J. Agric. Sci. Mansoura Univ., 27(11): 7313 - 7321, 2002

EFFECTS OF BREED AND SEASONAL VARIATIONS ON SEMINAL PLASMA ESSENTIAL AND NON ESSENTIAL AMINO ACIDS CONTENTS FOR RAMS AT NORTH WEST COAST OF EGYPT

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ABSTRACT

Fifteen rams of three breeds, five Barki, five local Awassi and five imported Awassi breed were used in the present study. The study was aimed to investigate the effects of breed and seasonal variations on seminal plasma amino acids concentration throughout the experimental year.

Results showed that there was a breed differences ($p<0.01$) in the concentrations of different amino acids. Glutamic acid (a non-essential amino acid) and threonine (an essential amino acid) were the predominant amino acids in all of the three studied breed. However, the concentration of the glutamic acid was significantly higher for local Awassi breed than the other two breeds. Threonine amino acid was significantly high concentration for the Barki rams than the other two breeds. In general it was found that total free amino acids in local Awassi seminal plasma significantly higher than in seminal plasma of the other two breed. This trend was also reflected on the essential and non-essential amino acids.

Total free amino acids as well as the total essential ones were increased significantly during summer season as compared to the other seasons. On the other hand, total non-essential amino acids were significantly increased throughout spring months. The increased value was more pronounced in therionine in summer and glutamic acid in spring. Results also showed that Barki and local Awassi breed had higher values of total amino acids at summer time which may suggest their suitability for breeding at the semi-arid of the North West Coast of Egypt.

INTRODUCTION

The biochemical and enzymatic components of seminal plasma play a pivotal role in providing substrates, energy and forming an essential link in the energy generating cycles in sperm metabolism. They are also play an important role in the process of fertilization and the maintenance of constant cellular osmotic pressure during sperm preservation. Free amino acids were found to serve as oxidizable substrate for aerobic metabolism by spermatozoa, create a favorable conditions for nucleic acid synthesis and enhance sperm survival (Mann, 1964; Setchell *et al.*, 1967 and Sexton *et al.*, 1971).

Tyler and Rothschild (1951) indicated that amino acids may also act by virtue of their ability to bind certain heavy metals presented in usual salt solution extenders in trace amount and which are toxic to spermatozoa.

Fraser (1985) also indicated that seminal plasma proteins play an important logical role in the development of sperm motility and fertilizing ability. The important of the free amino acids in seminal plasma of sheep in semi-arid areas like in Egypt and the factors affecting these components have not fully

determined. Moreover, the relationships of these components with the meteorological constituents have not fully studied. Therefore, the objective of this study was to investigate the variations in free amino acids composition of seminal plasma of three breed of sheep (Barki, local Awassi and imported Awassi) reared at the north west coast of Egypt. The seasonal variations in seminal plasma amino acids for the different breed used in the present study were also studied.

MATERIALS AND METHODS

This study was carried out at Bourg El-Arab Research Station, located 50 km west of Alexandria (31°15′ N and 30°10`E), Egypt. Chemical analysis of samples was carried out at the animal physiology laboratory of the Animal Production Department, Faculty of Agriculture, Alexandria University.

Animals and management :

Fifteen sexually mature rams of three different breed were used in the present study. Five Awassi (imported from Syria) of average weight 87±3.79kg, five Awassi (locally born) of average weight 60.8±5.40kg and five barki rams (native breed) of average weight 72.2±9.00kg, were used to study the free amino acid content of the seminal plasma throughout 12 months (from December, 1999 to November, 2000). All rams were between 3 and 4 years of age and kept outdoor with shelter during day time while were housed in a semi-open barn at night. Animals were offered roughage and concentrate supplement according to their body weight as was suggested by Morrison (1959). Animals were given berseem Egyptian clover (*Trifolium alexandrinum*) in winter and spring, and chopped green maize in summer and autumn in addition to hay. Each animal also received 500g/day of a pelleted concentrate mixture that contained (per kg) 610g total digestible nutrients (TDN) and 180g of crud protein. Daily temperature, humidity and photoperiod length data were obtained from a governmental meterological station near the research station and presented in Table (1).

Semen samples were collected monthly from all rams using an artificial vagina. Ejaculates were placed in a water bath at 38°C. Seminal plasma was separated from ejaculates by centrifugation at 5000 r.p.m for 10 min. The recovered seminal plasma fraction was further centrifuged at 1000 r.p.m. for 15 min at 4°C and the supernatant was stored at –20°C until analysis. One ml of seminal plasma after thawing was mixed with 50 mg of sulfosalicylic acid and centrifuged for five min. at 3500 r.p.m. The supernatant was obtained and diluted at a rate of 1:1 with diluting citrate buffer of 2.2 pH. Individual free amino acids were then estimated by the method described by Speakman *et al.* (1958) using a Beckman 119 CL amino acid analyzer. The response of the amino acid analyzer was checked by analyzing a standard mixture of seventeen commonly occurring amino acid in protein and ammonia and the obtained recoveries were used to calculate the amounts of the individual amino acid in various samples.

Statistical analysis :

Statistical analysis was performed using the general linear model procedure of the statistical analysis systems (SAS, 1989). A fixed effect model was assumed to underlay each observation in each trait studied. These effects were breed (B_i) season (S_j) and the interaction (BS_{ij}) between them.

The statistical model underlying this analysis was :

$$Y = \mu + B_i + S_j + BS_{ij} + e_{ijk}$$

Where Y is any trait (amino acid) and e_{ijk} is a random error assuming NID $(0,\sigma_e^2)$

Significant differences among means were detected using Duncan's multiple range test proposed by SAS (1989).

Table (1) : Mean seasonal variation in climatological data throughout the experiment year

Month	Air temperature (°C)	Relative humidity (%)	Photoperiod (h)
December	14.2	75	10.2
January	13.3	77	10.3
February	14.6	70	11.2
Average for Winter	14.0	74.0	10.6
March	16.2	79	12.4
April	17.8	65	12.9
May	21.8	69	13.7
Average for Spring	18.6	71.0	13.0
June	24.5	69	14.2
July	26.1	71	14.0
August	27.2	70	13.3
Average for Summer	25.9	70.0	13.8
September	25.9	68	12.4
October	22.2	64	11.4
November	17.3	67	10.6
Average for Autumn	21.8	66.3	11.5

RESULTS AND DISCUSSION

I. Effect of breed :

Overall means of various essential and non-essential amino acids in seminal plasma of rams as affected by breed are presented in Table (2), respectively and Figures (1 and 2). Results indicated that seminal plasma of Awassi local breed had the highest ($p<0.01$) total free amino acids, followed by Barki and finally Awassi imported breed. This trend was also observed with total essential and non-essential amino acids (Table 2). With regard to essential amino acids, thereonine was the most predominant amino acid in all breed followed by histidine, lysine, leucine and arginine in descending order. However, there was a significant ($p<0.05$) variations in the concentration of the essential amino acid concentrations between different breed. Thus, histidine and leucine were the most predominant in Barki seminal plasma, while lysine and histidine were the most occurring in both local and imported Awassi seminal plasma (Table 2).

Table (2) : Effect of breed on free amino acid concentration (mg/100ml) of rams seminal plasma.

	Barki	Awassi Local	Awassi Imported	Overall average
Essential amino acid				
Threonine	103.59±12.73^{a}	99.44±12.79^{b}	43.39±2.12^{c}	82.14
Methionine	4.60±0.33^{a}	4.03±0.34^{b}	3.45±0.49^{c}	4.03
Lysine	19.76±2.13^{c}	34.85±2.23^{a}	22.17±2.63^{b}	25.59
Valine	11.47±1.14^{a}	9.09±1.54^{c}	9.49±0.58^{b}	10.02
Isoleucine	13.24±1.62^{a}	10.44±0.59^{b}	9.65±0.19^{c}	11.11
Leucine	32.19±2.31^{b}	26.89±2.31^{a}	12.66±0.68^{c}	23.91
Arginine	16.42±2.24^{c}	28.70±1.86^{a}	18.13±1.06^{b}	21.08
Histidine	37.44±1.82^{a}	31.69±1.15^{b}	22.9±2.88^{c}	30.68
Phenylalanine	3.76±0.33^{b}	3.85±0.43^{a}	2.95±0.06^{c}	3.52
Non essential amino acid				
Glutamic	117.60±6.29^{b}	143.77±18.34^{a}	88.22±2.31^{c}	116.53
Proline	19.21±1.08^{c}	34.46±4.00^{a}	30.30±1.70^{b}	27.99
Tyrosine	4.71±0.43^{a}	4.57±0.25^{b}	3.11±0.09^{c}	4.13
Serine	33.43±2.10^{b}	40.40±1.67^{a}	29.89±0.25^{c}	34.57
Glycine	14.72±0.41^{a}	13.88±0.48^{b}	9.02±0.53^{c}	12.54
Cysteine	2.67±0.39^{a}	2.44±0.33^{b}	2.73±0.22^{a}	2.61
Aspartic	25.41±1.07^{b}	31.58±1.38^{a}	23.77±1.46^{c}	26.92
Alanin	19.41±0.72^{b}	20.87±0.74^{a}	14.38±0.81^{c}	18.22
Ammonia	37.68±3.23^{a}	35.09±2.05^{b}	21.18±1.97^{c}	452.25
Total free amino acid	469.61±29.85^{b}	540.93±34.91^{a}	346.22±10.20^{c}	212.08
Essential amino acid	242.47±23.17^{b}	248.97±13.17^{a}	144.80±7.20^{c}	243.51
Non essential amino acid	237.16±9.43^{b}	291.96±22.84^{a}	201.42±4.93^{c}	0.86
Essential / non essential	0.97±0.08^{a}	0.88±0.03^{b}	0.72±0.03^{c}	452.25

^{a,b,c} means with different superscript litters within any row differ significantly ($p<0.01$).

With regarded to the non-essential amino acids glutamic acid was the most predominant in the seminal plasma of the three breed of rams used in the present study. However,its value was significantly ($p<0.01$) higher for Awassi local breed which was followed by Barki breed then comes Awassi imported breed. Results also indicated that, serine and aspartic acids, in this order, were found in greater amount in seminal plasma of all breed used after gluatmic acid (Table 2). It was also found that seminal plasma of Barki breed contained a high level ($p<0.05$) of essential to non-essential amino acid ratio as compared to the other breed. Also, the essential amino acids concentration in seminal plasma of the Awassi breed was significantly ($p<0.05$) higher as compared to that of the Awassi imported breed (Table 2). I it can be concluded that the Awassi local breed is the best to be reared under the semi-arid condition of Egypt that prevailing in the north west coast area of Egypt. Barki breed can also be bred but is not as good as the local Awassi breed. This conclusion was drawn as the seminal plasma of the first breed had a high concentrations of total free amino acids as well as from the essential and non-essential amino acids especially that of glutamic acid. This predominant of glutamic acid in seminal plasma was also reported by Flipse *et al.* (1969) and Al-Hakim *et al.* (1970).Which proved the correlation between gluatmic acid and good ram fertility.

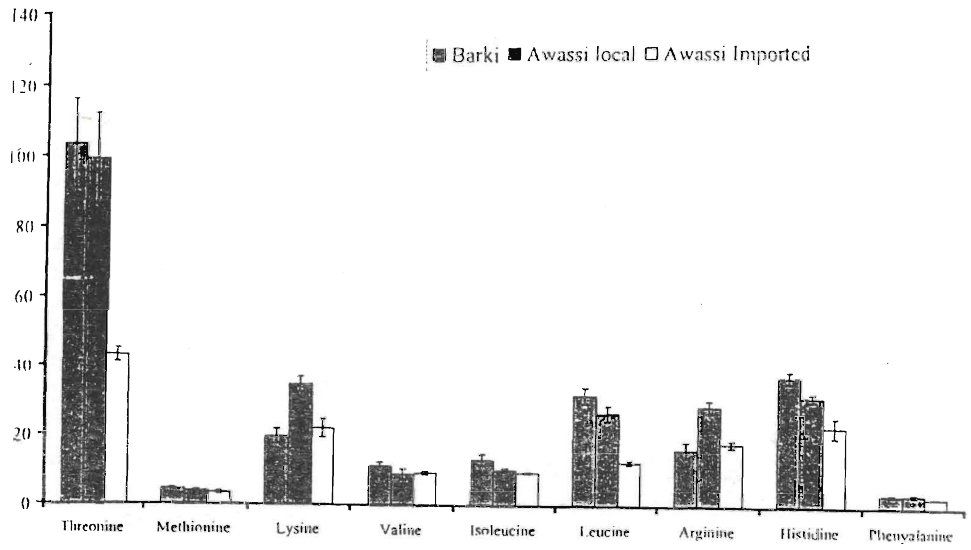

Fig (1) : Effect of breed on essential amino acids concentrations (mg/100ml) in seminal plasma of rams.

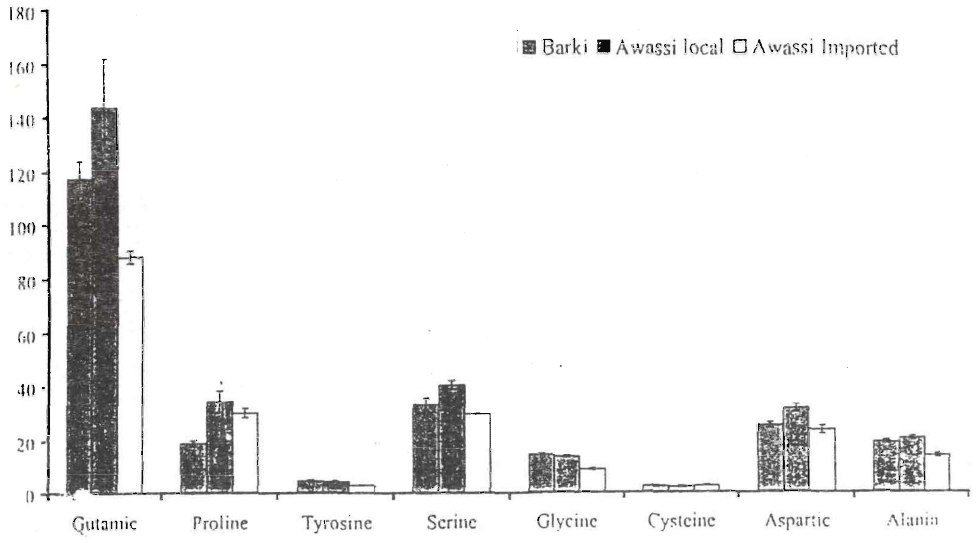

Fig (2) : Effect of breed on non essential amino acids concentrations (mg/100ml) in seminal plasma of rams.

II. Effect of seasons

Seasonal variations in free amino acids concentration in seminal plasma of rams used in the present study are shown in Table 3 and Figures 3 and 4. Data indicated that total free amino acids concentration was highly significant ($p<0.05$) during summer months than any other season of the year. This was followed by values of spring, autumn and winter seasons in descending order (Table 3).The difference between any two values was significant ($p<0.05$). This trend was also observed for the essential amino acids concentration but not for non-essential amino ones. Non-essential amino acids concentration was high ($p<0.05$) during spring season which was followed by summer, autumn and finally winter. In general, values for total, essential and non essential amino acids concentration showed a lower values during winter season. Results showed that the essential amino acids concentration had prevailed over that of the non-essential ones during autumn season (essential : non essential = 1.05).

Table (3): Effect of season on free amino acids concentrations (mg/100ml) of ram seminal plasma.

	Winter	Spring	Summer	Autumn
Essential amino acid				
Threonine	46.42±2.28^{c}	89.33±17.81^{b}	103.56±15.90^{a}	89.24±13.30^{b}
Methionine	3.07±0.58^{c}	4.40±0.21^{a}	4.39±0.37^{a}	4.24±0.54^{b}
Lysine	24.97±3.41^{c}	17.32±3.64^{d}	30.86±3.45^{a}	29.23±1.23^{b}
Valine	5.63±1.16^{d}	8.30±0.45^{c}	13.60±0.76^{a}	12.53±0.79^{b}
Isoleucine	10.36±0.38^{c}	7.64±0.30^{d}	14.11±1.65^{a}	12.34±0.92^{b}
Leucine	16.74±2.05^{c}	13.72±0.81^{d}	24.09±2.43^{b}	27.76±3.39^{a}
Arginine	21.30±3.04^{c}	14.28±2.11^{d}	23.88±3.10^{b}	24.88±0.70^{a}
Histidine	32.29±1.52^{b}	23.59±3.26^{c}	34.48±3.43^{a}	32.36±2.85^{b}
Phenylalanine	3.85±0.51^{a}	3.36±0.26^{b}	3.45±0.20^{b}	3.41±0.47^{b}
Non essential amino acid				
Glutamic	83.98±2.17^{d}	155.56±22.31^{a}	122.36±9.38^{b}	104.20±4.46^{c}
Proline	21.02±0.91^{d}	32.07±3.79^{b}	37.49±3.58^{a}	21.38±2.15^{c}
Tyrosine	4.10±0.48^{c}	3.29±0.13^{d}	4.74±0.52^{a}	4.39±0.27^{b}
Serine	32.70±1.03^{c}	29.99±1.84^{d}	41.51±2.75^{a}	34.09±1.29^{b}
Glycine	12.30±1.34^{c}	12.59±0.62^{b}	12.31±1.31^{c}	12.95±0.69^{a}
Cysteine	2.23±0.21^{c}	2.42±0.34^{b}	3.80±0.22^{a}	2.00±0.36^{d}
Aspartic	23.99±1.34^{c}	27.49±1.98^{b}	32.43±1.57^{a}	23.76±0.89^{d}
Alanin	16.51±1.62^{d}	18.34±0.94^{c}	18.62±1.62^{b}	19.40±0.56^{a}
Ammonia	23.93±3.04^{d}	39.18±5.60^{a}	31.97±1.97^{b}	30.18±0.96^{c}
Total free amino acid	361.45±16.15^{d}	463.70±55.36^{b}	525.69±40.62^{a}	458.16±24.36^{c}
Essential amino acid	164.62±8.04^{d}	181.94±26.07^{c}	252.42±25.13^{a}	235.99±20.07^{b}
Non essential amino acid	196.83±8.14^{d}	281.76±29.29^{a}	273.27±17.01^{b}	222.17±4.60^{c}
Essential / non essential	0.83±0.01^{c}	0.63±0.02^{d}	0.91±0.06^{b}	1.05±0.07^{a}

^{A,b,c,d} **means with different superscript litters within any row differ significantly ($p<0.01$).**

With regarded to individual essential amino acids, results revealed that the concentration of all of the amino acids had increased ($p<0.05$), throughout summer season as compared to values of other seasons except for leucine and arginine (Table 3). The last two amino acids concentration were high ($p<0.05$) during Autumn season.

On the other hand, most of the non-essential amino acids were significantly ($p<0.05$) increased during summer season (Table 3). However ,the glutamic acid level, increased ($p<0.01$) during spring season, but the glycine and alanin levels increased ($p<0.01$) during Autumn months (Table 3). The increased level in each of total free amino acids concentration and most of the essential and non-essential amino acid during summer season

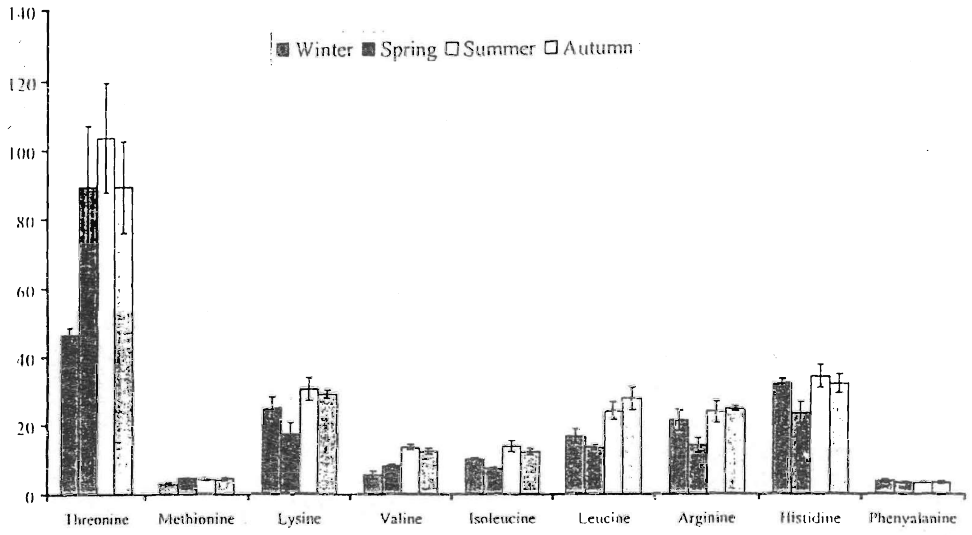

Fig (3) : Effect of season on essential amino acids concentrations (mg/100ml) in seminal plasma of rams.

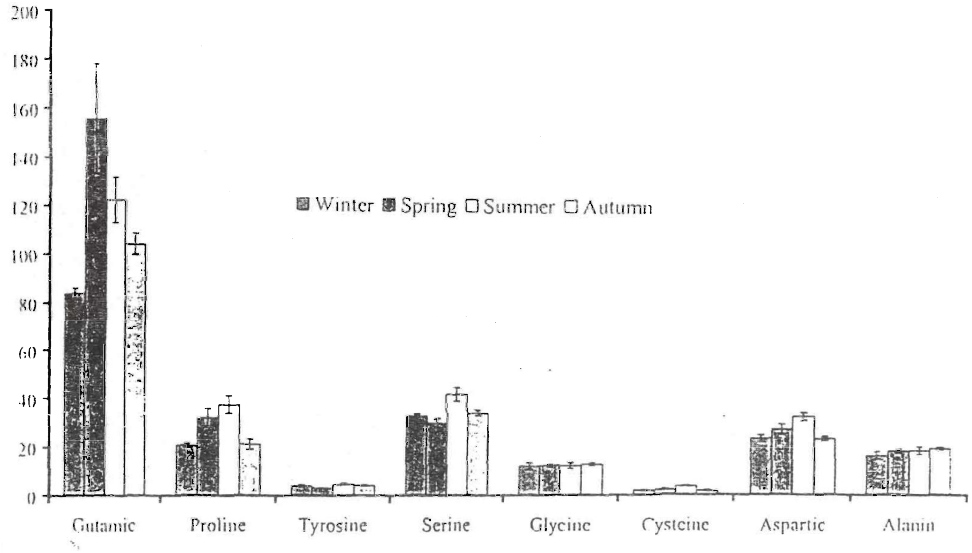

Fig (4) : Effect of season on non essential amino acids concentrations (mg/100ml) in seminal plasma of rams.

coincided with the increased in ambient air temperature and photoperiod. Thus, it can be conduced that during hot month of the year, the increased amino acid concentration in seminal plasma may be of importance in testicular activity for the well-being of spermatozoa. This, however, was proposed also by Gomes and Joyce (1975) and Lincoln *et al.* (1990) who showed a maximum testicular activity of southern sheep breed in summer and lowest in winter.

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J. Agric. Sci. Mansoura Univ., 27(11), November, 2002

تأثير السلالة والظروف المناخية علي تركيزات الأحماض الأمينية الأساسية والغير أساسية في بلازما السائل المنوي لذكور الأغنام في الساحل الشمالي الغربي

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معهد بحوث الإنتاج الحيوانى – مركز البحوث الزراعية

أستخدم فى هذا البحث عدد ١٥ ذكر أغنام بالغ تنتمي إلي أنواع البرقي والعواسي المحلي والعواسي المستورد (خمسة ذكور من كل نوع)وذلك لتقدير كمية وأنواع الأحماض الأمينية الموجودة في السائل المنوي لهذه الذكور وعلاقتها بالنوع وكذلك التغيرات الموسمية طوال العام و ذلك لما لهذه الأحماض الأمينية في السائل المنوي من أهمية في مكونات السائل المنوي وخصوبة وحيوية الحيوانات المنوية. وجد أن تركيز الحامض الأميني الأساسي الثريونين هو الغالب يليه الهستادين والليسين والليوسين والأرجنين في ترتيب تنازلي.

كذلك وجد فروق معنوية بين أنواع الأغنام حيث كان تركيز الحامض الأميني الأساسي الثريونين وكذلك الهستادين والليوسين أعلا في ذكور أغنام البرقي يليه العواسي المحلي وأظهر العواسي المستورد أقل تركيزاً لهذه الأحماض الأمينية. وكان تركيز الحامض الأميني الغير أساسي الجلوتامين غالباً يليه الثريونين والبرولين الأسبارتيك وأقلهم السيستين وكانت الفروق بين الأنواع معنوية حيث أظهرت تركيزات هذه الأحماض الأمينية الغير أساسية في بلازما السائل المنوي الخاص بذكور أغنام العواسي المحلية زيادة معنوية عن باقي الأنواع.

كما أظهرت الدراسة أن تركيزات حامض الجلوتاميك و المرتبط بحيوية الأسبرمات مرتفعة مما يشير إلي ارتفاع خصوبة هذه الأنواع بمنطقة الساحل الشمالي الغربي.

كما أظهرت الدراسة أن لفصل السنة تأثير علي تركيز هذه الأحماض حيث كانت تركيزات الثريونين والهستادين والليسين أعلاها في فصل الصيف وأقلهم تركيزاً في فصل الشتاء.

كان تركيز كل من الأحماض الأمينية (الجلوتاميك والبرولين والأسبارتيك) عالية في الصيف. إن الأنواع أظهرت اختلافاً معنوياً في تركيز هذه الأحماض تبعاً لفصل السنة حيث كانت تركيزات أحماض الثريونين والجلوتاميك مرتفعة في أغنام العواسي المحلية عن باقي الأغنام في فصل الصيف وإن الأغنام العواسي المستوردة أقلهم تركيزاً. وقد ترجع هذه الاختلافات بين أنواع الأغنام إلي تأثير الإختلاف في درجات الحرارة والرطوبة وكذلك طول فترة الإضاءة أو إلي الاختلاف ما بين الأنواع.و تؤكد الدراسة كفاءة تربية أغنام البرقي والعواسي في الساحل الشمالي الغربي من مصر نظراً" لزيادة تركيز الأحماض الأمينية صيفاً" في بلازما السائل المنوي لها.