CHANGES OF SOME HAEMATOCHEMICAL PARAMETERS IN DAIRY COWS DURING LATE GESTATION, POST PARTUM, LACTATION AND DRY PERIODS

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Abstract. The period of transition between late pregnancy and early lactation presents an important metabolic challenge to the high-yielding dairy cows. They need, especially for the high milk yield, more nutrients and energy supply than other animals. The aim of this study was to attempt at providing a complete picture of dynamics of selected biochemical blood parameters in dairy cows from late pregnancy to dry period, giving new and useful information about the guidelines for the management strategies during different physiological phases. The study was carried out on five clinically healthy dairy cows, breed Holstein Friesian, in good nutritional condition. All the experimental subjects were selected on the basis of their pregnancy and lactation status, so blood samples were collected two days before the expected parturition (Late gestation), during the post partum, in early lactation, during the 2nd, 5th and 15th weeks after parturition, at the end of lactation and at the dry period. On each serum sample urea, creatinine, total proteins, albumin, total cholesterol, triglycerides, NEFA, β-hydroxybutyrate, total and indirect bilirubins, calcium, phosphorus and magnesium were determined. On all data, normally distributed ($P<0.05$, Kolmogorov-Smirnov's Test), one-way Repeated Measure Analysis of Variance (ANOVA) was applied to evaluate the influence of the reproduction status on the considered parameters. A significant effect of the physiological phase was observed on urea, creatinine, total proteins, total cholesterol, triglycerides, NEFA, β-hydroxybutyrate, calcium and phosphorus. Our data confirm that the lactation period is the more sensible, by a metabolic point of view, for the high production dairy cow, so the information, provided in this paper, advance the continuous investigation in animal welfare and can be a useful tool in managing and preventing the deficiencies typical of high production ruminants.
Keywords: haematochemical parameters; dairy cow; parturition; lactation; dry period.

LABAI PRODUKTYVIŲ MELŽIAMŲ KARVIŲ KRAUJO BIOCHEMINIŲ RODIKLIŲ DINAMIKA SKIRTINGAIS LAKTACIJOS PERIODAIS

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Santrauka. Laikotarpis tarp paskutinės veršingumo stadijos ir laktacijos pradžios labai svarbus didelio produktyvumo melžiamoms karvėms. Norint pasiekti didelio produktyvumo, reikia daugiau didelės energijos pašarų. Šio tyrimo tikslas buvo nustatyti kai kurių kraujo biocheminių rodiklių dinamiką nuo paskutinės veršingumo stadijos ir laktacijos pradžios iki laktacijos pabaigos, taip pat paruošti šėrimo rekomendacijas šių skirtingų fiziologinių stadijų metui. Tyrimai atlikti su penkiomis kliniškai sveikomis Holšteino fryzų veislės gerai įmitusiomis melžiamomis karvėmis. Kraujo mėginiai paimti dvi dienas prieš numatomą veršiamąsi, po veršiavimosi, prasidėjus laktacijai, dvi, penkios ir 15 savaičių po veršiavimosi, laktacijos pabaigoje ir po užtrūkimo. Kraujyje tirtas šlapalo, kreatinino, bendras baltymų, bendras cholesterolio, trigliceridų, NEFA, beta hidroksibutirato, bendro ir netiesioginio bilirubino, kalcio, fosforo bei magnio kiekis. Tyrimo rezultatai statistiškai įvertinti ($p<0,05$) Kolmogorovo-Smirnovo metodu bei daugybine statistine analize (ANOVA). Nustatyta, kad fiziologinė gyvulio būklė turėjo statistiškai ženklios įtakos šlapalo, kreatinino, bendram baltymų, bendram cholesterolio, trigliceridų, NEFA, beta hidroksibutirato, kalcio, fosforo bei magnio kiekiui. Ištirta, kad laktacijos periodas turi įtakos labai produktyvių melžiamų karvių sveikatingumui. Tyrimų rezultatai yra naudingi norint išvengti labai produktyvių melžiamų karvių kraujo biocheminių rodiklių svyravimų skirtingais laktacijos periodais.
Raktažodžiai: kraujo biocheminiai rodikliai, melžiamos karvės, veršingumas, laktacijos periodai, užtrūkimas.

Introduction. Pregnancy and lactation are physiological status considered to modify metabolism in animals and induce stress (Iriadam, 2007; Tanritanir et al., 2009). The periparturient period is important in terms of its influence on the health and the subsequent performance of dairy cows, since cows develop serious metabolic and physiological changes during these periods (Tanaka et al., 2011). In fact, it is well known that during the pregnancy all the metabolic pathways are involved in sustaining the foetus growth (Bell, 2000). The period of transition between late pregnancy and early lactation presents a huge metabolic challenge to the high-yielding dairy cow and the haematochemical profiles are important in evaluating the health status of animals during this transition (Hagawane et al., 2009; Bell, 2000). Immediately after the calving, high rates of body condition score losses are associated with a severe negative energy balance status, indicated by alterations in blood metabolite and hormone profiles (Wathes et al., 2009). Specifically, high levels of non-esterified fatty acids (NEFA) and β-hydroxybutyrate concentrations are indicative of lipid mobilization and fatty acid oxidation (Wathes et al., 2009; Sakha et al., 2006). It is well known that to meet the nutritional demands of milk synthesis, dairy cows need to mobilize body reserves, causing negative energy balance until nutrient intake covers the demands (Kessel et al., 2008). Even the cholesterol serum level seems to be influenced from calving and milk yield, in lactating dairy cows (Kweon et al., 1986). All animals require minerals such as calcium (Ca), magnesium (Mg), and phosphorus (P) for growth, reproduction and lactation, which often affect specific requirements, and serve as catalytic components of enzymes or regulate several mechanism involved just in pregnancy and lactation (Samardzija et al., 2011; Tanritanir et al., 2009). Especially at the beginning of lactation, Ca homeostatic mechanisms have to react to a tremendous increase in demand for Ca (Liesegang, 2008). Mobilization of Ca from bone and increased absorption from the gastrointestinal tract are required to re-establish homeostasis (Liesegang, 2008). So, if it is well known that cows need, especially for the high milk yield, more nutrients and energy supply than other animals (Lohrenz et al., 2010), little information is available about how this need affects the physiological phase. During the last years, the average milk production increased, and conversely, due to the negative energy balance, the reproducibility decreased (De Garis et al., 2010). Our study is an attempt at providing a picture of dynamics of selected haematochemical parameters in dairy cows from late pregnancy to dry period, with the aim of providing new and useful information about the guidelines for the management strategies during different physiological phases.

Materials and Methods

The study was carried out in Sicily (Italy), in a farm site in Ragusa (36°55' N; 14°43' E), on five clinically healthy dairy cows, breed Holstein Friesian, in good nutritional condition (5 years old, mean body weight 650±50 kg, Body Condition Score 3≤4, mean daily milk yield 25 l/day during 305 days of lactation). During the trial, all animals were kept under natural photoperiod and ambient temperature. All animals were free from internal and external parasites and resulted negative to the coprologic examination carried out, using the flotation method, before experimental period. No treatments were administered before the start of the experiment. Their health status was evaluated during the experimental period by veterinarians.

Table 1. **Composition of the diet administered to the five Holstein Friesian cows during the late gestation period and the whole lactation period** (a), **and the detailed composition of the concentrate** (b)

a

Variable	Dietary content	Composition	Kg/ D.M.
Matter Intake (kg)	33.00	Alfalfa hay	4.00
D.M.I (kg/day)	21.00		
N.F.C.(% D.M.)	33.00	Triticosecale Silage	7.00
Crude Protein (%D.M.)	15.50	Concentrate	2.50
Forage/Concentrate	53/47		

b

Concentrate Composition	%
Corn meal 58%	17
Soy protein flour	16
Beet pulp	15
Barley flour 47%	13
Carob bean sprouts	7
Wheat bran	7
Sunflower f.e.35%	6
Carobs	5
Molasses	4
Extruded Soy	4
Rumen bypass fats (Megalac)	5
Calcium Carbonate	1

Table 2. **Composition of the diet administered to the five Holstein Friesian cows during the dry period** (a)**, and the detailed composition of the concentrate** (b)

a

Variable	Dietary content	Composition	Kg/ D.M.
Matter Intake (kg/day)	13.50	Alfalfa hay	4.00
D.M.I. (kg/day)	12.00		
N.F.C. (% D.M.)	22.00	Triticosecale Silage	7.00
Crude Protein (%D.M.)	12.10	Concentrate (A30)	2.50
Forage/Concentrate	82/18		

b

Concentrate Composition	%
Barley flour 47%	16
Sunflower f.e.35%	18
Wheat bran	5
Soy protein flour	32
Carob bean sprouts	8
Corn meal 58%	16
Dairy dry	5

During the lactation, they were milked twice (05:30 a.m. and 04:30 p.m.) by means of a milking machine and fed twice daily (07:00 a.m. and 06:00 p.m.) during the experimental period. Two diets, of different composition, were administered during dry period and lactation. The detailed compositions of the diets are presented in Tables 1 and 2. All the experimental subjects, were selected on the basis of their pregnancy status. Blood samples were collected two days before the expected parturition (Late gestation), during the post partum, in early lactation, at the 2nd, 5th and 15th weeks after parturition, at the end of lactation and at the dry period. Each sample, collected at the same hour in the morning from jugular vein into vacuum glass tubes containing no additive, consisted of 10 ml of blood. Following standing at room temperature for 20 minutes, the tubes were centrifuged at 3.000 rpm for 10 minutes and the obtained sera were kept at -20°C until pending analysis. On each serum sample urea, creatinine, total proteins, albumin, total cholesterol, triglycerides, NEFA, β-hydroxybutyrate, total and indirect bilirubin, Ca, Mg and P were determined by means of commercial kits (SEAC, Florence, Italy) and measured using the UV spectrophotometer (Slim, SEAC, Florence, Italy). All the results were expressed as mean values and standard deviation (SD). On all data, normally distributed (P<0.05, Kolmogorov-Smirnov's Test), one-way Repeated Measure Analysis of Variance (ANOVA), was applied to evaluate the influence of the physiological phases on the considered parameters. If ANOVA showed an acceptable level of significance (P<0.05), Bonferroni's test was applied for post hoc comparison. Data were analyzed using Statistica 7 software package (Statsoft Inc., USA).

Results

Tables 3, 4 and 5 show the mean (±SD) values of the parameters considered, expressed in their relative units of measurements with the statistical significances. A significant effect of the reproduction status was showed on urea (P<0.001; $F_{(7;28)}$=0.86), creatinine (P<0.0001; $F_{(7;28)}$=6.58), total proteins (P<0.0001; $F_{(7;28)}$=5.44), total cholesterol (P<0.0001; $F_{(7;28)}$=36.46), triglycerides (P<0.0001; $F_{(7;28)}$=7.45), NEFA (P<0.0001; $F_{(7;28)}$=0.62), β-hydroxybutyrate (P<0.0001; $F_{(7;28)}$=2.10), Ca (P<0.0001; $F_{(7;28)}$=9.13) and P (P<0.001; $F_{(7;28)}$=4.72). No statistical significances were found on albumin, total and indirect bilirubin and Mg.

Table 3. **Mean values** (±SD) **of total proteins, albumin, urea and creatinine serum contents in five Holstein Friesian cows, over eight reproduction periods**

Period	Total Proteins (g/dL)	Albumin (g/dL)	Urea (mg /dL)	Creatinine (mg/dL)	Indirect Bilirubin (mg/dL)	Total Bilirubin (mg/dL)
Late gestation	5.52±0.45	2.99±0.19	16.00±1.92	1.21±0.22	0.11±0.03	0.21±0.01
Post partum	4.83±0.88	3.17±0.36	20.83±4.30★	1.22±0.24	0.10±0.04	0.18±0.09
Early lactation	5.27±0.51	2.93±0.39	15.50±3.36○	1.06±0.09	0.06±0.02	0.13±0.06
2nd Week lactation	5.93±0.75	2.90±0.30	17.67±1.64	0.99±0.05	0.06±0.03	0.13±0.08
5th Week lactation	6.28±0.65○	2.86±0.22	15.00±2.34○	0.89±0.05★○	0.06±0.03	0.13±0.08
15th Week lactation	6.38±0.46○	3.06±0.16	23.50±1.30★□■●	0.85±0.17★○	0.04±0.01	0.11±0.05
End lactation	6.32±0.60○	2.82±0.18	23.00±1.67★□■●	0.83±0.06★○	0.06±0.04	0.15±0.11
Dry period	6.17±0.89○	2.85±0.16	18.00±2.30♦*	0.98±0.20	0.09±0.01	0.19±0.03

Significances: ★ vs Late gestation (P<0.001); ○ vs Post partum (P<0.0001); □ vs Early lactation (P<0.001); ■ vs 2nd week lactation (P<0.001); ● vs 5th week lactation (P<0.0001); ♦ vs 15th week lactation (P<0.001); * vs End lactation (P<0.001).

Table 4. **Mean values** (±SD) **of total cholesterol, triglycerides, NEFA and β-hydroxybutyrate serum content in five Holstein Friesian cows, over eight reproduction periods**

Period	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	NEFA	β-hydroxybutyrate (mmol/L)
Late gestation	79.17±12.30	14.83±4.08	0.27±0.04	0.39±0.06
Post partum	63.50±6.76	9.83±1.92★	0.60±0.08★	0.43±0.05
Early lactation	58.33±4.12	8.33±1.87★	0.42±0.10★○	0.49±0.08
2nd Week lactation	81.33±10.66	9.50±0.89★	0.20±0.05○□	0.59±0.02
5th Week lactation	111.50±8.43★○□■	8.50±2.58★	0.20±0.06○□■	0.51±0.08
15th Week lactation	149.00±29.16★○□■●	9.67±1.81★	0.07±0.02★○□■●	0.68±0.08★○
End lactation	80.33±8.01●	11.17±1.81	0.04±0.01★○□■●	0.90±0.08★○□■●♦
Dry period	83.50±7.49 □●♦	12.17±0.54	0.07±0.00★○□■●	1.02±0.27★○□■●♦

Significances: ★ vs Late gestation (P<0.001); ○ vs Post partum (P<0.0001); □ vs Early lactation (P<0.001); ■ vs 2nd week lactation (P<0.001); ● vs 5th week lactation (P<0.0001); ♦ vs 15th week lactation (P<0.001); * vs End lactation (P<0.001).

Table 5. **Mean values** (±SD) **of minerals serum content in five Holstein Friesian cows, over eight reproduction periods**

Period	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)
Late gestation	7.75±0.48	5.67±0.66	2.10±0.20
Post partum	5.78±1.00★	4.88±0.86	1.76±0.95
Early lactation	7.12±0.88○	5.13±0.55	1.93±0.19
2nd Week lactation	7.72±0.64○	5.43±0.45	2.15±0.20
5th Week lactation	7.52±0.68○	4.53±0.21★	2.07±0.08
15th Week lactation	8.15±0.32○	5.00±0.87	2.10±0.10
End lactation	8.03±0.80○	4.25±0.21★■	2.10±0.21
Dry period	7.83±0.76○	4.83±0.83	2.08±0.61

Significance: ★ vs Late gestation (P<0.001); ○ vs Post partum (P<0.0001); □ vs Early lactation (P<0.001); ■ vs 2nd week lactation (P<0.001); ● vs 5th week lactation (P<0.0001); ♦ vs 15th week lactation (P<0.001); * vs End lactation (P<0.001).

Urea serum levels showed a significant increase (P<0.01) during the post partum period compared to basal values. Instead, during the late and end of lactation periods, urea showed a statistically significant increase (P<0.001) compared to the late gestation and the beginning of lactation. Creatinine serum level showed a significant decrease during the 5th week of lactation and the end of lactation periods (P<0.0001), compared to the late gestation and post partum periods. Total proteins content showed a statistically significant increase during the 5th week after parturition and the end of lactation (P<0.001) compared with the late gestation and the beginning of lactation. The total cholesterol serum level showed a statistically significant increase during late lactation and a decrease during the end of lactation and the dry period (P<0.0001). Triglycerides serum level showed a significant decrease compared with the late gestation period, and its value increased during the end of lactation and the dry period (P<0.001). NEFA serum level showed a slight increase compared with the late gestation and an important decrease during the end of lactation and the dry period (P<0.001). An opposite trend was observed for the β-hydroxybutyrate serum levels, which increased just during the late lactation and the dry periods (P<0.001).

Ca serum levels showed a significant decrease during the post partum period, and a statistically constant increase during all the lactation periods (P<0.0001), whereas the P serum level decreased during the end of lactation (P<0.001).

Discussion

The pregnancy and lactation phases affect significantly the metabolic profile and so the variation recorded during different physiological phases is expected. The transition from gestation to lactation is a period of great metabolic stress for dairy cows (Rollin et al., 2010). In fact, the milk production and its composition are found to profoundly influence the metabolical status of dairy cows (Heck et al., 2009).

In our study, the total serum proteins levels were significantly affected from the physiological period and increased during lactation if compared to late gestation. The variations reflect the maternal requirements of proteins need for milking and providing immunoglobulins (Mohri et al., 2007; Roubies et al., 2006; Bell et al., 2000). The higher concentrate-to-forage ratio provided during the lactation is generally associated with lower levels of fibre and higher levels of starch in the diet, which gives rise to an increased production of propionic acid in the rumen and an increased microbial protein supply (Heck et al., 2009). This is reflected, in our study, by an increase of total serum protein during the lactation and a slight decrease during the dry period.

The renal function, principally represented by urea and creatinine concentrations, was significantly affected during the different physiological phases. In fact, as suggested from other studies, carried out in small ruminant, the increase in urea serum levels during the lactation period despite the late gestation is strictly dependent on the dietary intake of proteins, more relevant during lactation because of the increased requirements (Roubies et al., 2006). The creatinine serum level was also significantly affected by the physiological phase and showed the higher levels during the late pregnancy and early lactation. It is recognized that during the late gestation, the mother, for the foetal maternal circulation, assumes the load of organic waste of the newborn (Ferrell, 1991). So, the increase in serum creatinine levels could be attributed to the development of the foetal musculature, which is well documented in sheep and ewes too (Roubies et al., 2006).

Total cholesterol and triglycerides, in our study, resulted significantly affected by the physiological status, in fact, both showed substantial increases during the mild lactation. Probably because, during the puerperal period, there is an increase in the demands for regulatory mechanism, responsible for all the processes involved with milking (Krajnicakova et al., 2003). At this purpose, characteristic changes in lipid metabolism were found during pregnancy and lactation in most mammals (Roche et al., 2009). Endocrine profiles change and lipolisis and lipogenesis are regulated to increase lipid reserve during pregnancy, and, subsequently, these reserves are utilized following parturition and the initiation of lactation (Roche et al., 2009; Nazifi et al., 2002). Similar results, however, were found by other researchers, demonstrating that concentrations of total lipid and triglycerides increased at parturition, despite the kind of fed administered (Douglas et al., 2004).

Conversely, other researchers suggested that in dairy cows immunological conditions after calving are related to serum total cholesterol values during the dry period (Hiromichi et al., 2001). This is suggested also by the activity of some haematochemical parameters liver derived, used as indicator of physical stress that indicated the parturition and the post partum periods as the most stressful phases in the dairy cow productive life (Tanaka et al., 2011; Piccione et al., 2010).

Increased plasma NEFA concentrations, as showed during the early lactation, are useful for the animals to maximize milk synthesis with lower glucose consumption, moreover, the high Growth Hormone concentrations and the low insulin levels, present in bloodstream during this period, stimulate a marked mobilization from adipose tissues, as confirmed by the increase in NEFA plasma levels (Wheelock et al., 2010; Accorsi et al., 2005). Ketosis commonly results either from the lack of sufficient glucose precursors available for energy production or from a reduced gluconeogenic capacity by the liver and it is characterized by elevated concentrations of the ketone bodies acetoacetate, acetone and β-hydroxybutyrate in the blood, milk and urine (Rollin et al., 2010).

The association between elevated serum β-hydroxybutyrate concentrations and postpartum disease, milk production and reproductive efficiency is the source of recent and ongoing research efforts (Rollin et al., 2010). In practice, serum β-hydroxybutyrate concentrations are typically dichotomized to distinguish between normal and hyperketonemic cattle, with frequently recommended cutpoints of 1.000 to 1.400 μmol/L (Rollin et al., 2010). In our results, all the β-hydroxybutyrate levels are within these ranges, the higher mean value is recorded just during the dry period.

Concerning the electrolytes serum levels, all animals require minerals for growth, reproduction and lactation (Samardzija et al., 2011). The passage of calcium across the placenta is unidirectional; back transfer of this element is very limited, so, the mobilization from bone and the increased absorption from the gastrointestinal tract are required to re-establish homeostasis (Liesegang, 2008; Szenci et al., 1994). Also it is true that the requirement of calcium and phosphorus depends also on the physiological status and on the animal's productivity (Brezezinska and Krawczyk, 2009). Milk phosphorus and calcium output is directly related to milk yield, as milk phosphorus concentration is constant (Valk et al., 2002). In fact, increasing the milk production, more phosphorus from the ingested amount is transferred to milk and less is excreted with faeces (Valk et al., 2002). In our study, the calcium and phosphorus serum levels were high during all the lactation period and began to decrease when the lactation was over.

Conclusions

Our data confirm that from the metabolic point of view the lactation period is the most sensible for the high production dairy cows, therefore the information, provided in this paper, advances the continuous investigation in animal welfare. Moreover, since most economical losses occur due to wrong management of the dairy cows, this study could be a useful tool in preventing the deficiencies typical of high production ruminants. Further investigations should be conducedt to evaluate how the haematochemical parameters vary in dairy cows different for average milk yield.

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