Relationship of the genotypes resulting from the mutation (233069) of the POU1F1 gene with milk production and body dimensions in Iraqi camel females (*Camelus dromedarius*)

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Abstract

The study was carried out by using 50 Iraqi single-humped camel females which belong to the private fields in the AL-Furat AL-Awsat region involving three cities (Babylon, Diwaniyah and Muthanna). This research was conducted to determine the genotypes and their distribution ratios for the POU1F1 gene, and the relationship of the polymorphism of the gene with some productive traits, growth characteristics and blood biochemical parameters. The mutation 233069 was in the region of third intron of third studied segment, which included second intron, third exon and third intron, with a length of 909 bp using DNA sequencing technology, as this mutation resulted in three genotypes wild GA, hetero GA and mutant AA . The results indicated that there were highly significant differences in the distribution ratios of the genotypes resulting from the mutation. The results showed that there were no significant effects between the genotypes with each of the length of the milk season, daily and total milk production, its chemical components and animal weight. The results of the study also showed that there was no significant relationship with body dimensions with the exception of body height from the front and length of the tail, as it outperformed. Individuals with hetero GA genotype on wild GG and mutant AA genotypes in terms of body height were (223.50±1.50, 215.12±0.88, 213.43±2.55) cm and tail length (58.50 ±1.50, 51.73 ±0.57, 52.28 ±0.86) cm for the genotypes hetero, wild and mutant genetics respectively. The results also showed significant differences between the genotypes and blood parameters with the exception of total protein, The individuals carrying the wild and mutant genotypes outperformed the individuals carrying the hetero genotypes in all of the traits glucose (103.34 ±0.73, 100.41 ±2.35, 55.31 ±15.08), cholesterol (90.09 ±0.64, 90.91 ±1.06, 47.18±1.92), and triglycerides (55.30 ±0.60, 55.43 ±1.46, 74.64 ±18.77) for the genotypes wild, mutant and hetero genetics respectively.

Keywords: Genotypes, wild, intron and blood parameters.

Introduction

Camels are semi-ruminant placental mammals, and belong to the family Camelidae, which includes one-humped camels (*Camelus dromedaries*), two-humped camels (*Camelus bactrianus*), llama, Alpaca, Vicuna and Guanaco [1].The number of chromosomes in camels is 74 chromosomes, which are almost identical with only slight differences in the amount and patterns of heterochromatin distribution [2; 3]. The number of camels was estimated at 25.89 million heads in 2013, with the proportion of camels with one hump is 89%, and two-humped camels amounted to 11% [4].

Determining the genotypes of any gene and studying the quantitative trait loci (QTL) and detecting the presence of mutations are of great importance in the application of selection programs and increase the genetic yield, and the application of molecular technologies in evaluating the performance of ruminants through the study of genetic homogeneity within the breed and the relationship between genes with multiple effect to predict productivity as well as study its genetic diversity for the purpose of preserving it as a genetic source [5;6].Quantitative traits are affected by many factors, including genetic, non-genetic factors , and the composition of the herd and the location of the study which has significant effect on some economic traits of ruminants especially milk production and composition [7].

Genetic characterization at the level of DNA was also chosen to research some of the phenotypic and genetic characteristics of camels due to the large and important role of DNA in this animal,and that biological, molecular evolution and discovery of genetic maps led to the identification of programs and means that lead to improving the performance of animals [8]. The development in molecular genetic techniques made it possible to identify differences between individuals at the level of molecular genetics [9]. Recently, it has been used [10;11] the technique of sequencing the nitrogenous bases of genes or parts of them for the purpose of determining the genetic structure, discovering mutations for using them as markers and their effect on different traits. Among the genes with multiple genotypes is the POU1F1 gene, which is one of the transcription factors of the POU-family, the POU1F1 gene in camels is located on the first chromosome. It consists of 9 exons and has a size of 237287 nitrogen bases [12]. The POU1F1 gene is one of the first transcription factors identified in vertebrates that plays a specific role in the growth, proliferation and development of cells [13;14] and this gene encodes proteins called POU-domain, which in turn plays its role in the final differentiation of Somatolactotroph and Thyrotroph cells during development of the pituitary gland , its physiological role lies in regulating and activating the gene expression of the pituitary gland hormones genes and their receptors, including the growth hormone gene (GH),the prolactin gene (PRL) and the beta thyroid hormone-stimulating hormone gene (TSHß) through its association with the actuator region promoter of DNA , this gene also plays an active role in the body's ability to perform vital functions such as growth, development of mammary glands, production and secretion of milk [15]. Despite the growing interest in the genetic polymorphisms of the POU1F1 gene and their association with productive traits in

different agricultural animals during the past years, and since there are no studies on polymorphism The genetic forms of this gene in camels, so the study aimed at the determination of the genotypes of the POU1F1 gene in Iraqi she-camels, determining the relationship between the genotypes of the POU1F1 gene, milk production and the length of the milk season, the chemical components of milk and the biochemical parameters of blood.

Materials and Methods

Experimental animals and study site.

We selected 50 Iraqi, single-humped camel females which belong to private fields in the AL-Furat AL-Awsat region, 20, 15 and 15 animals were randomly selected from Babylon , Diwaniyah and Muthanna cities, respectively, whose ages range from 8-15 years.

Blood Samples Collection and PCR application

Blood samples were drawn and collected from the jugular vein and the abdominal milk vein, with one sample for each camel and an amount of 10 ml for each sample, using a medical syringe with a capacity of 20 ml. DNA was extracted from blood samples drawn using the kit according to the manufacturer's instructions attached to this kit Presto™ Mini gDNA Kit, Geneaid, Taiwan . The primers (Forward and Reverse) were supplied by Macrogen company in a lyophilized powder. lyophilized primers were dissolved in a nuclease free water to give a final concentration as stock solution, which is 100 pmol/µl . A working solution of these primers was prepared by adding 10 µl of stock solution (stored at -20 °C) to 90 µl of nuclease free water to obtain the final concentration of the working solution of 10 picmols/µl (Table 1). The materials and PCR reaction condition showed in Tables 2 and 3.

Table (1): Primer sequence

Primers	Start	Stop	Length	Tm	GC %
Forward 5`-GGAGGTTCCCAGGAGTAAA-3`	772	791	19	60	52.6
Reverse 5`-GGAAGGACAAACAGAAGGAATA-3`	1659	1681	22	60	40.9

Table (2): Master Mix components and volumes

No.	Components of Master Mix	Volume	
		1 Sample	50 Sample
1	Master Mix	12.5 µl	625 µl
2	Forward primer	1 µl	50 µl
3	Reverse primer	1 µl	50 µl
4	Nuclease Free Water	7.5 µl	375 µl
5	DNA	3 µl	150 µl
6	Total volume	25 µl	1250 µl

Table (3): Thermal cycling protocol

Stages	Temperature	Time	Number of courses
Initial Denaturation	95	5 Minute	1
Denaturation	95	30 Second	30
Annealing	60	30 Second	
Extension	72	1 Minute	
Final Extension	72	7 Minute	1
Hold	10	10 Minute	

DNA Sequencing

PCR product wewre sent to the Microgene Corporation – South Korea in order to read the sequences of nitrogenous bases and detect genetic mutations in them. The results were obtained and analyzed using geneious software .

Milk production

The total milk production was also calculated according to the following equation:

Total milk production = daily milk production rate **x** number of milking days

Animal body dimensions and weight

The dimensions of the body were taken by measuring tape in centimeters and for each animal in a state of natural standing as much as possible on a flat floor (19). The body weight was also estimated according to the method (37) through the following equation:

Live weight (kg) = shoulder height (m) x Heart girth (m) x hump girth (m) x 50

Statistical Analysis.

The data was analyzed by using Statistical Analysis System–SAS to study the effect of the genetic phenotypes of the POU1F1 genes on the studied traits, and significant differences was compared by used of least square means method .The Chi-square-$\chi 2$ test was also used to compare the percentages of the genotypes distribution in the POU1F1 gene.

Results and Discussion

Identification of POU1F1 Gene polymorphisms

In the current study, three SNPs of POU1F1 gene were detected in fragment 909 bp of POU1F1 gene in Iraqi camel females by direct DNA sequencing (Figs.1and 2).

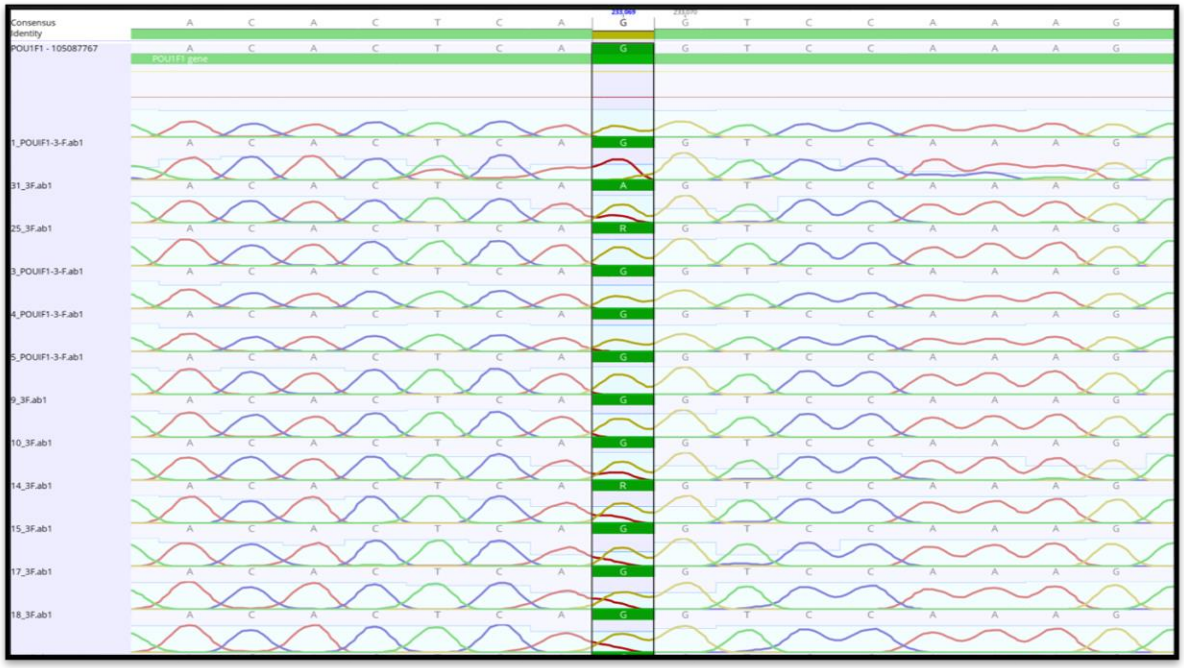

Figure (1): The site of the mutation (233069) in the third studied segment of the POU1F1 gene

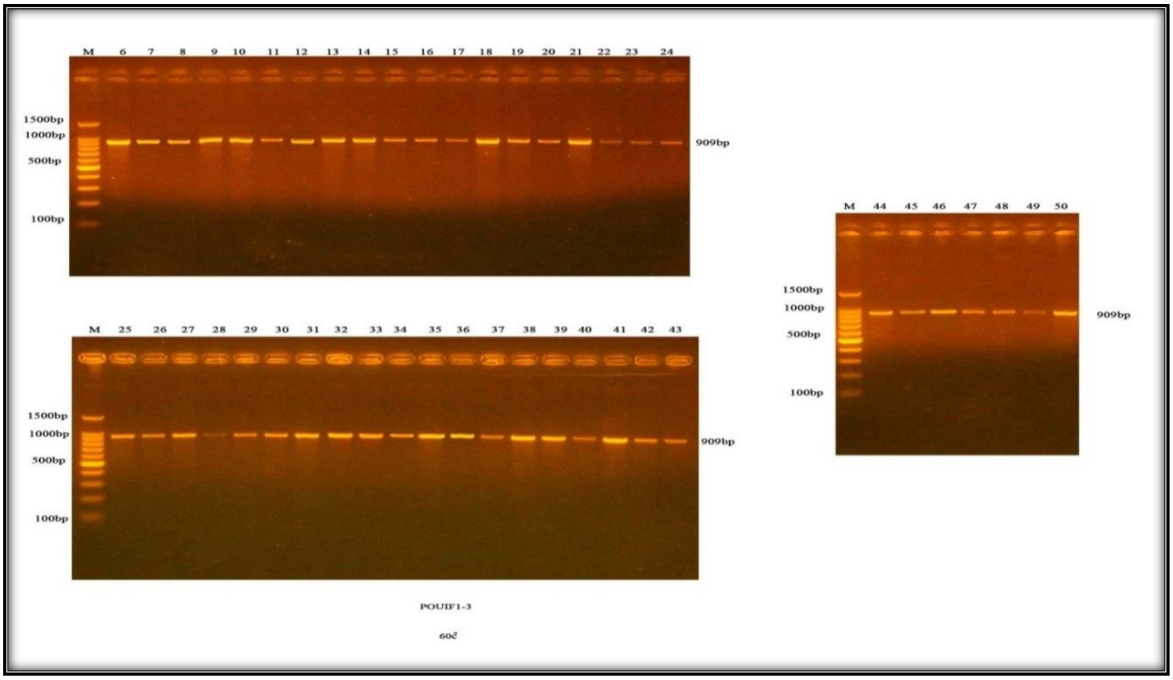

Figure (2): Result of the amplification of POU1F1 gene of Iraqi camels' female samples

Percentages, number and allelic frequency of the mutation (233069)

The results in Table (4) show that the percentages of the genotypes of the mutation (233069), which are wild GG, hetero GA and mutant AA in Iraqi camel females were 82, 4 and 14%, respectively, as we find that there are highly significant differences (P≤0.01) between the percentages of the distribution of genotypes, while the allelic frequency of the two alleles was wild (G: 0.84) and mutant (A: 0.16).

Table (4): Number, percentages of genotypes and allelic frequency of the mutation (233069) in POU1F1 gene

Genotype	No.	Percentage (%)
Wild : GG	41	82.00
Hetero : GA	2	4.00
Mutant : AA	7	14.00
Total	50	% 100
Chi-square value (χ^2)	----	** 88.56
Allele	Frequency	
G	.084	
A	0.16	
Chi-square value (χ^2)	23.047 **	
.(P≤0.01) **		

The relationship of the mutation (233069) with milk production and its chemical components

The results in Table (5) indicate that there are no significant differences between individuals carrying the genotypes resulting from this mutation, namely wild GG, hetero GA and mutant AA, in the productive traits represented by the length of the milk season, total and daily milk production and its chemical components.

Table (5): Relationship of the genotypes of the POU1F1 gene (233069/G>A) with milk production and its chemical components

Traits	Genotypes (Mean ± Standard error)			Significant level
	GG	GA	AA	
Length of the milk season (day)	251.56 ±4.16	243.50 ±19.50	234.85 ±4.63	N.S.
Milk production for the third month (kg/day)	4.13 ±0.17	3.00 ±0.02	4.27 ±0.19	N.S.
Milk production for the fourth month (kg/day)	4.33 ±0.15	3.50 ±0.00	4.47 ±0.14	N.S.
Milk production for the fifth month (kg/day)	4.73 ±0.16	4.00 ±0.02	5.02 ±0.21	N.S.
Total Milk Production (kg)	1119.67 ±39.02	882.34 ±51.30	1105.76 ±52.86	N.S.

Lactose in milk (%)	4.89 ±0.15	5.08 ±1.61	4.48 ±0.37	N.S.
Milk Triglycerides (g/l)	33.90 ±0.68	37.95 ±1.83	33.53 ±0.82	N.S.
Milk Cholesterol (mg/l)	43.18 ±2.50	49.41 ±27.03	33.74 ±5.34	N.S.
Milk protein (%)	3.44 ±0.12	2.81 ±1.50	2.95 ±0.37	N.S.
Total Solids in Milk (%)	13.03 ±0.33	11.71 ±0.28	12.51 ±0.69	N.S.
Fatty acids in milk (%)	0.089 ±0.01	0.162±0.07	0073 ±0.02	N.S.
N.S. : Not significant				

Relationship of the mutation (233069) with the dimensions of the body and animal weight of Iraqi camels females

The results in Table (6) showed that there were no significant differences between individuals carrying the genotypes resulting from this mutation, namely wild GG, hetero GA and mutant AA with animal weight, and the results also did not show any of the resulting genotypes distinguished in the studied body dimensions, with the exception of body height from the front and the length of the tail , It is clear from the results of the table that the individuals carrying the hetero GA genotype were significantly superior to the wild GG and the AA mutant ones, as the average body height from the front was 223.50, 215.12 and 213.43 cm, while the average tail length was 58.50, 51.73 and 52.28 cm for the genotypes hetero, wild and mutant sequentially.

Table (6): Relationship of the genotypes of the POU1F1 gene (233069/G>A) with body dimensions and animal weight

Traits	Genotypes (Mean ± Standard error)			Significant level
	GG	GA	AA	
Front Body Height (cm)	215.12 ±0.88 b	223.50 ±1.50 a	213.43 ±2.55 b	*
shoulder height (cm)	193.63 ±1.24	200.00 ±0.10	192.14 ±3.78	N.S.
body length (cm)	178.71 ±1.25	188.00 ±2.00	177.85 ±3.17	N.S.
head length (cm)	51.17 ±0.51	51.00 ±3.00	50.57 ±0.94	N.S.
neck length (cm)	111.39 ±0.57	119.50 ±0.50	112.00 ±2.28	N.S.
tail length (cm)	51.73 ±0.57 b	58.50 ±1.50 a	52.28 ±0.86 b	*
Heart girth (cm)	207.61 ±1.55	200.00 ±2.00	205.85 ±4.71	N.S.
hump girth (cm)	281.04 ±2.83	262.00 ±5.00	281.43 ±7.37	N.S.
animal weight (kg)	566.82 ±10.96	524.10 ±15.24	560.60 ±36.88	N.S.
The averages with different letters within the same row differ significantly between them * (P≤0.05) , N.S. : Not significant.				

Relationship of mutation (233069) with biochemical blood parameters

The results of the table (7) showed a significant relationship between the genotypes resulting from this mutation with some blood parameters. The individuals carrying the wild GG and mutant AA genotypes were significantly superior to the individuals carrying hetero GA genotype in each of the glucose ratio, with an average of 103.34, 100.41 and 55. 31 mg/dL and cholesterol ratio, which averaged 90.09, 90.91

and 47.18 mg/dL for the wild, mutant and hetero genotypes, respectively, while it was not clear that there were significant differences between wild GG genotype and mutant AA genotype in both traits, and the results showed the same table outperformed individuals carrying the mutant AA genotype significantly in triglyceride ratio 55.43 mg/dL over the rest of the individuals carrying both the wild and hetero genotypes, as the average triglycerides reached 55.43 and 74.64 mg/dL, respectively. While the same table showed that there was no significant relationship between this mutation with total protein in the blood.

Table (7): Relationship of the genotypes of the POU1F1 gene (233069/G>A) with biochemical blood parameters

Traits	Genotypes (Mean ± Standard error)			Significant level
	GG	GA	AA	
Glucose (mg/dL)	103.34 ±0.73 a	55.31 ±15.08 b	100.41 ±2.35 a	*
Total Protein (g/dL)	6.46 ±0.13	47.97 ±43.92	6.21 ±0.27	N.S.
Cholesterol (mg/dL)	90.09 ±0.64 a	47.18 ±41.92 b	90.91 ±1.06 a	*
Triglycerides (mg/dL)	55.30 ±0.60 a	74.64 ±18.77 b	55.43 ±1.46 a	*
The averages with different letters within the same row differ significantly between them * (P≤0.05) , N.S. : Not significant .				

The results of the study showed the mutation 233069 was in the region of the third intron of the third studied piece. With regard to the length of the milk season and the total and daily milk production, the results of the study showed the absence of a clear significant relationship between it and all the genotypes resulting from this mutation. As the results of the current study were different [16] in total milk production, it reached 1559 kg. It also differed from [17] in each of the daily milk production (4.21 kg / day), and the total milk production (1388.41 kg). This result can be explained by the fact that the effect of the multiple manifestations of this gene in milk production is due to the role of the proteins of this gene in regulating the gene expression of the prolactin gene, in addition to the fact that the gene expression of the POU1F1 gene is confined to the anterior lobe of the pituitary gland, in which lactotrope cells responsible for secreting the hormone prolactin are located, as well as the presence of expression of this gene in the mammary gland cells in lactoblasts [18].

As for the components of milk, the results of our study were in agreement with what was found by [19], where the percentages of lactose, protein, fat and non-fat solids were 4.86, 3.46, 4 and 12.2%, respectively. The results also matched with [20] who found that the percentages of protein, lactose and solid non-fat in milk were 3.52, 4.14 and 11.05%, respectively. Also, the results were close with [21] with respect to percentage of protein 3.1% and lactose 4.4%, while it was higher in the percentage of total solids 11.9% . Also, our results were close with [22] in each of the percentage of

protein and lactose, which amounted to 2.95 and 4.19%, respectively, while it was higher in the percentage of total solids, which amounted to 10.46%, and our results differed with [17] in each of the percentage of protein and total solid amounted to 2.67 and 10.75%, respectively. This difference is also due to the difference in the animal's breed, the health status of the herd, the type of feeding and the geographical location. The results of the current study differed from the findings of [23] regarding body weight, when they found that the average weight of Nigerian camels was 754.8 kg. Our results also differed with those of [24], who found that the average weights of camels for some Ethiopian breeds were 439.76, 533.95, 567.00 and 375.14 kg in each of the Jijiga, Hoor and Shanili breeds, respectively. Our results were higher with the results of [25], who classified the Al-Shanbali, Al-Kinani, Malia and Al-Majanin breeds as being of the Arabic heavy type with a weight of 506, 492, 479 and 473 kg, respectively. With regard to shoulder height, heart girth and hump girth, our results differed from what was found by [26], that shoulder height was 192.2 and 178.1cm, Heart girth was 181.5 and 190.1 cm, and hump girth was 220 and 163.8 cm for both the Tarji and the Saharan breeds respectively. While our results were higher and lower than the Libyan Ethiopian breed in shoulder height, Heart girth and hump girth 219, 193 and 263 cm, respectively, while our results were close to the Ethiopian Jijiga breed in shoulder height 198 cm and were higher in Heart girth and hump girth 176 and 248 cm on the respectively [24]. Also, our results in shoulder height were very close to the results of [27], as the average was 200.29, 190.06 and 194.80 cm for the Judi, Khawar and Hurra breeds, respectively. The studied camel females were distinguished by recording high values for both the height and length of the animal compared to [28] who recorded the animal’s height 194, 193, 190, 186 and 192 cm, and the animal’s length 147, 145, 142, 136 and 138 cm in both the Gueoudi, Guiloufi, Merzougui, Tataouine, Medenine and Tunisia breeds respectively. Part of the variation in the height of the animal may be due to the difference in the size of the hump according to the degree of the animal's body condition [29], The hump is the main store of fat in camels, which represents on average 85% of adipose tissue [30].

In terms of head and neck length, our results were similar with what was found by [26] for head length 51 and 52 cm, while it differed in neck length, reaching 109 and 102 cm for the Targi and Saharawi Algerian breeds respectively. While it was very close to what was found by [31] for head length of 50 cm and higher in neck length of 87.6 cm in the Pakistani Kohi breed. While it differed with the results of [32], who found that the head length is 42.1, 42.4, 42.3, 41.5, 46.5, 46.9, 42.8, 46.9, 42.8, 46.9 and 39.3 cm, while they were less and close to each other in neck length 87.8, 97.6, 94.3, 92, 107.1, 110.7, 96.2, 104.5, 92.3, 98.7 and 108.6 cm for each of the Hadhana, Aouadi , Asail, Awrk, Homor, Majaheem, Saheli, Shaele, Sofor, Waddah and Zargeh breeds respectively. In terms of tail length, our results were higher than those of [28], who found that the tail length was 38 and 41 cm in both the Gueoudi and Guiloufi breeds of Tunisia respectively, while the results of our study were less compared to 55.80 cm Nigerian camels [23].

The differences between the breeds of Arab camels in the expression of anthropometric measurements may be due to the difference in the genotype of each breed and to the geographical location in which it is found [33; 34]. According to [35] who clearly demonstrated that phenotypic diversity is a good reflector of ecological selection systems and breed history. The phenotypic structure is the genetic performance of the traits and adjusted according to the environmental conditions, and that the sum of genetic and environmental variance affects the phenotypic variance [36].

As for the biochemical blood parameters in our current study, the results were close to what [37] who found that the averages were 7.02 g/dL for total protein concentration and 88.65 mg/dL for total cholesterol concentration in camels, also our results were close with [38] in his study on the Iraqi local camels, and stated that the overall average of total protein was 7.18 g/dL and glucose 98.12 mg/dL, while our results were lower in cholesterol concentration, which averaged 114.23 mg/dL and higher in fat concentration. Triglycerides amounted to 36.17 mg/dL, and it differs with [38] in his study on camels, where the concentration of total protein was 6.16 g/dL and glucose 110.04 mg/dL, and it differed with the concentration of total cholesterol 38.30 mg/dL and triglycerides 34.30 mg/dL.

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