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High urea and pregnancy or conception in dairy cows: A meta-analysis to define the appropriate urea threshold

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ABSTRACT

Dietary proteins play an important role in reproduction, and increased dietary crude proteins, increased degradability of dietary proteins, and elevated blood or milk urea have been associated with decreased conception and pregnancy in many studies. The aim of this work was to provide a meta-analysis on the relationship between high milk or blood urea and pregnancy or conception, with a focus on defining the appropriate urea threshold associated with this issue. The meta-analysis included 61 different models from 21 papers. The thresholds of urea tested in the various models were built by steps of 1 mM urea. This constructed variable reduced heterogeneity by 61% in the meta-regression. The meta-analysis showed 43% lower odds of pregnancy or conception (odds ratio = 0.57; 95% confidence interval = 0.45–0.73) in cases where urea was ≥ 7.0 mM in the blood (plasma urea nitrogen = 19.3 mg/dL) or where urea was ≥ 420 mg/L in the milk compared with where urea values were lower. This threshold is the most suitable with regard to pregnancy or conception success, even if a threshold of 6.5 mM cannot be excluded with certainty. The results also highlighted the possibility of a stronger association between high urea concentrations and pregnancy or conception when high nitrogen exposure occurs before artificial insemination compared with after artificial insemination, but this possibility needs to be further studied. Whether the present results also apply to extensively pasture-based countries remains to be determined.

Key words: dairy cow, urea, nitrogen, reproduction

INTRODUCTION

Protein intake is an important determinant of dietary balance. Negative energy balance (NEB) is recognized as a significant challenge that dairy cows face during the early postpartum period. Diets that exceed the

requirements for rumen degradation or contain nondegradable protein are often observed in the field (Butler, 1998). Urea is a good indicator of energy or protein imbalance and is a sensitive indicator of protein utilization efficiency (Kenny et al., 2002). Its small molecular size allows it to easily circulate in all fluids, and its concentration values are well correlated between milk and blood. Both milk and blood urea concentrations can be used to evaluate the nitrogen diet status of animals (Oltner and Wiktorsson, 1983; Baker et al., 1995). Dietary proteins play an important role in reproduction, and increased dietary CP, increased degradability of dietary proteins, and elevated blood or milk urea have been associated with decreased conception and pregnancy despite studies failing to highlight this association. Several previous studies (Westwood et al., 1998b; Leroy et al., 2008a,b) reviewed these relationships as well as the physiopathology behind the epidemiological link. In brief, diets high in dietary protein may interact with reproductive efficiency through (1) increased NEB linked to higher production and energy cost of desamination in the liver, (2) potential toxicity of the direct by-products of protein catabolism for the oocyte and the embryo, (3) prevention of the natural increase in uterine pH after ovulation and changes in the ionic composition of uterine fluid, (4) changes in PGF_{2α} secreted by the endometrial tissue, and (5) changes in the motility of spermatozoa (Butler, 1998; Westwood et al., 1998b; Melendez et al., 2003; Leroy et al., 2008a,b). The NEB promoted by high dietary protein also affects reproduction, including changes in the IGF-1, LH, and progesterone profiles (Leroy et al., 2008a). Interestingly, it seems that changes in dietary protein levels rather than high dietary protein levels alone may be involved in decreased reproductive performance. Lactating dairy cows can metabolically adapt to a prolonged high intake of quickly degradable protein, probably by opposing the adverse effects of long-term high concentrations of protein on embryo growth (Dawuda et al., 2002; Laven et al., 2004). There is still some uncertainty regarding whether the effect of high urea on reproduction occurs mostly during the period before, surrounding, or after AI (Hammon et al., 2000;

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Leroy et al., 2008b). The success of embryo transfer was decreased when the donors were fed high levels of dietary protein, whereas the result was not related to the urea nitrogen status of the recipients, suggesting a higher sensitivity of reproductive success to the factors preceding AI (Rhoads et al., 2006). The timing and duration of exposure need to be further studied, and their association with reproduction may depend on the parameter measured.

Many of the studies analyzing the association between dietary protein levels and reproduction used blood or milk urea (or urea nitrogen) as the gold standard to evaluate dietary nitrogen status. These studies were not specifically designed to address the association between urea concentration and pregnancy or conception, and the thresholds of urea used or defined in these studies were heterogeneous and arbitrary. Thus, the threshold of urea to be used in the field is ambiguous. Reviews on this topic have not focused on the threshold of urea or on the relative risk of decreased reproductive performance. This omission is concerning because these same studies are typically used to define the threshold to be achieved and to determine the relative risk of outcomes. This question has been intensively studied for subclinical ketosis (Raboisson et al., 2014). A quantitative review published in the 1990s included a small number of studies available on the topic. This work focused on conception (Westwood et al., 1998b). A review performed in 2003 gathered the available studies and highlighted the heterogeneity in the proposed thresholds of BUN or plasma urea nitrogen (**PUN**) for fertility but did not perform a formal meta-analysis (Melendez et al., 2003). Another study on reproduction performance did not evaluate urea but did evaluate dietary protein (Lean et al., 2012). The aim of this work was to provide a meta-analysis regarding the relationship between high milk or blood urea and reproductive performance, to propose the urea threshold for consideration in the field, and to clearly quantify the association between urea and pregnancy or conception.

MATERIALS AND METHODS

A literature search and screening process was conducted using the PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), CABI (www.cabi.org), and Google Scholar (<http://scholar.google.com/>) search engines to create a data set of papers using the key words “crude protein,” “urea,” “plasma urea nitrogen,” “serum urea nitrogen,” “reproduction,” “conception,” and “pregnancy” separately or in combination. New papers referenced by at least 1 of the papers identified in the previous step were also included.

Inclusion and Exclusion Criteria

To be included in the data set, the papers must have examined the odds ratios (**OR**) or equivalent of various reproductive performances, such as conception at service, pregnancy success, and so on, for cows or groups of cows with diets containing varying levels of urea or urea nitrogen. At this stage, no criteria regarding experimental design, including the number of animals per group, breed, urea measure (milk or blood), or use of hormones or synchronization protocols, were considered. Two types of studies were included: some studies reported the OR of reproduction performance change in the event of urea change (case 1), whereas others expressed the association differently but allowed us to obtain an expression for the OR through the construction of contingency tables (case 2). For this second situation, a temporary data set was implemented with the records of the mean urea values of groups of animals and the corresponding reproductive performances. Studies with in vitro experimental designs or with results without a clear estimation of the mean urea value for the different groups (with or without exposure to urea) were not included. Publications through June 2015 were included. All urea or nitrogen values were standardized (by dividing the urea nitrogen by 0.46 if BUN, PUN, or MUN) and are expressed in millimoles per liter. The models included at this stage refer to various outcomes: pregnancy after service ($n = 28$), conception after service ($n = 18$), conception after the first service ($n = 15$), number of AI attempts before successful AI ($n = 8$), interval from calving to first AI ($n = 6$), percentage of oocytes with division ($n = 12$), percentage of embryos that reach the blastocyst stage ($n = 5$), and viability of embryos ($n = 7$). Because raw data must be distributed as a 5-class categorical variable for the meta-regression, only outcomes with a minimal number of raw data (i.e., only the first 3 outcomes) were retained for the subsequent part of the study (Supplemental Tables S1 and S2; <https://doi.org/10.3168/jds.2016-12009>). They were collected and labeled “pregnancy or conception.”

Twenty-one papers were selected. Most of the papers studied several outcomes or included more than 1 urea threshold, and 61 different models were recorded directly from the literature (case 1, $n = 23$) or through the implementation of a contingency table (case 2, $n = 38$). The final data set included the numbers of cows and herds studied, the energy density of the diet, the CP of the diet, the average milk production, the period (before, after, or both) of exposure to a high nitrogen level with respect to AI, the duration of high nitrogen level exposure, the date of sampling with respect to AI, the source used (blood or milk), whether pregnancy or

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