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Prepartum blood lead concentrations linked to subsequent cyclicity in high-producing dairy cows in a non-industrial area

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ABSTRACT

This study sought to identify the possible presence of lead (Pb) in blood and if detected to examine the relationship between blood Pb concentrations during the transition period and subsequent reproductive performance in high-producing dairy cows reared in a non-industrial area. Forty seven multiparous dairy cows were examined and/or sampled on Days 251–257 of gestation (visit 1,V1), the day of calving (V2) and on Days 8–14 (V3), 15–21 (V4), 22–28 (V5), 29–35 (V6), 36–42 (V7) and 50–56 (V8) postpartum. A mean level of 130 ± 17 ppm (± SD) of Pb was detected in feed samples. Blood samples were collected for Pb determination from V1 to V5 and lead was present in all collected blood samples. One unit increase in blood Pb concentration in the V1 sample led to a 0.3-fold reduction (P=0.02) in the likelihood of a cow being cyclic. Mean blood Pb concentrations were 0.97 ± 0.11 and 2.6 ± 0.1 µg/L for cyclic (n=24) and non-cyclic (n=23) cows, respectively. Cows with a body condition score (BCS) loss of ≥ 0.75 units between V1 and V4 (n=24) showed higher Pb concentrations throughout the study period than the remaining cows (n=23; P < 0.001). In conclusion, blood Pb levels were detected in all cows. Prepartum blood Pb concentrations were negatively related to subsequent cyclicity. Cows with higher Pb levels experienced a greater BCS loss during the transition period. Routine blood Pb tests could indicate a higher risk of anoestrus in cows with higher Pb concentrations.

1. Introduction

Developments in genetics and nutrition have increased milk production in dairy cows though this increase has been accompanied by a decline in reproductive performance (Lucy, 2001; López-Gatius, 2003). The transition from dry-off to early postpartum is a delicate period and poor health or well-being during this interval can affect the subsequent reproductive performance of lactating dairy cows. During the transition period, cows have to deal with reduced dry matter intake (DMI) and a negative energy balance (NEB) before they become cyclic again (López-Gatius et al., 2003; Roche et al., 2009). Thus, any external factor that negatively affects animals during the transition period will have serious repercussions on dairy economy.

Heavy metals occur naturally in the environment though human activities such as industry have led to their increased concentrations in air, crops and water. Lead (Pb) is an ubiquitous heavy metal with known negative effects such as inducing reactive oxygen species (ROS) which in turn, increases lipid peroxidation (Upasani et al., 2001). This pollutant is considered furthermore an important reproductive toxic chemical in humans (Kumar and Mishra, 2010; Buck Louis, 2014). For example, there is evidence indicating that lead may be behind up to 5% of the cases of unexplained human male infertility (Benoff et al., 2003). Plants may bio-accumulate this toxic metal and when grazing animals consume such plants it enters the food chain with possible impacts on both animal and human health (Miranda et al., 2005; Aslani et al., 2012). Lead poisoning is more common in farm ruminants than wild ones (Aslani et al., 2012). In farm animals, a blood Pb concentration of up to 0.25 μg/mL is considered safe, whereas levels in excess of 0.35 μg/mL can be toxic (Radostitis et al., 2000). Recently, chronic exposure to low Pb levels has been linked to high blood Pb concentrations in cows reared in an industrial area (Mohajeri et al., 2014). However, to our knowledge, no study has examined the

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possible clinical effects of low (considered non-toxic) blood Pb levels on reproductive variables in dairy cows. The present study was performed in high-producing dairy cows reared in a non-industrial area of Spain. Its aims were: (1) to identify the possible presence of Pb in food and blood and (2) to examine the possible relationship between blood Pb concentrations during the transition period and subsequent reproductive performance.

2. Material and methods

2.1. Cattle and herd management

The study was performed from March 2014 to January 2015 on a single commercial Holstein-Friesian dairy herd in northeastern Spain comprising 820 cows. The farm was located in an agricultural area without industries 25 km around. Mean annual milk production and culling rates for the study period were 11,940 kg and 27%, respectively. Herd management included housing in free stalls with cubicles with concrete slatted floors, and the use of fans and water sprinklers in the warm season. The herd was subjected to a reproductive health programme including meticulous postpartum checks. The cows calved all year round, were milked three times daily and were fed complete rations. Feeds consisted of cotton-seed hulls, barley, corn, soybean, and bran. Roughage was mainly provided as corn but also as barley or alfalfa silage and alfalfa hay. Rations were in line with NRC recommendations (National Research Council, 2001). Presence of mycotoxins (aflatoxin B1, deoxynivalenol, zearalenone, ochratoxin A and T-2 toxin) was determined monthly in each feed and in the complete ration given to the cows. No samples exceeded the maximum level established in the EU for aflatoxin B1 (Commission Regulation 574/2011 amending Annex I to Directive 2002/32/EC 2011), neither the recommended values of the other mycotoxins tested (European Comission, 2006, 2011, 2013a).

All animals were tuberculosis and brucellosis free as indicated by yearly tests from 1985 to 2015. Vaccination programmes for the prevention of bovine viral diarrhoea (BVD) and infectious bovine rhinotracheitis (IBR) included modified live vaccines (Cattlemaster, Pfizer, New York, USA) for animals 6–8 months old. Pregnant animals were given killed vaccines (Triangle 4, Boehringer Ingelheim, Barcelona, Spain) during the 7th month of each gestation period. Parous cows that were not pregnant on Day 150 postpartum received a further killed vaccine.

2.2. Reproductive health management

Dry cows were kept in a separate group and transferred to a “calving group” 7–25 days before delivery depending on their body condition score (BCS) (López-Gatius et al., 2006) and whether they were carrying twins (López-Gatius and Garcia-Ispierto, 2010). An early postpartum, or “fresh cow” group was established for postpartum daily checks and nutrition tests. At 7–20 day postpartum, primiparous and multiparous lactating cows were transferred to separate groups. In the postpartum checks, the following puerperal diseases were treated until resolved or until culling: signs of injury to the genital area (i.e., vaginal or recto-vulvar lacerations), metabolic diseases such as hypocalcemia and ketosis (the latter, diagnosed during the first or second week postpartum), retained placenta (foetal membranes retained longer than 12 h after parturition), or primary metritis (acute puerperal metritis diagnosed during the first or second week postpartum in cows not suffering placental retention).

The herd was maintained on a weekly reproductive health programme. This involved examining the reproductive tract of each animal by ultrasound from 15 to 21 and 50–56 days postpartum to check for normal uterine involution and ovarian structures. The entire reproductive tract was examined by ultrasound using a portable B-mode ultrasound scanner (Easy-Scan with a 7.5 MHz transducer). Scanning was performed carefully and slowly along the dorsal/lateral surface of the cervix and each horn and then the ovaries. Cranial cervical size and endometrial thickness were measured using the internal calipers of the ultrasonographer. Reproductive disorders diagnosed at this time such as endometritis or ovarian cysts were treated on a weekly basis until resolved. Cows were classed as suffering endometritis according to the following criteria: the presence of echogenic intrauterine fluid, cervical diameter ≥ 4 cm, or endometrial thickness ≥ 0.75 cm (López-Helguera et al., 2012). An ovarian cyst was diagnosed when a follicular structure larger than 20 mm in diameter (external diameter including the wall) was detected in either one or both ovaries in the absence of a corpus luteum (CL) and uterine tone (Hanzen et al., 2007). The presence of a CL in one or both ovaries was also recorded.

All postpartum reproductive disorders were resolved before 50 days in milk. Since a retained placenta or puerperal metritis were previously related to subsequent pregnancy loss in cows (López-Gatius et al., 1996), both disorders were always treated by introducing oxytetracycline boluses into the uterus plus cefquinome sulphate i.m. and prostaglandin $F_{2\alpha}$ at the end of treatment. Prostaglandin $F_{2\alpha}$ or a synthetic analogue was also used to treat endometritis and ovarian cysts. In the latter case, treatment was subsequent to manual rupture of the cystic structure per rectum (Hanzen et al., 2008).

Cows 60 days in milk and not detected to be in oestrus in the preceding 21 days were examined weekly by ultrasound until oestrus following a 5-day progesterone-based treatment (Garcia-Ispierto and López-Gatius, 2014) or until artificial insemination (AI) was performed during a spontaneous oestrus. All cows were artificially inseminated. Although oestrus detection using pedometers started on day 14 postpartum (López-Gatius et al., 2005), the voluntary waiting period for the herd was 50 days.

Over the study period, means ($\pm$SD) for the interval parturition-first insemination, milk production at 50 days postpartum and lactation number were 69 ± 11 days (51–97 days), 45 ± 9.8 kg (25–72 kg) and 5 ± 1 lactations (4–9 lactations), respectively. Rates of placenta retention, *endo*metritis, cyclicity and conception at first AI were 38.3%, 23.4%, 51.1% and 40.4%, respectively. Oestrus was recorded at least one time in 22 (91.7%) of the 24 cyclic cows, whereas non-cyclic cows did not show oestrus signs.

2.3. Insemination and pregnancy diagnosis

Oestrus was confirmed by palpation per rectum (López-Gatius and Camón-Urgel, 1988, 1991) in cows deemed to be in oestrus using the pedometer system and the animals inseminated at this time. Only cows showing oestrus signs with strong uterine contractility (determined by uterine tone) and copious, transparent vaginal fluid were inseminated (Roelofs et al., 2010; López-Gatius, 2012). If cows returned to oestrus, their status was confirmed by examination per rectum, and the animals were recorded as non-pregnant. In the remaining cows, pregnancy diagnosis was performed by ultrasound 28–34 days post-insemination. Cows diagnosed as non-pregnant were either returned to the reproductive programme or scheduled for culling.

3. Experimental design

Trace and heavy metals such as Cu, Zn, Mn, Se, Cd and Pb were analysed in food samples in three different herds. Since only Pb was present in all samples, possible effects of Pb was analysed in a