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Biochemical markers of peripartum nutritional status in postpartum anoestrous ewes grazing natural pasture in north eastern Algeria

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ABSTRACT

The relationship between peripartum nutritional status and the resumption of postpartum ovarian activity was studied in thirteen ewes bred traditionally on littoral pastures of the extreme north-eastern province of Algeria. In order to follow up their peripartum nutritional status, 147 blood samples were collected once during antepartum and eleven times during the first two months postpartum at five to six days interval. Plasma level of total cholesterol, triglycerides, albumin, Gamma-glutamyl transferase (GGT), and Aspartate amino transferase (AST) was measured. In addition, body condition was scored. The interval from lambing to onset of postpartum luteal activity was obtained via radioimmunoassay of plasma progesterone. The resumption of postpartum luteal activity, beyond 65 days in 88.33 % of monitored ewes, was found to be dependent on peripartum nutritional deficit indicated by a strong significant relationship with postpartum body condition score, antepartum GGT level and postpartum cholesterol concentrations ($P < 0.01$), and by a slightly significant correlation ($P < 0.05$) with postpartum levels of AST and triglycerides. The use of nutritional status indicators could contribute to the control of ewe's peripartum through a better understanding of their sensitivity and adaptability to their breeding constraints. This would lead to the improvement of their reproduction performance.

Keywords: biochemical parameters; body condition; nutrition, postpartum anoestrus.

INTRODUCTION

Algeria is a sub Mediterranean agricultural country where sheep farming is predominant. It has about 21 million heads [1]. Natural grasslands fulfill most of their nutritional requirements. The province of El-Tarf, located on the Mediterranean coast in the far north-eastern part of Algeria, may be taken as a representative model to the extent that the percentage of its natural grasslands is the highest nationwide (4700 ha or 19.14%, [1]). In the southern Mediterranean region, the reproductive system of sheep livestock is not clearly defined; lambing is distributed throughout the year with autumnal and vernal peaks [2, 3].

In "Ouled Djellal" ewe breed, the main representative of the dominant Algerian white breed sheep, it was reported that the breeding season extends from early April to the end of November, with greater concentration of mating between June and August. One major constraint to improve productivity is the low fertility level, recorded in field conditions, during early spring (anoestrus season), period farmers consider most suitable to breed their flocks [4].

The frequency of lambing and the overall lifetime productivity of sheep are determined by several factors, one of which is the postpartum-1st ovulatory oestrus interval [5]. This latter is greatly affected by nutritional level [6, 7].The resumption of postpartum luteal activity is important for first normal heat to occur which renders progesterone a pre-requisite [8].

The non existence of studies on factors influencing the interval from lambing to resumption of normal ovarian activity in ewes grazing pasture in Mediterranean humid zone of Algeria and under traditional breeding conditions has led us to carry out the present work. To do so, the influence of the nutritional status and feeding management (grazing) during critical and physiological stage of peripartum has been investigated. Well-known subjective useful marker, the body condition score (BCS), and some biochemical indicators (gamma-glutamyl transferase, Aspartate amino transferase, Total Cholesterol, Triglycerides and Albumin) of nutritional status (energy and protein) reported by several authors [9, 10, 11, 12, 13] have been used in order to better understand the interaction of nutritional level with the onset of pospartum ovarian activity, determining factor of reproduction performances of ewes.

MATERIALS AND METHODS

Animals, location and period of study

The study was carried out from October 2010 to June 2011. Thirteen ewes, aged from 2.5 to 5 years, belonging to a semi-extensive farm, located in the north-east Algerian province of El-Tarf (latitude 36°45'08.58"N and, 7°55'41.41"E) were followed-up. Ewes were monitored once in the last 3 weeks of antepartum (AP), and eleven times during the first two months postpartum at five to six days interval (day 5, 11, 17, 23, 28, 34, 40, 46, 52, 58 and 65). One ewe died on the fifth week postpartum. During the study period, ewes were allowed to suckle their lambs and to run with rams continuously. Grazing natural pasture, sometimes complemented with concentrate during rainy days, made up the essential of feeding. Food stock shortages were frequently observed. The use of anthelmintic was occasional and did not obey to any planning.

Samples

One hundred forty-seven blood samples were collected at 7:30 am after 12 hours of fasting. They were centrifuged (4000 t/min 10) and transferred to the laboratory in the next half hour. The collected plasma was stored at -20 °C for a period of stability consistent with the recommendations of manufacturer of assay kit.

Parameters investigated

Body condition score: The scoring of body condition (1 to 5) was done according to the method described by Suiter [14].

Biochemical parameters: Five biochemical indicators of nutritional status were targeted in collected plasma via commercial kits (Spinreact, SA, Spain). A quantitative colorimetric enzymatic method was used to measure the concentration of: Gamma-glutamyl transferase (GGT: U/l), Aspartate amino transferase (AST: U/l), Total Cholesterol (CHOL: mg/dl), Triglycerides (TG: mg/dl), and Albumin (ALB: g/l).

The postpartum luteal activity profile was established by plasma progesterone (P4) radio immunoassay measurement (RIA) using the Immunotech kit (A Beckman Coulter Company Marseille Cedex, France). The resumption of postpartum ovarian activity is defined as the time of the first P4 rate increase over 1 ng/ml, and which is maintained for at least two successive samples [15]. This step was used to parameter the lambing to onset postpartum luteal activity interval (L - OLA) and to sort the females in two groups: the first (“IN” group) comprises those with ovarian inactivity (P4 < 1ng/ml) and the second (“LA” group) those who resumed postpartum luteal activity.

Statistical analysis

Data were performed by analysis of variance (ANOVA) using SPSS Statistics 17.0 for Windows Statistical Software. The "t" test of Student and Pearson correlation “r” were calculated. Given the small size (2 ewes) of the “LA” group, only the values of «IN» group were compared with the reference values.

RESULTS

Flock Reproductive status

In the studied flock, lambing was recorded in two seasons, 38.46% at the end of autumn and 61.54% in spring; each ewe gave birth to one lamb and once annually. Resumption of postpartum luteal activity was resumed only in two ewes at 31.5 ± 10.5 days postpartum in autumn and beyond 65 days postpartum in spring in 83.33 % of ewes.

Peripartum nutritional status

The BCS values recorded during peripartum (Figure 1) were outside the target scores. A moderate body condition loss during the transition period (from the antepartum to the second week postpartum) was recorded in all groups (average variation of about 0.26 points); whereas weight regain were noted at the fourth week postpartum. This indicator showed comparable kinetics in ewes that had or not resumed luteal activity.

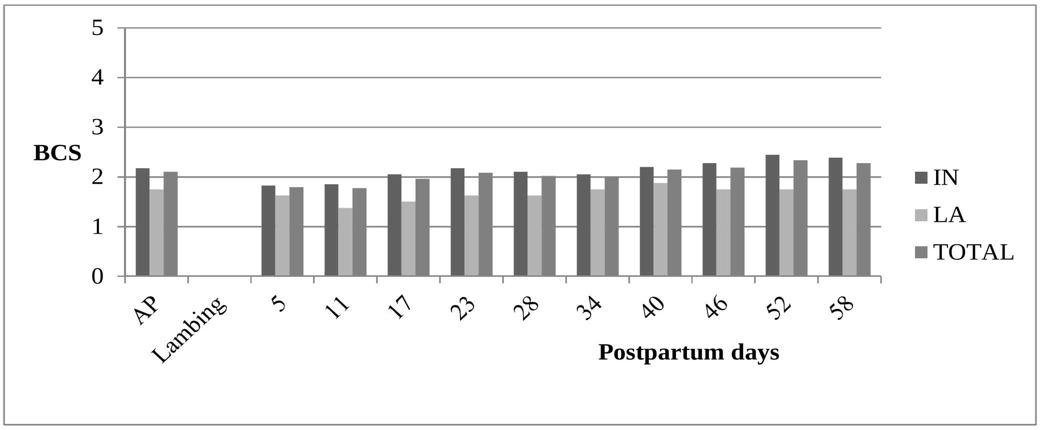

Figure 1: **Comparison of BC scores between groups**

From the biochemical point of view (Table 1), the GGT level of all ewes was significantly higher ($P < 0.05$) than the reference average although exceeding slightly the reference threshold [16]. The AST level, however, was not significantly different from the reference values; the cholesterol concentration exceeded slightly the average reference values reported by Kaneko et al. [16] but only during the postpartum period (significantly higher on day 17, 23 and 46 postpartum); the triglycerides value obtained were within the reference limits described by Aitken [17] and those recorded on day 58 and 65 postpartum were close to the lower limit reported by the latter author. The plasma albumin concentration, from the third week postpartum, was significantly lower than the normal values described by Kaneko et al. [16].

Table 1: Comparison (t test) of peripartum biochemical parameters in monitored ewes with reference values (results are expressed as mean ± standard deviation)

Indicators	GGT[1] (IU/l)	AST[1] (IU/l)	CHOL[1] (mg/dl)	TG[2] (mg/dl)	ALB[1] (g/l)
Reference values[1,2] (means and ranges)	33.5 ± 4.3 (20 - 52)	307 ± 43 (60 - 208)	64 ± 12 (52 - 76)	17.7 - 88.5	27 ± 1.9 (24 - 30)
AP	51.71 ± 17.72[a]	63.00 ± 34.18[a]	61.70 ± 4.30	45.20 ± 13.60	28.9 ± 5.5
Day 5	58.12 ± 34.42[a]	66.58 ± 58.00[a]	70.58 ± 13.53	26.50 ± 7.31	29 ± 4.2
Day 11	52.12 ± 22.53[a]	59.44 ± 40.16[a]	64.67 ± 16.16	24.56 ± 5.81	26.4 ± 4.2
Day 17	54.68 ± 39.44	108.43 ± 43.35[a]	49.86 ± 13.48[a]	26.71 ± 4.82	25.1 ± 2.9
Day 23	53.59 ± 29.35[a]	125.27 ± 22.49[a]	52.55 ± 11.28[a]	24.36 ± 6.04	23.4 ± 3.3[a]
Day 28	49.31 ± 28.27	134.33 ± 29.24[a]	64.17 ± 16.95	26.42 ± 7.18	24.2 ± 3.8[a]
Day 34	59.42 ± 29.67[a]	147.08 ± 40.37[a]	58.75 ± 8.94	30.33 ± 8.57	22.9 ± 2.7[a]
Day 40	65.67 ± 37.13[a]	148.58 ± 50.75[a]	59.67 ± 10.24	28.25 ± 5.53	22.9 ± 2.0[a]
Day 46	58.83 ± 44.35	180.08 ± 78.40[a]	56.42 ± 10.48[a]	30.33 ± 10.61	24.2 ± 3.4[a]
Day 52	66.25 ± 46.62[a]	209.42 ± 105.05[a]	59.00 ± 11.05	24.67 ± 8.11	25.3 ± 2.6[a]
Day 58	81.42 ± 75.87	251.83 ± 196.25	58.25 ± 9.96	22.25 ± 9.29	25.1 ± 2.2[a]
Day 65	101.67 ± 114.48	278.00 ± 257.62	60.33 ± 11.11	22.00 ± 7.68	24.7 ± 2.7[a]

[1] : Kaneko et al. (2008); [2]: Aitken (2007); [a]: Significant difference between obtained mean and reference mean ($P < 0.05$); AP= Antepartum; NC: Not Calculated

Comparative study of biochemical indicators according to the resumption of postpartum luteal activity

During antepartum, ewes with luteal activity (“LA” group) showed significant greater activity of GGT exceeding the thresholds values described by Kaneko et al. [16] compared to those of “In” group (Table 2). A high activity of GGT was present in the “In” groups at the end of the second month postpartum (on day 58 and 65) coincidently with an increase of plasma AST.

AST’s activity lay within the reference range reported by Kaneko et al. [16] in all groups except at the end of the second postpartum month where it showed an increase (Table 2). During peripartum, this activity was not different between “LA” and “In” groups; one significant difference was noted on day 23 postpartum when ewes with luteal activity showed lower levels.

Table 2: Comparison (t test)of plasma levels of liver enzymes during peripartum between groups

Indicators	GGT[1]			AST[1]		
Reference value	33.5 ± 4.3 (20 - 52) IU/l			307 ± 43 (60 - 208) IU/l		
Groups	IN	LA	Sig.	IN	LA	Sig.
AP	44.98 ± 12.03	78.61 ± 1.96[a]	*	56.75 ± 32.95[a]	88 ± 36.77	NS
Day 5	51.45 ± 32.38	91.48 ± 30.43	NS	57.90 ± 59.26[a]	110 ± 29.70	NS
Day 11	47.76 ± 22.89	67 ± 17.82	NS	58.71 ± 39.14[a]	62 ± 60.81	NS
Day 17	46.95 ± 44.42	74.00 ± 19.80	NS	106.60 ± 52.95[a]	113 ± 1.41	NS
Day 23	51.72 ± 31.22	62 ± 25.46	NS	132.33 ± 17.96[a]	93.50 ± 3.54	**
Day 28	47.57 ± 29.75	58.00 ± 25.46	NS	140 ± 28.82[a]	106 ± 1.41	NS
Day 34	58.31 ± 32.68	65 ±.00	NS	155.20 ± 39.15[a]	106.50 ± 13.44	NS
Day 40	65.70 ± 40.48	65.50 ± 20.51	NS	154.80 ± 52.40[a]	117.50 ± 36.06	NS
Day 46	64.10 ± 47.01	32.50 ± 9.19	NS	197.10 ± 73.27[a]	95 ± 43.84	NS
Day 52	69.40 ± 50.36	50.50 ± 21.92	NS	231.80 ± 100.73[a]	97.50 ± 2.12	NS
Day 58	89.80 ± 80.46	39.50 ± 28.99	NS	284 ± 200.43	91 ± 4.24	NS
Day 65	107.50 ± 120.94	NC	NC	303.63 ± 262.86	NC	NC

*[1]: Kaneko et al. (2008); [a]: Significant difference between obtained mean and reference mean (P < 0.05); *P < 0.05; **P < 0.01; NC: Not Calculated; NS: not significant (P > 0.05); Sig: Significance*

Cholesterol levels lied within reference limits given by Kaneko et al. [16] in all groups. Ewes without luteal activity showed significantly higher values when compared with those of "LA" group, only on day 46 and 52 postpartum (Table 3). Peripartum triglycerides' values observed in all groups were within the reference limits reported by Aitken [17]. There was no significant difference between ewes with or without luteal activity, with the exception in the triglycerides level of the «IN» group which was significantly lower on day 58 postpartum.

Unlike the cholesterol and triglycerides levels, that of plasma albumin was found to be significantly lower than the average reference values reported by Kaneko et al. [16], between day 23 and day 46 postpartum in «IN» groups (Table 3).

Table 3: Comparison (t test) of plasma levels of CHOL, TG and ALB during peripartum between groups

		AP	Day 5	Day 11	Day 17	Day 23	Day 28	Day 34	Day 40	Day 46	Day 52	Day 58	Day 65
CHOL[1] (mg/dl)	IN	62.25 ± 4.62	73.80 ± 11.77[a]	67.14 ± 17.54	50.80 ± 16.21	53.78 ± 11.86[a]	65.40 ± 17.58	60.80 ± 7.93	60.50 ± 10.80	60.00 ± 6.78	61.80 ± 9.66	60.10 ± 9.75	61.88 ± 10.80
	LA	59.50 ± 2.12	54.50 ± 12.02	56.00 ± 7.07	47.50 ± 4.95	47.00 ± 8.49	58.00 ± 16.97	48.50 ± 7.78	55.50 ± 7.78	38.50 ± 4.95	45.00 ± 5.66	49.00 ± 5.66	NC
	Sig.	NS	NS	NS	NS	NS	NS	NS	NS	**	*	NS	NC
TG[2] (mg/dl)	IN	46.50 ± 13.41	27.50 ± 7.47	25.14 ± 6.57	26.40 ± 5.32	25.11 ± 6.47	25.50 ± 7.34	29.20 ± 8.70	28.50 ± 5.10	29.00 ± 11.06	23.00 ± 7.85	19.90 ± 7.95	21.13 ± 7.72
	LA	40.00 ± 18.38	21.50 ± 4.95	22.50 ± 0.71	27.50 ± 4.95	21.00 ± 1.41	31.00 ± 5.66	36.00 ± 7.07	27.00 ± 9.90	37.00 ± 5.66	33.00 ± 1.41	34.00 ± 7.07	NC
	Sig.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NC
ALB[1] (g/l)	IN	29.0 ± 5.1	29.0 ± 3.6	26.0 ± 3.3	25.8 ± 3.1	23.6 ± 3.6[a]	23.9 ± 4.1[a]	22.5 ± 2.7[a]	22.6 ± 2.1[a]	24.1 ± 3.3[a]	25.4 ± 2.5	25.2 ± 2.4[a]	24.7 ± 2.9
	LA	28.5 ± 9.2	29.0 ± 8.4	28.0 ± 8.4	23.5 ± 2.1	22.5 ± 0.7	25.5 ± 2.1	25.0 ± 1.4	24.5 ± 0.7	24.5 ± 4.9	24.5 ± 3.5	24.5 ± 0.7	NC
	Sig.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NC

*[1]: Kaneko et al. (2008); [2]: Aitken (2007); [a]: Significant difference between obtained mean and reference mean (P < 0.05); *P < 0.05; **P < 0.01; NC: Not Calculated; NS= not significant (P > 0.05); Sig = Significance*

Relationship between the studied markers and the commencement of postpartum luteal activity

According to Table 4, L-OLA interval was significantly and negatively related to BCS in postpartum (high significance, P < 0.01, from the third week postpartum), to triglycerides on day 58 postpartum (r = -0.63) and to GGT in antepartum (r = -0.77). However, there is a significant and positive correlation of L-OLA interval with a high activity of AST (r = 0.64 on day 23 postpartum) and with cholesterol level (r = 0.61 and 0.80 at day 5 and 46 postpartum, respectively). This interval seemed to be independent of albumin (*P* > 0.05).

Table 4: Relationship (Correlation of Pearson) between L-OLA interval and different variables during ante and postpartum

	BCS	Chol	TG	Alb	GGT	AST
AP	-0.46	0.31	0.041	0.22	-0.77**	-0.48
Day 5	-0.41	0.61*	0.37	0.20	-0.52	-0.38
Day 11	-0.59*	0.34	0.20	0.04	-0.46	-0.21
Day 17	-0.56	0.06	-0.25	0.47	-0.39	-0.07
Day 23	-0.71**	0.15	0.29	0.15	-0.22	0.64*
Day 28	-0.56	0.06	-0.36	-0.21	-0.22	0.42
Day 34	-0.48	0.42	-0.37	-0.40	-0.08	0.48
Day 40	-0.50	0.12	-0.08	-0.38	0.06	0.34
Day 46	-0.75**	0.80**	-0.23	0.10	0.28	0.53
Day 52	-0.78**	0.51	-0.44	0.26	0.10	0.47
Day 58	-0.74**	0.36	-0.63*	0.15	0.21	0.36
Day 65	NC	0.42	-0.34	0.09	0.15	0.30

**P < 0.05; **P < 0.01; NC: Not Calculated*

DISCUSSION

Nutritional status of monitored ewes

The body condition scores were below the target score (Figure 1) described by Caldeira et al. [11]. These authors have suggested that a BCS of 3 appears to be ideal to assure nutritional and metabolic welfare and with a BCS below 2, ewes seem more susceptible to metabolic imbalances. The BCS change in peripartum probably reflects a state of nutritional deficit due to food shortage frequently noted in the studied flock. The Decrease of body condition score around lambing was probably the result of body fat mobilization in response to negative energy balance as described by Cannas [18].

Peripartum GGT activity has slightly exceeded ($P < 0.05$) the threshold reported by Kaneko et al. [16]. This activity seems to be related to exposition of all periparturient ewes to a longer process of fatty infiltration of liver accompanying the mobilization of body fat reserves [19] previously suggested by a decreasing of body condition scores.

The increase in AST activity, observed after parturition (Table 1), was also recorded by Taghipour et al. [20] during the same physiological stage in Iran. Activity of this enzyme was considered by Caldeira and Portugal [9] as a good indicator of mobilization of body protein reserves when ewes are in negative energy balance. These latter authors have revealed a gradually increasing of AST activity after loosing of body condition as well as showed in the present study from day 17 postpartum (Table 1). Higher concentrations of GGT and AST activities determined in all ewes in the postpartum period were also noted by Antunovic et al. [13].

Cholesterol and triglycerides showed values within reference range. Cholesterol concentrations were slightly below the average reference on day 17, 23 and 46 postpartum. Changes in these two parameters were reported to be related to nutritional status [21]. Decrease in concentrations of cholesterol and triglycerides, was suggested as indicator of energetic deficiency of ewes in early lactation [13].

Postpartum cholesterol levels were similar to those recorded in winter grazing ewes in Souk-Ahras, a province bordering El-Tarf [22] and to those reported by Carcangiu et al. [21] in lean sheep (BCS < 2.5). These latter also observed a lower TG level in lean sheep than in those with good body condition.

Depending on the physiological state, a drop in cholesterol and triglycerides levels was recorded during the transitional period from antepartum to postpartum [20], whereas we found that only that of the triglycerides fell. Postpartum triglycerides levels (Table 1) were greater than those recorded by Ouanes et al. [22] and lower than those of lactating ewes bred in arid south - eastern Algeria, reported by Deghnouche et al. [23].

From day 23 postpartum, a significantly lower level of plasma albumin than the average reference values reported by Kaneko et al. [16] was recorded (Table 1). Postpartum concentrations of Albumin in all ewes were lower than values obtained by Safsaf et al. [24] in early lactating Ouled Djellal ewes in steppic region of eastern-Algeria and than those recoded by Antunovic et al. [13] in lactating Tsigai ewes.

The traditional breeding system most widely found through the Algerian coast is characterized by an irrational management affecting mainly feeding level. This finding is enhanced by the investigated indicators (Table 1, Figure 1), signing probably a nutritional deficit, inevitably leading to a negative peripartum energy balance.

Effect of nutritional status on Lambing – onset of luteal activity interval (L-OLA)
The peripartum nutritional status has influenced the resumption of ovarian activity.

Body condition effect
Lambing-OLA interval reveals to be negatively related to BCS. An effect of body condition on ovarian activity was described by several authors [6, 7]. This could be due to the direct effect of glucose and metabolic hormones (insulin and leptin) at the ovarian level [25].

However, it was also noted that spring-lambing ewes (61.54%) have not shown a luteal activity in spite of a marked weight improvement comparatively to LA group (notably at the end of the 2nd month postpartum) following a more feeding availability in the spring in the studied province (Figure 1). Some authors (reviewed by Landau and Molle [26]) found no relationship between body condition and resumption of cyclic activity in “Aragonesa” ewes lambing in spring and in an unfavorable photoperiod.

Biochemical indicators
Significant differences between groups (“In” and “LA”) were reported using indicators namely total cholesterol (on day 46 and 52 postpartum), triglycerides (on day 58 postpartum), GGT (in antepartum) and AST (on day 23 postpartum) (Table 4).

A positive correlation between plasma levels of cholesterol and “L-OLA” interval on day 5 ($P < 0.05$) and 46 ($P < 0.01$) postpartum, with a decrease in total cholesterol on day 52 and 46 postpartum in the "LA" group, could be, probably, related to its use as a precursor substrate for the synthesis of ovarian steroids [27], progesterone notably [28].

The low level of triglycerides, noticed during the postpartum in the different groups (Table 3), was also reported by other authors [12, 29]. It was probably due to the mobilization of fat stores to meet energy requirements [30]. Postpartum energy deficit indicated by low level of TG, as well as suggested by Antunovic et al. [13], has probably delayed the resumption of ovarian activity as revealed by a negative and significant correlation ($r= -0.63$) between this parameter and the interval L-OLA L on day 58 postpartum (Table 4)

A significant increase in the GGT activity was only found during antepartum in the “LA” group. It was associated to an earlier return of postpartum luteal activity (negative correlation with the “L-OLA” interval). This increase reflected a probable mobilization of body fat during late pregnancy leading to a possible fatty infiltration of liver, which remains reversible and physiological given that a return to normal GGT levels has occurred from day 42 postpartum without being accompanied by a pathological increase in AST concentration. This process of fatty infiltration, indicated by the GGT level, was also reported by other authors [10, 19].

A high AST activity, on the contrary, was significantly related, on day 23 postpartum only, to an extension of “L-OLA” interval (Table 4). This relationship seems to be explained by probable effect of negative energy balance of ewes in early lactation (postpartum) indicated by increasing of this enzyme activity as described by Caldeira and Portugal [9] and Antunovic et al. [13] on ovarian function through probably some sufferance of hepatic cells during early lactation.

The plasma levels of albumin was found to be significantly lower than the average reference values reported by Kaneko et al. [16], between day 23 and day 46 postpartum (Table 3), in ewes without ovarian activity (IN groups) which have maintained a moderate (environ 2) BCS (figure 1). Caldeira et al. [11] have found that ewes in undernutrition state ($BCS \leq 2$) had a lower serum albumin. However, in the present study (Table 4), the onset of postpartum luteal activity was not correlated to albumin level.

CONCLUSION

Reproductive performance, modulated by a return to normal postpartum ovarian activity in ewes under traditional breeding conditions in the far north- eastern province of Algeria seems to be related to their sensitivity to a nutritional deficit, mainly energetic, during transition period. Therefore, the use of more practical subjective indicators and more sensitive biochemical markers of metabolic changes, to manage the critical period of peripartum and to control the adaptability to breeding conditions, proves to be an interesting way of enhancing the Algerian ewe reproduction aptitudes.

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