cheese yield is calculated and expressed as kg of cheese/100 kg milk.

2.4 Fatty acids and free fatty acids analysis

The first step for fatty acids profile determination is fat extraction from milk and cheese (Hamilton *et al*., 1992). Fat was extracted from the cheese by the method described by Folch *et al.* (1957), by using a mixture of chloroform water. Free fatty acids have been estimated based on the method described by Bills *et al.* (1963). The fatty acid profile (FA) for milk and cheese samples cooked was analyzed according to Nudda *et al.* (2005), using gas chromatography (GC).

2.5 Statistical analysis

Data were analyzed statically using the GLM procedure of the SAS, (2011) program, and the significance between means was estimated using the Duncan test (1955).

3. Results and discussion

3.1 Impact of inclusion on milk quality standards

As shown in Table 2, The average daily production of milk was affected by treatments, milk yield increased with feeding CALTP in the diet compared to the control diet, it was 418 and 492 g/d for the CON and CALTP,

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respectively. This increase is believed to be caused as a result of the improvement in nutrient digestion and the palatable diet. Abdollahzadeh *et al.* (2010), noted the increase in milk production in Holstein cows that feed on a mixture of tomatoes and apple pomace compared to the control sample. The fat content rose in the sample of CALTP-fed sheep milk which can be associated with the high-fat content of citrus leaves (Fernández *et al.,* 2018), and the inclusion of soybean oil in the diet, or because tomato pomace contains a quantity of pectin which shift the fermentation toured increase acetate production and, which in turn increases milk fat synthesis (Del Valle *et al.,* 2006) The fat ratio was 4.51 and 5.83 for both the CON and CALTP, respectively. The total solids increased in the CALTP sample compared to the control sample, where it reached 13.72 and 15.02 for the CON and the CALTP samples, respectively This is due to the high-fat content in the CALTP milk compared to the control sample. While there were no differences in the somatic cell count for both transactions.

Table 2. Effect of *Citrus aurantium* leaves and tomato pomace on milk and composition.

Parameters	CON	CALTP	p-value
Milk yield (g/d)	418±2.00[b]	492±1.73[a]	*
Fat (%)	4.51±0.017[b]	5.83±0.021[a]	*
Protein (%)	3.78±0.026	3.77±0.024	N.S
Lactose (%)	4.56±0.012	4.54±0.012	N.S
Ash (%)	0.87±0.011	0.88±0.012	N.S
SNF (%)	9.21±0.011	9.19±0.014	N.S
Total Solids (%)	13.72±0.134[b]	15.02±0.129[a]	*
SCC (10^3 cells/mL)	115.5±0.78	114±0.81	N.S

Values are presented as mean±SD. Values with different superscript within the same row are statistically significantly different (P<0.05). N.S: No Significant, CON: Control, CALTP: *Citrus aurantium* Leaves and Tomato Pomace, SNF: Solids Non-Fats, FFA: Free Fatty Acids, SCC: Somatic Cell Count.

3.2 Impact of inclusion on the composition and yield of matured cheese

Table 3 explains the effect of the inclusion of *Citrus aurantium* leaves and tomato pomace on the composition and yield of 60-day matured cheese. The cheese manufactured from CALTP-fed sheep's milk contains significantly higher concentrations of fat and total solids than CON. This is due to the high proportion of fat and total solids in milk used in the industry, where the fat ratio is 31.24 and 34.86, while the ratio of total solids is 61.72 and 63.94 for the CON and the CALTP sample, respectively These results concurred with Sánchez-Macías *et al*. (2010), who showed that when making cheese from goat milk containing high levels of fat gave high-fat cheese and total related substances. Also detected were protein content in cheese decreased in CALTP treatment, where it reached 26.37 and 24.26 for the CON and the CALTP samples, respectively. The lower the fat content in cheese the higher the protein ratio, where fat acts as a material filled with casein in cheese and therefore when the fat rate decreases lead to a higher rate of routine (Rudan *et al.,* 1999). However, there were no significant differences in ash and pH. At the same time, cheese yield was higher for CALTP cheese than CON, this may be due to the significant increase in total solids. In addition, FFA decrease in CALP treatment as compared to control.

3.3 Fatty acid profile

Tables 4 and 5 show the fatty acid profile of both milk and matured cheese after 60 days, respectively. It was noted that short-chain fatty acids (c4-c8) did not differ between treatments. While the proportion of fatty acids (c10-c16) decreased in CALTP compared to CON, the content of C18:1, C18:2 and C18:3 increased in CALTP as compared to CON for both milk and matured cheese. This could be the consequence of a lower biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen and a higher rumen and duodenum flow of biohydrogenation intermediates (Kucuk *et al.,* 2004). Fatty acids produced in the rumen through PUFA biohydrogenation may prevent the formation of de novo from the synthesis of FA (Chilliard *et al.,* 2001). This inhibition can affect the final concentrations of mid-chain fatty acids (c10-c16) derived mainly from de novo synthesis. The high-fat content in milk and mature cheese from the CALTP diet may also be due to the high PUFA content in the CALTP diet. Thus, the PUFA of lemon leaves and soybean oil may have inhibited

Table 3. Effect of *Citrus aurantium* leaves and tomato pomace on the cheese yield and composition after 60 days of maturation.

Parameters	CON	CALTP	p-value
pH	5.11±0.023	5.13±0.020	N.S
Fat (%)	31.24±0.22[b]	34.86±0.24	*
Protein (%)	26.37±0.19[a]	24.26±0.21[b]	*
Ash (%)	2.24±0.024	2.17±0.021	N.S
Total solids (%)	61.72±0.51[b]	63.94±0.52[a]	*
FFA (meq/100 g fat)	5.64±0.019[a]	3.08±0.013[b]	*
Cheese yield (kg cheese/100 kg milk)	14.68±0.125[b]	15.21±0.119[a]	*

Values are presented as mean±SD. Values with different superscript within the same row are statistically significantly different (P<0.05). N.S: No Significant, CON: Control, CALTP: *Citrus aurantium* leaves and tomato pomace, FFA: Free Fatty Acid.

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Table 4. Effect of *Citrus aurantium* leaves and tomato pomace on milk's fatty acids (mg/100 mg fat).

Fatty Acids	Milk		p-value
	CON	CALTP	
C4:0	2.66±0.018[a]	2.58±0.020[a]	N.S
C6:0	1.64±0.011[a]	1.57±0.012[a]	N.S
C8:0	1.82±0.010[a]	1.78±0.011[a]	N.S
C10:0	6.82±0.022[a]	5.86±0.020[b]	*
C12:0	4.08±0.011[a]	3.89±0.013[b]	*
C14:0	10.26±0.015[a]	9.85±0.013[b]	*
C16:0	25.19±0.11[a]	22.43±0.13[b]	*
C16:1	1.21±0.014[a]	1.20±0.010[a]	N.S
C17:0	0.26±0.010[a]	0.26±0.010[a]	N.S
C18:0	9.08±0.021[b]	9.31±0.019[a]	*
C18:1	20.34±0.14[b]	21.68±0.16[a]	*
C18:2	3.31±0.010[b]	3.69±0.010[a]	*
C18:3	0.98±0.010[b]	1.23±0.010[a]	*
C20:0	0.21±0.021[a]	0.20±0.019[a]	N.S
C20:1	0.08±0.009[a]	0.09±0.009[a]	N.S
SFA	62.02±1.14[a]	57.73±1.21[b]	*
MUFA	21.53±0.34[b]	22.97±0.28[a]	*
PUFA	4.39±0.071[b]	4.92±0.064[a]	*

Values are presented as mean±SD. Values with different superscript within the same row are statistically significantly different (P<0.05). N.S: No Significant, CON: Control, CALTP: *Citrus aurantium* leaves and tomato pomace, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

microbial synthesis and biohythetism, resulting in reduced contents of medium-chain FAs and increased PUFA levels in mature milk and cheeses (Chilliard *et al.* 2003). Some researchers (Bodas *et al.,* 2010; Gómez-Cortés *et al.,* 2011) noted that soybean oil supplements are more effective than flaxseed oil in increasing CLA content in sheep's milk and cheese. This suggests that the trans unsaturated FA biohydrogenation was more complete in linseed-fed ewes than those fed on soybeans. The amount of vaccenic acid and conjugated linoleic acid flowing across the duodenum was higher in sheep-fed diets supplemented by linoleic acid-rich oils compared to oils fed on sheep rich in linolenic acid. The values of fatty acids in cheese were close to what you get (Saadi, 2018).

4. Conclusion

Many agricultural and industrial by-products can be used as feed for farm animals, especially as due to the high prices of concentrated feed. The results indicate that the inclusion of *Citrus aurantium* leaves and tomato pomace in the feed of Awassi ewes increases the fat content in milk and cheese. It has also improved the properties of milk fat by reducing the proportion of saturated fatty acids (SFA) and increasing the proportion of UFA, so the use of crop residues can be interesting to reduce feeding costs in production areas.

Table 5. Effect of *Citrus aurantium* leaves and tomato pomace on the fatty acids profile (mg/100 mg fat) of 60-day matured cheese.

Fatty Acids	Cheese		p-value
	CON	CALTP	
C4:0	2.48±0.022[a]	2.43±0.019[a]	N.S
C6:0	1.69±0.014[a]	1.61±0.015[a]	N.S
C8:0	1.70±0.023[a]	1.67±0.017[a]	N.S
C10:0	5.98±0.044[a]	5.41±0.035[b]	*
C12:0	4.07±0.022[a]	3.48±0.026[b]	*
C14:0	9.97±0.032[a]	8.72±0.028[b]	*
C16:0	23.21±0.082[a]	19.61±0.094[b]	*
C16:1	1.29±0.012[a]	1.24±0.010[a]	N.S
C17:0	0.19±0.010[a]	0.21±0.010[a]	N.S
C18:0	8.34±0.042[b]	9.58±0.038[a]	*
C18:1	18.86±0.14[b]	21.27±0.12[a]	*
C18:2	3.01±0.012[b]	3.43±0.011[a]	*
C18:3	0.95±0.008[b]	1.19±0.010[a]	*
C20:0	0.20±0.009[a]	0.19±0.009[a]	N.S
C20:1	0.07±0.008[a]	0.09±0.009[a]	N.S
SFA	57.83±1.09[a]	52.91±1.11[b]	*
MUFA	20.22±0.87[b]	22.60±0.90[a]	*
PUFA	3.96±0.021[b]	4.62±0.026[a]	*

Values are presented as mean±SD. Values with different superscript within the same row are statistically significantly different (P<0.05). N.S: No Significant, CON: Control, CALTP: *Citrus aurantium* leaves and tomato pomace, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Conflict of interest

The authors declare no conflict of interest.

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