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Potassium Polymorphism and Its Relation with Mastitis Resistance and Semen Quality in Iraqi Sheep

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Abstract: ***Background and Objectives: The study conducted to indicate the association among blood potassium concentration with some reproduction and physiological traits of Awassi Iraqi sheep. Methods: ninety nine Awassi ewes and (20) Rams were enrolled. The ewes were examined for mastitis affection. Semen quality traits were conducted on 20 Awassi rams. Blood potassium was estimated by atomic absorption flame photometer. Results: Potassium level of Awassi ewes showed a bimodal distribution, the low potassium (LK) and the high potassium (HK). Blood concentration in LK was11.69 m.eq./L. blood ; while the HK type means recorded 33.99 m.eq./L. blood. Potassium level in Awassi rams showed a bimodal distribution also, the low potassium (LK) and the high potassium (HK),and their potassium concentration were 10.62 and 34.97 m.eq./l blood, respectively. No significant differences in mastitis affection between HK and LK types of Awassi ewes, in spite of mathematical high level in LK ewes was noticed. Awassi LK rams showed significantly higher semen volume than that in Awassi HK rams, 0.60cm and 0.55cm respectively. Awassi HK rams had significantly higher individual motility and high livability sperms percentage (68% and 74.13%) than those found in Awassi LK rams (60% and 70.4%) respectively. Conclusions: The blood potassium concentration useful in election of Awassi sheep breed of high productivity and reproductively also useful in selection of the sheep for breeding with age of two months because the level of potassium concentration in red blood cells be fixed in this age.***

Keywords: Awassi sheep; blood potassium; mastitis; semen quality, Iraq

1. Introduction

There are a number of studies that deal with the biochemical and their relationship to efficient production of sheep and one of these studies done by Lehninger[1], who explained the importance of high intracellular concentration of potassium in order to accomplish several vital stages of animal cell and they believed that union with potassium leads to morphological changes in the Pyruvate Kinase enzyme to make it more effective. Omer et al.[2]in (Tuj) orientation in, Turkey, where he studied the various and different blood value and their relation with biochemical traits.

These relationships are important for clarifying the causes of physiological differences, and to be exploited as the basis accurate and early for the election of animals depending on productivity characteristics, and one of the most important of these biochemical variations is the concentration of potassium level in the blood. The study of potassium level in sheep blood started at 1954, when Evans[3], found two types of potassium levels in the Black face Scottish sheep and Chevot sheep dividing them into high potassium level "HK" at a rate of 36 m.eq. / Liter of blood (m.eq/L. of blood) and low potassium level "LK" at a rate of 13 m.eq. / liter of blood, and since then used to divide the sheep on the basis of the level of this element in the blood to high potassium HK and low potassium LK based on Evans study, which is the starting point in this scientific field.

The genetic bases of this trait in red blood cells, has been studied directly after this discovery by Evans and King[4], and they explained that the character affected by a gene of two alleles, the dominant allele is K^L and the recessive one is K^H those give rise to two types of phenotypes; high potassium level HK which is homozygous recessive (HKHK), and low potassium level LK and this either homozygous (LKLK) or heterozygous (HKLK).

several scientists studied in the years followed Evans and King the gene frequencies that control potassium level in the blood, and reached different results for the gene frequency and potassium concentration according to the differences in breeds and geographic locations and the size of herds, all the studies have supported what we have said about the presence of high and low potassium alleles and the low potassium allele is dominant.[5-9]

In a study conducted by Arora and Acharya [10], to see the effect of these different phenotypic traits according to potassium concentration level in weight at birth, and weights after three months, and six months, and years, as there were no significant differences due to potassium difference but noted a slight increase in the weights rate mentioned for HK sheep. Lazoviskii & Spiridonov [11] was found an increase at weight at birth in LK sheep at birth and at weaning and at the age of six months on those of HK type.

There may be a direct relationship between the level of potassium concentration on the one hand; and some of productivity traits of these sheep on the other hand. Watson and Khattab[12], was found in a study of 1594 of Welsh mountain sheep that LK sheep characterized by neonatal growth speed higher than that in HK sheep, also showed a slight increase in birth weight of sheep in LK sheep compared with HK one, these results has been endorsed by many researchers [13-15]

Regarding the effect of the level of potassium concentration level in reproductive efficiency, the results of Meyer [13], study has shown that the HK ewes characterized by higher fertility compared with LK ewe. While the (HK) rams has better fertility than that of heterozygous genotypes. In contrast to those results; Bernoco [14], in a study of 438 sheep found different results, while Yatsenko[15]' showed that the HK rams more fertile than LK rams and the differences were significant. Also Krishnamurthy[16],has shown that HK sheep produce more number of lambs compared to LK sheep, but the differences did not reach significance level.And many researchers have continued to work in this field and obtained different results [17-19].

2. Materials & Methods

2.1. The Animals

This study was conducted on the local Awassi breed with the adoption of 99 ewes in lactation period and their age were more than two years and tested for mastitis affection, were also distributed on the basis of potassium concentration level in the blood, the high and low potassium level.20 Awassi rams placed to this study in the sexual period, with more than two years old, for the purpose of testing the semen quality, were also distributed on the basis of potassium concentration level in the blood, the high and low potassium level.

2.2. Estimate of potassium concentration in the blood:

Khattab[20], method were followed for estimating potassium concentration in the blood. Potassium concentration were measured by m.eq./liter of blood, using the device Atomic absorption flame photometer 10 AL"Tokyo-photoelectric-LTD-Japan"

2.3. Mastitis

Clinical examination adopted for milking Ewe for the purpose of diagnosing cases of mastitis, as well as the adoption of special papers for milk examination (Indication paper for udder tests) and relied on to change the yellow color on the kart to greenish yellow color for the purpose of confirmation of affection.

2.4. Semen Quality Tests

The rams accustomed on the electrical stimulation device during their reproductive season[21], and then samples of semen were collected within a week, their ages were within two years, taking into account that the rams were under a uniform diet, and then studied the characteristics of semen where the following criteria were adopted primarily to evaluate the efficiency: the color, and size of the ejaculate, and pH, and the mass movement ratio, and individual movement, and the concentration of sperm, and the percentage of live sperm, and the percentage of sperm distorted, and the proportion of acrosome deformed sperm according to Evans and Maxwell[22],Taking into account the adoption of grades 1 to 4 to indicate the color of the seminal fluid, as follows: 1 degree for creamy color, 2 degree for un-transparent milky color, 3degree shimmering color and. 4degree for red or yellowish color.

2.4 (a) The size of ejaculate:
The size took read directly through the measuring tube and the measurement was to the nearest decimal 0.1 ml. It was saved in a special container from cork to protect it from external factors

2.4 (b) pH.:
PH meter was used and the gradient of up to 0.1.

2.4 (c) Mass active percentage
small drop of semen had been taken and placed on a glass slide, and then placed into a clean container and placed in a water bath at 37-35^{o}c, and then examined under a microscope, in on the basis of wave motion, and then converted to a percentage [22].

2.4 (d) Individual motility percentage
Where Chemineau method was adopted[23],they took a small drop of semen and placed on a glass slide and put 4-3 drops of 2.9% sodium citrate, and examined under microscope to see the speed and quantity of forward movement of sperm.
Sperm concentration/ml. According to Mohan [24], using Neumbauer chamber, and then applied to the following equation:

$$\textbf{sperm conc.}\,(\textbf{cm3}) = \frac{\boldsymbol{number\ of\ sperm/5squars}}{\mathbf{80}}\ \boldsymbol{X\ 400X200X100}$$

2.4 (e) The proportion of live sperm.
And estimated according to Chemineau method[23]

2.4 (f) The percentage of deformed sperm.
which was used to calculate the live sperm by the way of Mohan[24].

2.4 (g) Abnormalities of acrosom.
It was calculated by the method of Watson[25], and the types of defects are: inflation body, Dent headdress, broken head of the sperm, the erosion of the head of the sperm and the separated aspects head

2.5. Statistical Analysis:

Animals were divided according to the level of potassium each strain separately, and adopted the minimum area of 99% at a confidence interval between high potassium and low for HK and LK potassium followers, in the statistical design[26].Data using (Chi-square), (t test) and Analysis of variance. and use a Least significant differences to compare the averages between the different groups as well as finding correlation coefficients and regression between traits as indicated by Steel and Torrie[27].

3. Results and Discussion

Potassium level:
The level of potassium concentration divided into two groups in Awassi ewes (Table 1), LK and HK groups. The mean of potassium concentration in LK and HK ewe were 11.69 and 33.99m.eq./l. blood respectively. potassium concentration level showed bimodal distribution within Awassi

ram sheep, low potassium level (LK) and high potassium level (HK) and their potassium concentration were 10.62 and 34.97 respectively (table 2).The differences were highly significant ($p < 0.01$) between high and low potassium level. These results come in concordance with other studies[6, 14, 28]

Table 1: Numbers of ewes that tested for mastitis and the mean range of potassium concentration

DATA	Awassi ewes		
	Over all (HK+LK)	HK	LK
Number of animals	99	82	17
Mean ± SE	30.16 ± 1.07	33.99 ± 0.77	11.69 ± 0.94
Range	5.5 – 51.2	51.2 – 21.9	5.4 – 19

Table 2: Numbers of rams that tested for semen quality and mean and the range of potassium concentration

DATA	Awassi Rams		
	Over all(HK+LK)	HK	LK
Number of animals	20	15	5
Mean ± SE	28.89 ± 2.61	34.97 ± 1.07	10.62 ± 2.23
Range	2.8 – 43	26.9 – 43	2.8 – 18

The relationship of potassium level with mastitis

Clinical diagnosis by palpation test used with the adoption of indication paper for udder tests for the purpose of diagnosis of mastitis cases in all 99 Awassi ewes under study, and the result was 14 affected by mastitis (table 3).

Table 3: Numbers of ewes that tested for mastitis and the percentage of the affection of each type of potassium level

Potassium level	Awassi ewes			
	Numbers of animals	Healthy ewes	Diseased ewes	Mastitis %
Whole blood potassium	99	85	14	14%
HK	82	72	10	12%
LK	17	13	4	23.5%

When the incidence of mastitis was distributed according to the basis of potassium level; in 82 HK ewes there were 10 sheep infected with mastitis, in 17 LK ewes the total ewes infected with mastitis were 4 ewes. In comparison between the affection in HK and LK, appeared to increase in the proportion of LK ewes compared to HK, but the differences did not reach significance level.

The incidence of mastitis in LK Awassi ewes higher than that found in HK ewes, and the differences were not significant. The reason may be due to the small number of Awassi ewes with LK blood type that examined or tested for mastitis affection. And the fact that the arithmetic difference between the two groups is high which gives a clear indication that the genotype of HK type have a preference against mastitis disease taking into consideration the lack of LK sheep as the reason for not achieving a significant difference, i.e., that the HK sheep have a genetic susceptibility against stress and diseases compared with LK sheep, this result confirms by Taneja[29],Barillet[30], explained the possibility of selective breeding against mastitis adoption of SCC in milk, and also concluded that the genetic equivalent of this trait is (0.15).

Semen quality

The results of the characteristics of the semen of male sheep Awassi, amounting to 20 straight studied recipes semen where (Table 4), which included the color, and sise of the ejaculate, and pH, and the mass movement ratio, and individual movement, and the concentration of sperm, and the percentage of live sperm, and the percentage of sperm distorted, and the proportion of acrosome deformed sperm; the results of semen efficiency of Awassi rams showed variation between HK rams (15 animals) and LK rams (5 animals), as shown in the table (4).

Table 4: Semen qualities in Awassi ram according to potassium level

Potassium level	Ejaculate size	Mass movement	Individual movement	concentration	Live sperm	Deformed sperm	Acrosome deformity
Whole blood K (20)	0.565 ± 0.02	65 ± 1.58	66 ± 1.60	121.35 ± 0.35	73.20 ± 0.60	19. 45 ± 0.39	18.75 ± 0.47
HK (15)	0.55 ± 0.26 A	65.30 ± 1.86	68 ± 1.81 a	121.67± 0.42	74.13 ± 0.62 A	19.07 ± 0.42	19.07 ± 0.37
LK (5)	0.60 ± 0.05 B	64 ± 3.23	60 ± 1.58 b	120.40 ± 0.40	70.40 ± 0.40 B	20.60 ± 0.75	17.60 ± 0.47

***The different capital letters in column indicates significant difference $p \leq 0.01$**
Different small letters indicates significant difference $p \leq 0.05$
*** The figures in brackets indicate the number of animals**

Where the size of ejaculate 0.55 cm^3; the degree of color 1.27 ; pH 6.89 ; 65.3% mass movement ; individual movement 68% concentration of sperm 121.67 (x106 / cm^3) ; the percentage of live sperm 74.13% ; the percentage of sperm distorted 19.07% ; the proportion of acrosome deformed sperm 19.07% in HK rams, as compared with LK rams : 0.6 cm 3, 1.6, 6.9, 64%, 60%, 120.4 (x 106 / cm 3), 70.4%, 20.6% and 17.8%, respectively. Where the LK rams recorded more volume of ejaculate than that in HK ram, ($p < 0.01$). The movement of individual sperm ratio in HK sheep were higher than found in LK sheep ($p < 0.05$), also HK breed recorded a highest proportion of live sperm than found in LK ($p < 0.01$).

LK awassi rams outperformed on those HK semen size with significant differences. In HK Awassi rams individual movement rates, and the percentage of live sperm was significantly better than that in LK rams, It gives an explanation about why the increase in the size of LK semen rams; may be contrary to concentration, as the volume of LK sheep semen was higher than that in HK sheep.

Tests of semen came in general approach as stated by Zakari[31], in many respects, and different from the results in other aspects, as the rates were comparable with the size of ejaculate and color, and pH, and the proportion of live sperm, and the percentage of deformed sperm, and acrosome deformity where they were 0.55cm, 1.3, 6.9, 0.74%, 17.5% and 18%. The differences were in the massive movement and individual movement, and sperm concentration of 73%, a 0.70% and 123 x 106/cm^3, respectively.

Correlation coefficients and regression between potassium concentration in the blood and the semen qualities was calculated as shown in (Table 5).

Table 5: Correlation and regression between potassium concentration in the sheep blood and some seminal quality traits

Semen quality	Statistical test	
	Correlation	Regression
Ejaculate size	- 0.07927	- 8.908
pH	0.014282	1.5185
Mass movement	0.051274	0.0847
Individual movement	0.457359 x	0.7442
Concentration(10^6/cm)	0.298116	2.2257
Live sperm	0.581249 xx	2.5463
Deformed sperm	- 0.38206	- 2.579
Acrosome deformity	0.17411	0.9577

Shows that there is significant correlation between the level of concentration of potassium and individual movement ($p < 0.05$), and the proportion of live sperm ($p < 0.01$), while the correlation coefficient between the level of potassium, and the concentration of sperm reached (0.30) did not reach a significant level, while the correlation coefficient between the potassium concentration with the percentage of deformed sperm negative (-0.38), while the correlation coefficient is positive between potassium ratio and acrosome deformity, but did not reach the significant level .Swenson [32], said that the blood supply to the testes has two major missions, first allowed creation of alduminal environment through which controls the metabolism of sperm; example: selective excretion of potassium ions to alduminal space and its important in maintaining sperm in a stable condition, and the second act as a barrier that protect movement of sperm in the testicles interstitum.

One of the most important benefits of the blood supply to the testicles is to create alduminal environment that helps to excrete potassium in selective form to the alduminal space and is important is to maintaining the sperm in a stable condition **[32]**. As explained Van Niekerk [33],that there is a positive and significant correlation between the level of some tress elements in the blood and the volume of ejaculate and sperm concentration and sperm movement and negative with the percentage of sperm abnormalities. Gundogan[34],showed that the volume of semen and the movement of sperm and its concentration is higher in the months of autumn than in the rest of the months of the year, in addition to the presence of moderation in the rate of abnormalities of sperm during the autumn months, Gundogan[35], confirms this results in their study on 10 local and foreign rams, and within a sequential research [36], found that there is a high correlation between some of the components of blood and movement of sperm concentration factor ($p < 0.01$) which may allow for adoption as an indicator of reproductive efficiency, and this is what has been observed under our study.

In conclusion, it is clearly evident from the foregoing that it is possible to adoption of the level of potassium concentration to elect breed or even sub breed of sheep that has a high productivity and reproductively, also it can be elected a new breed of Awassi sheep which is the most important and largest breed of sheep in Iraq have a resistant against some epidemic diseases, and taking in concentration that we can select the sheep for breeding with age of two months because the level of potassium concentration in red blood cells be fixed in this age.

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