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Original article

Seasonal variations of vitamin A, D and E levels in serum of female camels (*Camelus dromedarius*) and their calves raised in five geographic regions of Saudi Arabia

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ABSTRACT

The aim of this study was to investigate the serum level of fat-soluble vitamins A, D and E in clinically healthy lactating female camel (*Camelus dromedarius*) and suckling calf > one-year-old during winter and summer seasons in five main regions of Saudi Arabia. 60 sera samples were collected and tested for vitamins A, D and E levels and the results were statistically analyzed. The statistical mean value of vitamin A was within the reported range but for D and E, there were minor variations. The effect of season was insignificant ($p > 0.05$) for vitamins A and E in the combined results of the dam and newborn together. This seasonal effect was highly significant in dam serum ($p < 0.05$). Region effect was significant for vitamin A in the northern area ($p < 0.05$) and for vitamin E in the southern region ($p < 0.05$). Correlations analysis revealed significant results in the season vs vitamin A and E $p < 0.05$. Mean values of vitamins A, D and E in dam and newborn did not observe significant variations however, in the season and regions there were significant variations which can be attributed to the climate difference, availability of balanced rations and camel management in each location of the five main regions of Saudi Arabia. There is a great need for further studies and the consequent development of supplementation programs and camel feed manufacturers awareness of such results is highly recommended.

1. Introduction

In adult female camels and the newborn calf, vitamin A plays an important role in cellular differentiation and is involved in the formation and protection of epithelial tissues and mucous membranes (McDonald et al., 2010). In camels likewise other animals and humans, vitamin A has many physiological functions in vision by being important in the regeneration of visual purple and necessary for dim light vision (Mashhadi et al., 2013). The well-known role of vitamin A (Retinol) in reproduction performance is not fully investigated in camel (Clagett-Dame and Knutson, 2011). Vitamin A has been reported an important role in certain camel diseases such as Camel mange and (Fassi-Fehri, 1987; Lyaktini et al., 2013; Palanivelrajan et al., 2015) Night blindness (Faye et al., 2019; Faye and Mulato, 1991; Gebrehiwet, 1998; Mohamed et al., 2018).

Vitamin D (Cholecalciferol) deficiency was also reported in camels to cause bone disorders such as osteomalacia and rickets during the growth period (Riad et al., 1994). Investigators reported that the circulating level of 25-OH-D was lower in winter than in summer and since the rutting period in winter so this may have suggested that vitamin D doesn't appear to control the

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psychological-neurological-endocrinological processes of camel reproduction directly or indirectly (Mohamed et al., 2018). Riad et al. (1994) reported that the serum level value of vitamin D in non-lactating and non-pregnant camel was 83.5 ± 4.5 ng/100 ml and in lactating camel was 123.8 ± 7.5 ng/100 ml.

Vitamin E (tocopherol), is a fat-soluble vitamin which is widely distributed in fodders, young grasses, leaves and cereals. The main deficiency manifestation of vitamin E is white muscle disease (WMD) and young camels in the Gulf area are very susceptible to this deficiency (Al-Qarawi et al., 2001; El-Khouly et al., 2001; Seboussi et al., 2004). Vitamin E also protects the blood cell membranes from oxidation by free radicals (Bednarek et al., 1996). The normal level of vitamin E in camel plasma was analyzed in 1064 camels and it was 194 ± 70 ug/100 ml (Wernery et al., 2009).

Some of the research described the effect of season on the fat-soluble vitamins A, D and E such as the level of vitamin A in camel blood was 67.54 ± 6.76 ug/100 ml in winter and in summer it decreased to 48.7 ± 2.3 ug/100 ml (Baraka, 2012). One of the studies reported that vitamin A in the rainy season (July to October) in camel blood was 49.3 ± 8 ug/100 ml while in the dry season, it reduced to 40.0 ± 6.0 ug/100 ml. Vitamin D level variations in different seasons were observed higher in February- July and lowest in August –January (Mohamed, 2008). As for vitamin E variation according to the season was reported to be higher in the rainy season at 230 ± 50 ug/100 ml and dropped in the dry season to 140 ± 40 ug/100 ml (Mohamed, 2006). Moreover, geographical location also plays a key role in the milkconstituents of a camel (Faraz et al., 2020).

The objectives of this study were to investigate and analyze the three fat-soluble vitamins in the blood serum of the dam and newborn camel in five main regions of Saudi Arabia and in two main seasons and to give basic data and information about the current status of the levels of these vitamins. To investigate the effect of season and region on these vitamins and to assess the importance of the results obtained. According to a literature search, no studies comparing the amounts of fat-soluble vitamins in the serum of lactating female camels and their calves throughout different seasons and different locations have been done.

2. Materials and methods

2.1. Samples locations coordinates

Each region selected a known farm or camel collection enclosure with a stable location and owners cooperated and allowed blood samplings in two seasons and all five regions. They were as follows, Al-Jenadriyah location for the central, Dammam for the eastern, Khamis-Mushait for the southern, Sakaka for the northern, and Wadi- AlFora'a for the western region, (Fig. 1).

Locations of sampling sites. The background image obtained from the Natural Earth Raster Data (https://calysteau.fr/data/Natrural Earth Raster/Natural Earth 1/).

2.2. Weather summary

Saudi Arabia is the country with deserts except to the southwestern part where semi-arid lands are present. The central region represents severe hot and dry temperatures especially in summer, followed by inland regions ranging from 27 °C to 43 °C and the coastal areas with 27 °C to 38 °C. However, winter temperature falls between 8 °C and 20 °C in the interior areas whereas the coastal region of the Red Sea recorded higher temperatures (19 °C to 29 °C). The daily average high and low air temperature within the sampling sites, Source (https://weatherspark.com/compare/104953- 101,549 - 104,018 - 101175/Comparison- of-the-Average-Weather-in-Riyadh-Dammam-Khamis mushait-Sakaka and Madina), (Fig. 2A). The southwestern parts documented 400 – 600 mm of average annual rainfall while it was<150 mm in most of the areas of the country. Annual accumulated rainfall (mm/year) for Saudi Arabia based on ERA5 reanalysis, source: ERA5 global reanalysis https://www.ecmwf.int/en/forecasts/datasets/reanalysis-datasets /era5, (Fig. 2B).

2.3. Samples collection

Followed the ethical guidelines and obtained approval from the Deanship of Scientific Research, King Faisal University regarding the use of animals for the research work (KFU-REC-2023- JAN-ETHICS479). Actual seasonal sample collections were performed in December and January during winter, while June and July in summer, those two months selected in each season represented the average temperature readings of the related seasons (Fig. 2A). In each location we have selected a permanently settled breeder with at least 50 dams and 20 newborns at each collection time, with the help of local veterinarians and collaborations (Fig. 1). During the sample collection, the dams along with newborns were isolated for blood collection. In some locations with good farm management and friendly camels' sampling was done in a standing position from the jugular vein or milk vein with little effort from the camel shepherd. A sterile syringe of 20 ml with an 18G needle was used to puncture the vein and about 10 ml of blood was collected and instantly transferred to a sterile serum separation bottle. Sample data for each location containing date, season, location name and owner name and kept the samples in the ice box. Sera separation bottles were kept in an ice box and transferred to the laboratory. The blood samples were centrifuged at 3000 rpm for 5 min and the sera were collected and stored in a freezer at −20′C.

2.4. Laboratory analysis

Frozen Samples were sorted out to represent each location, season and camel type equally, so 30 samples for each season were received (6 samples from each location divided into 3 for a dam and 3 for a newborn). A total of 60 sera samples have been tested for serum value of vitamins A, D and E by High-performance Liquid Chromatography (HPLC) (Castle and Cooke, 1985). The test apparatus was Shimadzu HPLC, CR4A CHROMATOPAC with SCL6A system controller. The column was C18 ODS reversed phase with CTO oven. UV visible detector SPD 6AV. The pump was LC 20 AD model (McDowell, 2000).

2.5. Data analysis

Categorical variables were expressed as the number of cases and proportions. Continuous variables were expressed as mean ± standard deviation. Missing data were analyzed using multiple imputations under the missing at random assumption. Sensitivity analysis was used to test this assumption. For comparing mean vitamin levels in dams and newborns, and comparing the effect of season, we used Student's *t*-test. To assess the effect of region on vitamin levels, used Analysis of Variance (ANOVA) test. Correlation analysis was performed to explain the relationship between the parameters investigated. A α level of 0.05 was set, and a $p < 0.05$ was considered statistically significant. SPSS Statistics v.28 (IBM, USA) was used to analyze the data.

3. Results

Mean values ± SD of vitamins A, D and E in the blood sera of the dams were 37.6 ± 24.5 μg/100 ml, 82.4 ± 8.3 ng/100 ml and 80.8

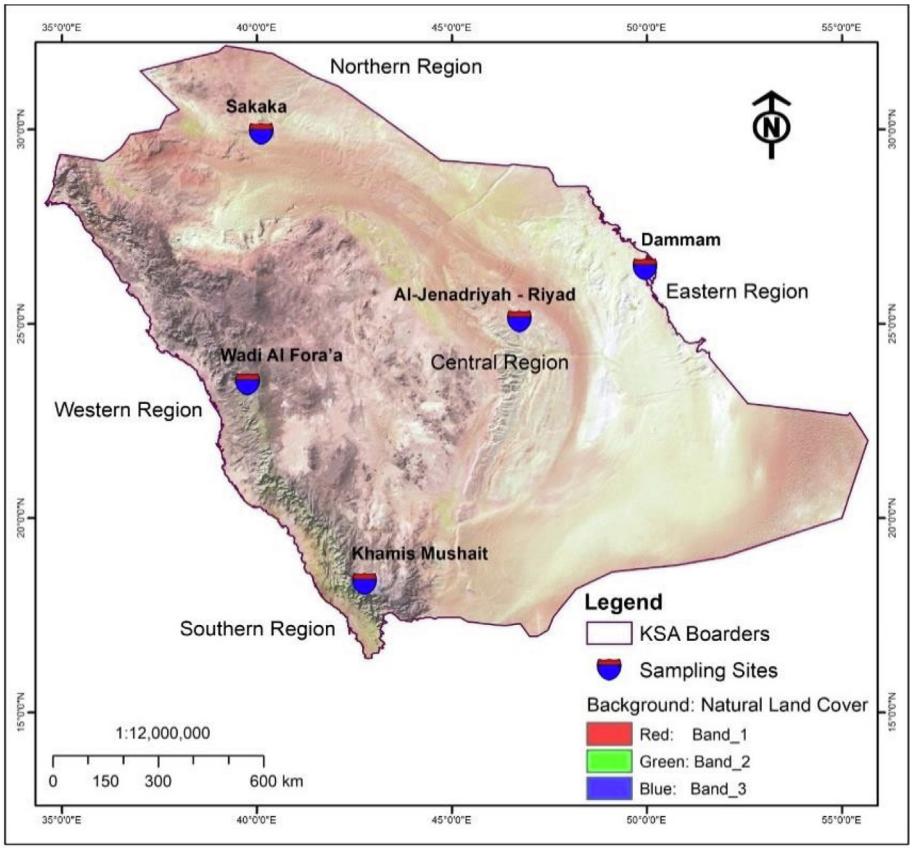

Fig. 1. Collection Locations Coordinates, Saudi Arabia.

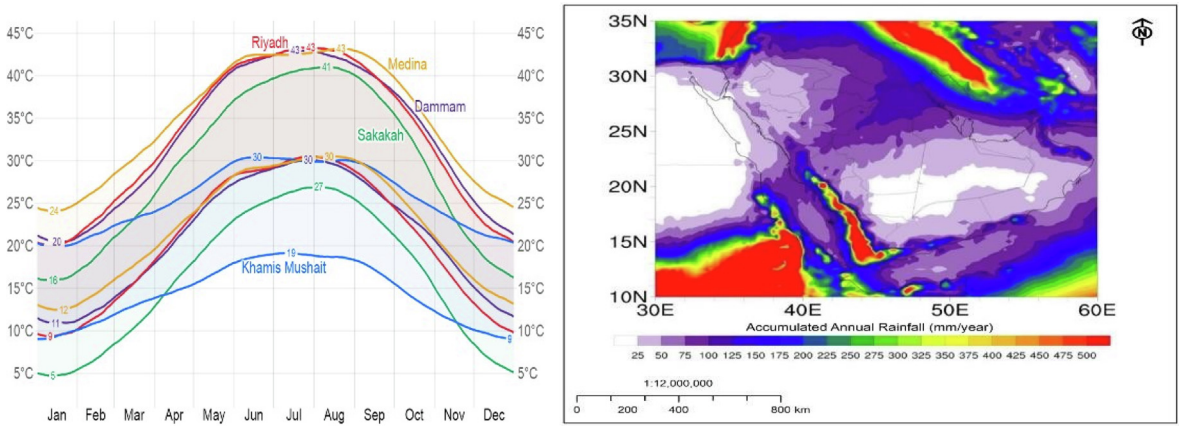

Fig. 2. **A**) Daily average, high and low air temperature. **B**) Annual accumulated rainfall (mm/year).

± 44.0 μg/100 ml respectively. The same vitamins levels in newborns were 36.7 ± 17.2 μg/100 ml, 80.2 ± 15.5 ng/100 ml and 74.7 ± 43.6 μg/100 ml respectively, (Table 1). When the $p > 0.05$ is considered an insignificant relation.

On analysis of vitamins A, D and E in blood sera of dams and newborns (combined together) and from all locations versus season (winter and summer) results of p-value revealed significant relation for vitamin A and vitamin E ($p < 0.05$), (Table 2). As mentioned earlier for comparing serum levels of vitamins A, D and E vs season and locations performed Student's t-test, and any result $p < 0.05$ was considered statistically significant.

Statistical analyses from the results of vitamins A, D and E of dam sera alone versus season, revealed high values of Vitamin E in winter $p < 0.05$ (Table 3). The same was done for the values of vitamins A, D and E in newborns sera but no significant difference was observed (Table 4).

Table 1
Mean values of vitamins A, D and E levels in blood serum of dams and newborns.

Animal Type	Vitamin A mean ± SD (n)	Vitamin D mean ± SD (n)	Vitamin E mean ± SD (n)
Dam	37.6 ± 24.5 (30)	82.4 ± 8.3 (30)	80.8 ± 44.0 (30)
Newborn	36.7 ± 17.2 (27)	80.2 ± 15.5 (27)	74.7 ± 43.6 (27)
p-value	0.874	0.508	0.607

* p-value calculated using unpaired Student's t-test. SD = standard deviation.

Table 2
Mean values of vitamins A, D and E in serum of both dam and newborn (combined) vs season.

Season	Vitamin A mean ± SD (n)	Vitamin D mean ± SD (n)	Vitamin E mean ± SD (n)
Winter	43.3 ± 24.7 (28)	81.0 ± 15.03 (28)	89.8 ± 40.1 (28)
Summer	31.3 ± 15.3 (29)	81.6 ± 8.9 (29)	66.4 ± 44.3 (29)
p-value	**0.03**	0.85	**0.04**

* *p*-value calculated using unpaired Student's *t*-test. SD = standard deviation.

ANOVA test was used to check the impact of location on the vitamins A, D and E serum levels of dams and newborns (combined). Vitamin A, which was a statistically significant value ($p < 0.05$), (Table 5).

To highlight further, the effect of different locations on the vitamins A, D and E in the dam and newborns separately, vitamin A in the northern location (Sakaka) and vitamin D in the southern location (Khamis-Mushait) revealed statistically significant values of $p < 0.05$, (Table 6).

Finally, vitamins A, D and E sera values vs camel (dams and newborns together), seasons and locations parameters, a positive correlation was detected of vitamin A and E with p value<0.05. These correlations have been summarized in Table 7.

4. Discussion

Camel milk has been reported with therapeutic against many diseases and rich source of nutrition which makes it in high demand (Konuspayeva et al., 2022, 2021). This study analyzed the variations of different vitamins in dromedary camel and their calves, seasonal and geographical impact on the vitamin composition.

The mean value ± SD of vitamin A was analyzed using the unpaired Student's *t*-test, a serum value of 37.6 ± 24.5 μg/100 ml for the dam and 36.7 ± 17.2 μg/100 ml for the newborn has been reported (Table 1). These values of both dam and newborn are within the suggested average range by Faye and Benogoumi which was between 30 and 70 μg/100 ml (Faye and Bengoumi, 2018). Also, these mean values were similar to those reported in some of the studies, (Baraka, 2012; Homeida et al., 2010; Mashhadi et al., 2013; Mohamed, 2006; Wernery et al., 2009). However, one of the studies reported that the weight of newborns is independent of serum minerals in dromedary camel (Essawi and Gouda, 2020). A mean value ± SD of 173 ± 5.1 μg/100 ml was reported in one of the studies however, such a high value is rare in research data (Al-Senaidy, 1998). A study comparison between dam versus newborn vitamin A serum values has resulted in a $p > 0.05$ (Table 1). Statistical analysis of both dam and newborn versus season has revealed $p < 0.05$ (Table 2) which is a significant one, however, when analyzed individually dam vs season and newborn vs season and were of no statistical significance ($p > 0.05$) (Table 3 and Table 4). Table 2 represents vitamin A value as higher in winter than in summer with a significance which may be attributed to the fact that in Saudi Arabia winter is the season of rain, so the grazing areas are of better desert plants and also the availability of cereals and cereals byproducts resulting in more sources of camel feed and almost in all Saudi Arabia locations. Serum vitamin A in the dam and newborn (combined) versus the five locations revealed a statistically significant relation $p < 0.05$ (Table 5). Analysis of the dam and newborn vitamin A versus each location separately, a statistical significance observed in the northern region 52 ± 24.3 μg/100 ml in the dam versus 21.8 ± 8.4 μg/100 ml in

Table 3
Mean values of vitamins A, D and E in serum of dam vs season.

Season	Vitamin A mean ± SD (n)	Vitamin D mean ± SD (n)	Vitamin E mean ± SD (n)
Winter	41.5 ± 17.8 (15)	78.6 ± 20.2 (15)	94.6 ± 40.3 (15)
Summer	30.8 ± 15.2 (12)	82.2 ± 6.6 (12)	50.0 ± 35.0 (12)
p-value	0.11	0.57	**0.006**

* *p*-value calculated using unpaired Student's *t*-test. SD = standard deviation.

Table 4
Mean values of vitamins A, D and E in serum of newborn vs season.

Season	Vitamin A mean ± SD (n)	Vitamin D mean ± SD (n)	Vitamin E mean ± SD (n)
Winter	45.5 ± 31.5 (13)	83.8 ± 4.1 (13)	84.4 ± 40.8 (13)
Summer	31.6 ± 15.9 (17)	81.3 ± 10.4 (17)	78.0 ± 47.3 (17)
p-value	0.12	0.41	0.70

* *p*-value calculated using unpaired Student's *t*-test. SD = standard deviation.

Table 5
Mean values of vitamins A, D and E levels in both dam and newborn (combined) vs Location.

Location	Vitamin A mean ± SD (n)	Vitamin D mean ± SD (n)	Vitamin E mean ± SD (n)
Central	45.5 ± 26.5 (12)	81.2 ± 26.5 (12)	60.9 ± 39.6 (12)
Eastern	31.6 ± 12.2 (12)	81.2 ± 12.2 (12)	86.4 ± 41.6 (12)
Southern	42.5 ± 23.3 (11)	82.9 ± 23.3 (11)	86.7 ± 40.1 (11)
Northern	34.2 ± 22.2 (10)	76.0 ± 22.2 (10)	80.7 ± 42.4 (10)
Western	32.3 ± 19.1 (12)	84.7 ± 19.1 (12)	75.9 ± 54.1 (12)
p-value	**0.03**	0.57	0.60

**p*-value calculated using ANOVA. SD = standard deviation.

Table 6
Mean values of vitamins A, D and E levels of blood serum in dam and newborn vs location.

Vitamin Type	Newborn mean ± SD (n)	Dam mean ± SD (n)	*p*-value*
Vitamin A			
Al-Jenadriyah	34.1 ± 19.0 (6)	56.8 ± 29.6 (6)	0.14
Dammam	31.4 ± 10.7 (6)	31.7 ± 14.5 (6)	0.96
Khamis-Mushait	41.3 ± 17.1 (5)	43.5 ± 29.1 (6)	0.88
Sakaka	52.7 ± 24.3 (4)	21.8 ± 8.4 (6)	**0.01**
Wadi- AlFora'a	30.2 ± 12.6 (6)	34.3 ± 25.1 (6)	0.72
Vitamin D			
Al-Jenadriyah	81.3 ± 7.1 (6)	81.1 ± 6.3 (6)	0.96
Dammam	80.0 ± 6.6 (6)	82.3 ± 11.4 (6)	0.68
Khamis-Mushait	86.9 ± 6.2 (5)	79.6 ± 8.9 (6)	0.16
Sakaka	63.7 ± 37.3 (4)	84.1 ± 10.8 (6)	0.23
Wadi- AlFora'a	84.7 ± 2.6 (6)	84.6 ± 2.7 (6)	0.98
Vitamin E			
Al-Jenadriyah	64.5 ± 45.3 (6)	57.2 ± 36.9 (6)	0.76
Dammam	85.6 ± 38.4 (6)	87.3 ± 48.3 (4)	0.94
Khamis-Mushait	55.6 ± 32.4 (5)	112.6 ± 24.5 (6)	**0.01**
Sakaka	94.7 ± 52.8 (4)	71.6 ± 36.0 (6)	0.43
Wadi- AlFora'a	76.7 ± 53.7 (6)	75.1 ± 59.6 (6)	0.96

* *p*-value calculated using unpaired Student's *t*-test. SD = standard deviation.

Table 7
Correlation between vitamins A, D and E vs parameters.

Parameters	Animal Type	Season	Location
Vit A	−0.162	−0.287	−0.162
P-value	0.228	**0.030**	0.228
Vit.D	0.033	0.025	0.033
P-value	0.809	0.854	0.809
Vit.E	0.081	−0.271	0.081
p-value	0.547	**0.041**	0.547

newborn and $p < 0.05$ (Table 6). Finally, a collective summary of correlation was done of serum vitamin A values versus all parameters namely (animal type, season and location) and a significant statistical correlation appeared in the season parameter with $p < 0.05$, this correlation indicates great importance for researchers, concerned veterinary authorities, camel farming owners and camel feed manufacturers.

Generally, in camel research limited references are available on vitamin D aspects (Faye and Bengoumi, 2018; Konuspayeva et al., 2022). The mean value ± SD of serum vitamin D for dams were 82.4 ± 8.3 ng/100 ml and 80.2 ± 15.5 ng/100 ml for newborns (Table 1) were mostly similar to the study of non-lactating camels (83.5 ± 4.5 ng/100 ml) and found less than the values for pregnant and lactating camels (89.7 ± 4.5 ng/100 ml and 123.8 ± 7.5 n g/100 ml) respectively (Riad et al., 1994). El-Khasmi (2000) analyzed 25-OH-D3 (25- hydroxycholecalciferol) in the plasma of a one-day-old camel calf and was 5.33 ± 3.3 ng/100 ml and his dam on the same day and the value was 48 ± 6 ng/100 ml, however, the dam result was less than from this study. One of the recent studies demonstrated that the lactating camel needs higher nutrition in winter as a supplement to improve productivity (Abdelrahman et al., 2022). Other analyses of 25-OH-D3 in camel serum was 390 ± 45 ng/ml (Khasmi et al., 2013) also studied the effect of transport stress on the 25-OH-D3 vitamin and values were 420 ng/ml and 370 ng/ml before and after transport stress (el Khasmi and Faye, 2011). Detailed comparisons of mean value and effect of seasons on vitamin D levels were studied by Student's *t*-test and the effect of the five locations based on the ANOVA test and there was no statistical significance of any variable, even for the outcome of the correlations. These results can be attributed to the color of most commercial milking camels in Saudi Arabia and since vitamin D main source is the sunlight which is continuously bright and shinning plus almost all camel owners never believed in shedding (building sun sheds for camels) even in the intensive commercial farms. However, some of the studies reported the highest value level of vitamin D in-between February and July (summer) and the lowest levels in-between August and January (winter) in Sudan (Mohamed, 2008) and one of the studies reported 448 ± 98 ng/ml in summer and 276 ± 13 ng/ml during winter (Shany et al., 1978).

Vitamin E was analyzed using HPLC and the results were statistically studied on the same methods as vitamin A and D. Dams mean ± SD serum values of our study was 80.8 ± 44.5 μg/100 ml newborns was 74.7 ± 43 μg/100 ml (Table 1). These values are similar to one of the studies ranging from 30 to 165 μg/100 ml values of D vitamin in young camels (Barri and Al-Sultan, 2007). Other researchers have reported different values than this study, Seboussi et al. (2009) have reported 116 ± 81 μg/100 ml in adult camel and 82 ± 106 μg/100 ml in young ones, while Wernery et al. (2009) reported 194 ± 70 μg/100 ml and Baraka (2012), have reported for an adult camel with 150 ± 120 μg/100 ml and 110 ± 01 μg/100 ml for young ones. This study analyzed the season effect on the serum vitamin values of both dam and newborn combined using Student's *t*-test, a $p < 0.05$ was obtained which is a significant effect of season on the vitamin value of both camel types and in the winter, value increased to 89.8 ± 40.1 μg/100 and in summer it was decreased to 66.4 ± 44.3 μg/100 ml. The seasonal effect on dam vitamin levels alone revealed a high significance ($p < 0.05$), except for newborn was $p > 0.05$ which is not a significant one. This variation in vitamin levels may be due to the availability of cereals, grasses and leaves in winter which is a rich source of vitamin E for the camel. The season from winter to summer has a great impact on camel feeding due to the availability of diet (Faye, 2020). Locations effect on vitamin level in both tested camel types revealed $p > 0.05$ despite when comparing dam and newborn separately with the five locations a $p < 0.05$. In the southern region alone reported $p < 0.05$ indicated that there is a significant effect of southern location on the vitamin levels of the dam and newborns. This result is similar to one of the studies in which camel milk with a higher proportion of polyunsaturated fatty acids decreases vitamin absorption and also declines the vitamin E level in camel newborns (Seboussi et al., 2010).

It was difficult to get the samples due to camel farms or enclosures were not stable in one location for one year period to collect samples for different seasons of the year, far geographical locations of the country and the emotional attachment between the owner and camel they didn't allow to draw many samples.

5. Conclusions

Fat-soluble vitamins have a significantly vital role in camel performance, reproduction and production, this study has revealed variations of fat-soluble vitamins A, D and E levels in camel serum in relation to camel type, season and camel locations in Saudi Arabia with statistical *P*-values indicating the need for more studies to be conducted. In Saudi Arabia, ongoing development and high growing interest in camel products need a careful study and attention to such study results and any other related ones when formulating camel rations and feeds. Vitamins A, D and E have a huge impact on camel reproduction and bodybuilding, especially with the well-known poor reproduction efficiency and imbalanced nutrition representing a main cause of this deficiency, especially with the very poor and limited grazing areas and limited knowledge camel owners' of instant formulated feeds on good scientific supervision and data. The emerging need for camel vitamin feed additives also shouldn't be neglected.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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