

## MONTHLY AND SEASONAL VARIATIONS IN SEMINAL PLASMA CONSTITUENTS OF BARKI, DAMASCUS MALE GOATS AND THEIR CROSSBREED UNDER SUBTROPICAL CONDITIONS

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### SUMMARY

Fifteen sexually mature bucks, 5 each of Barki (B), Damascus (D, imported from Syria) and their crossbred (BD) were used to study the changes in seminal plasma composition throughout one year. Results showed that seminal plasma total protein, albumin and globulins values were higher in August (end of summer), throughout fall months and in December (beginning of winter). The same trend was found for fructose and total fructose concentrations but their elevated levels were continued throughout winter months. Total lipids and cholesterol recorded the highest values in late summer (August), and were high in the fall and early winter. Aspartate amino transaminase (AST) activity showed the highest values in November and December, while alanine amino transaminase (ALT) activity recorded the highest values in late summer (August) and early fall (September). The above parameters showed the lowest values during spring months. The interaction between breeds and months of the year were significant for A/G ratio, total fructose, total lipids, cholesterol, AST and ALT/AST ratio; these results were discussed.

*Keywords: Goats, seminal plasma, season*

### INTRODUCTION

Although information is available about semen characteristics of several breeds of goats (Mann, 1981; Ali and Mustafa, 1986 and Roca *et al.*, 1992), little is known about the biochemical constituent levels and enzymatic activity of seminal plasma and their relation to semen quality parameters. Taha *et al.* (2000b) found that elevated levels of ram seminal plasma constituents were associated with high semen quality. The objective of the present study is to investigate the monthly variations in some seminal plasma biochemical parameters and their relation with semen quality parameters in Barki, Damascus and their crossbred goat bucks in Egypt.

### MATERIALS AND METHODS

This work was carried out at Bourg El-Arab Research Station, located 50 km west of Alexandria (31° 15'N and 30° 10'E). Chemical analyses of samples were carried out at the Animal Physiology Laboratory of the Animal Production Department, Faculty of Agriculture, Alexandria University.

#### Animals and management

Five native Barki breed, weighed between 40-55 kg; 5 Damascus, imported from Syria, weighed between 52-69 kg and 5 crossbred Barki X Damascus, weighed between 52-67 kg were used to study the biochemical composition of seminal plasma of these goat groups throughout 12 months and each animal was used as a replicate. All animals were between 2-3 years of age and were kept outdoors under shed during daytime and housed in a semi-open barn at night. Animals nutrition and their management were as described by Ayoub *et al.* (2000).

#### Determination of seminal plasma constituents

Semen collection was performed monthly using an artificial vagina. Determination of initial seminal fructose was carried out immediately after collection according to the method of Mann (1948), while total fructose was calculated by multiplying initial fructose concentration by semen ejaculate volume.

Seminal plasma was separated from ejaculates by centrifugation at 5,000 rpm for 10 min. The recovered seminal plasma fraction was further centrifuged at 10,000 rpm for 15 min at 4 °C and the supernatant was stored at -20 °C until analysis. Total seminal plasma protein was measured by the

Biuret method, and total albumin (A) concentration was determined by the method of Doumas *et al.* (1977). Total globulin (G) concentration was calculated as the difference between seminal plasma total protein and seminal plasma albumin, then A/G ratio was calculated. Total lipids were determined as described by Frings *et al.* (1972) and total cholesterol concentration was measured by a colorimetric method (Watson, 1960). Transaminase activities (aspartate amino transaminase, AST and alanine amino transaminase, ALT) were measured by colorimetric methods as described by Reitman and Frankel (1957) and AST/ALT ratios were calculated.

### Statistical analysis

Statistical analysis was performed using the general linear model procedure of the Statistical Analysis Systems Institute (SAS, 1989). A fixed effect model was assumed to underlay each observation in each trait studied. These effects were breed, month and the interaction between breed and month. Significant differences among means were detected using Duncan's Multiple Range Test of SAS (1989).

## RESULTS

Table 1 and Figure 1 show the overall mean values of seminal plasma total protein, albumin (A), globulin (G) and albumin/globulin (A/G) ratio throughout one year. Total protein, albumin and globulin concentrations showed lower values during spring months recording lowest values (1.61, 0.79 and 0.83 g/100 ml, respectively) in April, then increased during summer, fall and winter reaching highest values in August, September and October (Table 1). Breed and the interaction between month and breed had non-significant effects on seminal plasma total protein, A or G. There were significant effects ( $P < 0.01$ ) of breed and of the interaction between months and breed on A/G ratio, where Damascus breed showed the highest ( $P < 0.05$ ) values.

Table 1. Overall mean values of seminal plasma total protein, albumin (A) globulin (G) and A/G ratio in goat breeds throughout one year (least square means  $\pm$  S.E.)

Character	N	Total Protein (g / 100 ml)	Albumin (g / 100 ml)	Globulin (g / 100 ml)	A / G Ratio
Month (M) effect		**	**	**	**
Dec	15	4.14 $\pm$ 0.35 <sup>c</sup>	2.39 $\pm$ 0.20 <sup>ab</sup>	1.74 $\pm$ 0.17 <sup>cd</sup>	1.46 $\pm$ 0.11 <sup>a</sup>
Jan	15	3.78 $\pm$ 0.24 <sup>c</sup>	1.98 $\pm$ 0.16 <sup>bc</sup>	1.80 $\pm$ 0.10 <sup>cd</sup>	1.10 $\pm$ 0.06 <sup>cd</sup>
Feb	15	3.52 $\pm$ 0.20 <sup>c</sup>	1.93 $\pm$ 0.14 <sup>c</sup>	1.59 $\pm$ 0.10 <sup>dc</sup>	1.24 $\pm$ 0.10 <sup>bc</sup>
Mar	15	2.65 $\pm$ 0.20 <sup>d</sup>	1.34 $\pm$ 0.11 <sup>de</sup>	1.31 $\pm$ 0.10 <sup>ef</sup>	1.05 $\pm$ 0.06 <sup>de</sup>
Apr	15	1.61 $\pm$ 0.10 <sup>e</sup>	0.79 $\pm$ 0.07 <sup>f</sup>	0.83 $\pm$ 0.03 <sup>g</sup>	0.93 $\pm$ 0.05 <sup>de</sup>
May	15	2.13 $\pm$ 0.04 <sup>de</sup>	1.00 $\pm$ 0.03 <sup>ef</sup>	1.13 $\pm$ 0.02 <sup>fg</sup>	0.89 $\pm$ 0.03 <sup>e</sup>
June	15	2.38 $\pm$ 0.16 <sup>d</sup>	1.14 $\pm$ 0.07 <sup>def</sup>	1.24 $\pm$ 0.09 <sup>ef</sup>	0.94 $\pm$ 0.03 <sup>de</sup>
July	15	3.62 $\pm$ 0.22 <sup>c</sup>	1.45 $\pm$ 0.07 <sup>d</sup>	2.16 $\pm$ 0.18 <sup>bc</sup>	0.72 $\pm$ 0.05 <sup>f</sup>
Aug	15	5.59 $\pm$ 0.26 <sup>a</sup>	2.74 $\pm$ 0.14 <sup>a</sup>	2.85 $\pm$ 0.15 <sup>a</sup>	0.98 $\pm$ 0.05 <sup>de</sup>
Sep	15	5.19 $\pm$ 0.26 <sup>a</sup>	2.51 $\pm$ 0.14 <sup>a</sup>	2.68 $\pm$ 0.15 <sup>a</sup>	0.95 $\pm$ 0.06 <sup>de</sup>
Oct	15	4.97 $\pm$ 0.27 <sup>ab</sup>	2.51 $\pm$ 0.14 <sup>a</sup>	2.46 $\pm$ 0.18 <sup>ab</sup>	1.06 $\pm$ 0.07 <sup>de</sup>
Nov	15	4.29 $\pm$ 0.40 <sup>bc</sup>	2.40 $\pm$ 0.22 <sup>ab</sup>	1.89 $\pm$ 0.18 <sup>cd</sup>	1.29 $\pm$ 0.04 <sup>b</sup>
Breed (Br) effect		NS	NS	NS	**
Barki	60	3.71 $\pm$ 0.18	1.85 $\pm$ 0.10	1.86 $\pm$ 0.10	1.02 $\pm$ 0.03 <sup>b</sup>
Damascus	60	3.78 $\pm$ 0.18	1.97 $\pm$ 0.10	1.80 $\pm$ 0.10	1.14 $\pm$ 0.04 <sup>a</sup>
BXD	60	3.65 $\pm$ 0.22	1.82 $\pm$ 0.12	1.83 $\pm$ 0.11	1.01 $\pm$ 0.04 <sup>b</sup>
M $\times$ Br Effect	180	NS	NS	NS	**

<sup>a-g</sup> Means within each column, with different superscripts differ significantly ( $P < 0.05$ ).

\*\*  $P < 0.01$ ; NS = Non significant

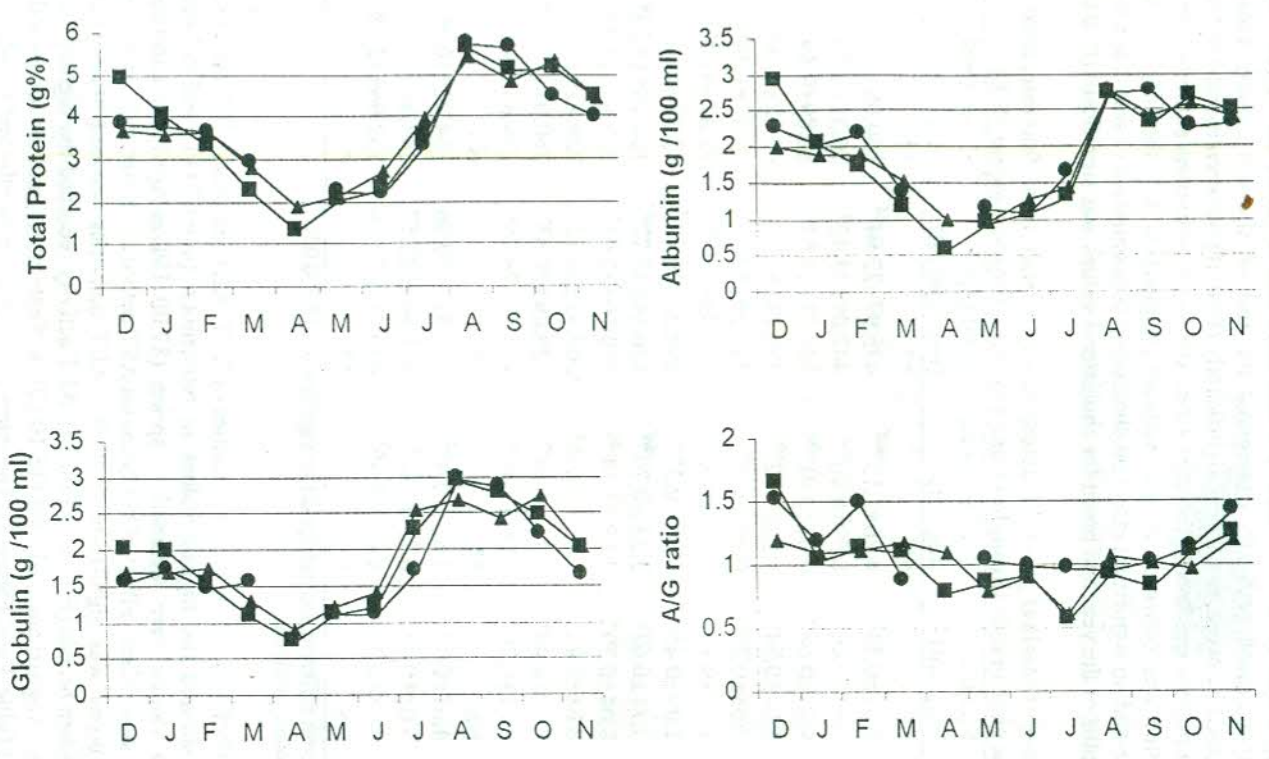


Figure 1. Monthly variations in seminal plasma total protein, albumin (A), globulin (G) and A/G ratio in goat breeds (Barki ▲ , Damascus ● and their crossbred ■ , ) throughout one year.

The overall mean fructose concentration recorded the highest values during late summer (8.04 mg/ml in August) and early fall (8.01 mg/ml in September), then decreased throughout winter and spring reaching lowest value (1.30 mg/ml) in May. Total fructose per ejaculate showed similar trend. The highest values were observed during late summer (7.55 mg/ejaculate in August) and fall (10.67 and 9.41 mg/ejaculate in September and October), then decreased throughout winter and spring reaching lowest value (0.74 mg/ejaculate) in May. The effect of breed on total fructose was significant ( $P < 0.01$ ), where Damascus breed recorded the highest value (Table 2 and Fig. 2). The interaction between M X Br on total fructose was significant ( $P < 0.05$ ). Total lipid concentrations recorded the highest values in late summer (August), and decreased gradually throughout the rest of the year months. Total lipid concentration was not affected by breed, but varied significantly ( $P < 0.01$ ) between months of the year and the interaction between months and breed (Table 2). Cholesterol concentrations exhibited the same trend where the highest value was observed in late summer (August) and fall, then declined during winter and spring. Breed showed no significant effect on cholesterol concentration (Table 2 and Fig. 2). The interaction between months of the year and breed for cholesterol values was significant ( $P < 0.05$ ).

**Table 2. Overall mean values of seminal plasma fructose concentration, total fructose, total lipids and cholesterol in goat breeds throughout one year (least square means±S.E)**

Character	n	Fructose (mg / ml)	Total Fructose (mg / ej)	Total Lipids (mg / 100 ml)	Cholesterol (mg / 100 ml)
Month (M) effect		**	**	**	**
Dec.	15	5.31±0.54 <sup>b</sup>	4.26±1.15 <sup>cd</sup>	475.97±22.94 <sup>cd</sup>	160.08±20.79 <sup>b</sup>
Jan.	15	5.53±0.46 <sup>b</sup>	6.59±0.96 <sup>bcd</sup>	447.09±23.42 <sup>d</sup>	76.31±6.73 <sup>c</sup>
Feb.	15	4.77±0.66 <sup>bc</sup>	4.03±0.91 <sup>cd</sup>	348.53±19.03 <sup>e</sup>	80.89±9.49 <sup>c</sup>
Mar.	15	3.64±0.54 <sup>c</sup>	4.50±1.12 <sup>cd</sup>	256.13±8.72 <sup>fg</sup>	99.32±6.10 <sup>c</sup>
Apr.	15	2.05±0.27 <sup>d</sup>	0.98±0.16 <sup>ef</sup>	213.20±7.87 <sup>g</sup>	53.53±1.49 <sup>c</sup>
May	15	1.30±0.13 <sup>d</sup>	0.74±0.08 <sup>f</sup>	210.93±4.38 <sup>g</sup>	77.82±6.64 <sup>c</sup>
June.	15	2.09±0.45 <sup>d</sup>	1.80±0.52 <sup>ef</sup>	298.22±30.62 <sup>ef</sup>	85.32±13.21 <sup>c</sup>
July.	15	3.71±0.62 <sup>c</sup>	3.33±0.70 <sup>def</sup>	436.44±43.77 <sup>d</sup>	155.18±15.23 <sup>b</sup>
Aug.	15	8.04±0.39 <sup>a</sup>	7.55±1.54 <sup>abc</sup>	710.19±32.01 <sup>a</sup>	267.63±13.33 <sup>a</sup>
Sep.	15	8.01±0.44 <sup>a</sup>	10.67±1.45 <sup>a</sup>	602.93±25.15 <sup>b</sup>	239.11±16.15 <sup>a</sup>
Oct.	15	7.68±0.48 <sup>a</sup>	9.41±1.34 <sup>ab</sup>	529.09±21.83 <sup>c</sup>	260.07±13.84 <sup>a</sup>
Nov.	15	6.14±0.70 <sup>b</sup>	6.06±2.48 <sup>bcd</sup>	460.59±28.75 <sup>d</sup>	234.65±30.39 <sup>a</sup>
Breed (Br) effect		NS	**	NS	NS
Barki	60	4.67±0.37	3.80±0.49 <sup>b</sup>	421.31±20.86	154.82±10.94
Damascus	60	5.02±0.43	6.73±1.02 <sup>a</sup>	411.98±25.57	147.52±14.06
BXD	60	4.88±0.33	4.45±0.40 <sup>b</sup>	430.61±22.99	152.96±12.78
M × Br effect	180	NS	*	**	*

<sup>a-b</sup> Means within each column, with different superscript differ significantly ( $P < 0.05$ ).

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; NS = Non significant

Months showed significant ( $P < 0.01$ ) effects on the activities of AST, ALT and AST/ALT ratio (Table 3 and Fig. 3). AST activity showed the highest values in November (446.67 U/L) and in December (519.80 U/L), while lowest values were recorded in spring (87.00 U/L in April) and early summer (193.73 U/L in June). No significant effect of the breed on AST activity was detected. However, the interaction of months and breed was significant ( $P < 0.01$ ). ALT activities varied ( $P < 0.01$ ) between months of the year and between breeds ( $P < 0.05$ ) (Table 3). ALT activity recorded the highest values in late summer (18.21 U/L in August) and early fall (15.19 U/L in September), and the lowest values in spring (2.72 U/L in May) (Table 3 and Figure 3). The interaction between months and breeds on ALT values was not significant. AST/ALT ratio values showed significant ( $P < 0.01$ ) effects between months, breeds and the interaction between months and breeds (Table 3). AST/ALT ratio recorded the highest value in spring (114.44 in May) and the lowest value in late summer (22.23 in August) and early fall (21.95 in September) (Table 3 and Figure 3). Damascus goats showed low sexual desire during March and April months, therefore seminal plasma samples were not sufficient to determine seminal plasma biochemical parameters (Figures 1, 2, 3).

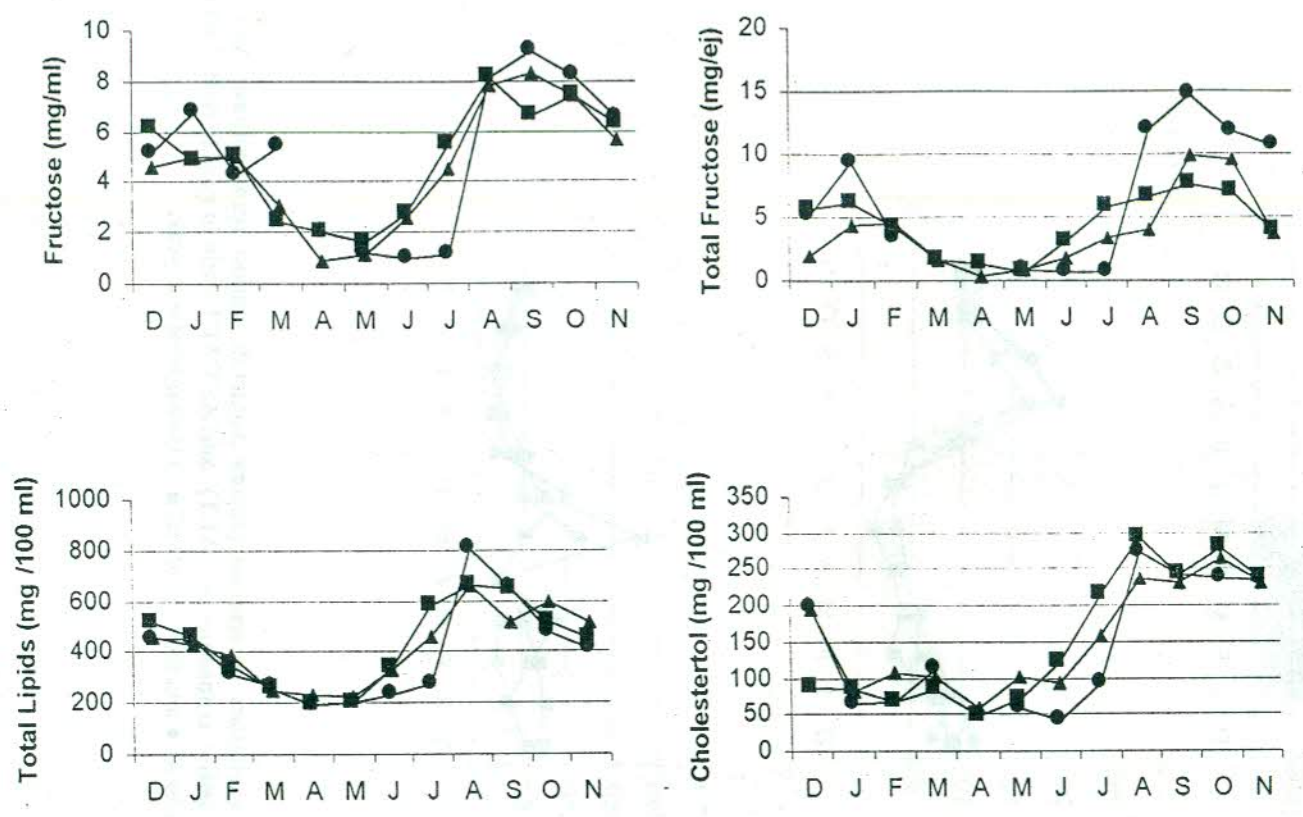


Figure 2. Monthly variations in seminal plasma fructose concentration, total fructose , total lipids and cholesterol in goat breeds (Barki ▲ , Damascus ● and their crossbred ■ , )throughout one year.

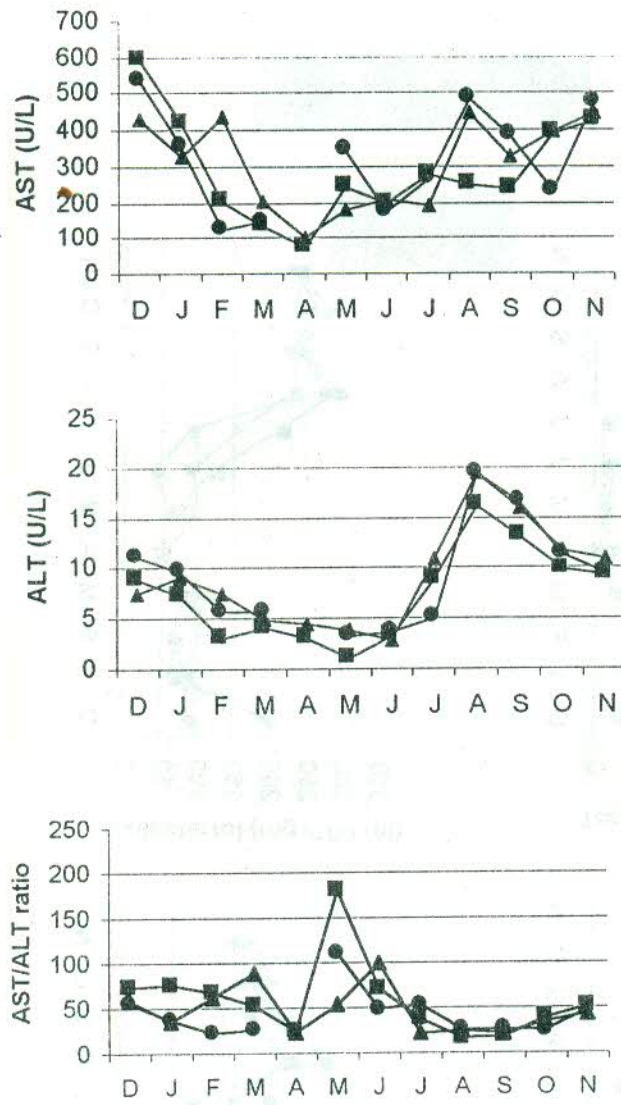


Figure 3. Monthly variations in seminal plasma aspartate amino transaminase (AST), alanine amino transaminase (ALT) and AST/ALT ratio in goat breeds (Barki ▲ , Damascus ● and their crossbred ■ , ) throughout one year.



**Table 3. Overall mean seminal plasma aspartate amino transaminase (AST), alanine amino transaminase (ALT) and AST/ALT ratio in goat breeds throughout one year (least square means±S.E)**

Character	N	AST (U/L)	ALT (U/L)	AST / ALT Ratio
Month (M) effect		**	**	**
Dec.	15	519.80±43.07 <sup>a</sup>	9.05±0.95 <sup>c</sup>	62.10±4.98 <sup>bc</sup>
Jan.	15	365.93±36.14 <sup>bc</sup>	8.71±0.94 <sup>c</sup>	48.90±8.64 <sup>cd</sup>
Feb.	15	252.27±50.50 <sup>bc</sup>	5.37±0.70 <sup>d</sup>	50.30±8.03 <sup>cd</sup>
Mar.	15	160.67±19.08 <sup>bc</sup>	4.87±0.54 <sup>d</sup>	55.63±15.10 <sup>cd</sup>
Apr.	15	87.00±4.33 <sup>b</sup>	3.70±0.23 <sup>d</sup>	23.70±0.32 <sup>f</sup>
May	15	257.60±21.45 <sup>bc</sup>	2.72±0.36 <sup>d</sup>	114.44±15.07 <sup>a</sup>
June	15	193.73±14.90 <sup>ef</sup>	3.25±0.35 <sup>d</sup>	72.90±11.28 <sup>b</sup>
July	15	244.20±19.14 <sup>def</sup>	8.29±1.09 <sup>c</sup>	37.30±4.38 <sup>def</sup>
Aug.	15	393.20±37.52 <sup>bc</sup>	18.21±1.44 <sup>a</sup>	22.23±1.97 <sup>f</sup>
Sep.	15	317.00±24.56 <sup>cd</sup>	15.19±1.39 <sup>b</sup>	21.95±1.87 <sup>f</sup>
Oct.	15	336.27±34.77 <sup>cd</sup>	10.97±1.27 <sup>c</sup>	32.82±2.94 <sup>ef</sup>
Nov.	15	446.67±41.23 <sup>ab</sup>	9.85±0.94 <sup>c</sup>	47.06±3.11 <sup>cde</sup>
Breed (Br) effect		NS	*	**
Barki	60	307.00±21.85	9.06±0.75 <sup>a</sup>	46.90±5.11 <sup>b</sup>
Damascus	60	320.11±22.26	9.13±0.87 <sup>a</sup>	43.35±3.50 <sup>b</sup>
BXD	60	285.90±21.40	7.31±0.66 <sup>b</sup>	58.71±6.29 <sup>a</sup>
M × Br Effect	180	**	NS	**

<sup>a-f</sup> Means within each column, with different superscript differ significantly ( $P < 0.05$ ).

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; NS = Non significant

## DISCUSSION

In the present study, we found positive correlation between semen quality parameters (Ayoub *et al.*, 2000) and levels of goat seminal total protein, albumin, globulin, fructose concentration, total fructose, total lipids and cholesterol. Many studies showed that low content of seminal plasma proteins is associated with poor semen quality (Verma *et al.*, 1985; Dhani and Kodagali, 1989). Seminal plasma proteins are mainly composed of albumin and globulin, in addition to small quantities of non-protein nitrogen, amino acids and peptides (Kulkarni *et al.*, 1996). These compounds make up the amphoteric property of seminal plasma proteins and, thus, low protein content in seminal plasma reduces its buffering capacity and in turn semen quality (Dhani *et al.*, 1994). This suggestion is confirmed in the present study where the lowest total protein and albumin level were noted in the first half of the year (winter and spring) (Table 1) and coincided with the highest percentages of dead sperm, abnormal sperm and altered acrosome spermatozoa as reported by Ayoub *et al.* (2000) in the same study. On the other hand, the observed increase in % sperm motility in March and April (spring) which was associated with increases in percentages of dead sperm, abnormal sperm and altered acrosomes spermatozoa could be due to the release of some acrosomal contents (i.e. acrosin) which were proved to stimulate sperm motility. Fritz *et al.* (1976) stated that acrosin increases sperm motility through kinin generation. Total protein contents in seminal plasma were highest in August and fall months and coincided with increases in albumin and globulin levels as well. Strezek *et al.* (1985) found that during the period of reproductive activity globulins were the major seminal proteins, while during the period of reproductive quiescence the level of globulin gradually decreased in red deer males. This result was in agreement with the present findings since globulins concentrations were low during winter and spring months than during the rest of the year.

Mann and Lutwak-Mann (1981) reported a close relationship between fructose and citric acid concentrations in the semen and the androgenic activity of the male. In the present study, fructose concentration showed lower levels during spring months until July (summer) then increased till it reached the highest levels during late summer and fall. This trend is similar to blood testosterone profile as reported in the same study by Ayoub *et al.* (2000). Thus testosterone level in males is correlated with semen content of fructose. Therefore, level of fructose in seminal plasma reflects testosterone activity and quality of semen. Moreover, our results are in agreement with those of Amir and Volcani (1965) in Awassi rams, and of Taha *et al.* (2000a) where minimum fructose concentrations in seminal plasma were found during spring months and the highest values were noted during August till November. The

overall mean values of total fructose varied ( $P < 0.01$ ) between the three groups of goats (Table 2). This could be due to variations which were found in ejaculate volume and sperm concentration between the groups of goats as reported by Ayoub *et al.* (2000) in the same study.

Kelso *et al.* (1997) reported that the reduction in sperm concentration and motility is associated with a decrease in seminal plasma content of lipids and also with sperm aging (poor semen quality). In the present study, we observed low level of total lipids and fructose during spring months (Table 2 and Fig. 2), as well as low percentage of live sperm and elevated percentages of dead, abnormal and altered acrosomes sperm (low quality semen) during the same period (Ayoub *et al.*, 2000).

Cholesterol levels were low during the first half than during the second half of the year (Table 2).  $T_3$  hormone reduced levels of total lipids and cholesterol in winter and spring seasons, while testosterone showed an opposite trend (Ayoub *et al.* 2000). Thyroid hormones stimulate cholesterol synthesis as well as the hepatic mechanisms that remove cholesterol from the circulation. Cholesterol is the precursor in the biosynthesis of sex hormones. The decline in plasma cholesterol levels may occur because the rate of the latter process exceeds that of the former, or it may be secondary to changes in the plasma lipoprotein levels. The plasma cholesterol level drops before the metabolic rate rises (Guyton, 1981). In addition, Guyton (1981) reported that male sex hormones (androgens) increase blood cholesterol. These findings are in agreement with findings in the present study.

Iritani and Nishikawa (1964) and Iritani *et al.* (1964) found that both enzymatic activity and chemical constituents of goat seminal plasma are increased during the breeding season. In the present study, seminal AST and ALT were lowest in spring season than those in other seasons of the year. However the ratio of AST/ALT was higher in spring than that in other seasons. The increase in the concentrations of AST and ALT enzymes in August (summer) till December (early winter) was associated with high percentage of live sperm and low percentages of dead, abnormal and altered acrosomes spermatozoa which suggested that these transaminases were of seminal plasma origin. Polakoski *et al.* (1976) stated that seminal plasma enzymes, which can be measured at higher concentrations in the first portion of the ejaculate such as aspartate aminotransferase are probably of prostatic origin. Contrary to our finding in goats, results in sheep (Taha *et al.*, 2000b) showed that AST and ALT enzyme activities in seminal plasma were lower during summer (good quality semen) than those during spring (low quality semen) which could be due to species difference. Furthermore, unpublished work on Egyptian buffalo semen showed positive correlation between transaminase activities and good semen quality.

## CONCLUSION

The biochemical analyses of goat seminal plasma indicated that the highest values of total protein, albumin, globulin, fructose, total lipids, cholesterol, AST and ALT enzymes were recorded in late summer and early fall, while A/G and AST/ALT ratios exhibited the lowest values. On the other hand, the lowest values of the same parameters were recorded in spring, while AST/ALT ratio showed highest value.

## ACKNOWLEDGEMENT

The authors are grateful to Prof. M. H. Salem for critical reviewing of the manuscript.

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