# Effect of some blood metabolites on the conception risk of Montbeliard cows

**F Chacha, Z Bouzebda[1], F Afri-Bouzebda[1], D Gherissi[1], R Lamraoui[2], A Djaout[3] and C Mouffok[4]**

*Biotechnology Research Center, Constantine, Algeria*
*chachafaycal@yahoo.fr*
*[1] Laboratory of Animal Productions, Biotechnologies and Health. Cherif Messaadia University, Souk-Ahras. BP 41000, Souk-Ahras, Algeria*
*[2] Departement of Biology of Living Organisms, faculty of Natural and Life Sciences.University of Batna 2, Batna (05110), Algeria*
*[3] Algeria's National Institute for Agricultural Research (INRAA). Setif 19000, Algeria*
*[4] Laboratory for the Valorization of Natural Biological Resources, Ferhat Abbas University 19000, Setif, Algeria*

## Abstract

The study was conducted on 74 Montbeliard cows raised in Northeastern Algeria under a semi-arid climate to compare the body condition score (BCS) status and some plasma concentrations of major substrates (urea, albumin, total cholesterol, glucose) and minerals (calcium, phosphorus, magnesium) to relate with reproduction performance. The blood samples were collected from cows at 1 month intervals for 3 months postpartum. An assessment of the body condition on the Edmonson grid and the reproductive events were recorded. At the third month post calving, the level of glucose and total cholesterol significantly increased ($P<0.05$), while the concentrations of magnesium and phosphorus significantly decreased ($P<0.05$). There was a significant interaction between the nutritional statuses and some reproductive parameters during the postpartum. The reduction of glucose, total cholesterol and urea levels and the increase of calcium and albumin scores were significantly associated to cows with longer intervals of calving-first service and open days (OD). In other hand, a reduction of blood magnesium and phosphorus concentrations and an increased calcium level were recorded in cows with number of services per conception (NSC) equal or greater than three. These results suggest that the postpartum metabolic pattern of change in Montbeliard dairy cows from semi-arid region estimated by the blood substrates and minerals offers a clear indication in the context of the reproduction monitoring. It can explain some factors of low reproductive performance such as the conception rate of the first insemination, insemination index and days open.

***Key words:*** *Algeria, body condition score, metabolic profile, postpartum, reproduction trait, semi-arid*

# Effets de quelques métabolites sanguins sur les chances de conception de vaches

# Montbéliardes


## Résumé

L'étude a été menée sur 74 vaches de race Montbéliarde, élevées dans le Nord-Est algérien sous climat semi-aride pour comparer l'état corporel et certaines concentrations plasmatiques de substrats majeurs (urée, albumine, cholestérol total, glucose) et de minéraux (calcium, phosphore, magnésium) en relation avec les performances de reproduction. Les échantillons de sang ont été prélevés sur des vaches à des intervalles d'un mois pendant 3 mois postpartum. Une évaluation de l'état corporel sur la grille d'Edmonson et les événements de reproduction ont été enregistrés. Cette étude a révélé qu'au troisième mois postpartum, les taux de glucose et de cholestérol total ont augmenté significativement ($P < 0,05$), tandis que les concentrations de magnésium et de phosphore ont diminué significativement ($P < 0,05$). Les résultats ont montré une interaction significative entre les statuts nutritionnels avec certains paramètres de reproduction pendant la période post-partum. La réduction des taux de glucose, de cholestérol total et d'urée et l'augmentation des scores de calcium et d'albumine étaient significativement associées aux vaches ayant un long intervalle vêlage-première saillie et vêlage-saillie fécondante. D'autre part, une réduction des concentrations sanguines de magnésium et de phosphore et une augmentation du taux de calcium ont été enregistrées chez les vaches nécessitant 3 inséminations et plus. Ces résultats suggèrent que le profil biochimique durant le post-partum chez les vaches Montbéliardes élevées dans la région semi-aride estimé par les substrats sanguins et les minéraux, offre une indication claire pour le suivi de la reproduction. Il permet d'expliquer en partie certains facteurs de faibles performances de reproduction tels que le taux de conception à la première insémination, l'indice coïtal et l'intervalle vêlage-saillie fécondante.



***Mots-clés:*** *Algérie, NEC, note d'état corporel, postpartum, profil métabolique, reproduction, semi-aride*


## Introduction

Anoestrus and repeat breeding are negatively affecting the productive and reproductive performance of cows and causing great economic losses for dairy farmers (Dutta et al 1988). The root causes of breeding problems in a herd are multiple and include management, nutritional and pathological factors. Nutrition often represents more than 50% of this variation (Short et al 1990).

Several studies showed that under-nutrition during the first phase of lactation is accompanied by disturbances in plasma concentrations of reproductive hormones (Westwood et al 2002; Mouffoket al 2011), low follicular development (Fassi Fihri et al 2005) and poor quality of oocytes (Jorritsma et al 2003). Buckley et al (2003) have reported that a severe negative energy balance causes metabolic disorders and decreased fertility. However, Grimard et al (2003) observed an improvement in the fertility by correcting the energy balance supported by improved dietary levels, even if the body condition score remained low.

In addition, the results of many authors (El-Azab et al 1993; Balakrishnan and Balagopal 1994; Qureshi 1998) suggest that normal blood levels of various biochemical constituents are essential for the normal functioning of various body systems, including the reproductive system. Indeed,

changes in various biochemical constituents have been proscribed for reproductive failure. Thus, the biochemical profile could be a potential help to characterize these problems.

Highly variable ratios were available on the levels of these biochemical constituents, from which the present study was planned to evaluate the levels of some biochemical compounds relating to the energy, nitrogen and mineral supply, during the postpartum and the consequences on the reproductive behavior of cows.

## Materials and method

### Topography of the Study Area

The present experimental work was carried out on a total of 74 multiparous and primiparous Montbeliard cows without any visible signs of clinical disease managededin ten commercial dairy farms in Ain El Kebira in Setim provnce of the North East of Algeria. These localities are characterized by a semi-arid continental climate with a very variable level of precipitation from one year to another and from north to south of 600 to 200 mm per year and the average temperatures oscillate from 5°C (January) to 26°C (July). The average altitude varies between 700 and 1300m. The farms have been selected for correct data recording, relatively good management and cooperation.

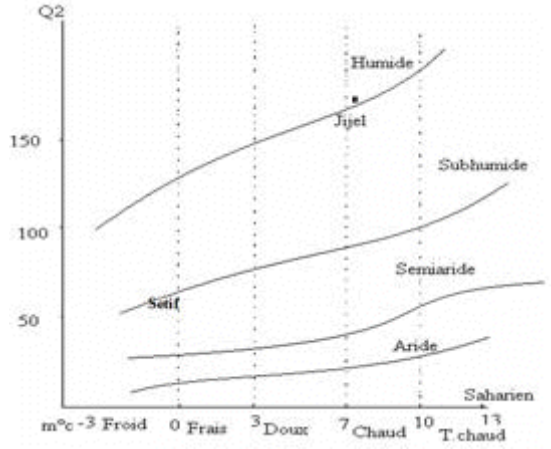



**Figure 1.** Bioclimatic properties of the study region according to different algerian ombrothermal stages

### Management of dairy cows

The studied animals were kept under similar dietary and managerial practices on a semi-intensive livestock system. The dietary calendar during the winter (November-February) depended on the distribution of hay (Meadows or oats), sorghum silage and commercial concentrate as complementary ration. During the spring, the herds exploited natural grasslands and fallows, whereas during both summer and autumn, residues were the effects of grass mowing and / or cereal stubble, provided part of the diet. Animals were kept in tie-stalls. Natural breeding was commonly used but sometimes artificial insemination was conducted.

**Measurement of body condition and milk production**

The body condition score (BCS) assessment was done by the same person on the score of 1: very thin to 5: very fat according to Edmonson grill of 0.25 deviations (Edmonson et al 1989). Measurements were made in the dry period one month before calving (BCS T), one month after calving (BCS 1), two months after calving (BCS 2) and three months after calving (BCS 3). The quantity of milk produced daily was recorded from the farm's production sheet at each visit.

**Laboratory blood tests**

Blood samples for biochemical analysis were taken once a month for three months postpartum from the selected groups of cows 2-3 hours after the morning feeding. From each animal, about 5 ml blood was taken by puncture of vena coccygea into the screw capped vacuum tubes.

In order to minimize the stress in the animal and to standardize the blood collection procedure, all the dairy cows were restrained with the same technique and the collection was made by the same person.

The tubes with blood were transported into a rolling fridge to the laboratory for biochemical analysis “Public hospital establishment of Ain El Kebira”. Each blood serum for biochemical analysis was separated after centrifugation of the sample blood at 3000 revolutions / min for 5 minutes and transferred into a sterilized plastic vial and labeled. Clean glassware, micropipettes of different capacities and analytical grade chemicals (Spinreact®) were used in this study

**Reproduction settings retained**

Seven reproduction parameters were selected:

- Calving to first service interval in days (C1stSI).

- Calving to conception interval in days (Open days).

- Calving interval (CI).

- Services per conception (SPC).

- First service to conception interval in days (S1-CI).

- Success rate; at 1st service, at 3rd service and more, at 70, 90 and 110 days.

- Rate of CI<380d and CI>440d.

### Statistical analysis

The statistical analyses of the data were performed using XLSTAT 2014 software. The descriptive statistics (mean, minimum, maximum, and standard error) were estimated. A one-way analysis of variance was applied (ANOVA) to observe the differences between the physiological periods. The significance levels *, **, *** were attributed when $p<0.05$, $p<0.01$ and $p<0.001$.

## Results

Our results are summarized in Tables 1-4, and Figures 2-5.

### Evolution of BCS

The body condition score decreased significantly at first month postpartum ($p<0.01$) (Table 1). The recovery of BCS was established from the 3rd month of lactation 3.6±0.4 ($p<0.05$). Thus, 64% of cows lost more than 0.5 points of BCS during the first month postpartum (Table 2).

**Table 1.** Evolution of BCS around calving

| | *n* | Mean | Std.Error |
|---|---|---|---|
| BCS T | 74 | $3.92^{a}$ | 0.38 |
| BCS1 | 74 | $3.28^{b}$ | 0.53 |
| BCS2 | 74 | $3.28^{b}$ | 0.45 |
| BCS3 | 74 | $3.60^{c}$ | 0.46 |
| Intra subject effect | | ** | |

**Table 2.** Percentage of cows according to the degree

of body condition loss at 1 month postpartum

| Degree of BCS loss | Numbers | % |
|---|---|---|
| Low (<0.5) | 19 | 26 |
| High (>0.75) | 20 | 27 |
| Middle [0.5-0.75] | 35 | 47 |

## Reproduction settings

The averages of C1stSI, open days, S1-CI, CI and SPC were: 102, 125, 24, 402 days and 1.62, respectively (Table3). Only 26% of cows were considered pregnant at 70 days and 41% at 110 days; 58% were pregnant after a single insemination and 13.5% required 3 inseminations and more.

**Table 3.** Reproduction parameters of Montbeliarde cows under semi-arid region

| Parameters | Mean | Std Error |
|---|---|---|
| $C1^{st}SI$ (d) | 102 | 46 |
| Open days | 125 | 59 |
| S1-CI (d) | 24 | 44 |
| CI (d) | 402 | 57 |
| SPC | 1.62 | 0.88 |
| CP at S1 (%) | 58 | - |
| R +3S (%) | 13.5 | - |
| CR at 70 d (%) | 26 | - |
| CR at 90 d (%) | 31 | - |
| CR at110 d (%) | 41 | - |
| RCI<380d (%) | 50 | - |
| RCI>440d (%) | 19 | - |

*$C1^{st}SI$: Calving to first service interval, S1-CI: First service to conception interval CI: Calving interval, SPC: Services per conception, CP at S1: Conception rate at 1 $^{st}$ service, R+3S:percentage of cows with 3 or more inseminations, RCI<380d: percentage of cows with CI<380d, RCI>440d: percentage of cows with CI>440d.*

## Dynamics of change and evolution patterns of blood metabolites

Serum levels of energy metabolites increased significantly from the first to the third month of lactation (Table 4); from 0.61±0.07 to 0.66±0.03g/l for glucose, and 1.21±0.38 to 1.51±0.4g/l for total cholesterol.

The averages of BUN and albumin were stable around; 0.18±0.06 to 0.17±0.06g/l and 2.84±0.46 to 2.86±0.57g/l respectively); even for calcium, which was of the order of 8.4±1.62 to 8.7±1.2 mg/dl. However, magnesium and phosphorus showed a significant decrease in the third month; 2.16±0.6 vs 1.97±0.38 mg/dl and 4.96±2.46 vs 4.00±1.7 mg/dl, respectively.

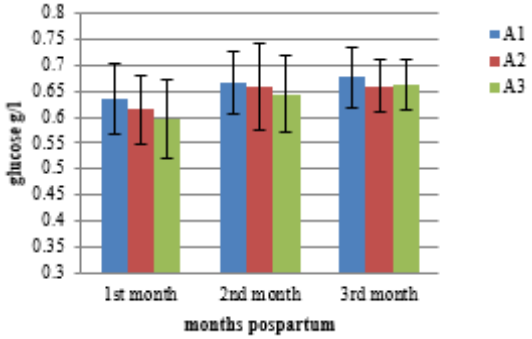
**Table 4.** Biochemical parameters during postpartum period in Montbeliard cows

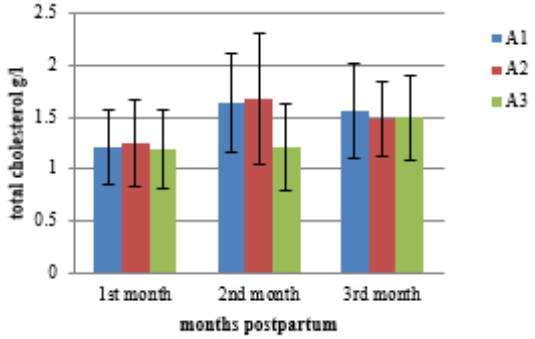
| Biochemical constants | 1st month PP | | 2nd month PP | | 3rd month PP | |
|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | Mean | Std Error |
| Glucose, g/l | 0.61[a] | 0.07 | 0.65[b] | 0.07 | 0.66[b] | 0.03 |
| **Tot**al cholesterol, g/l | 1.21[a] | 0.38 | 1.49[b] | 0.51 | 1.51[b] | 0.4 |
| Urea, g/l | 0.18 | 0.06 | 0.17 | 0.05 | 0.17 | 0.06 |
| Albumin, g/l | 2.84 | 0.46 | 2.73 | 0.54 | 2.86 | 0.57 |
| Calcium, mg/dl | 8.40 | 1.62 | 8.42 | 1.22 | 8.70 | 1.20 |
| Magnesium, mg/dl | 2.16[a] | 0.60 | 2.20[a] | 0.52 | 1.97[b] | 0.38 |
| Phosphorus, mg/dl | 4.96[a] | 2.46 | 4.93[a] | 2.40 | 4.00[b] | 1.70 |

*Different letters on the same line indicate significant difference at p <0.05.*

**Relationship between nutritional metabolites and reproductive performances**

The analysis of the impact of nutritional status on the cows' reproductive parameters showed a significant effect on at least one of the reproductive parameters studied. In fact, glucose, total cholesterol and BUN were significantly lower in cows with an extension of C1stSI during the first month for glucose and BUN, and during the second month for total cholesterol. In parallel, calcium and albumin show significantly elevated levels during the first and second months respectively (Figure 2).



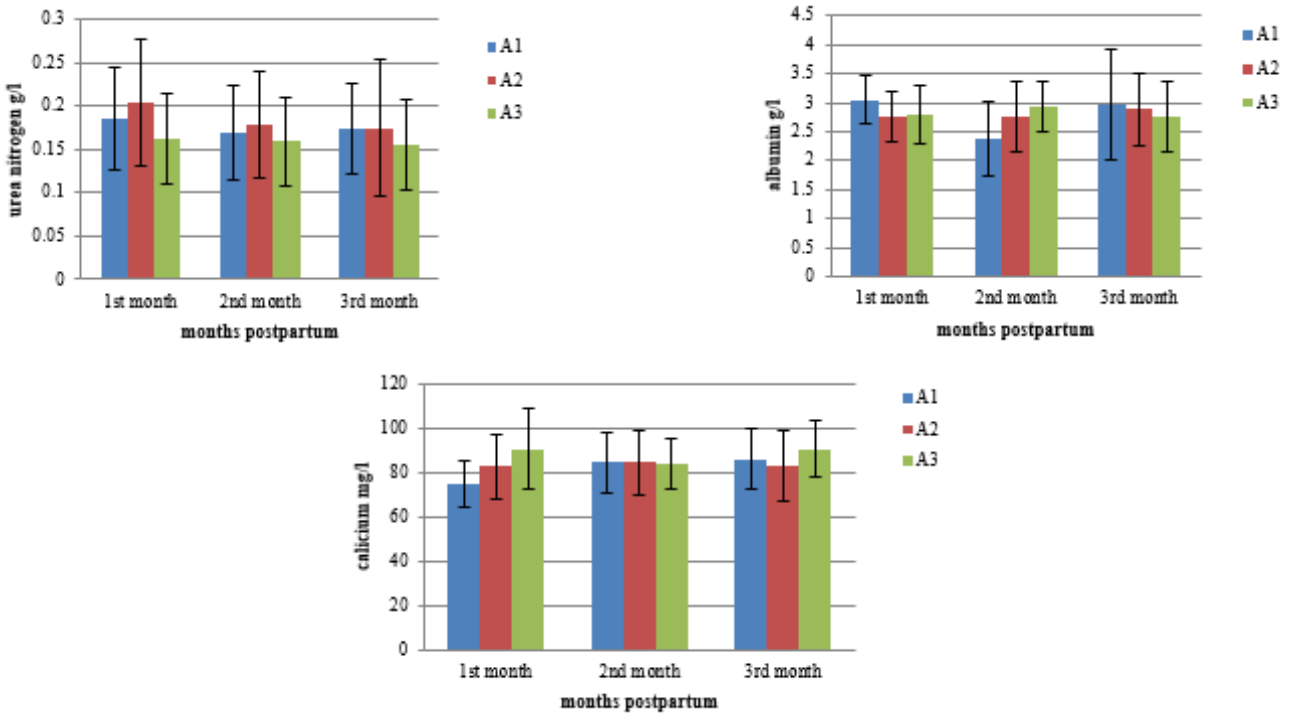



**Figure 2.** Dynamics of change of some blood metabolites affecting calving to first service during postpartum periods (A1 : <70d A2: [70-100d] A3: >100d)

Thus, total cholesterol and BUN showing significantly lower rates in cows having delayed fertilization during the second and third months, respectively (Figure 3).

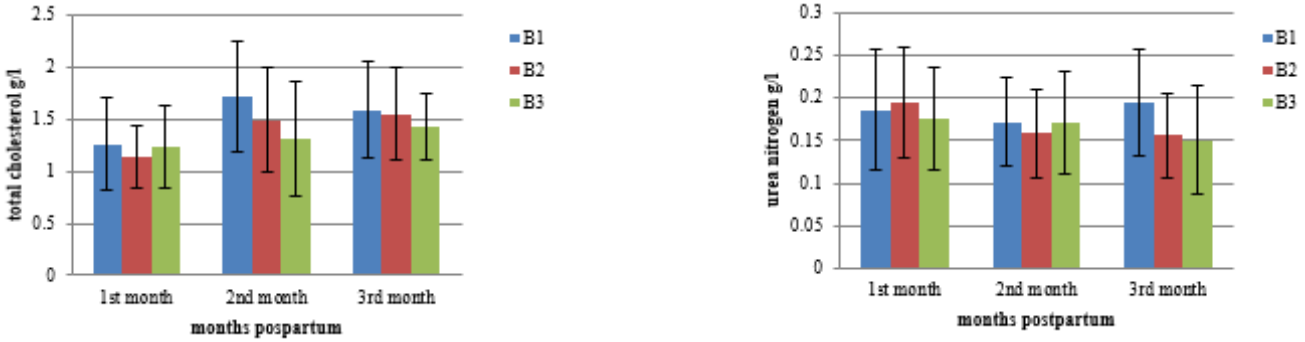



**Figure 3.** Dynamics of change of some blood metabolites affecting calving to conception rate

during postpartum periods(B1 : <90d, B2: [90-110d], B3: >110d)

In addition, Figure 4 shows a significantly higher concentration in the second month for albumin in cows that had an elongation of calving interval.

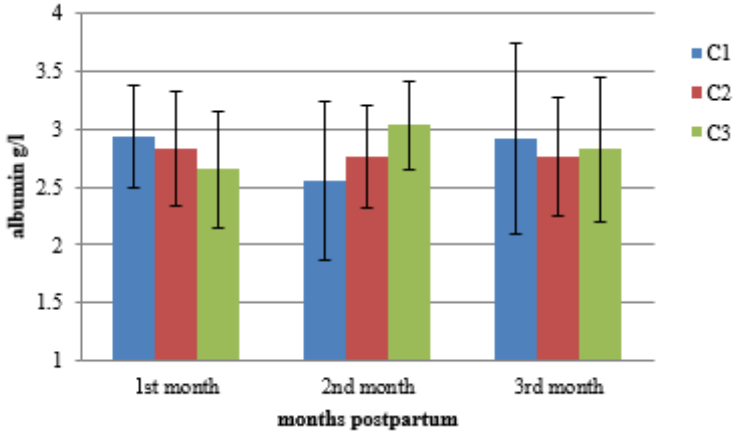
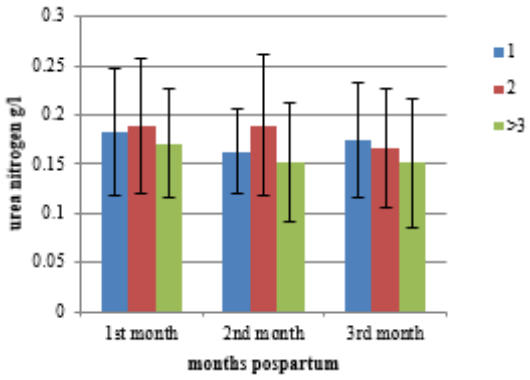
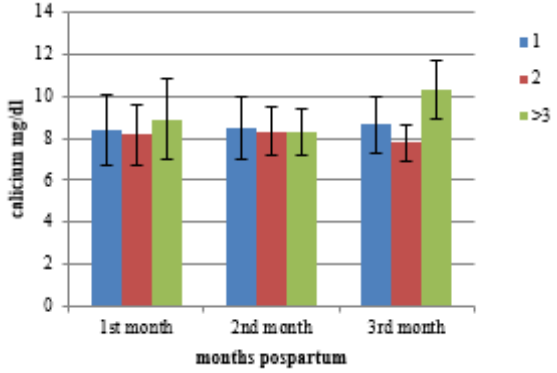



**Figure 4.** Dynamics of change of some blood metabolites affecting calving interval
during postpartum periods(C1 : <380d, C2: [400-440d], C3: >440d)

The nutritional status of the cows during postpartum also affects the number of inseminations per conception. As a result, the concentration of BUN, magnesium and phosphorus were low ($p$ <0.05) during the first and second postpartum months for BUN and Mg, and during the second and third month of lactation for P in cows requiring three inseminations and more (Figure 5). On the other hand, the level of calcium was higher ($p$<0.05) during the third month for these cows (Figure 5).



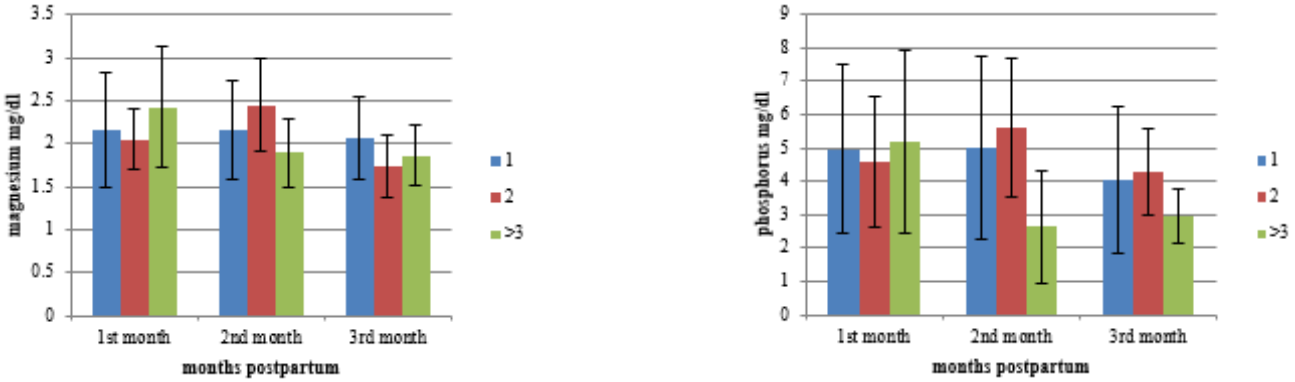



**Figure 5.** Dynamics of change of some blood metabolites affecting number of services per conception during postpartum periods(1 : 1 IA, 2: 2IA,>3: >3IA)

## Discussion

### Reproduction settings

The study of reproduction in the Montbeliard breed in the semi-arid region of Algeria showed performance a little far from the objective to have one calf per cow and per year according to the criteria of Wattiaux (2005). This was for the elongation of the cycle recovery and fertilizing insemination. Our results were similar to those found by Bouzebda et al (2003) in Holstein cows located in extra eastern Algeria, and to those registered in conditions close to ours (Van Sanh et al 1997; Sraïri et Baqasse 2000), and those recorded in temperate countries (Gillund et al 2001; Pryce et al 2001; Veerkamp et al 2001). Nevertheless, they were inferior to those found by Mouffok et al (2011) in the same region of our study. According to Dutta et al (1988), the anoestrus and repeat breeding are negatively affecting the productive and reproductive performance of cows and causing large economic losses for dairy farmers.

### Relationship between nutritional metabolites and reproductive performance

In our study, blood glucose levels changed significantly from the first to the third month postpartum. The same approach was described by most authors (Butler et al 2006; Graber et al 2010), and also with Mouffok et al (2011) in Montbeliard breed under same climate of our study.

The average mean of glucose concentration in our work was significantly lower during the first postpartum month in cows that had prolonged intervals to first insemination. Several studies have associated negative energy balance (EB) with prolonged intervals to first ovulation (Butler et al

1981; Canfield et al 1990; Staples et al 1990).

According to Ijaz Ahmad et al (2004), blood glucose appears to be one of the key nutrients affecting cyclicity in farm animals. Nadiu and Rao (1982) and Dutta et al (1988) reported significantly lower serum glucose level in anoestrus than normally cycling animals. Mouffok et al (2011) showed that cows had better performance when they had concentrations around 0.60 to 0.62g/l vs 0.58g/l.

Cholesterol is the most abundant sterol in animal tissues, and is the source of most steroids, especially progesterone (Ruegg et al 1992). In our results, total cholesterol was significantly lower in the first month PP. Our results were similar to those reported by Butler et al (2006) in the Holstein breed in Italy, by Graber et al (2010) for Brown Swiss and Fleckvieh in Switzerland and by Mouffok et al (2011) in the Montbeliard breed.

The cholesterolemia during the second postpartum month was inversely related to calving to first insemination interval and calving to conception; the same finding was reported by Kappelet al (1984) and Mouffok et al (2011). Beam and Butler (1997) and Villa-Godoy et al (1988) described that during an energy deficit, there was a reduction in steroidogenesis and therefore a decrease in circulating concentrations of progesterone and estrogen, causing the deterioration of reproductive performance. Increasing plasma cholesterol concentrations were measured in postpartum, as observed in other studies (Aeberhard et al 2001), reflecting the changes in lipid and steroid metabolism during decreasing body weight losses.

In our study, there was an alteration of all reproductive criteria in cows with nitrogen deficiency (<0.15 g /l). Our results are in agreement with those of Miettinen (1990), Wolter (1992) and Tillard (2007); also, with those described by Mouffok et al (2011), who observed that cows with better performance had concentrations around 0.19 and 0.21 g/l. According to Zhangrui Cheng et al (2015) both high and low circulating urea concentrations have been associated with reduced fertility.

In addition, the average concentration of blood urea slightly changed during the postpartum, which in agreement with Butler et al (2006) and Graber et al (2010). However, Tillard (2007) and Mouffok et al (2011) reported that the BUN concentration significantly increased during the first month of lactation and declined thereafter.

Albumin has a relatively short half-life and can reflect protein deficiency problems over a period of a month or two (Vagneur 1992). Changes in plasma albumin concentrations in the present study were similar as reported by Blum et al (1983), Aeberhard et al (2001) and Graber et al (2010), who found no significant change in plasma albumin concentration during postpartum cows. Moreover, in our study we found significant increases in serum albumin concentration in cows with open days over 120 days and calving interval >440d, which in agreement with those reported by Randel (1990) and Ijaz Ahmad et al (2004). Van Saun (2004) considered that fresh cows which could maintain serum albumin concentrations ≥3.5 g/dl were less likely to have postpartum disease.

Early lactation imposes sudden high demand for $Ca^{++}$ and P vital for the synthesisof milk ingredients. Some cows exhibit a marked decrease in blood $Ca^{++}$ and P levels in early lactation, resulting in a dramatic decline (<1.5 mmol/L), leading to hypocalcemia, reduced neuromuscular excitability and milk fever/puerperal paresis (Sharma et al 2006). Puls (1988) recommended classification of blood $Ca^{++}$, P and Mg values in dairy cows based on their concentrations, as deficient, limiting, adequate and high.

On this basis, 40% of the cows in our study had calcium deficiency (<75 mg/l) in the first month, 35% in the second month and 23% in the third month. P was <35 mg/l in proportions of 36 to 40%. Then, 11% of cows had Mg concentration of less than 15 mg/l at 17% in the third month (Radostits et al 1997; Wolter1992).

In our study, cows with high levels of $Ca^{++}$ in the first month postpartum cows had longer calving to first insemination interval and required more than three inseminations to be fertilized, which agrees with the reports by Zollner et al (2001) and Poppe and Velkeniers (2003) that hyperprolactinemia impairs the pulsatile secretion of GnRH and, hence, interferes with the occurrence of ovulation.

The average phosphorus and magnesium levels were significantly lower in cows requiring three and more inseminations, as described by several authors (Meschy 1994; Paragon 1995; Tillard2007; Youssef 1989). According to Atherton (1994), infertility caused by phosphorus deficiency occurs due to other symptoms such as hair loss and decreased appetite. However, many authors observed that there was no relationship between serum phosphorus and fertility (Ingraham et al 1982; Larson et al 1980). On the other hand, Danvin (1988), Paragon (1995) and Poncet (2002) reported that a magnesium deficiency was associated with a decrease in the success of insemination.

## Conclusion

- From the foregoing therefore the results of this study have demonstrated reliable relationships between the pattern of blood biochemical status postpartum and the reproductive parameters.
- The low levels post partum of the following substrates: glucose, total cholesterol, urea; and high scores of calcium and albumin were associated with increased intervals of calving-first service and open days.
- Likewise, a low concentration of magnesium, phosphorus and a high level of calcium were recorded in cows with higher number of services per conception.
- Based on these findings, we recommend that for the feeding of dairy cows, during the period post-calving, the components of the diet can be used to modify the physiology of the animal, in order to promote its health and its reproduction.
- Our study shows that the deficit of energy, nitrogen and minerals had some effect on fertility. To avoid this deficit, we must verify that the ration is properly balanced (adequate in vitamins and minerals), well preserved and consumed in sufficient quantities.

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*Received 2 April 2018; Accepted 28 April 2018; Published 1 May 2018*

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