EFFECT OF SEASON AND PARITY ON BLOOD PLASMA METABOLITES, MILK YIELD AND MILK COMPONENTS AND SOMATIC CELL COUNT IN NEWLY PARTURITION EGYPTIAN BUFFALO FEMALES

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SUMMARY

total of 24 newly parturition female buffaloes weighted above 450 kg were selected randomly and directly after parturition from APRI station herd which belonging to Animal Production Research Institute, Mahhalet Mousa, Ministry of Agriculture, Egypt and directly distributed into two groups of experimental animals as twelve of them were selected as expected to give birth in winter months (October to March, S1) and the other twelve were selected as expected to give birth in summer months (April to September, S2). Two sub-groups were allotted and they were in different parturition sequences from the 2rd parturition to the 14th one (P1 and P2), where up to 4 parity was in sub group 1 (P1) and the sub-group 2 (P2) were the animals from parity 5th and up till parity 14th. The aim of this investigation was to study the effect of seasons of the year expressed as Temperature-Humidity Index (THI) and parities on some blood plasma metabolites of female buffaloes as indicators to the animal health, milk yield and milk components The effect of season and parities on somatic cell count (SCC) in the milk were also aimed.moderate heat stress was recorded in S2 as proved by THI value which was 78.2 vs 60.6 in S1. Concentrations of total protein (7.6 g/100 ml in S1 vs 5.2 g/100 g/100 ml in S2) and globulins (4.5 g/100 ml in S1 vs 2.3 g/100ml in S2) in the plasma of healthy buffalo females were significantly (P< 0.01) influenced by season of calving the albumin concentrations were slightly higher (P>0.05) in plasma of the buffalo females that gave birth in the winter (3.1 g/100 ml) compared with summer calving (2.9 g / 100 ml). Plasma urea concentrations in S1 (winter calving) were more (P < 0.05) than its concentration in S2 (27.6 mg / 100 ml) in summer S2 versus (35.0 mg/100 ml) in winter S1). Higher (P < 0.01) AST activity was detected in the winter calves (S1) compared with the summer calves (S2). No significant effect (P > 0.05) for parity was detected among all plasma blood parameters investigated in the present study. Numerical higher values were recorded in winter season (S1) than in summer season (S2) in milk yield and milk components. No significant effect was observed by parities on both of them. Somatic cell count (SCC) proved to be affected by seasons but not by parities.

Keywords: Egyptian buffaloes females, season, parity, THI, blood metabolites, milk yield, milk components, somatic cell count (SCC) in the milk.

INTRODUCTION

Egyptian buffaloes are exposed to seasonal variation which naturally governed by environmental temperature, humidity and day light hours that may affect open–day interval. To assess animal nutritional status and health blood plasma metabolites are usually used as tools of diagnostic procedures based on determining the various parameters in the blood of animals (Antunovic *et al.*, 2009 and Ashmawy, 2015). Significant variations in these parameters reflect the physiological conditions that affect their nutritional and health status (Van Saun, 2000). Understanding the variability in milk yield and milk composition is important when making management decisions and in milk-recording programs (Quist, *et al.*, 2008). Also, Somatic cell count is usually used to detect udder infection, especially the sub-clinical mastitis (Cziszter, 2003). The total bacteria count shows how the milk was harvested manipulated and stored before reaching the milk processing plant (Cziszter *et al.*, 2012). Therefore, the aim of this study was to provide an overview of the effect of seasons of the year and parities on some blood metabolites, milk yield and milk composition and somatic cell count (SCC) in the milk of newly parturition Egyptian buffalo females.

MATERIALS AND METHODS

The present study was carried out at Mahallet Moussa Experimental Station, Kafr El-Sheikh Governorate, which is belonging to Animal Production Research Institute (APRI). The experimental study started from October, 2014 up to September, 2015. It was aimed to investigate the effect of season of the year and parities on some blood metabolites, milk yield and milk composition and somatic cell counts of the milk of newly parturition Egyptian buffalo females.

Experimental animals

The present study was conducted on a total of 24 newly parturition lactating buffalo cows. Those buffalo females were chosen randomly after parturition. Twelve of them were selected as expected to give birth in winter months (October to March, S1) and the other twelve were selected as expected to give birth in summer months (April to September, S2). All animals were allotted randomly from the farm's herd and they were in different parturition sequences (P1 and P2), from the second parturition to the 14^{th} one as shown in Table (1).

Table (1): Parturition seq	uences of the experiment	al animals according	to seasons (S) and	parities (P).
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Parity	Season				
	S1	S2	Total		
P1	6	6	12		
P2	6	6	12		
Total	12	12	24		

S1, S2 winter and summer seasons, orderly; P1, number of animals in 2 to four parities, P2, number of animals from 5 parities and more. The initial body weights of the two experimental groups of animals were 500 ± 25 and 460 ± 35 kg for S1 and S2, respectively.

Housing and feeding systems:

The animals were kept in loose housing under sheds while the day. The animal's house in Mahallet Moussa station was a free-stall barn has an asbestos roof, earthy land, the nutrition, drinking basins and walls from cement. The height of the ceiling was nearly about 4.5-5 m from land and nearly 3.0 to 3.5 m from animal to roof. Buffalo females in the present study were fed according to Animal Production Research Institute allowances (APRI, 1997) concentrate feed mixture in feeding groups according to their live body weight and milk production. The concentrate feed mixture and the roughage as Egyptian clover (*Trifolium alexandrinum*) and rice straw were offered (ad lib) during cold season, while in hot season, clover hay, rice straw and the concentrate feed mixture were introduced.

Experimental procedure

This study focused on the first 150 days post–partum for all experimental groups (S1P1, S1P2, S2P1 and S2P2). All Buffalo females were hand milked twice daily at 6.00 a.m. and 4.00 p.m. during the whole experimental period. Milk production was recorded daily for all of the experimental animals. The routine mating system is heat detection by using experienced bull, where the bull was allowed to run with the buffalo females at the proper time after parturition throughout the day during the experimental period for detecting the buffalo cows in heat which showed by standing behavior of the female. Buffalo cows which showed standing behavior were served at the 1st detected heat post-partum.

Determination of Temperature-Humidity Index (THI)

The daily ambient temperature and relative humidity percentage were obtained from the local weather station data throughout the experimental period. The temperature-humidity index (THI) was calculated by the formula of Mader*et al.* (2006) which was stated as follows: THI = (0.8 T [(0.01 RH) (T-14.4)] + 46.4

Where: T is air temperature in centigrade and RH is the relative humidity%

The THI at which cows begin to experience heat stress is 68, Mild moderate stress respiration rate exceeds 75 bpm and rectal temperature exceeds 38 $^{\circ}$ C. Moderate severe stress respiration rate exceeds 85 bpm and rectal temperature exceeds 40 $^{\circ}$ C. Severe stress respiration rate exceeds 120 bpm and rectal temperature exceeds 41 $^{\circ}$ C.

Blood metabolites assay

Blood samples were collected twice a week by jugular venipuncture (between 7.00 and 8:30 am) for determination of blood metabolites concentrations. The start of collection of blood samples was at 15 day postpartum. The blood sample collection was ended when the buffalo female proved pregnant. Blood samples were collected in heparinized test tubes, after collection the blood samples were centrifuged at 4000 rpm/15 minutes. The clear plasma was then aspirated and stored at -20 ^oC, until time of analysis. Blood metabolites was analyzed monthly along whole experimental period. Plasma total proteins were determined using a calorimetric method as described by Armstrong and Carr (1964) with minor modifications. The determination of plasma albumin was carried out according to the method of Doumas *et al.* (1971) with some modifications. Globulin values were determined by difference of plasma total protein and plasma albumin. The reaction between substituted diketons and the amino groups of urea constitutes the basis for the colorimetric determination of the latter, the most used compound is diacetylmonoxime (Curtius and Marce, 1972). Plasma transaminases were determined calorimetrically by the method of Reitman and Frankel (1957).

Assay of milk components

Samples of milk were collected from each animal at morning and evening milking, immediately after each milking, samples were taken for acidity determination according to Ling (1963). The sample of each animal represents a mixed sample of constant percentage of the evening and morning yield. Fat content,

total protein and ash were determined according to Ling (1963). Lactose content was determined calorimetrically according to Barrnett and Abd El-Tawab (1957). Solids not fat content was calculated by the differences between total solids and fat content. Somatic cell count (SCC) was determined according to Schukken *et al.* (2003).

Statistical Analysis:

Data of blood plasma parameters was analyzed using SAS (2002) and SPSS (2007) to study the effect of season (winter,S1 and summer,S2), parities as sub-groups(P1and P2) and the interaction between them. The statistical model was as follows:

Model 1: $Y_{ijkl} = \mu + S_i + P_j + (S^*P)_{ij} + an_{ijkl} (S^*P)_{ij} + B(X)_{ijkl} + e_{ijkb}$

 Y_{ijkl} is the dependent variable (studied trait; albumin, alt_sgpt, ast_sgot, urea, total-protein, globulin) of the lth record on the kth animal, jth parity, ith season; , μ the overall mean of studied trait; S_i the effect of the ith season, i=1 to ...; P_jthe effect of the jth parity, j=1 to;

an_{ijkl} the effect of animal; $(S*P)_{ij}$ the effect of interaction between season and parity; $b_{y/x}$ the regression coefficient of the studied trait on THI; X_{ijkl} is the independent variable of THI; e_{ijklmn} the effect of random error, associated with each observation assumed to be normally and independently distributed with 0 mean and variance I σ_e^2 .

Model2: where data of monthly milk production were analysed using repeated measurements $:Y_{ijkl} = u + S_i + P_j + (S^*P)_{ij} + an_{ijkl}(S^*P)_{ij} + M_k + (S^*M)_{ik} + (P^*M)_{ik} + e_{ijkl}$

 Y_{ijkl} is the dependent variable (studied traits; milk production of the first to the fourth month) of the lth record on the kth animal, jth parity, ith season; μ the overall mean of studied trait;

S_i the effect of the ith season, i=1 to ...; P_jthe effect of the jth parity, j=1 to; an_{ijkl} the effect of animal; (S*P)_{ij} the effect of interaction between season and parity; an_{ijkl} (S* P)_{ij} the effect of animal within season*parity (error1);M_k the effect of month (k= 1,2,...);(S*M)_{ik} the effect of interaction between season and month; (P*M)_{jk} the effect of interaction between parity and month; e_{ijkl}the effect of random error, associated with each observation assumed to be normally and independently distributed with 0 mean and variance I σ_{e}^2 .

RESULTS AND DISCUSSION

In this study, THI during winter season (S1) was 60.6 while in summer (S2) was 78.2 (Table 2). The difference was significant at P < 0.05 level. Animals in THI more than 68 are considered to be exposed to moderate heat stress as reported by Mader *et al* (2006).

Table ((2)	: The	ambient	attributes	in win	ter.	summer	seasons	during	the	exper	imental	period	I.
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Ambient Attributes	S1	S2
Average Temperature	16.1±1.10	28.3±0.92
Average Relative Humidity	75.0±2.10	65.8±3.81
Temperature Humidity Index	60.6 ± 1.65^{b}	78.2±2.41 ^a

Superscript a and b in small form represent a significant difference at P <0.05 orderly.

Seasonal Effect on Buffalo Metabolic Profile

Plasmaprotein profile

The results of blood metabolites of the healthy buffalo females calved either on winter or summer seasons (winter, S1 and summer, S2), are presented in Table (3). Total protein (g / 100 ml), albumin (g/100), globulin (g / 100 ml), urea (mg/ 100), ALT and AST (U/L) were determined as an average \pm S.E.

Plasma total protein reflects the nutritional status of the animal and it has a positive correlation with dietary protein (Kumar *et al.*, 1980). It has been noted that heat exposure causes initial haemo-concentration followed by haemo-dilution (Gudev *et al.*, 2007). The female's metabolic profile has been related to the reproductive activity as plasma lower concentration of total protein was correlated with abnormal cycling activity and longer interval to post-partum conception Amanullah *et al.* (1997) and Kumar *et al.* (2010).Concentrations of total protein in the plasma of healthy buffalo females were significantly (P< 0.01) influenced by season of calving and ranged between 5.2 to 7.6 mg / 100 ml,with an average of 6.4 ± 0.28 as shown in Table (1). The current results are in accordance with the results of El-

Masery and Marai (1991) who found that TP were 4.4 g/100 ml and 5.1 g/100 ml in summer and winter seasons, respectively. Also, Omran *et al.* (2011) indicated that total proteins in Egyptian buffalo calves exposed to direct solar radiation were decreased by 11.9 %. Moreover, Gudev *et al.* (2007); Haeeb *et al.* (2007); Abou-Zeina *et al.* (2009); El-Khashab (2010); and Das *et al.* (2013) reported that plasma total protein level tended to be lower during maximum heat load and it showed a significant decrease with cooling or supplementing anti-heat stress compounds. Additionally, Al-Saeed *et al.* (2009) in dairy cattle reported that the plasma total proteins in the winter season showed significantly higher values than that of the same animals during the summer season. Heat exposure and dehydration during heat stress results in a sharp increase in ADH level, which was associated with a significant decrease in urine output and a significant increase in plasma protein (El-Nouty*et al.* 1980).

Parameter	S1	S2	Overall mean
Total protein (g/100 ml)	$7.6^{A} \pm 0.28$	5.2 ^B ±0.28	6.4
Albumin(g/100 ml)	3.1 ±0.08	2.9 ± 0.08	3.0
Globulin(g/100 ml)	$4.5^{A} \pm 0.29$	$2.3^{B}\pm0.29$	3.4
Urea(mg/100 ml)	$35.0^{a} \pm 1.87$	$27.6^{b\pm}1.87$	31.3
Alanine Transaminase (U/L)	45.0±2.79	36. 2±2.79	40.6
Aspartate Transaminase (U/L)	105.2 ± 9.94	$76.5^{\rm B} \pm 9.94$	90.8

 Table (3): Effect of season on blood plasma metabolites during postpartum period in newly parturition female buffaloes.

Values are shown as mean \pm standard deviation. S1 refers to experimental animals that delivered in winter season; S2 experimental animals that delivered in summer season. Superscript a and b in capital or small form represent a significant difference at < 0.01 and 0.05 orderly.

Plasma albumin is known as a key element in transporting metals, fatty acids, cholesterol, bile pigments, and drugs, in addition to regulation of osmotic pressure and distribution of fluid between different compartments as reviewed by Peters (1996). It also plays a vital role in the scavenging activity (anti-oxidant) to remove free radicals from the system during thermal stress (Koubkova *et al.*, 2002). In the current study, the albumin concentrationswere slightly higher (P> 0.05) in plasma of the buffalo females that gave birth in the winter (3.1 g / 100 ml) compared to summer calvers (2.9 g / 100 ml) as shown in Table (3). It might be worthy to note that Rowlands *et al.* (1977) reported no considerable difference between summer and winter calvingin serum albumin concentrations. Also, the plasma albumin concentration between buffalo females calved either in S1 or S2 may be attributed to the feeding system since buffalo females calved during summer season depending only on concentrates.

Plasma Globulins usually indicate thestatus of the animal's immune system (Bush, 1991). Plasma globulins in the current study werefound to be influenced by season of calving (S1: 4.5 and S2: 2.3) with significantly higher (P<0.01) concentrations in winter (S1) than in summer (S2). In dairy buffaloes, focusing on the effect of season on globulin concentration are rare and also contradictory (Atallah and Abd-Alla, 1998). The effect of climatic conditions on globulins in dairy cows is controversial: some studies did not find a seasonal influence on blood globulin concentrations (Chan *et al.* 2004), while other studies found higher concentrations in summer than in winter (Wenz *et al.* 2010).

Plasma urea (BUN)

During transition period, buffaloes are known to be highly prone to nutrient drainage (Mandali *et al.*, 2002).Urea concentration is an indicator of energy protein balance (Campanile *et al.*, 1998; Dhali et al., 2006) and is typically increased in animal's deficient in energy. In the current study (Table 3). Plasma urea concentrations in S1 (winter calving) were more (P<0.05) than its concentration in S2 (27.6 mg / 100 ml) in summer (S2) versus (35 .0 mg/100 ml) in winter (S1). Khan *et al.* (2011) reported an increase in BUN during the post-partum period in winter season calves reflecting better availability of feeds and fodders supplying sufficient nutrients for optimum performance.In line, the level of BUN in the study of Omran *et al.*, (2011) on Egyptian buffalo was also significantly lowered in response to heat stress. Similar

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significant seasonal variations were obtained in BUN concentration in dairy cows by Dhali *et al.* (2006). Higher BUN in winter calves during the post-partum period indicates a deficiency in energy as sufficient quantity of leguminous fodder is supplied during this period. However, higher serum urea was reported in the summer season and spring than autumn and winter (Querchi *et al.*, 1999). Also, El-Masry and Habeeb (1989) reported that blood urea nitrogen level was decreased due to heat stress condition in Friesian cows and calves. The lowered trend in urea levels probably associated with the use of urea for protein synthesis on rumen-hepatic pathway due to compensation of the low protein uptake in summer (Yokus *et al.*, 2006), and plasma urea concentration increases as a result to the increase in rate of protein breakdown as explained by Bush (1991).

Enzyme activities (ALT and AST concentrations)

In this study, it was found higher (P<0.01) AST activity in the winter calves (S1) compared to the summer calves (S2) was found. The values measured in this study were 105.2 U / L for S1 compared to 76.5 U / L for S2 females; (Table 3). No significant difference (P>0.05) was detected in ALT concentration (U/L) as affected by both seasons (S1: 45.0 and S2: 36.2 U/ L). Al-Saeed *et al.* (2009) in dairy cattle reported that AST and ALT in the winter season showed significantly higher values than that of the same animals during the summer season. However, contradictory to our results, the activity of ALT and AST were found significantly elevated during summer season compared to winter (Pandey *et al.*, 2009). Blinco and Dye (1958) reported a normal healthy cattle value of ALT ranged 19 to 99 U / L . The values measured in ourstudywere in that range as shown in Table (1), but are lower than the values reported by Khawaskar *et al.* (2012) in Surti buffaloes. These results might indicate the integrity and of liver tissue was obviously maintained during the current experiment. Moreover, the increase in ALT and AST activities in the blood of buffaloes may be related to variation in dry matter intake around parturition and in some cases may lead to hepatic lipidosis to alter the normal function of the liver (Greenfield *et al.*, 2000).

Effect of parity on postpartum metabolic activity

Results in Table (4) show the effect of parity (as p1 represents the experimental animals that have parity two to four parities, and p2 represents experimental animals that have more than four parities) on the concentration of some plasma metabolites. No significant effect (P > 0.05) for parity was detected among all plasma blood parameters investigated in the present study as shown in Table (4).

Parameter	P1	P2	LS Mean	Overall mean
Total protein (g/100 ml)	6.5	6.3	0.13	6.4
Albumin (g/100 ml)	3.0	2.9	0.04	3.0
Globulin (g/100 ml)	3.5	3.4	0.13	3.4
Urea (mg/100 ml)	31.3	31.2	0.88	31.3
ALT (U/L)	41.4	39.7	1.31	40.6
AST(U/L)	91.5	90.1	4.68	90.8

Table (4). Effect of buffaloes'	narity on blood plasm	na metabolites durin	g nostnartum neriod
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P1 refers to experimental animals in parity 1 to parity 4; P2 experimental animals in parity 5 and more, no significant differences were found amongst all parameters (P>0.05).

These findings were in line with the findings of Elgabban (2007) and Khazanehei *et al.* (2015) who worked on Holstein cows and stated that parity order didn't affect the level of blood plasma metabolites. While contradictory results were reported with the findings of Blum *et al.* (1983); Cozzi *et al.*, (2011) working on Holstein lactating cows and found that parity affected blood concentration of total protein, globulins, creatinine and alkaline phosphatases.

Also, Abdulkareem (2013) found that in buffaloes, AST activity was numerically higher but not significant (P>0.05; $96.06^{-1}8.75 \cdot 102.61^{-1}11.62$ Unit L⁻¹) during postpartum periods in comparison with its value at calving ($90.06^{-1}8.04$ Unit L⁻¹) indicating that hepatic metabolism might be more stressed and tissue catabolism was more.

In general aspect and from the obtained results, it was noticed that all blood biochemical components were significantly or numerically lower in S2 and in parity 2. These findings may indicate that moderate environmental conditions influence some blood biochemical components in dairy buffalo during postpartum period.

Seasonal effect of milk components and somatic cell count (SCC) in milk

Seasonal and parity influence of milk yield

The buffalo milk yield and components are predetermined genetically (IDF,2010), but it also depends upon many factors, including stage of lactation, parity, feeding regimens, age, udder health and season (Kilic and Kilic, 1994 and Haenlein, 2003). A negative correlation between environmental temperature and the amount of milk fat and protein. The rise in environmental temperature during the summer season was found to result in a decrease of milk production and the solid fat. As we stated earlier, in this study (Table 2), the average THI value, during winter season (S1) was 60.6 while in summer (S2) was 78.2. Animals in THI more than 68 are exposing to moderate heat stress as reported by Mader *et al* (2006).

In the current study, it was found no significant differences (P > 0.05) for the effect of seasons and parity on the amount of milk yield and 4% FCM (Kg) in the different experimental groups as shown in Table (4).

Parameter	S1±S.E	S2± S.E	Mean± S.E
P1	307 ± 16.13	267 ± 13.9	287 ± 15.07
	(404.2)	(345.9)	(375.1)
P2	286± 13.5	290 ± 16.5	288 ± 15.07
	(365.8)	(386.6)	(376.2)
mean	297 ± 14.8	279 ± 15.2	288 ± 4.7
	(385.0)	(366.3)	(375.7)

Table (4):	Amount of milk yield and 4% fat corrected milk (FCM) /month /animal (kg) in the
	experimental groups as affected by season of the year and parity number in Egyptian
	buffalo.

S1 represents experimental animals that delivered in winter season, ,S2 represents experimental animals that delivered in summer season, P1: animals that had 2 to 4 parities, P2: animals that had 5 parities and more.

*Number between brackets are FCM estimates based on: FCM $_{(Kg)} = 0.4$ (milk yield in kg) + 15 (amount of fat in milk in kg).

Our results agrees with the results of Dular and Lebelle (1977) who found no significant differences in milk yield between summer and winter seasons, where they attributed that to prolactin content of secretory granules in the interior pituitary which was lower in winter than in summer. Contradictory results were obtained by Kassab *et al*(1976); Romcevic *et al*. (1984); Ahmad *et al*. (2001); Bufano *et al*. (2006), and Pawar *et al*. (2012) where the highest milk yield was obtained in animals calving in winter season. These differences may be attributed to the difference in both maximum environmental temperature and daily photo period length during the study which alternate the neuro- endocrine system (Schams *et al.*, 1980). Also, reduction in milk yield might further intensified by decrease in feed consumption by the animals to compensate high environmental temperature.

In line with our findings regarding the parity influence on total milk yield and FCM, Cady *et al.* (1983), Pandya *et al.* (1984); Ahmad *et al.* (1993); Javed *et al.* (2001); Pawar *et al.* (2012) and Pawar *et al.* (2012), did not find any significant effect due to parity on total milk yield. Yet, Anderson (1985) reported that production of cow reaches the peak around fifth parity, when animal is seven to eight years old and gains to adult body size. Therefore, increase in production is caused by maturation and increase in body weight and mammary gland. He added that Mammary gland grows and develops parallel to growing, maturation and recurring pregnancies and lactations. Moreover, Bagnato (1994) showed that cows of the same age but different parity have different production, and that differences are particularly evident for the first and second parity. However, these in consistent variations among results may be attributed to environmental factors among the study and the individual variation that exist within experimental animals.

Seasonal changes of milk components and somatic cell counts

The results in Table (5) shows the effects of season (winter; S1 and summer; S2) on chemical composition of milk and somatic cell count (SCC).

Parameter	S1	S2	LS Mean
Fat%	6.11	6.01	0.319
Protein%	3.85	3.21	0.148
Lactose%	5.34	5.20	0.093
Total Solids (TS)%	15.83	15.13	0.372
Solids Not Fat (SNF)%	9.81	9.07	0.206
Somatic Cell Count(SCC)	4.5 X 10 ^{5 B}	9.8 X 10 ^{5 A}	0.032

Table (5): Effect of season on chemical composition of milkand somatic cell count (SCC).

SI represents experimental animals that deliver in winter season, S2 represents experimental animals that deliver in summer season, The superscript a and b letters in capital form represent a significant difference < 0.01..

No significant differences (P > 0.05) between both seasons among all corresponding chemical parameters investigated. Yet, it is worth mentioning that numerical higher values were recorded in winter season (S1) than those in summer season (S2). Similar feeding regime may explain the insignificancy (P > 0.05) detected among chemical composition data (The concentrate feed mixture and the roughage as Egyptian clover (Trifolium alexandrinum) and rice straw were offered (ad lib) during the cold season, while in hot season, clover hay, rice straw and the concentrate feed mixture were introduced according to APRI (1997). In support of this view, Nateghi et al. (2014) reported that the different seasons of the year is often related to different food regimes for dairy cattle. They added that food intake and kind and quality of fodder are connected to the food regime which offers different possibilities to the breeder because using suitable diets according to the needs of the cows which on the other hand effects on the milk composition. Lyatuu and Eastridge (2003) approved that changes in milk component are more correlated to feeding than to genetic ones, so for better correlations among different composition the food regime is more pronounce than the level of nutrient in a diet. However, numerical higher values detected in \$1 than S2 in chemical composition of milk might be due to increased feed intake in winter season than in summer. That was in agreement with the results of Azad et al. (2007). The average fat percentage was found to be significantly affected by season of the year (Pawar et al., 2012). They also found that solid not fat and fat content of milk was little highest during winter. Bernabucci et al. (2010) studied the effects of hot season on milk protein component in 40 mid-lactating Holstein. Their results showed that the decreased of milk protein level in the hot season was due to the decrease in the casein that it means reduction in β -casein and α s-casein. However, Frelich *et al.* (2012), investigated relation about feeding system and seasonal diffidence in fatty acid composition of milk. The seasonal decreased in saturated fatty acids against the unsaturated fatty acids omega-6 against omega-3 polyunsaturated fatty acid confirmed that the cow's milk in summer was more beneficial to humans' health than that cow's milk in winter. Concerning the effect of season on somatic cell count, significant increase (P > 0.01) was detected in summer (S2) compared to winter (S1).

These Results were in agreement with the results of Saravanan *et al.* (2015) who found significant increase value in flush season (summer) than in lean season (winter). This change in somatic cell counts during different seasons in their study was also highly significant (p<0.01). They attributed The higher somatic cell count observed during the flush (hot humid) season to harsh climatic condition of high humidity and ambient temperature leading to stress condition and an increase in the susceptibility to infection as reported earlier by Dohoo and Meek (1982) and Hogan *et al.* (1989).

Parity influence on milk components and somatic cell counts

The effect of parity on milk composition and somatic cell counts was shown in Table (6). No significant differences (P > 0.05) were detected among results in all parameters tested. Our results are in agreement with results of Vasilios *et al.* (2012) who worked on buffalo cows and found no significant difference by parity (P> 0.05) on milk composition and somatic cell counts regardless the significant difference (P<0.01) they detected in milk yield. They configured that milk fat (80.8 g/kg), protein (45.9 g/kg), lactose (51.2 g/kg) and ash (8.2 g/kg) contents, as well as somatic cell counts (82.9 ×1000/ml) and colony forming units (44.9 ×1000/ml), were not affected (P>0.05) by parity.

In addition, Yadav et al. (2013) investigated the characteristics of lactation curves and effect of lactation stage, parity and season on milk yield and level of its constituents in 100 buffaloes varying from

parities 1 to 7. They found that data on levels of milk constituents varying over the lactation stages and parities.

Parameter	P1	P2	LSMean
Fat	6.06	6.22	0.150
Protein	3.61	3.45	0.069
Lactose	5.28	5.25	0.044
Total Solids (TS)	15.36	15.61	0.175
Solids Not Fat (SNF)	9.50	9.38	0.097
Somatic Cell Count(SCC)	5.9 X 10 ⁵	5.5 X 10 ⁵	0.022

Table (6): Effect of parity on milk chemical composition and somatic cell counts (SCC).

P1: animals in 2 to 4 parities, P2: animals from 5 parities and more *In this study, the average THI value during winter* season (S1) was 60.6 while in summer (S2) was 78.2. Animals in THI more than 68 are exposing to moderate heat stress as reported by Mader et al (2006).

CONCLUSION

From the obtained results, there were significant or numerical effects by seasons on all blood biochemical parameters and milk components. No effect by parity was detected. These findings may indicate that moderate environmental conditions influence blood biochemical and milk components in dairy buffalo during postpartum period. All blood values were in normal ranges and in good health condition although exposing during summer season to moderate heat stress. Somatic cell count (SCC) proved to be affected by seasons but not by parities.

REFERENCES

- Abdulkareem, T.A. (2013). Some hematological and blood biochemical attributes of Iraqi riverine buffaloes (*Bubalus bubalis*) around calving and postpartum periods. Al-Anbar J. Vet. Sci., 6: 143-150
- Abou-Zeina, A.A.A.; S. G. Hassan, H. A. Sabra and A M. Hamam (2009). Trials for elevating adverse effect of heat stress in buffaloes with emphasis on metabolic status and fertility. Global Veterinaria 3 (1): 51–62.
- Ahmad, M.; R. Hussain; K. Javed (2001). Effect of some environmental factors on milk production traits on murrah buffalo. The J Ani Pl Sci; 11(4): 147-49.
- Ahmad, S.S., M.A. Khan, A. Ali, M.S. Muhammad (1993). Effect of environmental factors (year, season and parity) on milk production traits in Murrah buffaloes The J Ani Pl Sci 3: 110-12.
- Al-Saeed, E.A., E.M. Zubeir, O.A.O. Owni (2009). Antimicrobial resistance of bacteria associated with raw milk contaminated by chemical preservatives. World Journal of Dairy and Food Sciences, 4(1), 65-69
- Amanullah, M., M.K. Tandle, S.S. Honnappagol, S.D. Sonwane, S.M. Kartikesh, B.C. Das and Jagjiwaniram (1997). Serum cholesterol, calcium, phosphorus and total protein in relation to estrus and anoestrus in non-descript buffaloes. Indian J. Dairy. Sci., 50: 410-412. 32.
- Anderson, R.R. (1985). Mammary gland. In: Lactation (Ed.: Larson, B. L.). Ames, The Iowa State University Press; p. 276
- Antunovic, Z., M. Speranda, B. Mioc, J. Novoselec and T. Speranda (2009). Zum ernahrungszust und von ziegen unter organischen produktionsbedingungen. Tierarztliche Umschau, 64: 18-23. (Arabic abstract).

- APRI (1997). Animal Production Research Institute allowances, Ministry Of Agriculture, Dokki, Giza, Egypt.
- Armstrong W. D. and C. W. Carr (1964). Physiological chemistry: lab. Direction, 3 ded. Page 75, Burges publishing company, Minneapolis, USA.
- Ashmawy, Neama A. (2015). Blood Metabolic Profile and Certain Hormones Concentrations in Egyptian Buffalo during Different Physiological States. Asian Journal of Animal and Veterinary Advances, 10: 271-280
- Atallah, S.A. and O. A. Abd-Alla (1998). Relationship Between Fertility And Some Blood Metabolites In Buffalo . Assut Vet. Med. J. 39:78,175
- Azad, S., R. Narasimha and S.K. Sett (2007). Multiresolution analysis for separating closely spaced frequencies with an application to Indian monsoon rainfall data. International Journal of Wavelets, Multiresolution and Information Processing 5(5): 735–752
- Bagnato, A. O. (1994). Phenotypic evaluation. J. Dairy Sci.; 77: 874-82.
- Barrnett, A. J. G. and G. Abd El-Tawab (1957). Determination of lactose in milk and cheese. J. Sci. Food Agric., 8: 437.
- Bernabucci, U., N. Lacetera, L.H. Baumgard, R.P. Rhoads, B. Ronchi and A. Nardone (2010). Metabolic and hormonal adaptations to heat stress in domesticated ruminants. Animal. ;4:1167–1183
- Blinco, C. and W. B. Dye (1958) Serum transaminase in white muscle disease. J. Animal science, 117:774
- Blum, J.W., P. Kunz, H. Leuenberger, K. Gautschi and M. Keller (1983). Thyroid hormones, blood plasma metabolites and haematological parameters in relationship to milk yield in dairy cows. Anim Prod. ;36:93–104.
- Bufano, G., D. Carnicella, P. De Palo, V. Laudadio, G. Celano and C. Dario (2006). The effect of calving. Arch Lat. Prod. Ani.; 14(2): 60-61.
- Bush, B.M. (1991). Interpretation of laboratory results for small animal clinicians. Oxford Blackwell scientific publication, London
- Cady, R.A., S.K. Shah, E.C. Schermerhorn and R.E. McDowel (1983). Factors affecting performance of dairy cattle. J. Dairy Sci.; 66: 578-86. <u>.</u>
- Campanile, G., C. De Filippo, R. Di Palo, W. Taccone and L. Zicarelli (1998). Influence of dietary protein on urea levels in blood and milk of buffalo cows. Livest. Prod. Sci. 55:135-143.
- Chaiyabutr, N., W. Chanchai, D. Boonsanit, S. Sitprija and S. Chanpongsang (2008). Different responses of oxidative stress index in the plasma of crossbred Holstein cattle during cooling and supplemental recombinant bovine somatotropin. J. Anim. Vet. Adv. 10(8), 1045-1053.
- Chan, J.P., C.C. Chu, H.P. Fung, S.T. Chuang, Y.C. Lin, R.M. Chu and S.L. Lee (2004). Serum haptoglobin concentration in cattle. J. Vet, Med. Sci., 66: 43-46
- Cozzi, G., L. Ravarotto, F. Gottardo, A.L. Stefani, B. Contiero, L. Moro, M. Brscic and P. Dalvit (2011). Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production [†]Journal of Dairy Science, Volume 94, Issue 8, Pages 3895–3901
- Curtius, H. C. and R. Marce (1972). Clinical biochemistry principles and methods volume 2 page 1123
- Cziszter, L.T. (2003). Dirijareafuncțieiglandeimamare, Eurostampa, Timișoara, , pp.50-77, 123-147, 228-238 (abstract in Arabic)
- Cziszter, L. T., S. Acatincă, F. C. Neciu, R. I. Neamţ D. E. Ilie, L. I. Costin, D. Gavojdian and I. Tripon (2012). The Influence of Season on the Cow Milk Quantity, Quality and Hygiene Animal Science and Biotechnologies, 45 (2).
- Das, K. S., J. K. Singh, G. Singh and V. Nayan (2013). Effect of heat stress alleviation on plasma protein, metabolites and lipid profile in lactating Nili-Ravi buffaloes under tropical climate. Indian J. Anim. Sci., 83(5): 546–549

- Dhali, A., R. K. Mehla, S. K. Sirohi, A. Mech and M. Karunakaran (2006). Monitoring feeding adequacy in dairy cows using milk urea and milk protein contents under farm conditions. Asian Aust. J. Anim. Sci., 19(12):1742-1748.
- Dohoo, I.R. and A. H. Meek (1982). Somatic cell counts in bovine milk. Can Vet J., 23(4): 119–125.
- Doumasm, B.; W. Wabson and H. Biggs (1971). Albumin standards and measurement of serum with bromochresol green. Clinical Chemistry Acta, 31:87
- Dular, R. and F. Labella (1977). Isolated prolactin and growth hormone granules from bovine pituitary contents and stability are seasonally dependent. Endocrine Res. Commun., 4 : 195
- Elgabban, S. Y. S. (2007). Effect of breed, parity and lactation stage on some blood metabolites in dairy cows in Sudan, M.Sc. Thesis, Department of Dairy Production, Faculty of Animal Production University of Khartoum
- El-Khashab, Mona A. (2010). Physiological and productive responses to amelioration of heat stress in lactating buffaloes under hot summer conditions in Egypt. Available at: http://www.spsaegy.org/?p=835. cited from Conf. of sustain. Agric. and Develop. Fac. of Agric, Fayoum Univ., 27-29- December, (2010).
- El-Masery K.A. and I.F.M. Marai (1991). Comparison between Friesians and water buffaloes in growth rate, milk production and some blood constituents, during winter and summer conditions. Egypt J. Anim. Prod. 53, 39-43
- El-Masry, K. A. and A. A. Habeeb (1987). Thyroid function in lactating Friesian cows and water buffalo and its relationship with their milk yield in winter and summer Egyptian conditions. Proc. 3rd Egyptian - British Conf. on Animal, Fish and Poultry Prod., 7-10 October, 1989, Alex. Univ.
- El-Nouty, F.D., I.M. Elbanna, T.P. Davis and H.D. Johnson (1980). Aldosterone and ADH response to heat and dehydration in cattle. J. Appl. Physiol. 48, 249-255.
- Frelich, M. Šlachta, O. Hanuš, J. Špička, E. Samková, A. Węglarz and P. Zapletal. (2012). Animal Science Papers and Reports. 30(3), 219-229.
- Greenfield, C.L., J.B. Messick, P.F. Solter, D.J. Schaeffer. (2000). Results of hematologic analyses and prevalence of physiologic leukopenia in Belgian Tervuren. J. Am. Vet. Med. Assoc. Mar., 15; 216(6):866-71
- Gudev, D., S. Popova-Ralcheva, P. Moneva, Y. Aleksiev, T. Peeva, Y. Ilieva and P. Penchev (2007). Effect of heat stress on some physiological and biochemical parameters in buffaloes. Italian Journal of Animal Science, 6 (Supple. 2): 1325–28.
- Haeeb, A. A. M., F. I. T. Fatma, S F. Osman (2007). Detection of heat adaptability using heat shock proteins and some hormones in Egyptian buffalo calves. Egyptian Journal of Applied Science 22 (2A): 28–53.
- Haenlein, G. (2003). Nutritional value of dairy products of ewe and goat milk. Retrieved January 28, J. Dairy Science, 69: 32-37.
- Hogan J. S., K. L. Smith, K. H. Hoblet, D. A. Todhunter, P. S. Schoenberger, W. D. Hueston, D. E. Pritchard, G. L. Bowman, L. E. Heider and B. L. Brockett (1989). Conrad bacterial counts in bedding materials used on nine commercial dairies. J Dairy Sci., 72: 250-258.
- IDF (2010). Bulletin of the International Dairy Federation (IDF) no . 446: The World Dairy Situation 2010. Buffalo Milk, pp: 8-9.
- Javed, K., M. Afzal, M. Ahmad, R. Hussain (2001). Genetic studies. The J. Ani. Pl. Sci.; 11(2): 48-52.
- Kassab, S. A.; S. M. B. Slal; F. H. Gharib and G. A. R. Kamar (1976). Physiological aspects in milk production in semi – arid conditions of graded cattle in Iraq. Egypt. J. Anim. Prod. 16:1
- Khan, F.A., G.K. Das, Pande, Megha, M.K. Pathak and M. Sarkar (2011). Biochemical and hormonal composition of follicular cysts in water buffalo (Bubalusbubalis). Animal Reproduction Science 124: 61.
- Khawaskar, M., M. T. Panchal, A. J. Dhami, K. K. Hadiya and S. B. Patel (2012). Seasonal variation in seminal biochemical constituents in Surti buffalo bulls. Ind. J. Anim. Repro., 33 (2): 41-46.

- Khazanehei, H., E. Khafipour and J. C. Plaizier (2015). Effects of dry period management and parity on rumen fermentation, blood metabolites, and liver triacylglyceride (TAG) in dairy cows Canadian Journal of Animal Science 95(3):150513045450001 · May 2015
- Kilic, A. and S. Kilic (1994). Feeding and Milk. Bilgehan Press. Izmir
- Koubkova, M., I. Knizkova, P. Kunc, H. Hartlova, J. Flusser and O. Dolezal (2002). Influence of high environmental temperatures and evaporative cooling on some physiological, haematological and biochemical parameters in high yielding dairy cows. Czech J. Anim. Sci. 47, 309-318
- Kumar, S., A. Saxena and R. Ramsaga (2010). Comparative studies on metabolic profile of anestrous and normal cyclic Murrah buffaloes. Buffalo Bull., 29(1): 7-10.
- Kumar, N., U. B. Singh and D. N. Verma (1980). Effect of Different Levels of Dietary Protein and Energy on Growth of Male Buffalo Calves. Indian J. Anim. Sci. 51,513
- Ling, E. R. (1963). "Text Book of Dairy Chemestry" Vol. II. Practical Chapman and Hall, L.T.D., London. 3 rd ed.
- Lyatuu E. T. and M.L. Eastridge (2003). Research and Reviews: Dairy. Ohio State University., , pp:6
- Mader, T. L.; M. S. Davis and T. Brown-bandle (2006). Environmental factors influencing heat stress in feedlot cattle. J. Anim.. Sci., 84:712.
- Mandali, G. C., P. R. Patel, A. J. Dhami, S. K. Rawal and K. S. Christi (2002). Biochemical profile in buffaloes with periparturient reproductive and metabolic disorders. Indian J. Anim. Reprod. 23(2):130-134.
- Nateghi, L., M. Yousef, E. Zamani, M. Gholamian and M. Mohammadzadeh (2014). The effect of different seasons on the milk quality. Euro. J. Exp. Bio., 4(1):550-552
- Omran, Fayza I., G. Ashour, M. M. Youssef and M. M. Shafie (2011). Responses of hematology, blood metabolites, mineral ions and hormonal profile to heat stress for Egyptian buffalo-calves. Egypt. J. Agric. Res., 89 (3),
- Pandey, A.K., P. Jain, G.K. Podila, B. Tudzynski, M.R. Davis (2009). BcEnol-1 functions as a transcriptional regulator and controlled by cAMP. Mol Genet Genomics 281(2):135-46
- Pandya, D. K.; K. J. Johar and A. Singh (1984). Study of some economic traits of Jersey in tropics . Livestock Adviser 9:19
- Pawar, H.N., G.V.P.P.S. Ravi Kumar and Raman Narang (2012). Effect of year, season and parity on milk production traits in murrah buffaloes. Journal of Buffalo Science, 1, 122-125
- Peters, T.J. (1996). All About Albumin. Academic Press, San Diego. Ca, USA.
- Quist, M.A., S.J. LeBlanc, K.J. Hand, D. Lazenby, F. Miglior, D.F. Kelton (2008). Milking-to-milking variability for milk yield, fat and protein percentage, and somatic cell count, J. Dairy Sci., 91(9), 3412-3423.
- Qureshi, M.S., H.A. Samad, N. Nazir Ahmad, G. Habib, A.D. Anjun, M.M. Siddiqui (1999). Reproductive performance of dairy buffaloes under peri-urban commercial farming in VWFP, Pakistan. Pakistan Vet. J. 18, 197–201.
- Reitman, S. and S. Frankel (1957). Calorimetric determination of pt activity according to Reitman and Frankel method. American J. Clinical Pathology,28-56
- Romcevic, L. J.; M. Katic; S. Vasovic ; R. Lazorevic and N. Nikitovic (1984). The effect of calving season on dairy performance of cows. Stocarstov. 38:171 (Arabic abst.)
- Rowlands, G. J.; W. Little and B. A. Kitchenham (1977). Relationships between blood components and fertility in dairy cows. j. dairy res., 44,1-4
- Saravanan, R., D.N. Das, S. De and S. Panneerselvam (2015). Effect of season and parity on somatic cell count across zebu and crossbred cattle population. Indian J. Anim. Res., 49 (3) : 383-387
- SAS (2002). Statistical Analysis System, Copyright (c) 2002 by SAS Institute Inc., Cary, NC, USA. for windows version 6.1.7600, SAS information Version 9.00 TS level 00M0.
- Schams, D.; E. Stevan and R. D. Hooley (1980). The effect of heat exposure on blood serum level of anterior pituitary hormones in calving, heifers and bulls. Acta Endocrin. 94: 309

- Schukken, Y.H.; Wilson, J. David; Welcome, Francis; Garrison-Tikofsky, L. Gonzalez, N. Ruben (2003). "Monitoring udder health and milk quality using somatic cell counts". Vetenary Research (EDP Sciences) 34 (5): 579–596.
- SPSS (2007). Statistical package for social science, release 18, copyright 2007 polar engineering and consulting.
- Van Saun, R. (2000). Blood profiles as indicators of nutritional status. Proceedings of the 18th Annual Western Canadian Dairy Seminar, March 1-20, 2000, Alberta, Canada, pp: 1-6.
- Vasilios, A. B., N. Eleonora, B. Vasileios, C. Vladimiros, C. Dimitrios, M. Ioanis and L. Vassiliki (2012). Effect of parity and calving month on milk production and quality of greek buffalo (*Bubalus bubalis*). Bampidis /Scientific Papers: Animal Science and Biotechnologies, 2012, 45 (2).
- Wenz, J.R., L.K. Fox, F.J. Muller, M. Rinaldi, R. Zeng, D.D. Bannerman (2010). Factors associated with concentrations of select cytokine and acute phase proteins in dairy cows with naturally occurring clinical mastitis. J. Dairy Sci., 93: 2458-2470.
- Yadav, S. P.; P. Sikka, D. Kumar and R. Sethi (2013) Variation in milk constituents during different parity and seasons in Murrah buffaloes. <u>The Indian journal of animal sciences</u> 83(7):474.
- Yokus, B., D. U. Cakir and Z. Kanai (2006). Effects of Seasonal and Physiological Variations on the Serum Chemistry, Vitamins and Thyroid Hormone Concentrations in Sheep. Journal of Veterinary Medicine Series A 53(6):271-6.

تأثير موسم السنة وعدد مرات الولادة علي تركيز معالم بلازما الدم، محصول اللبن، مكونات اللبن والعد البكتيري في اللبن في اناث الجاموس المصري الوالدة حديثًا

> امال عبد المجيد عبده¹ ، عبد الكريم السيد² ، احمد سليمان² ، انعام مخلص¹ و حسين الرجالاتي¹ 1 معهد بحوث الانتاج الحيواني - مركز البحوث الزراعية- الدقي - الجيزة – مصر. 2 قسم الانتاج الحيواني - كلية الزراعة – جامعة بنها.

تم اختيار عشوائي لعدد 24 جاموسة والدة حديثا تزن الواحدة منها في المتوسط أكثر من 450 كجم من قطيع محطة بحوث محلة في موسي التابع لمعهد بحوث الانتاج الحيواني وزارة الزراعة بمصر. وتم تقسيم الحيوانات التجريبية الي مجموعتين تجريبيتين اساسيتين في كل منهما 12 جاموسة، المجموعة الاولي21 تضم مجموعة الحيوانات التي يتوقع لها أن تلد في الشتاء (اكتوبر الي مارس) في كل منهما 12 جاموسة، المجموعة الدوانات التي يتوقع لها أن تلد في الشتاء (اكتوبر الي مارس) والمجموعة الحيوانات التي يتوقع لها أن تلد في الشتاء (اكتوبر الي مارس) الي مجموعة الثانية 22 تضم مجموعة الحيوانات التي يتوقع لها أن يلد في الشتاء (اكتوبر الي مارس) الي مجموعة الثانية 23 تضم مجموعة الحيوانات التي يتوقع لها أن تلد في الشتاء (اكتوبر الي مارس) الي مجموعتين فرعيتين تضم كل منهما ستة حيوانات بحيث ضمت المجموعة الفرعية الاولي إلا الحيوانات ذات عدد مرات ولادة من الي مرتين إلى اربعة عشرمرة. وقد كان مرتين الي اربعة مرات وضمت المجموعة الفرعية الأولي إلالي الدوانات ذات عدد مرات ولادة من الي مرتين الي اربعة عشرمرة. وقد كان مرتين الي اربعة مرات وضمت المجموعة الفرعية الثانية/22 الحيوانات ذات عدد مرات الولادة من خمسة الي اربعة عشرمرة. وقد كان الي مرتين الي اربعة مرات وراحي لعلي علي اداء و سلامة و صحة الحيوانات التجربيبة وكذاك تأثير ذلك علي محصول اللبن وتركيبه و العد لان لان الائي إلان التجربيبة وكذاك تأثير ذلك علي محصول اللبن وتركيبه و العد الان لاولي 31 بقوس حرارة الأولي 32 مقال مراري لموسم ألحيوانات المحموعة الاساسية الثانية 22 مقاس حرارة ورطوبة 2.87 في 22 أما في 31 في معربي ما وقد خلائ ألولي 31 للجروبين الكي ملحون في يعض معالم بلازما الدي لاولي 32 معرار معاوي أل في 30 مال 60 وقد تأثير البروتين الكلي لبلازما لدم والجلوبيولين بموسم الاولي 31 في 32 في 32 في 32 في 30 في 30 في 30 في 30 في ما في عام مولي وي أل ما حمو حين في يعض معالم بلازما المن معنوي الي مولي الن التجرمع 23 مال مال من الخروبي 32 في 30 في 32 في 30 في 3 الموني مع دراسة تأثير الدوان علي علي 32 معالم حيوانات المجموعة الاساسية الثانية 23 مقان ما لدم والجلوبيولين بموسم اللاولي 31 في من ذلي مع 23 مقان فا قد مع 32 معالم الانزيمي ووفي نفس الاتجاه لكل من انزيمي مي ون في ما الذم مع 23 كل من ا