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Influence of lipoproteins at dry-off on metabolism of dairy cows during transition period and on postpartum reproductive outcomes

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ABSTRACT

High-yielding dairy cows are metabolically challenged during transition, when intense mobilization and hepatic oxidation of lipids is achieved, thus leading to fatty infiltration, ketosis and generalized inflammation. The condition is associated to periparturient diseases and poor fertility. The aim of this study was to assess whether serum lipoprotein concentrations in the dry period could influence the occurrence of postpartum diseases and reproductive performance in dairy cows. The study was carried out on 30 multiparous Holstein Friesian cows. Blood samples were collected at dry-off (−60 days), 30 days after dry-off and within 12 h after parturition for biochemical and serum lipoprotein assays. From 10 to 60 days after parturition milk was collected twice weekly after feeding, for milk whey progesterone assay. The Optimal Cutpoint package identified a threshold of 89% for serum High Density Lipoprotein (HDL) concentration at the beginning of the dry period with 95% of confidence interval. Cows with serum HDL greater than 89% (High group, n = 10) showed better reproductive performance when compared to those with low values (Low group, n = 18). The odds ratio for reproductive disorders in High group was 0.6875, however, differences were not significant probably due to both the reduced number of animals per group and overall low incidence of postpartum reproductive disease. First postpartum luteal activity occurred around day 23, while the second one between days 40 and 48. The average calving to first AI interval was 64.00 ± 3.95 days and 94.50 ± 12.32 days in High and Low group, respectively ($P < 0.05$). The calving-conception interval was 129.86 ± 24.42 days and 199.18 ± 24.73 days in High and Low groups, respectively ($P < 0.05$). Low group displayed an increase in liver markers, that is total bilirubin, with 0.46 ± 0.09 mg/dL and 0.23 ± 0.08 mg/dL, in Low and High group respectively ($P < 0.05$), and NEFA/cholesterol ratio, with 0.30 ± 0.06 and 0.14 ± 0.03, in Low and High groups, respectively ($P < 0.05$), at parturition. Concentrations of HDL >89% at dry-off could be suggestive of improved liver adaptation to the transition, and probably of enhanced fertility in High group.

1. Introduction

In the last weeks of gestation, cow's metabolism has to sustain fetal growth, mammary gland development and after calving the energy demand further increases due to lactation. Usually, during this period the dry matter intake is not sufficient to meet the requirements. In order to cope with this imbalance, dairy cows mobilize great amounts of body reserves, especially lipids [1]. Lipids are released from periphery into the circulation in the form of non-esterified fatty acids (NEFA) and then they are used in liver for gluconeogenesis and ketogenesis. If negative energy balance is excessive, the intake of NEFA overcomes the possibility of complete oxidation in the liver. In this case, NEFA are re-esterified, turned into triglycerides and stored into the cytoplasm of hepatocytes as lipid droplets. This condition, known as fatty liver, is usually associated to impairment of liver function, subclinical or clinical ketosis, periparturient metabolic diseases and poor fertility, which deeply influence the herd profitability [2–5]. Human and bovine hepatocytes are able to synthesize very low-density lipoprotein (VLDL), in order to export triglycerides towards peripheral tissues [6]. However, bovine liver is not able to adjust VLDL synthesis based on NEFA absorption and re-esterification into cytoplasmic droplets [2];

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therefore, the prevalence of lipidosis among transition dairy cows can reach 50% [7,8]. Very-low density lipoprotein represents only 3–5% of all circulating lipoproteins in cattle, while other classes, such as high-density (HDL) and low-density lipoprotein (LDL), account for 80–85% and 10–15%, respectively. As liver can export triglycerides only through VLDL, it is generally assumed that low levels of circulating VLDL are representative of increased fatty acids infiltration [6,9].

It has been reported a decrease of pregnancy at first artificial insemination when more than 50% of cows in a herd have serum NEFA ≥0.5 mEq/L one week before calving, as indicative of intense lipids mobilization [3]. An increased odds ratio for retention of fetal membranes and metritis in cows with prepartum NEFA ≥0.3 mEq/L was reported [4]. It has also been highlighted that the peculiar metabolic status of the dairy cows during periods of lipids mobilization could cause lipid accumulation in oocytes and the regenerating endometrium, which impairs fertility via reduction in embryo survival and increased inflammatory changes, respectively [10]. The majority of studies concerning the association between biochemistry profiles of dry cows in late gestation and postpartum performance are focused on the last two or three weeks before calving [3,4,9]. However, Dervishi et al. [11] reported alterations in inflammatory and metabolic profiles as early as eight weeks before calving, in dairy cows that will develop metritis. Brscic et al. [12], recently reported some reference limits for metabolic profiles in Holstein late-pregnant heifers and dry cows, but postpartum performance was not assessed.

Moved by the need of finding new predictive indexes of reduced reproductive efficiency, we hypothesized that the level of circulating lipoproteins during the last period of pregnancy could be indicative of the degree of adaptation to transition of dairy cows and of their susceptibility to both postpartum reproductive diseases and reduced fertility. Moreover, we evaluated biochemistry profiles from dry-off until calving as a tool to verify the overall health status in our experimental animals and to exclude alterations in lipoprotein metabolism due to subclinical pathological conditions.

2. Materials and methods

2.1. Animals and husbandry

In the present study, 30 healthy multiparous Holstein Friesian cows were selected with parity ranging from 2 to 5; they belonged to a commercial dairy farm located in Central Italy (42°95′ N, 12°39′ E), with a herd size ranging between 100 and 120 lactating cows. Each cow was randomly selected through the herd-management software before entering into the dry period. Mean heard intercalving period was 410.27 ± 15.87 days, the mean dry period length was 60.25 ± 10.14 days and the voluntary waiting period averaged 50.16 ± 7.08 days. Average milk production was above 10,900 kg/lactation/cow. All lactating cows were housed in free stalls with cubicles and milked with two Automatic milking systems (DeLaval S.p.A., VMS, Milano, Italy); dry cows were kept in a free stall barn with straw. The dry cows had free access to a total mixed ration (TMR) offered *ad libitum*, composed of 4.5 kg wheat straw (4.60% CP, 78.90% NDF, 48.40% ADF), 4.5 kg oat hay (8.70% CP, 61.30% NDF, 38.20% ADF), 3.0 kg concentrate (28.50% CP, 20.20% NDF, 9.70% ADF) per head. During the close-up period this ration was supplemented with 10 kg of fresh cow TMR/head. The overall TMR composition was: 4 kg alfalfa and lolium mixed hay (7.67% CP, 37.86% NDF, 20.84% ADF), 3.5 kg alfalfa hay (14.91% CP, 42.70% NDF, 33.10% ADF), 11.5 kg concentrate (28.50% CP, 20.20% NDF, 9.70% ADF), 22 kg corn silage (9.20% CP, 45.90% NDF, 27.00% ADF) and 0.8 kg molasses (4.30% CP, 0% NDF, 0% ADF). Mycotoxins content in feed was within the legislative established limits (Italian Law 149/2004). Contents of organic acid in silage and total mixed ration from dry cow feedbunk were evaluated by HPLC analysis. Only butyric acid was fairly present in both silage and total mixed ration, with mean values of 0.02 ± 0.01 and 0.03 ± 0.001 g/100 g, respectively.

2.2. Experimental procedures and samples collection

The study was conducted from January to December 2015; all experimental cows calved before the end of April 2015. Two cows were excluded from the experiment due to abortion and premature calving. Body condition score (BCS), assessed through a five-point scale [13], and blood sampling were performed at 60 (T1) and 30 days (T2) before the expected calving and within 12 h postpartum (T3). Samples were obtained by coccygeal vein puncture into plain and EDTA vacuum tubes (BD Vacutainer Systems, Plymouth, UK) between 5.30 and 6.00 a.m., half an hour before feeding. Samples were stored at 4 °C and delivered within 1 h to the Laboratory; serum was obtained through centrifugation at 1300 g for 10 min and immediately processed. An aliquot of serum from each sample was stored at 4 °C until electrophoresis analysis. From 10 to 60 days after parturition milk was collected twice weekly after feeding, between 6.00 and 7.00 a.m., in empty eppendorf tubes and immediately frozen at −20 °C, until analysis.

To evaluate healthy condition of the newborn calf, the APGAR score described by Vannucchi et al. [14] was employed. Briefly, the following symptoms were considered and scored: mucous color (0 = cyanotic; 1 = pale; 2 = normal), heart rate (0 = absent; 1 = bradycardia, < 80 beats per minute or irregular; 2 = regular, > 100 beats per minute), muscle tone (0 = flaccid; 1 = slight flexion; 2 = flexion), activity (0 = absent; 1 = some movement; 2 = active calf), and respiration (0 = absent; 1 = irregular < 24 respiration per minute; 2 = regular > 36 respiration per minute).

Postpartum uterine diseases were diagnosed according to Sheldon et al. [15]. Briefly, animals were monitored once weekly after calving until complete uterine involution was achieved. The fetal membranes were considered retained when not released within 12 h after parturition. Cows that presented an enlarged uterus with watery red-brown to viscous off-white purulent uterine discharge, from 0 to 21 days after calving were considered suffering from metritis. Clinical endometritis was defined by the presence of pathological uterine discharge 21 days or more postpartum.

Reproductive parameters such as calving to first insemination interval, calving to conception interval and number of inseminations per pregnancy were retrieved from the herd management software, at least until 200 days after parturition.

The experimental activity was carried out in accordance to the guidelines of animals experiments as set by the Italian Law 26/2014 (national application of EU Directive 2010/63/EU) and has been approved by the Ethical Committee of the University of Perugia.

2.3. Serum lipoprotein's electrophoresis, biochemical profiles and CBC

Electrophoresis of serum lipoproteins was obtained with the Hydrasys – LC Sebia automatic system and Hydragel 7 LIPO + Lp(a) Kit (Sebia® Electrophoresis, Sebia Inc., Norcross, GA USA). Briefly, lipoprotein classes were separated by electrophoresis on agarose gel buffered plates (pH 8.5); different bands were then read with densitometry scanner at 570 nm (Epson Perfection V700 PHOTO, Seiko Epson Corporation, Japan) and lipoproteins were expressed in term of relative percentage. Three main bands were identified, namely HDL (or α-lipoproteins), VLDL (or pre-β-lipoproteins) and LDL (or β-lipoproteins).