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Article *in* Livestock Research for Rural Development · May 2018

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Abstract

This study aimed to compare 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 study was conducted on 74 Montbeliard cows raised in northeastern Algeria under a semi-arid climate. Blood samples were collected at 1 month intervals for 3 months postpartum, assessment of the body condition on the Edmonson grid and reproductive events were recorded. The statistical analyses of the data were performed using Excel Stat 2014 software. The results revealed significant increase ($P<0.05$) of glucose and total cholesterol in the third month of lactation. However, magnesium and phosphorus were decreased ($P<0.05$). The analysis of the impact of nutritional status on the reproductive behavior of dairy cattle postpartum showed a significant effect of the latter on at least one of the reproductive parameters studied. In effect, glucose, total cholesterol and urea were displaying significantly lower rate in cows that had a longer calving - first insemination interval and calving - conception. At the same time, calcium and albumin are increased. Thus, significantly lower concentrations of magnesium and phosphorus and significantly higher serum calcium in cows requiring 3 or more inseminations were revealed. In conclusion, this study showed the effect of the nutritional status of cows during postpartum on the resumption of the physiological cycle and the success of insemination.

Key words: *BBCS, metabolic profiling, post-partum, reproduction traits, semi-arid*

Introduction

Anoestrus and repeat breeding, negatively affect the productive and reproductive performance of cows and cause 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 show that under nutrition during the first phase of lactation is accompanied by disturbances in plasma concentrations of reproductive hormones (Westwood et al 2002 ; Mouffok et al 2011), low follicular development (FassiFihri et al 2005) and poor quality of oocytes (Jorritsma et al 2003). Buckley et al (2003) have reported that it is the 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 level even if the body condition score remains 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 reproductive system. Indeed, changes in various biochemical constituents have been proscribed for reproductive failure. Thus, the biochemical profile could be a potential helped to characterize these problems.

Highly variable ratios were available on the level of these biochemical constituents, from which the present study was planned to evaluate the levels of certain biochemical compounds relating to the energy supply, nitrogen and mineral, during the postpartum and their 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 reared in ten commercial dairy farms in Ain El kebira in north east Algeria. These localities were 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 oscillated from 5 °C (January) to 26 °C (July). The average altitude varies between 700 and 1300m. Farms have been selected for correct data recording, relatively good management and cooperation.

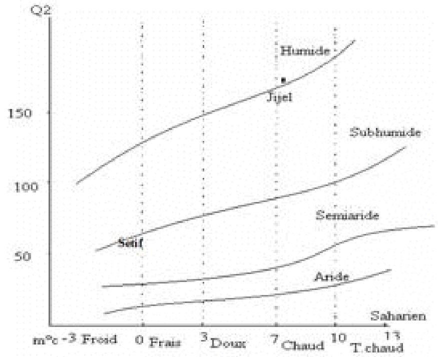

Figure 1. Bioclimatic properties of the study region according to different algerian ombrothermal stages

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) that depended on the distribution of hay (Meadows or oats), sorghum silage and commercial concentrate as complementary ration. During Spring, herds exploit natural grasslands and fallows, whereas during both summer and autumn, residues were the effects of grass mowing and / or cereal stubble provide 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 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). In dry period one month before calving (BCS T), one month after calving (BCS 1), two months after calving (BCS 2), three months after calving (BCS 3). The quantity of daily milk produced 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 month postpartum from the selected groups of cows 2-3 hours after the morning feeding. From each animal, about 5 ml blood was performed 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 the rolling fridge to the laboratory for biochemical analysis “public hospital establishment of Ain El kebira”. 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 Excel Stat 2014 software. The descriptive statistics (Mean, minimum, maximum, and error standard) were estimated.One-way analysis of variance was applied (ANOVA) to observe the differences between the physiological periods. The significance was 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 decrease significantly at first month postpartum ($p<0.01$) (Table 1). The recovery of BCS was established from the 3rd month of lactation 3.6±16 ($p<0.05$). Thus, 64% of cows were lost more than 0.5 point 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	3.6^c	0.46
Intra subject effect		**	

Table 2. Percentage of cows according to the degree of body condition loss

Degree of BCS loss	Numbers	%
Low (<0.5)	19	26
Hight (>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 from : 102, 125, 23.5, 402 days and 1.62, respectively (Table3). Moreover, Only 26% of cows were considered pregnant at 70 days and 41% were at 110 days. While, 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	45.8
Open days	125	59.4
S1-CI (d)	23.5	44
CI (d)	402	57.4
SPC	1.62	0.88
Rat S1 (%)	58	-
R+3S (%)	13.5	-
Ratat70 d (%)	26	-
Ratat90 d (%)	31	-
Ratat110 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, Rat S1: Success 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 increase significantly from first to third months of lactation (Table 4); from 0.61±0.07 to 0.66±0.03g/l for glucose, and of 1.21±0.38 to 1.51±0.4g/l for total cholesterol.

The mean average of BUN and albumin were stable around; (0.18±0.06 to 0.17±0.06g/l and 2.84±0.46to 2.86±0.57g/l respectively). Even for calcium, which was in 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, at; 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	1^{st} month PP		2^{nd} month PP		3^{rd} 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
Total 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 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).

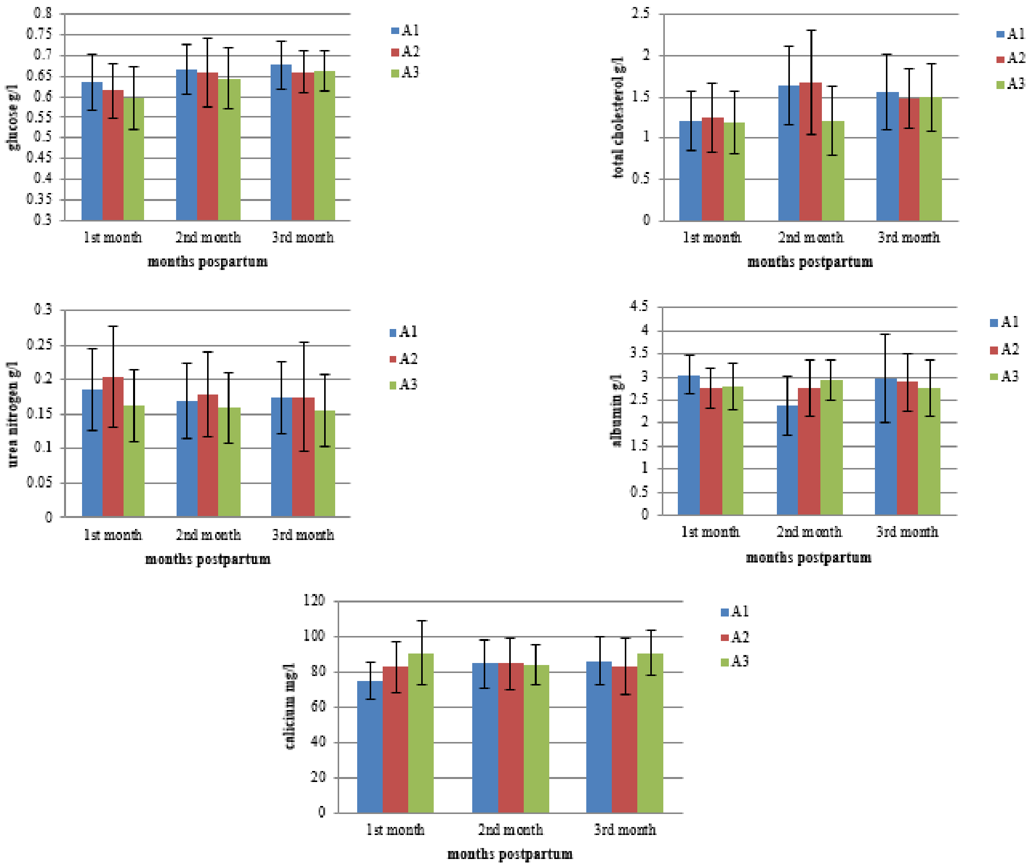

Figure 2. Dynamics of change of some blood metabolites affecting calving to first service during postpartum periods(A1 : <70d A2: [70-100d] A3: >100d)

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

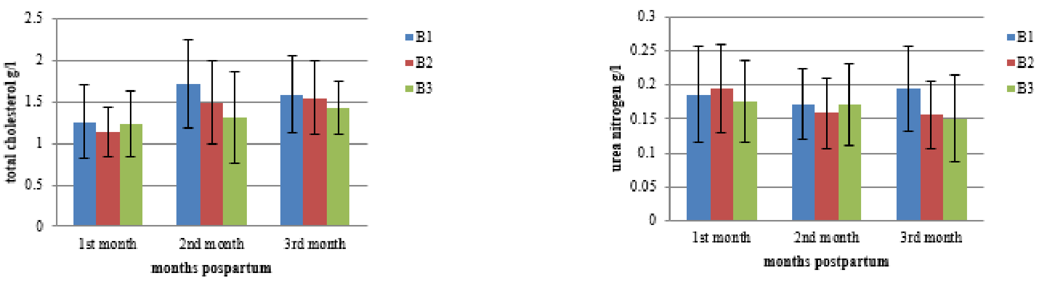

Figure 3. Dynamics of change of some blood metabolites affecting calving to conception rate during postpartum periods(B1 : <90d, B2: [90-110d], B3: >110d)

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

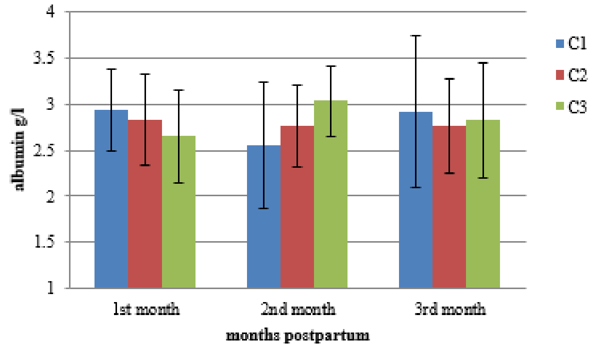

Figure 4. Dynamics of change of some blood metabolites affecting calving interval during postpartum periods(C1 : <380d, C2: [400-440d], C3: >440d)

The nutritional status of 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).

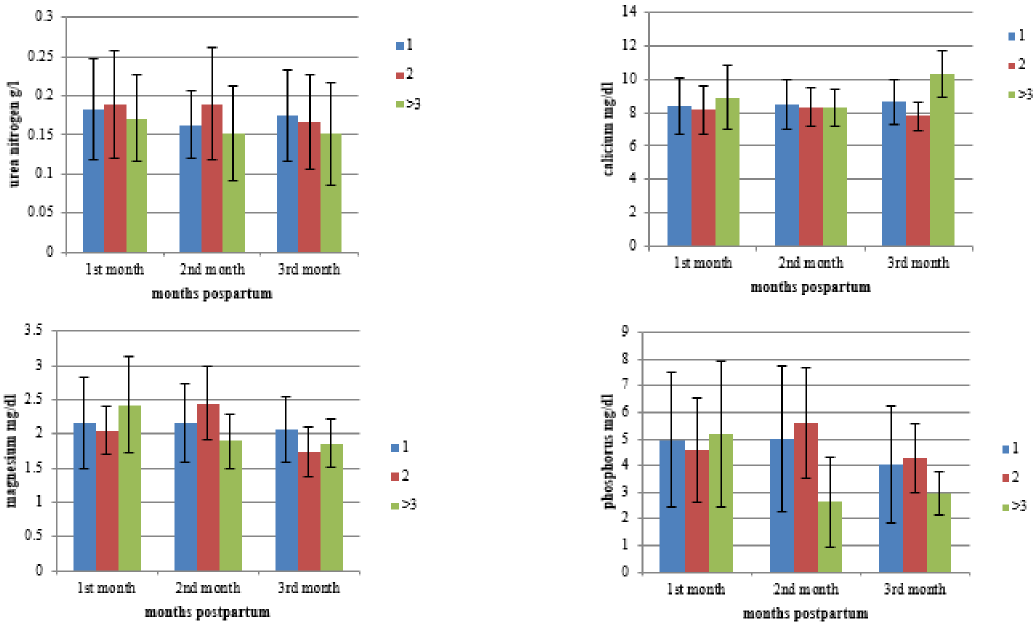

Figure 5. Dynamics of change of some blood metabolites affecting number of services per conception during postpartum periods(1 : 1 IA, 2: 2IA,>3: >3IA)

Discussion

Reproduction settings

The study of reproduction in the Montbeliarde breed conducted 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 located in extra eastern Algeria, 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, 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 performances of cows and causing large economic losses for dairy farmers.

Relationship between nutritional metabolites and reproductive performances

The results of many authors (El-Azab et al 1993; Balakrishnan and Balagopal 1994; Qureshi1998) suggest that normal blood levels of various biochemical constituents were indispensable for the normal functioning of the various systems of the body, including the reproductive system. In fact, Many papers have been published on the effect of specific endocrine, metabolic, immune, uterine health, and nutritional factors involved in the achievement of key reproductive targets and pregnancy in pp dairy cows (Aungier et al 2014).

The relationship between EB and reproductive performance has been well documented. For Banoset al (2004), negative energy balance is associated with the difficulties encountered by the cow to receive and maintain a pregnancy. These problems are more noticeable in high yielding cows. Royalet al (2002) reported that in dairy cows, the negative energy balance was the result of a high activity of hormones that regulate the intermediate metabolism through the mobilization of body reserves. This activation disrupts and impairs the flow of reproductive hormones. Adequate energy supplementation before and after calving can correct this negative balance (Staples et al 1998; Pruitt 2001). However, Cavestany et al (2009) reported that the correction of the EB was valid only in multiparous cows. Doreau et al (1983) related positively blood glucose to energy balance during the first six weeks of lactation. The relationship between blood glucose and fertility was also controversial. It appears that blood glucose is associated with infertility when it was well below its usual values (Miettinen 1991). On the other hand, the postpartum concentrations of blood metabolites were a reflection of the instantaneous energy balance, while the body score was more reflective of the cumulative energy balance of previous weeks (Chilliard et al 1998; Reist et al 2002).

In our study, blood glucose levels change significantly from the first to the third month post-partum. Same approach described by most authors (Butler et al 2006; Graber et al 2010), and also with Mouffok et al (2011) in Montbeliarde breed under same climate of our study. That can be explained by direct use of most glucose for lactose synthesis (Bauman and Currie 1980). In fact, the lower concentrations of glucose, in +4wk compared to the other time-points reflect a poorer energy status of the cows in +4wk (Graber et al 2010). However, Kronfeld et al (1982) reported that glucose was under the regulation of homeostasis and was no longer considered a very sensitive indicator of energy status.

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 EB with prolonged intervals to first ovulation (Butler et al 1981; Canfield et al 1990; Staples et al 1990) and metabolic status as early as the first 4 to 10 wk after parturition has been related to the return of cyclic ovarian activity and subsequent reproductive performance in postpartum dairy cows.

According to Ijaz Ahmad et al (2004), blood glucose appears to be one of the key nutrients affecting cyclicity in farm animals. For Van Knegsel et al (2005), low blood glucose may be associated with infertility. Nadiu and Rao (1982) and Dutta et al (1988) reported significantly lower serum glucose level in anoestrus than normally cycling animals. Negative energy balance is linked with decreased GnRH pulse frequency and, hence, reduced LH pulses from the pituitary gland, as glucose is a preferred substrate for neuronal energy metabolism, an inadequate supply inhibits the GnRH pulse generator (Castâneda-Gutiérrez et al 2009). As well as reducing the maturity and production of estrogen by the ovarian follicles (Butler 2000). Low blood insulin concentrations are responsible for low IGF-I production from the liver (Butler et al 2003) which together reduce responsiveness of ovarian follicles to gonadotropins. As well, decreasing DMI is associated with lower blood concentrations of the metabolic hormones, insulin and IGF-I (Butler et al 2006). Mouffok et al (2011), exposed that cows had better performance when have concentrations around 0.60 and 0.62g/l vs 0.58g/l. However, Ganzalez et al (1998) and Kappel et al (1984b) reported that plasma glucose concentration was not associated with poor fertility.

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

Cholesterolemia increases significantly (+ 20-25%) during the first third of lactation, along with the amount of milk produced. This increase was higher in cows with positive energy balance than in cows with negative balance (Beam and Butler 1997). Cholesterol levels were lower when the cow was fat at calving and loses body condition (Ruegg et al 1992). However, Ruegg et al (1992) found that cholesterol is inversely correlated with the loss of body condition; more energy deficit was important,more cholesterol was low. (Beam and Butler 1997).

Cholesterolemia during the second postpartum month was inversely related to calving to first insemination interval and calving to conception, same finding reported by (Kappel et al 1984b).Thus, Mouffok et al (2011)found that the lowest levels of reproductive success were those with low BCS associated with relatively low blood glucose and cholesterol levels. 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. Burle et al (1995) reported lowest serum concentration of cholesterol in anoestrus than in cycling cows. Reduced concentrations of estradiol are associated with ovulation failure during the first follicular wave postpartum in noncystic dairy cows (Beam and Butler 1999). Nevertheless, Gonzalez and Rocha(1998) reported elevated levels of total cholesterol in the first week of the Holshtein cow in Brazil which has an elongation of calving to conception interval, it could be associated with a greater mobilization of lipid involved in a restricted energy diet (Kaneko et al 1997). Accordingly, the cow therefore tries to cover glucose requirements by increased hepatic gluconeogenesis (Greenfield et al 2000; Reynolds et al 2003). 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.

Energy balance is not the sole nutritional factor that affects reproduction (Lucy 2003). Specific nutrients that act independently of energy balance have been reported to directly or indirectly alter reproductive efficiency and fertility. Among these are protein (Armstrong et al 2001), starch (Armstrong et al 2001; Burke et al 2006 ; Roche et al 2006) and minerals (Underwood and Suttle 2001).

Blood urea nitrogen (BUN) has been described as the most sensitive indicator of inadequate nitrogen (Tillard 2007) or carbohydrate intakes. While, the influence of high protein intake and the effect of urea on dairy cow fertility remain ambiguous (Laven et al 2007; Jackson et al 2011).There is also no single measurable metabolite which directly reflects protein status. Rather, multiple parameters are utilized including blood urea nitrogen (BUN), creatinine, total protein, albumin and creatine kinase (Van Saun 2006). A high mean plasma urea concentration was associated with a significant reduction in pregnancy rate in dairy cows (Butler et al 1996). In contrast, high plasma urea concentrations due to high levels of dietary nitrogen had no effect on parameters of fertility in other studies (Ordonez et al 2007; Law et al 2009). Moreover, high blood urea and the metabolic indicators of NEB often occur simultaneously in high-yielding cows making it difficult to separate out any individual effects on subsequent fertility (Jackson et al 2011).

Although the effects of protein and its metabolites are unclear. Both Sinclair et al (2000) and Armstrong et al (2001) reported a reduction in oocyte quality when dietary protein was increased, consistent with the reduction in conception rate with increased RDP (Canfield et al 1990). Butler et al (1996) concluded that an average plasma urea concentration more than 3.14 mmol/l on the day of artificial insemination around 60 days postpartum results in a decreased pregnancy rate. Additionally, deleterious effects of high concentrations of PUN on conception and pregnancy rates in cows (Canfield et al 1990; Carroll et al 1988), as well as on embryo quality (Dawuda et al 2002) were reported. Moreover, there is a strong positive association between urea nitrogen in the follicular fluid and PUN (Roseler et al 1993).Thus, one would expect that a diet that generates high concentrations of PUN may impair oocyte competence, thereby suppressing embryonic development rate and decreasing reproductive performance(Hammon et al 2005). Moreover, Ferreiraa et al (2011) rapported high PUN concentrations decreased oocyte competence in heifers.

However, in our investigation, there is an alteration of reproductive performance in cows with nitrogen deficiency (<0.15 g /l), even number of inseminations per conception. Our results were in agreement with those of Tillard (2007) for the farms of the island of Reunion, which showed that a restriction of nitrogen intake before calving induced a prolongation of calving first insemination intervals, while a restriction during the first month of lactation induces a prolongation of calving conception interval. No association between nitrogen deficiency and number of insemination per conception was found (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. Our results were in line with those described in other studies. Nitrogen-deficient intakes are associated with delayed recovery of ovarian cyclicity and longer calving conception interval (Carlsson 1989; Seegers and Malher 1996) and in conjunction with a decrease in the secretion of proteinaceous gonadotropic hormones (Randel 1990). Miettinen (1990) and Wolter (1992) reported that reproductive function is impaired as soon as uremia is less than 0.14 g /l ; the case of our study. According to Zhangrui Cheng et al (2015) both high (>7.5 mmol/L) and low (<4.5 mmol/L) circulating urea concentrations have been associated with reduced fertility.

The pathogenesis of nitrogen deficiency depends on the nature of deficit nitrogen and energy resources.If there was a deficiency of degradable nitrogen, complicated or not with an energy deficiency, the microbial proteosynthesis decreased, as well as the appetite of the animals, the digestibility of the ration and the efficiency of the use of the energy metabolizable. These result in a decrease in blood glucose and insulinemia inhibiting the hypothalamic secretion of GnRH, the pathogenesis was then similar to that of an energy deficit, with its attendant underproduction, weight loss, risk of ketosis and infertility (Kaur and Arora 1995). In addition, in our work we reported a chronic acidosis with under production and therefore infertility; as well as, when high blood glucose >0.6 g /l and low blood urea <0.2g /l there is a risk of acidosis, under-production and infertility. With a moderate glycemia between 0.55 and 0.6 g/l and weak uremia there is a poor production and infertility (Vagneur 1994). Although, ureogenesis depends on the degradability of nitrogen intake and energy availability: it reflects the ratio (PDIN-PDIE) / UFL. It is stimulated when the nitrogen inputs were important and the microbial syntheses limited by the energy (Fekete et al 1996).

The average concentration of blood urea slightly changed during the postpartum in all the cows of our investigation, which in agreement with Butler et al (2006) and Graber et al (2010). However, Mouffok et al (2011) reported an increase in urea in the first month PP, then a significant decline to the third month PP. Moreover, Tillard (2007) reported that the BUN concentration significantly increased during the first month of lactation in under-weight females and declines thereafter, while evolution is regular in fat cows.

Albumin reflects availability of protein and their concentration decline in the face of protein deficiency. However, this occurs over a period of time. Albumin has a relatively short half-life and can reflect protein deficiency problems over a period of a month or two (Vagneur 1992). As, according to Van Saun (2004), albumin was found to be associated with postpartum disease and can be used to predict disease risk in close-up and fresh periods. 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. In spite of this, the lower albumin concentrations in first month postpartum could be explained by colostrum building in the udder for which protein is used (Blum et al 1983). As well, albumin can be affected by nutritional inadequacies, gut malabsorption, dehydration, or liver impairment (Grummer 1993). In fact, higher level of total serum albumine was associated with low fertility, as reported by Hewett (1974). The mechanism by which high level of protein adversely affects reproduction in dairy cows is unknown (Randel 1990). It can be related to excessive intake of protein in the feed, thus as reviewed by Ijaz Ahmad et al (2004) excessive intake of protein in the feed can reduce fertility and increase the number of services per conception. This agrees with our findings; in which we found significant increases in serum albumin concentration in cows with open days over 120 days. Also, fertility is impaired more by feeding excessive protein to older cows (Ijaz Ahmad et al 2004).

On the other hand, a protein deficiency will cause metritis, mastitis, foot rot and other problems without antibiotic treatment. Thus El-Azab et al (1993) reported significantly higher serum protein in cyclic cows than the non cycling one's. Van Saun (2004) reviewed that fresh cows which could maintain serum albumin concentrations ≥3.5 g/dl were less likely to have postpartum disease. Other authors reported low values of plasma albumin have been related with poor reproductive performance in beef cows (Gregory and Siqueira 1983) and dairy cattle (Ferguson and Chalupa a 1989), possibly reflecting availability of amino acids from the labile protein pool (Van Saun 2004). In spite of this, Tegegne et al (1993) found inconsistent trends.

Macro- and micro-nutrients are essential to maintain the normal function of vital biochemical processes in the dairy cow's body. Different degrees of deficiency of these inorganic substances can lead to clinical and subclinical symptoms and significantly reduce productive and reproductive performance in dairy cows (Ballantine et al 2002; Spears 2003; Dobrzański et al 2005).

In early lactation, the regulatory mechanisms for Ca++ and inorganic P homeostasis in dairy cows are adapted to markedly increased Ca++ and P demands placed by the mammarygland. Calcium mobilization from bone and calcium absorptionfrom the gastro-intestinal tract increase to enable the cow tocreate homeostasis. High-yielding cows were found to mobilize larger amounts of Ca++ and P from bone compared to low-yielding cows (Liesegang et al 2007).

Early lactation imposes sudden high demand for Ca++ and P vital for the synthesis of 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/puerperalparesis (Sharma et al 2006). Puls (1998) 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 low (<35 mg/l) in proportions of 36 to 40%. Then, 11% of cows had Mg concentration of less than 15 mg/l and 17% in the third month (Radostits et al 1997; Wolter 1992). The deficiency of macro minerals causes major problems related to reproduction such as; retained placenta, metritis, delay in eduterine involution, discrete heat, anoestrus and decreased fertility (Meshy 1994). According to Tillard (2007) ante-partum calcemia less than 112 mg/L is associated with a prolongation of the calving conception interval. Serum calcium beyond 30 days of lactation below 100 mg/L is associated with a prolongation of the calving to first insemination.

In our study, cows with high levels of Ca++ in the first month postpartum had longer calving to first insemination interval and required more than three inseminations to be fertilized. According to Zollner et al (2001) and Poppe and Velkeniers (2003), hyper prolactinemia impairs the pulsatile secretion of GnRH and, hence, interferes with the occurrence of ovulation.

P is component of the metabolic profile that is highly affected by change in feeding in ruminants (Payne and Payne 1987). Nevertheless, a study in lactating cows (Knowlton and Herbein 2002) suggested when dietary phosphorus (0.34%) was inadequate but dietary calcium sufficient, bone was resorbed to fulfill the P deficit. Infertility caused by phosphorus deficiency occurs due to other symptoms such as hair loss and decreased appetite (Atherton 1994). However, for phosphorus deficiency, it results in irregular estrus and decreased ovarian activity (Tillard 2007). In our study, the average phosphorus level was significantly lower in cows requiring three and more inseminations. A decrease in phosphorus intake generally induces a decline in fertility or an increase in the period of anoestrus (Meschy 1994; Paragon 1995; Youssef 1989), but not always. Some authors did not observe any relationship between serum phosphorus and fertility (Ingraham et al 1982; Larson et al 1980). In the same way (Valk and Sebek 1999; Wu and Satter 2000) it was found no deterioration of reproductive performance in dairy cows fed a diet deficient in phosphorus over a long period; although these authors suggest the existence of a concentration "threshold" below which the effects were noticeable.

Magnesium homeostasis depends on an optimal supply from alimentary sources; accordingly, Mg concentration is dependent on Mg absorption in the rumen (Fontenot et al 1989; Kurćubić et al 2010). In dairy cows, milk production and reproductive performance are reduced, muscle tone in the uterus is impaired, placental retention is a frequent disorder, uterine involution is delayed, and the service period is prolonged (Daniel 1983). Magnesium deficiency in feeds for dairy cows inhibits the synthesis and secretion of parathormone, reduces the absorption of calcium and inorganic phosphorus in the digestive tract, and inhibits the production of the active form of vitamin D (1.25 (OH)2 D3) (Djokovic et al2014).

In our study, magnesium was significantly decreased in cows requiring 3 inseminations and more, which was in agreement with those found by Poncet (2002). According to Poncet (2002), a magnesium concentration of less than 2 mg / L is associated with a decrease in the success of insemination. Inadequate intake results in lower AI success rate (Danvin 1988; Paragon 1995), an extension of the calving conception interval (kappel et al 1984a) or a higher frequency of uterine involution delays or retained placenta (Meschy 1994; Paragon 1995; Serieys 1997). Although, magnesium supplementation was not systematically beneficial (Ingraham et al 1987), which relativizes the impact of deficiency. However, according to (Poncet 2002), magnesium was an insensitive indicator of magnesium intake, but Wolter (1992) also considers the reliability of the chemical analysis of the ration. Moreover, Goff and Horst (1997) indicated low blood levels of magnesium in dairy puerperium cows contribute to insufficient insulin production, leading to a disorder in organic nutrient metabolism and predisposition to ketosis and fatty liver.

Conclusion

- It is important to specify that biochemical profiles do not predict a priori the occurrence of infertility, except when they highlight major imbalances. But they are useful a posteriori, during the analysis of reproduction performance. Indeed, biochemistry has contributed to the dietary origin of infertility: it supported the hypothesis of chronic acidosis common to all cows, and it widened the field of possible etiologies by revealing hepatic dysfunctions, that would promote or trigger reproductive disorders. The imbalances in minerals are not to be neglected during fertility disorders.

- In fact, we can conclude in our study the deficit effect of energy, nitrogen and mineral status on fertility. For this, we must verify that the ration is properly balanced (vitamin mineral adequate), well preserved and sufficiently consumed.

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Received 2 April 2018; Accepted 10 April 2018; Published 1 May 2018

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