Impact of Oxytocin-Milking Method on Lactation Performance and Lactation Length of Sheep

Research Article

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ABSTRACT

Determination of milk yield potential using an accurate method is essential for assessing nutritional requirements, evaluation of genetic potential, lamb growth and survival, management decisions and improving performance traits of sheep flocks. Exogenous oxytocin injection is applied to estimate milk secretion rate in sheep. Oxytocin is a neurohormone produced in the hypothalamo-neurohypophysial system, which is released into the blood in response to teat stimulation. It has a galactopoietic effect and plays an important role in lactation acting on milk ejection via the secretory activity and contraction of myoepithelial cells. One hundred singleton-bearing and rearing Makui breed ewes were assigned into two treatments: group I (hand milking and stripping following the intramuscular injection of 2.5 IU of oxytocin) and group II (hand milking and stripping). The study aimed to examine the effect of exogenous oxytocin injection on milk yield, milk composition and lactation length of ewes during the entire lactation period. Data were recorded at fortnightly intervals from post-partum day 14 onward. Oxytocin treatment group produced significantly more average daily milk yield (562.3 *vs*. 301.4 g) and total milk yield (93.6 *vs*. 38.5 kg) compared with non-treated animals (P<0.05). Lactation length was 145.7 days and 115.2 days for the oxytocin and the control group, respectively (P=0.038). Oxytocin injection increased milk peak yield and lactation persistency (P<0.05) while shorter peak time (34.1 *vs*. 39.2 days) was observed for oxytocin group (P=0.043). Oxytocin administration increased milk fat percentage (6.73 *vs*. 5.86) in treated compared to control ewes (P=0.029). However, no difference was detected in the percentage of protein and lactose associated to the oxytocin injection (P>0.05). The development of milk ejection inhibition throughout lactation during the exclusively suckling period, followed by hand-stripping, entails the need for oxytocin to sustain mammary cells and lactation persistency in ewes. It further confirms that oxytocin allows overcoming the negative feedback mechanisms exerted on milk ejection, enhancing the intracellular transfer of secretory vesicles and contraction of myoepithelial cells and thereby increasing milk production and lactation length, which is a useful technique to ensure complete alveolar milk let-down and optimum milk withdrawal from the cistern.

KEY WORDS lactation length, lactation persistency, Makui sheep, milk components, milk yield, oxytocin method.

INTRODUCTION

There is growing interest in sheep´s milking and sheep´s milk products worldwide, especially in the Mediterranean area, Africa and the developed European countries, e.g., France, Italy, Spain, and Greece (Haenlein, 2001). World total sheep milk productionis over than 10 million tonnes, the Asian and the Mediterranean basin countries accounting for more than 80% of this production (FAO, 2012). Sheep produce 25% of the total milk yield during early lactation;

this production potential has the greatest effect on lamb growth and survival. Estimates of the milk produced by lactating ewes is an important factor for determining sheep milk yield, lamb growth rate, establishing breeding programs and improving sheep management in intensive, extensive and agro-pastoralism production systems. There are disparate methods to estimate the sheep´s milk production, which include the "Plunket" method, double "oxytocin"-milking method, body-water dilution techniques, offspring live weight gain and udder scoring appraisal method (Doney *et al*. 1979; Dove, 1988; Unal *et al*. 2007; Iniguez *et al*. 2009; Fernández *et al*. 2013). However, accurate estimates of sheep milk production is difficult, the milk yield potential influenced by the natural behavior of the dam and lamb.

Oxytocin is synthesized in the paraventricular and supra-optic nuclei of the hypothalamus. In response to suckling, it is released from the posterior pituitary gland, via a neuro-endocrine reflex and causes myoepithelial cell contraction, reducing intra-alveolar pressure and re-establishing normal mammary blood flow that elicit stored milk in the alveoli to the mammary ducts and the gland cistern (Lefcourt and Akers, 1983), resulting in the milk ejection. *In vitro* research shows that oxytocin enhances cell proliferation and acceleration of intracellular transit of casein, regulates the secretory activity of the lactating mammary gland, which subsequently cause complete and fast milk descent (Sapino *et al*. 1993; Lollivier *et al*. 2006). The release of oxytocin by tactile teat stimulation is necessary for the removal of milk during the short course of milking; although residual milk and fat fractions remain in the udder, these can be obtained by oxytocin injection. Zamiri *et al*. (2001), Ribeiro *et al*. (2007) and Bencini (1995) reported that administration of oxytocin increases milk yield and milk fat concentration of sheep.

Archaeological excavations (Tamtama, Ganji Dareh, Ali Kosh, Warwasi, Asiab-western Iran) indicates the Mesopotamia is the original area of sheep and goat domestication (Hesse, 1978; Braidwood *et al*. 1983; Zeder *et al*. 2006). Iran is the leading sheep rearing country in Middle East, with a population of about 50 million heads of sheep, producing 465000 tonnes of milk; Iran is the fourth ranked country of the world in sheep number, the 5th in greasy wool and the 6th in sheep milk production (FAO, 2012). Sheep produce about 6.3% of total milk production in Iran and 4.6% of the world's sheep milk production (FAO, 2012), which is mainly processed for cheese, yoghurt, butter and ghee. Sheep management systems in Iran comprise intensive, extensive and traditional pastoral systems (nomadic, transhumance and ranching), for mutton, milk, wool and pelt production, thus playing a significant economic role in rural and nomadic livelihood.

Makui sheep are reared in the northwest region of Iran, mainly under nomadic or semi-nomadic systems or under sedentary rural management systems. The number of Makui sheep in west and east Azerbaijanis about 2500000 to 3500000. Makui are multi-purpose (milk, meat and wool) fat-tailed animals, producing 35-45 kg of milk per lactation; the annual greasy fleece weight of Makui sheep is 1.2-1.5 kg. This breed is well suit to harsh environmental conditions of the highlands, semi-arid zones in Ararat foothills and karst areas, hot in summer and cold in winter. Animals are well adapted to walk long distances on rangeland, possessing high endurance and adaptability; moreover, they are able to graze on poor quality herbage in a transhumance system: in winter they range in meager heterogeneous grassland and undesirable pasture in rough terrains (the winter-quarters), while during summer they are kept in the highlands, summer-quarters under nomadic or ranching management systems. Makui sheep is usually milked once a day during a period of four to six months in which lambs suckle once-a-day, about 20 to 30 min; sheep is shorn once per year. The lamb is removed from their dam between 3 and 5 days after lambing; the length of the suckling period in the ranching system is 90 days. Hogget first mate at 18-19 months of age. The lamb feedlotting program in use for this breed is accomplished under intensive production systems based on dry roughage, creep grazing and supplementary feeds in autumn and winter seasons. The aim of this study was to examine the effect of oxytocin injection on milk yield and composition and lactation length of Makui sheep during the whole lactation period.

MATERIALS AND METHODS

Experimental conditions, animals and diets

The experiment was carried out at the Production and Breeding Center of Makui sheep (Iran-Europe International Road), 15 km southeast of Maku city, West Azarbaijan, Iran. To evaluate the effect of oxytocin on milk yield, composition and lactation length during the entire lactation period, one hundred mixed parities, singleton bearing and rearing of Makui ewes were selected from local husbandry farms. Ewes had an average of 45 kg body weight and were of similar days in lactation, the animals were randomly assigned in two treatment groups. In group I, hand milking and stripping followed an intramuscular injection of 2.5 IU of oxytocin (n=50, distributed according to age as follows: 15 ewes aged 2 years old, 10 ewes aged 4 years old, 15 ewes 5 years old and 10 ewes aged 6 years old); in group II (control), hand milking and stripping was performed without oxytocin administration, control group, (n=50, distributed according to age as follows: 15 ewes aged 2 years old, 10 ewes aged 4 years old, 15 ewes 5 years old and 10 ewes

aged 6 years old) (Doney *et al.* 1979; Fernandez *et al.* 2013). In order to assess the changes in milk production through lactation, its length was divided into three periods: early lactation (days 14 to 56 after lambing), middle lactation (days 57 to 98) and late lactation (days 99 to 168).

In the first 60 days of lactation ewes had *ad libitum* access to a complete mixed lactation diet composed of 50% alfalfa, 20% wheat straw, 25% barley and 5% cottonseed meal. The diet, formulated to meet NRC (2007) requirements for lactating ewes, contained 14% crude protein (CP) (dry matter (DM) basis) and was estimated to contain 2.5 Mcal/kg of metabolizable energy (ME). Pens and feeders were arranged so that the lambs had no access to the ewes feed. The animals received food in the trough, as complete mixture, twice daily in the morning and afternoon; water and mineral salt were offered *ad libitum*. Deworming was performed twice during the experiment. Ewes and lambs were vaccinated against enterotoxaemia disease during the trial; ewes and lambs received 2.5 and 1 mL, respectively, of the vaccine in the form of a subcutaneous injection. Lambs were allowed to suckle *ad libitum* for the first 3 days after birth. There after, suckling was restricted to daytime, from 08.00 to 16.00, the lambs being separated in the evening in a nearby pen. Weaning was performed at day 60. Until then, with exception of the days 14, 28, 42 and 56, lambs had free access to their dam's milk in daytime. Lambs had access to water *ad libitum*, good quality alfalfa and concentrate. After weaning, the animals of the two groups were transferred to good quality pasture in spring and summer (June to September), as semi-nomadic system; all animals were hand milked once daily according to the tradicional milking routine until the end of lactation. After cleaning of the teats and teat ends, ewes were hand-milked and hand-stripto to remove residual milk from the udder.

Milk yields estimation and sample analysis
For individual ewes daily milk yield was measured at fortnightly intervals beginning on post-partum day 14 (i.e., on days 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154 and 168) by hand milking. On the days of milk recording, the lambs were separated from dams at 09.00 a.m. and the ewes maintained in a holding pen. At 10.00 a.m. each ewe was hand milked. Animals in group 1 received an intramuscular injection of 2.5 IU of oxytocin (Scanpharm-Denmark® 10 IU/mL) befor milking. Four hours later, each ewe was milked once more (in group 1 after second intramuscular injections of 2.5 IU of oxytocin) and his weight of this milk collection was recorded to the nearest gram on a sensitive laboratory scale. The amount of milk produced during a 4 h period, was multiplied by a factor of 6 as the estimation of 24 h milk yield (Fernandez *et al.* 2013). The milking period ended for each ewe when milk yield was < 100 mL on a recording day. Sheep with lactation shorter than 100 days were omitted from the analysis according to the procedure of milk recording scheme (ICAR, 2010). Daily milk yield was calculated using the following formula:

[(24/time between milkings) × (milke yield at 2nd milking)]

The sampling procedures were carried out during each milking. Preservative (potassium dichromate, Normapur, VW International, Strasbourg, France) was added to the milk samples, which were stored at -4 °C until analysis. The milk samples were thawed in a water bath (60 °C) and the percentages of fat, protein, lactose, ash and solid non-fat (SNF) were measured in duplicate (10 mL aliquots) using MilkoScan (Foss Electric Hillerød, Denmark).

Statistical analyses
Milk yield was recorded in each milking and total milk yield throughout lactation was calculated using Fleischmann method (ICAR, 2010).

$$TMY = y_1 t_1 + \sum ((y_i + y_{i+1})/2) \times (t_{i+1} - t_i)$$

Where:
TMY: total milk yield.
y_1: milk yield at first test day.
t_1: number of days between lambing and first test day.
y_i: milk yield of the test-day i^{th}.
t_i: number of days between the test day i and test day (i+1), (i=1,…, k).

Lactation persistency (P) was calculated (Keskin and Dag, 2006) by the following formula:

$$P\,(\%) = \frac{\sum_{i=1}^{k}\left(pi + 1\right)/pi}{k}\,100$$

Where:
Pi: yield of the record i that start at peak time.
k: record number from peak time to the end of lactation. Data were statistically analyzed by the MIXED procedure of SAS (SAS, 2009). Milk yield and composition were analyzed using repeated measures over time. Ewe weight at lambing was fitted as a covariate. The general model used was:

$$Y_{ijk} = \mu + A_i + B_j + (A \times B)_{ij} + E_{ijk}$$

Where:
Y_{ijk}: dependent variable.
μ: verall mean.
A_i: effect of evacuation method.
B_j: fixed effect of the stage of lactation j.

(A×B)$_{ij}$: interaction between treatment and stage of lactation.

Eijk: random residual error.

Least squares means, standard errors of the means and P-value in the model were reported and effects were considered significant at the probability of P < 0·05.

RESULTS AND DISCUSSION

Milk yield and lactation length
Least squares means of average daily milk yield, total milk yield, lactation length, peak yield, peak time and lactation persistency comparing oxytocin treated and controls are shown in Table 1. Average daily milk yield, total milk yield and lactation length were affected by oxytocin treatment. Oxytocin treatment group produced more average daily milk yield and total milk yield than the control group (P<0.05). Control ewes, milked without oxytocin, had shorter lactation periods than those milked with an oxytocin group (P=0.038). Total milk production calculated by Fleischmann method was 43% greater for oxytocin treated than controls (P<0.001). There was an increase in milk peak yield and lactation persistency in animals administrated by oxytocin (P<0.05), whilst shorter peak time was observed in the same group (P=0.043). The shape of lactation curves of Makui sheep after fitting the Fleischmann method is shown in Figure 1. The shape of the curves shows the development of an ejection inhibition throughout lactation.

As seen in the figure, in late lactation only slightly differences were detected between the groups because the ewes were more adapted to hand stimulation and higher amount of milk in the oxytocin group, corresponding to the volume of alveolar milk removed by oxytocin.

Stage of lactation and milk yield
Least squares means of an average milk production for oxytocin and control groups at three stages of lactation are presented in Table 2.

In the present study, stages of lactation had a significant effect on daily milk yield of ewes (P<0.001). Interaction between stages of lactation and treatment was significant (P<0.001). The average daily milk production for the three stages of lactation (early, middle and late) was 989.2, 580.1 and 235.7 g, respectively, in the oxytocin treated group, while it was 371.4, 286.6 and 150.3 g, respectively, for the control group.

Milk composition
Least-square means and standard errors of milking methods and stages of lactation on milk composition are presented in Tables 3 and 4. Oxytocin treated ewes possessed higher fat (P=0.029) and solid non-fat (SNF) percentage (P<0.05) in milk, whereas percentage of protein and lactose was similar among treatment groups (P>0.05). The lactation stages (early, middle and late) had a significant effect on milk fat, protein and SNF percentage (P<0.05) in either group (Table 4). Treatment did not affect the lactose percentage in milk at the three stages of lactation (P>0.05). However, with the progress of lactation, lactose percentage slightly decreased.

Hand milking is often routinely used in Mediterranean, Asia and Africa regions. The sheep udder morphology and milk partitioning, makes milk removal difficult because of the outlet duct (teat orifice) of the teat, which is often deviated from the edge of the gland cistern. Therefore, some milk might remain stored in the cistern and without hand milking is not expelled into the teat (Bruckmaier *et al.* 1997). In addition, suckling and hand milking stimulates nerve activity more efficiently than the machine milking, thus strengthen the release of oxytocin (Gorewit *et al.* 1992). Therefore, hand milking and hand stripping are more efficient for expulsion of milk from alveolar and cisternal fractions or residual milk withdrawal due to the indirect effect of oxytocin on stimulating milk ejection. Ewes easily adapt to routine milking in nomadic and intensive dairy production systems, although in ranching and in intensive lamb production systems ewes may be nervous, as it happens with unaccustomed ewe with out milking experience or with suckling or younger ewes. Therefore, to measure milk yield by hand milking, injection of oxytocin is recommended (Unal *et al.* 2007; Geenty, 2010). Oxytocin is a suitable auxiliary method to measure milk production, which is uncomplicated by the lamb appetite, particularly during early lactation and is useful for ensuring complete milk ejection (Doney *et al.* 1979). In the present study, oxytocin injection produced 43% more milk in treated than non-treated ewes (Table 1), in agreement with Awoniyi (2003) who showed that the total milk obtained with oxytocin injected sheep 54.71% was greater than control treatment. Banda *et al.* (1992) reported that throughout lactation period, oxytocin increased milk yield by 40.5% compared with weigh-suckle-weigh method or hand-milking without oxytocin injection. Aboul-Naga *et al.* (1981) indicate that milk obtained by hand milking without oxytocin administration was 31.6% less than weigh-suckle-weigh or oxytocin milking methods. Zamiri *et al.* (2001) showed that ewes' treatment with oxytocin before and after weaning, increased milk yield by 56% and 25% respectively. In another study, the administration of 3 IU oxytocin to ewes originated 17% more milk and an increase of 28% in the average total milk yield (kg/day) (Ribeiro *et al.* 2007). In our study, the oxytocin-treated group had longer lactation period (30 days) compared to the control ewes.

Table 1 Effect of oxytocin injection over the lactation performance and length in Makui ewes

Parameters	Control	Oxytocin	SEM	P-value
Total milk yield (kg)	38.5	93.6	16.46	0.019
Average daily milk yield (g)	301.4	562.3	115.29	0.001
Lactation length (day)	115.2	145.7	11.84	0.038
Peak yield (g)	393.5	1023.2	285.53	0.001
Peak time (day)	39.2	34.1	1.18	0.043
Persistency (%)	55.3	59.4	1.56	0.045

In the previous study by Zamiri *et al.* (2001) who stated that oxytocin treatment increased lactation length by 22%, corresponding to a 31 days longer lactation than control ewes.
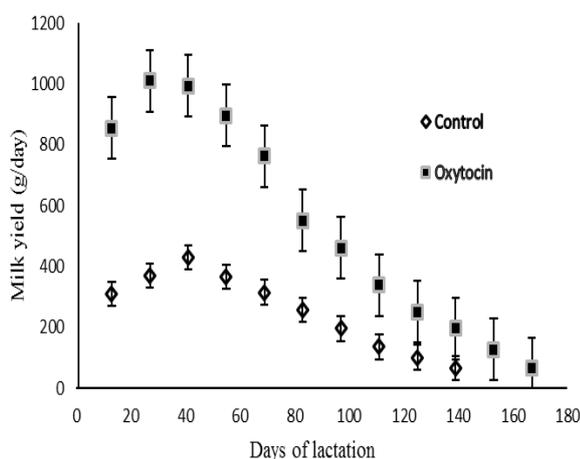
Figure 1 Lactation curve in oxytocin treated and control groups of Makui ewes

These results indicates that oxytocin is necessary for maintenance of mammary cells and lactation persistency as well as to induce the alveolar and residual milk ejection and thus completely empty the udder, which subsequently stimulates additional milk secretion through the alveoli emptying (Silanikove *et al.* 2010). It also confirms previous research by Marnet *et al.* (1998) who found that oxytocin stimulates milk flow from the lumen of alveoli to the cistern between milking. However, other studies showed oxytocin binds to specific receptors, located on mammary myoepithelial cells, which surround the alveoli and the small intralobular ductules that increase the secretory processes (Lollivier *et al.* 2006). In the literature, the effects of oxytocin over the mammary epithelial cell proliferation have been mentioned (Sapino *et al.* 1993; Wagner *et al.* 1997). The results of the present experiment and other results (Aboul-Naga *et al.* 1981; Zamiri *et al.* 2001; Awoniyi, 2003; Ribeiro *et al.* 2007; Antonic *et al.* 2013) demonstrate that, oxytocin directly affects productivity and the metabolism of the mammary epithelial cells. On the other hand, the osmotic balance of the secretory epithelium and secretory activity of the mammary epithelial cells may determine milk volume.

Perhaps oxytocin, by modulating glucagon and insulin secretion (Wallin *et al.* 1989), increases glucose availability to the mammary gland; alternatively, the direct stimulation of mammary blood flow may increase the mammary nutrient uptake. Some evidences suggest the existence of a plasminogen activator-plasminogen-plasmin and FIL based negative feedback that interferes with the casein secretory pathway and inhibits alveoli milk secretion (Burgoyne and Wilde, 1994; Silanikove *et al.* 2010) Moreover, it has recently been suggested that serotonin (Hernandez *et al.* 2008) and α-lactalbumin (Sharp *et al.* 2008) are participants of additional inhibitory feedback pathways of lactation.

Possibly, the normal physiological dose of exogenous oxytocin increases mammary blood flow, which in turn inhibits milk-borne negative feedback mechanisms around the alveoli and decreases tight junction permeability, thus preventing ion exchange and tight junction leakage and consequently increasing milk production.

Therefore, increase in lactation length and the galactopoietic effect associated to oxytocin injection might be explicated by the proliferation of mammary epithelial cell and its secretory activity, limiting the effects of inhibitory feedback mechanisms and increasing the intracellular casein transit that stimulates the contraction of myoepithelial cells (Sapino *et al.* 1993; Lollivier *et al.* 2002; Bencini *et al.* 2003; Lollivier *et al.* 2006).

Milk yield generally increases to the peak of lactation (about 25-40 days after lambing); afterwards it gradually declines until the end of lactation, depending on the breed, genotype, season, nutrition and management,. In the present study, peak time was observed at 34 and 39 days of lactation, respectively for the for oxytocin and control treatment groups. These findings were similar to those reported by Ribeiro *et al.* (2007), who refer that lactation peak occurred on the 37th and 35th days of lactation for controls and the oxytocin-treated group, respectively, though Zamiri *et al.* (2001) located the milk peak time at the 7th and 4th week of lactation, respectively for ewes injected with oxytocin and control ewes. In addition, Keskin and Dag (2006) established the real peak time for Akkaraman sheep at 35 days of lactation, using hand milking twice daily. Belgin *et al.* (2010) using the same method, reported the milk peak time for Awassi, Morkaraman and Tushin sheep breeds at 4.94, 5.68 and 5.23 weeks of lactation, respectively.

Table 2 Effects of oxytocin injection on milk production (g) at the three lactation stages (early, middle and late)

Parameters	Daily milk yield per ewes (g)		SEM	P-value	
	Control	Oxytocin		Treatment	Treatment × lactation stage
Lactation stages					
Early lactation (day 14 to 56)	371.4	989.2	237.52	0.001	0.001
Mid lactation (day 75 to 98)	286.6	580.1	183.84	0.001	0.001
Late lactation (day 99 to 168)	150.3	235.7	26.35	0.001	0.001

SEM: standard error of the means.

Table 3 Effects of udder evacuation methods over milk composition in Makui ewes

Parameters	Control	Oxytocin	SEM	P-value
Fat (%)	5.86	6.73	0.512	0.029
Protein (%)	4.80	4.82	0.003	0.471
Lactose (%)	4.53	4.50	0.001	0.673
Solid non-fat (SNF) (%)	11.24	12.66	0.927	0.034

SEM: standard error of the means.

Table 4 Oxytocin effects on milk composition at the three stages of lactation in Makui ewes

Lactation stage	Fat (%)		Protein (%)		Lactose (%)		Solid non-fat (SNF) (%)	
	Control	Oxytocin	Control	Oxytocin	Control	Oxytocin	Control	Oxytocin
Early	5.33	6.35	4.44	4.53	4.55	4.58	10.74	11.35
Middle	5.69	6.75	4.74	4.77	4.56	4.52	11.88	11.32
Late	6.58	7.81	5.16	5.28	4.45	4.43	11.76	12.85
SEM	0.359	0.625	0.129	0.094	0.021	0.015	0.869	0.975
P-value	0.026	0.033	0.045	0.049	0.726	0.845	0.044	0.037

SEM: standard error of the means.

This contrasts with data gathered in the present research, which showed that oxytocin treated ewes have shorter lactation peak time than control ewes.

Milk production was largely related to the shape of the lactation curve. The lactation pattern includes the peak yield, as the maximum daily yield reached during lactation, and lactation persistency that defines the rate of milk yield decrease after the lactation peak, which is an indicator of lactation performance. Figure 1 shows the lactation curves of two milking methods along milk yield, peak time, peak yield and days of lactation, which are affected by oxytocin treatment.

In both methods, milk production rapidly increased from the beginning to peak time; there after, the milk yield gradually decreased to the end of lactation. Lactation persistency is the ability of the sheep to maintain milk production at a high level after peak yield and is affected by some options, which include hormones, mammary secretory activity, number of mammary cells and nutritional status. In this survey, the lactation persistency was significant higher in the oxytocin group (P=0.045) than in control ewes (Table 1).

Nevertheless, oxytocin-mediated milk ejection is necessary to maintaining lactation persistency, peak milk production and total milk yield, while the consistent deficiency in milk ejection reflex reduces milk production by 35% throughout the lactation period (Marnet and McKusik, 2001). Accordingly, oxytocin administration, with increased cell numbers and maintenance of mammary epithelial cells secretory activity, increases lactation persistency.

In comparison to the control group, differences in the stages of lactation (early, middle and late) were highly significant (P<0.001), which agrees with other works (Maria and Gabina, 1993; Kuchtik *et al*. 2008; Novotna *et al*. 2009) reporting the significant effects of the lactation weeks on the total and daily milk yields. However, mammary tissues structural changes during pregnancy, lactation and the dry off as well as the stages of lactation significantly influence the dimensions of sheep udder. In fact, milk production is always positively linked with the width, length and depth of the udder (Fernandez *et al*. 1995). Even so, between 50 and 80% of ewe milk stored in the udder cistern compartment and larger cisterns plays an important role for milk collection and storage, which significantly affects milk ejection during milking (Marnet and McKusik, 2001). Increased milk yield from early lactation to lactation peak time is due to the increases in mammary epithelium differentiation while the decreases in milk yield after peak time are due to the loss of epithelial cells, decrease in the total DNA content of mammary parenchyma and in the mammary cell number (Capuco *et al*. 2001; Boutinaud *et al*. 2004). Sapino *et al*. (1993) and Wagner *et al*. (1997) suggested that oxytocin directly induces myoepithelial cell growth, cell differentiation and proliferation in the mammary gland by enhancing the effect of lactogenic hormones. All the same, prolactin is a lactogenic hormone, released during milking and suckling which is important in regulation of mammary cell growth and gene expression. It is possible that oxytocin, acting by stimulation of prolactin, may exert direct effects on the mammary gland structural

integrity, cell renewal and mammary epithelium secretory activity during lactation. However, oxytocin as a promoter, transfers milk from the alveolar lumen to the cistern gland (Lollivier *et al.* 2002). Carretero *et al.* (1999) found that mammary gland alveolar growth occurs in dairy sheep between week 1 and 5 of lactation and according to stage of lactation and the tubulogenic structure changes that result in an extensive proliferation of the canalicular system. Investigation shows that, during the suckling period at week 1 of lactation 'intussusceptive growth' occurs at lobular ducts, which increases the number of tubules (Carretero *et al.* 1999). Thus, the early lactation was expected to have greater daily milk yields than late lactation during the trial. It appears that oxytocin causes cell proliferation and regulates the secretory activity of mammary epithelial cells because mammary epithelial cells express oxytocin receptors and as oxytocin binds to these receptors (Lollivier *et al.* 2006) Thus, increases in epithelial cells of the mammary gland and their secretory activity do influence the shape of the lactation curve (Capuco *et al.* 2003).

In the present study, fat and solid non-fat (SNF) percentage differed in the oxytocin treated group (P<0.05), whereas protein and lactose percentage were unaffected. These values were consistent with Zamiri *et al.* (2001), Ribeiro *et al.* (2007) and Antonic *et al.* (2013) who reported an increase in fat percentage associated to oxytocin treatment.

According to Lollivier *et al.* (2002) oxytocin would transfer fat globules from the apical part of the epithelial cell into the cistern. *In vitro* study showed that oxytocin stimulates the agglomeration of milk components in the apical part of mammary epithelial cells and increases secretion rate of intracellular casein (Lollivier *et al.* 2006). Therefore, the fat content of alveolar milk is higher than cisternal milk and consequently oxytocin, due to the better alveolar milk removal, increases milk fat concentration. Our results and the increased fat and protein percentage in fresh milk (Zamiri *et al.* 2001) indicate that oxytocin with increased intracellular casein secretion directly or indirectly by stimulating prolactin secretion, regulates the fat and protein concentration of milk (Ollivier-Bousquet, 2002). However, differences were observed in the different stages of lactation concerning changes in milk composition in the oxytocin group from early to late lactation, whereby fat percentage increased from 6.35% in early lactation to 7.81% in late lactation and milk protein percentage increased from 4.53% in early lactation to 5.28% in late lactation (P<0.05). It was also variable in the control group, which was in agreement with available reports. Ploumi *et al.* (1998), Kuchtik *et al.* (2008) and Novotna *et al.* (2009) reported that fat, protein and SNF content of milk increased gradually with the progress of lactation.

Nevertheless, sheep milk composition is influenced by several non-genetic factors, including: breed, nutrition, stages of lactation, milking technique, lactation number, mastitis, management, climate, litter size and body condition. Nonetheless, fat and protein levels progressively decline to mid lactation, but then increase in the final weeks, while lactose shows a gradual decline across lactation. Da Costa *et al.* (1995) reported that oxytocin, acting by stimulation of myoepithelial cells, releases milk products already secreted in the mammary alveoli or removes the residual milk which presents higher fat content (Morgan *et al.* 2000). This behavior could be due to different regulatory mechanisms in milk secretion and in milk components; hence, short intervals between sheep milking would increase milk and fat secretion rate whereas after ewe milking, fat secretion rate decreases while protein increases (Bencini *et al.* 2003). Heesom *et al.* (1992) reported that feedback inhibitory pathways might regulate the lactose and casein synthesis while medium chain fatty acids act as inhibitors of fat synthesis. In contrast, Peaker and Taylor (1994) concluded that milk fat is not an inhibitor of milk secretion.

Yet, composition of milk is affected by changes in interval and frequency of milking (Bencini *et al.* 2003). However, in the absence of oxytocin, 75% of the milk fat remains in alveolar fraction (Marnet and McKusik, 2001). Moreover, oxytocin stimulates the intracellular transit of caseins in mammary epithelial cells and increases their secretion. Hence, it seems that oxytocin stimulates milk fat and proteins secretion rate (Lollivier *et al.* 2002; Ollivier-Bousquet, 2002). Maybe oxytocin and prolactin directly regulate milk and the milk component secretion rate, or perhaps other feedback mechanisms exist for regulating milk and milk composition that have yet to be discovered. However, the possible regulating effect of oxytocin on milk and component secretion needs the further investigation.

CONCLUSION

Oxytocin administration significantly increased daily milk yield, fat percentage and lactation length; producing 43% more milk throughout the lactation. Hand milking and hand stripping, due to the indirect effect of oxytocin, stimulate milk ejection, withdrawal of residual milk; therefore, hand milking and hand-stripping after oxytocin injection, are more efficient for complete expulsion of milk from alveolar and cistern fraction. This result demonstrates the necessity of oxytocin for removal of alveolar milk to increase milk yield in lactating ewes and confirms that oxytocin presents a galactopoietic effect on lactating mammary tissue, firstly due to the acceleration of the intracellular transfer of secretory vesicles and secondly due to the contraction of

myoepithelial cells, limiting the milk-borne negative feedback mechanisms on milk secretion. However, in non-dairy sheep breeds, the milking procedure should be applied carefully with accurate results. Meanwhile, when oxytocin technique is applied for measuring milk secretion rate of lactation ewes, the following options should be considered: 1) Isolation of lambs from the dams may affect milk production. 2) Oxytocin has a short half-life in the body, therefore milking should be done as fast as possible without causing stress or pain. 3) Oxytocin method may overestimate milk yield during early lactation. 4) Milk secretion rate may be affected by short or long test period. 5) Frequently administration of the oxytocin may not give reliable results. 6) For removal complete of alveolar milk, ewes must be trained to milking routines. 7) At the start and at the end of milk recording period, the udder should be similarly evacuated. 8) During the milk recording period, to achieve an uniform milk secretion rate, the procedure must be performed at a specified time. 9) Using high dosage of oxytocin may have negative effect on milk yield and changes milk composition.

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