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Impact of parity, stage of lactation, and subclinical mastitis on the concentration of vitamin c in Shami camel milk

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Abstract

A study was conducted at Deir Al-Hajar Research Station for Shami Camels, Administration of Livestock Research, General Commission for Scientific Agricultural Research (GCSAR) during 2019 and 2020 to find the relation between vitamin c concentrations in milk, and either stage of lactation, parity (lactation number) or the presence of mastitis. For this purpose, thirty lactating Shami camels from different parties were used. Milk samples were collected monthly over one entire lactation season lasting 11 months. The concentration of vitamin c was 35.01±9.81 mg/l milk during considered parities without significant differences in its concentration. However, vitamin c concentration was affected by lactation month P<0.0.5, and the values ranged between 18.99 and 53.03 mg/l milk during lactation. An evident decline in vitamin c concentration in camel milk appeared with positive interactions for the California test. The average concentration of vitamin c in milk produced from healthy and infected udders was 35.01±9.81 mg/l and 22.99±1.30 mg/l, respectively. It has been concluded that vitamin c in milk is crucial indicator of udder health status

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Introduction

Vitamin c (ascorbic acid) is one of the water-soluble vitamins (1), and its composition in mammals depends on glucose content in the liver (2). It is available in two forms of biological importance, one of which is reduced and called L-Ascorbic acid and is the most biologically active form, and the other is the oxidizing form L-dehydroascorbic acid, which has a biological value as well (3). Due to the intense oxidation process in the rumen, a large part of vitamin c is converted to diketogulanic acid which has no vital functions, making them more susceptible to diseases associated with vitamin c deficiency as scurvy and mastitis (4). Camel milk has a relatively high vitamin c content compared to that of other farm animals, and it is 3-5 times that of cow's milk, which gives it high nutraceutical values, especially for people living in desert areas (5).The importance of this high content of this compound is that it is a powerful antioxidant that prevents bacterial growth in milk and keeps it from spoilage as well as its essential role in stimulating collagen protein secretion by hydrogenating lysine and proline amino acids (6). Vitamin c concentration in camel milk varies 15-435 mg/l (7), and this variation in vitamin c concentration is due to the effect of several factors such as the breed. Thus, vitamin c concentration in the milk of double-humped camels (*Camelus bacteriaus*) was higher than those of dromedary (*Camelus dromerdaries*) and hybrid camels. These concentrations valued at 169, 146 and 133 mg/l in double-humped, dromedary and hybrids, respectively (8), in addition to other factors such as quality change of feed consumed per season (9) and stage of lactation, as the colostrum has a relatively high content of vitamin c compared to regular milk (10). The health status of the udder is one of the most influential factors in the variation of

vitamin c content in camel milk by more than 45%, as was mentioned by Mohamed *et al.* (10). This study aims, in light of the pervious, to study the effect of milk production month, parity, and subclinical mastitis on vitamin c level in the milk of Shami camels in order to establish an identity for vitamin c level, whether as an essential indicator of the health status of the udder or as a nutraceutical element of Shami camels.

Materials and methods

Study location and animals

The study was conducted at Deir Al-Hajar Research Station for Shami Camels of Livestock Research Administration at the General Commission for Scientific Agricultural Research during the 2019 and 2020 seasons. It was carried out on 30 Shami milking camels ages 5-20 years from different milking seasons; from the first to the sixth parity, with five animals in each group. Animals were selected at the end of the gestation period and randomly from the original herd.

Feeding the study animals

Maintenance and production nutritional requirements were provided for the camels according to milk amount produced during milk production season until drying off. So, camels were fed twice daily at 9.00 am and 6 pm with concentrate (barley, extracted cottonseed meal, bran, minerals and vitamins), and hay was provided in the evening and grazing the animals daily the fodder barley crop for 5 hours.

Sheltering animals

Animals are housed freely in semi-open pens, where free water and concentrated feed are provided in two batches, morning and evening.

Milk sampling

Milk samples (25 ml) were taken from each camel separately at a rate of once a month until the end of milking season (lasting approximately eleven months in lacing Shami camels), after mixing the milk produced by manual milking. Vitamin c level in those samples was estimated.

Estimation of vitamin c in milk samples

Vitamin c was estimated by chemical titration (11), which is based on oxidation-reduction using 2.6 chlorophenol indophenol dye in which ascorbic acid is oxidized to dehydrogenated ascorbic acid and the dye (blue color) is reduced to pink color.

Methodology

Titration of milk sample: 5 ml of milk sample is diluted by adding 15 ml of 6% trichloroacetic acid, and then filtered. Later, 10 ml of filtrate is taken and 10 ml of 0.4% oxalic acid is added, then dye-titration is done until the appearance of pink color. Titration of vitamin c: A standard solution of vitamin c at a concentration of 0.2 mg/l is prepared by dissolving 20 mg of ascorbic acid in 100 ml of oxalic acid at 0.4% concentration, then 10 ml of that standard solution is taken and dye-titrated until the appearance of pink color. Titration is repeated three times for the sample and the control, and the average volume is taken. The following relation calculates the concentration of ascorbic acid in the samples; Ascorbic acid mg/l = (average volume of dye consumed by sample / average volume of dye consumed by the standard solution of vitamin c) x concentration of the standard solution of vitamin c.

California mastitis test (CMT)

1 ml milk was placed in a test cup, 1 ml California CMT test reagent (volume of milk = volume of reactant) was added and stirred well to mix milk with CMT reactant. The results were read according to Brookbanks (12) method which was either; negative: when the milk remains liquid and grey, the milk is from udders not infected with mastitis, or positive with varying degrees: when there is a soft or thick sticky jelly, sticky lumps, or a gel of white egg texture with a change in color of milk between light violet pink and dark violet, and this implies that the milk is produced from udders infected with subclinical or clinical mastitis in varying degrees.

Statistical analysis

The study indicators were compiled in unique tables in Excel, and SPSS v.25 was used to perform the statistical analysis according to variance analysis ANOVA. The least significant difference at confidence level 5% (L.S.D 5%) was estimated. The results were expressed as arithmetic mean X ± standard deviation S.D.

Results

The general average of vitamin c

The average concentration of vitamin c in milk produced from healthy udders was 35.01 ± 9.81 mg/l, and ranged between 18.99-53.03 mg/l milk during its measurement period. Its average concentration in camel milk that showed positive reactions to the California test was 22.99±1.30 mg/l.

Analysis of variance for vitamin c

The results of the analysis of variance in table 1 revealed a significant effect ($P<0.05$) for the stage of lactation and health status of the udder on the average concentration of vitamin c.

Table 1: The effect of lactation stage and health status of udder on vitamin c concentration

Variance sources	*fd*	The average sum of deviation squares	LSD	SE
parity	5	1.16	-	0.54
Stage of lactation	10	1356.42*	0.76	
Health status of udder	4	414.77*	1.06	1.65

* Implies that there is a significant difference at level P<0.05.

The effect of parity on vitamin c concentration

Table 2 shows vitamin c concentration by considered parities. It is noticed that vitamin c concentration was not affected by parity.

Table 2: Vitamin c concentration (mg/l ± S.D.) from the first to sixth parity

Parity	Mean ± SD
1	33.01±10.05
2	33.83±9.90
3	34.79±9.81
4	35.49±9.74
5	36.15±9.67
6	36.80±9.60

The effect of milk production month on vitamin c concentration

Table 3 shows vitamin c concentration by milk production month. It is noticeable that there is a significant change P<0.05 in vitamin c concentration by milk production month, where the highest value reached 53.03 mg/l in the first month and the lowest was 18.99 mg/l in the sixth month of lactation.

The effect of the health status of the udder on vitamin c concentration

Table 4 shows variation in vitamin c content according to the California test results conducted on all milk samples collected to detect subclinical mastitis of milking camels.

Table 3: Vitamin c concentration (mg/l ± SD) according to milk production month

Milk production month	Mean ± SD
1	53.03k±1.70
2	33.14e±2.32
3	31.90d±2.57
4	25.04c±1.13
5	22.25b±1.20
6	18.99a±1.90
7	41.69i±1.20
8	44.53j±0.93
9	36.03f±0.58
10	38.05g±0.67
11	40.50h±0.82

Different letters indicate significant differences at P<0.05.

Discussion

The average concentration of vitamin c of health status camel milk corresponds to that of some other international studies carried out on one-humped camel milk. Average concentration reached 33±1.7 mg/l (9), 37.4 mg/l (13), and 25-60 mg/l (14), while it was about 44 mg/l (10). Parity did not significantly affect its concentration, and its level remained normal ranging between 33.01 mg/l and 36.80 mg/l, and this was also shown by Mohamed *et al*. (10) on the Arabian camel in Sudan that a slight effect of parity was observed.

The results showed that the highest concentration of vitamin c in camel milk was in the first month of lactation season. This was also stated by Konuspayeva *et al*. (15), reporting that the first weeks of lactation are associated with high vitamin c content in the milk of considered camels, and this may be because milk is an essential source to meet the newborn's needs of this element during that age stage. It was also found that the newborns cannot synthesize vitamin c with high self-efficacy until they reach 4-week age (16).

Table 4: Vitamin c concentration (mg/l ± S.D.) in experimental animals according to California test scores

Milk texture	Symbol	Interaction	Mean ± SD
Unchanged	O	Negative	35.02a±9.81
Formation of a light viscous residue that disappears with continuous movement	T	Impact	30.09b±0.46
distinct, separate mucus residue disappears with continuous movement	+1	Poor positive	24.50c±0.70
Direct thick mixture with the appearance of a symptom of gel formation	+2	Apparent	21.90d±0.80
Jelly (convex gel form)	+3	Strong	17.52e±0.42

Figures with different letters within the same column indicate significant differences at the 5% level using the L.S.D test.

It should also be noted that vitamin c content decreased significantly during the period extending from the fourth to the sixth month, and this may be due to the increase in milk produced during that stage of lactation and got the same result Konuspayeva *et al.* (7). In Kazakhstan, on different breeds of camels, the lowest concentrations of vitamin c were recorded in spring, when milk production reached its highest levels. There are also similar results on the *Anafi* camel breed in Sudan (17), where milk production declined in dry seasons and increased vitamin c concentrations in the considered camel milk compared to those in the wet seasons. This may be because vitamin c composition depends on D-glucose or D-galactose through the glucuronic acid cycle. The demand for sugar lactose increases in high-producing animals for increasing milk production, negatively affecting the vitamin c content of milk (18).

It is noticeable that the concentration of vitamin c decreased by 20-50% in the milk samples that showed positive results for the California test compared with those that gave adverse reactions during the same stage of lactation. This result is in line with that of Mohamed *et al*. (10) on the Arabian camel in Sudan, where there are significant differences in vitamin c content in camel milk according to the health status of the udder, and its average concentration reached 47.4 mg/l in the milk produced from healthy udders versus 26.8 mg/l in the milk produced from udders infected with mastitis. In another study conducted on Indian cows to reveal the relationship between vitamin c concentration and health status of the udder, it was found that the animals showed clinical or subclinical symptoms of mastitis when treated by injecting doses of vitamin c under the skin for five consecutive days (at the rate of 25 mg ascorbic acid/kg live weight). The health status of their udder has improved with an efficiency exceeding that of antibiotics (19). Researchers think that high concentrations of vitamin c have an essential role in activating the defensive neutrophil functions in terms of migration, adherence, phagocytosis and cytocidal actions, as it is a powerful antioxidant capable of donating an electron, thus neutralizing the highly electronegative free radicals released by neutrophils while performing their defensive functions (20).

Conclusions

It is possible to benefit from camel milk at early lactation season, as vitamin c concentrations are high, whether for nutrition or treatment of some diseases. In addition, subclinical mastitis symptoms in camels are associated with a deficiency of vitamin c in milk, so the level of vitamin c in milk is an important indicator to predict the health status of the udder. More extensive research is required to confirm the critical role of camel's supplementation with vitamin c either by nutrition or by injection in immune function and thus preventing mastitis, which causes losses in lactating herds.

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Conflict of interest

All the authors declare that there is no conflict of interest regarding the publication of this manuscript, and it is to the advantage of the General Commission for Scientific Agricultural Research that was the only authority funding it.

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تأثير موسم الحلابة ومرحلة إنتاج الحليب والتهاب الضرع تحت السريري في تركيز فيتامين ج في حليب الإبل الشامية

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الخلاصة

نفذت الدراسة في محطة بحوث دير الحجر للإبل الشامية التابعة لإدارة بحوث الثروة الحيوانية في الهيئة العامة للبحوث العلمية الزراعية خلال عاميّ ٢٠١٩ و ٢٠٢٠. بهدف تحديد تركيز فيتامين ج في حليب النوق. استخدم لهذا الغرض ثلاثون ناقة من سلالة الإبل الشامية من مواسم حلابة مختلفة. جُمعت عينات حليب شهرياً خلال موسم حلابة كامل والذي امتدَّ إحدى عشر شهراً. لم يلاحظ وجود فروق معنوية في تركيز فيتامين ج بين مواسم الإدرار المدروسة، في حين لوحظ أن تركيز فيتامين ج تأثر بشهر الإنتاج وتراوحت القيم خلال موسم الحلابة بين ١٨,٩٩ و ٥٣,٠٣ ملغم/لتر. كما ظهر تراجعاً واضحاً في تركيزه في حليب النوق التي أظهرت تفاعلات إيجابية لاختبار كاليفورنيا، إذ بلغ متوسط تركيز فيتامين ج في الحليب الناتج عن الضروع السليمة والمصابة نحو ٣٥,٠١±٩,٨١ و ٢٢,٩٩±١,٣٠ ملغم/لتر على التوالي. ومن هنا يمكن القول إن مستوى فيتامين ج في الحليب قد يكون مؤشراً هاماً في الكشف عن الحالة الصحية للضرع.

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