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Elevated non-esterified fatty acid and β-hydroxybutyrate in transition dairy cows and their association with reproductive performance and disorders: A meta-analysis

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ABSTRACT

A meta-analysis including 36 different results of statistic models from 14 papers was conducted. It evaluated the association between elevated non-esterified fatty acids and/or β-hydroxybutyrate (BHB) on the reproduction outcomes that were pregnancy at first insemination, estrous cyclicity, time to pregnancy, metritis and placental retention. Each association between BHB or NEFA and an outcome reported in literature was a model considered as raw-data for the meta-regression. For each outcome, the meta-regression adjusted the odds ratio, relative risk or hazard ratio with various moderators to reduce the heterogeneity among the studies. The relative risk for metritis and placental retention in case of high BHB or NEFA was in accordance to previous meta-regression and was 1.91 (IC95 = 1.72 to 2.12) and 1.51 (95%CI = 1.19 to 1.92), respectively. The relative risk for pregnancy at first insemination in case of high BHB was assessed to be 0,62 (95%CI = 0,41 to 0,93). The hazard ratio for time to pregnancy in case of high BHB and NEFA was 0.77 (95%CI = 0.61 to 0.97). The present meta-analysis failed to clearly conclude on the association between estrus cyclicity and high BHB or NEFA. The present work allowed a new overview on the association between hyperketonemia and reproductive performance and disorders. It updated the previous meta-regression and included new outcomes. It highlighted the urgent need of further intensive epidemiologic studies on this topic.

1. Introduction

During early lactation, high yielding dairy cows are unable to meet their tremendous energy demand by the mammary gland from dietary intake alone [1]. The cows must accommodate this increase in energy demand by fat mobilization from adipose tissue to provide non esterified fatty acids (**NEFA**) as an energy fuel [2,3]. Excessive fat mobilization leads to increased concentration of NEFA in the blood. NEFA can partly be used by final tissus but the main part is metabolised by hepatocytes via β-oxidation to acetyl-coenzyme A (Acetyl-CoA). Acetyl-CoA is also shunted to de novo cholesterol synthesis [4] or may be metabolised into ketones. Circulating ketone bodies can be used to a certain extent as a fuel source by heart, brain, liver, and mammary tissue [5], but too much ketogenesis and low tissus uptake may result in increasing circulating ketone bodies and occasionally hyperketonemia [6]. β-hydroxybutyrate (**BHB**) is the predominant circulating ketone body in ruminants [7] and is considered a gold standard for diagnosing sub-clinical ketosis (**SCK**) due to its stability in blood [8]. Nonetheless, NEFA can also be used as markers of negative energy balance [9,10].

In the last years, many studies have shown that increased concentrations of BHB or NEFA are associated with various illness including reproductive disorders such as placental retention, metritis, endometritis, purulent vaginal discharge, delayed cyclicity, decreased conception at service … [11,12]. Amazingly, the associations between increased concentrations of BHB or NEFA and

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reproductive disorders remain inconsistent and the overall positive or negative association is still difficult to evaluate [9]. Some possible explanations for the inconsistency between the results of individual studies are the heterogeneity of the design, how the trials have conducted and the heterogeneity of the enrolled populations.

A recent meta-analysis [23] was performed on disorders associated to hyperketonaemia, but there remains some issues to be fixed. First, the precision of the effect size (i.e. the risk for disorder in case of SCK) depended on the outcomes. It was reported to be good for disorders such as abomasum displacement, early culling or placental retention but the authors of the previous meta-analysis clearly highlighted the limits of some outcomes related to reproduction due to a low number of papers available for the meta-regression. Second, the previous meta-analysis adjusted the effect size on several co-variables including BHB or NEFA thresholds, but did not distinguish whether NEFA or BHB were used to define SCK. Third, outcomes like estrous cyclicity and time to pregnancy were not included in the previous meta-analysis although they are key parameters for reproduction management. Fourth, (few) new papers reporting the association between high BHB or NEFA concentrations and reproductive disorders are available since the previous has been done and analysing how they impact the previous results may be useful. The present work aims to extend the above mentioned work [23] for reproductive performance and disorders, thanks to a new meta-analysis with updated literature, new reproductive parameters and new way to adjust for co-variables and definition of SCK.

2. Materials and methods

2.1. Literature search and selection of papers

All English-language papers published between 1980 and June 2016 that analyzed the association of elevated NEFA and BHB in early lactation and productive and reproductive performance were included. The search was carried out using PubMed (the National Library of Medicine, Bethesda, MD, USA; http://www.ncbi.nlm.nih.gov/pubmed/), CAB (CAB Abstracts, Cab International, Oxon, UK; http://cabi.org/), and Google Scholar (Company—Google, California, USA; http://scholar.google.com/). The following key words in different combinations were used for the search: BHB, NEFA, subclinical ketosis, fertility, conception, calving rate, peri-partum, reproductive performance, reproduction, cow, early lactation and reproductive disorders. To be included in the data set, the papers must have examined the effect of elevated NEFA and/or BHB on reproductive performance and disorders (named outcomes) in peri-partum dairy cows. The papers were excluded from the meta-analysis in cases of (i) no original research papers published in a peer-reviewed journal, (ii) no study design described, (iii) risk not calculable thanks to the results of the paper (e.g., relationship only measured by correlation coefficient), (iv) lack of disease-free controls and association estimated by comparing cases according to severity, (v) analyses carried out at the herd level and (vi) data collected from countries with traditionally non intensive dairy farming (Table S1).

2.2. Data organization and abstracting

The literature review process ultimately identified 48 studies, of which 34 were considered unsuitable for inclusion. 36 different results of statistical models out of the 14 remaining studies were extracted. Each result of model was included in the database (one line of the database per model) which contains the type of study (observational and clinical trials), the country, the number of cows and herds studied, the average 305d milk production, the statistical method used (logistic regression, Poisson regression, or raw data with contingency table), the expression of risk [odd ratio (OR), relative risk (RR) and hazard ratio (HR)], the metabolite used for the diagnose (BHB and NEFA), the threshold used, the peripartum week of sampling, the total number of samplings per cow, the prevalence of the outcome or of the mean value if relevant, the mean, standard error or standard deviation value of the risk or the change in the outcome and its 95% confidence interval. Because HR, OR or RR were used in the various analyses, the term “risk” refers to any of these terms when at least 2 of them were used in the meta-regression.

2.3. Meta-analysis procedures

All the analyses were computed in R [24] by using the Meta [25] and Metafor [26] statistical packages. For easier interpretation, the logarithmic-scale observed outcomes in the meta-regression has been transformed back to the risk scale through exponentiation [27]. A fixed-effects model was first used. In this model, it was assumed that the true effect sizes were the same for all studies and any difference observed was simply due to sampling variation.

A random-effects model was then conducted for each metabolite to estimate the logarithmic effect size, its 95% confidence interval, and its statistical significance (p value). The inconsistency of results among trials was quantified using both $\chi 2$ test of heterogeneity (Cochran's Q-test) and the I^2 statistic to assess the fixed-effects assumption. The I^2 index describes the percentage of total variability across studies due to true heterogeneity rather than chance, with a value of >75% indicating medium-to-high heterogeneity [28]. If evidence of heterogeneity was found (I^2>75%), a meta-regression analysis (mixed-effects model) was subsequently performed to explore the sources of heterogeneity, using the logarithmic individual effect size for each trial as the outcome. The following mixed-effects model was used:

$$\theta_j = \gamma_0 + \gamma_1 \times mod + u_j + \varepsilon_j$$

where γ_0 is the expected effect for a study when the moderator is zero, γ_1 the fixed-effects, mod is the moderator introduced, u_j the random-effects and ε_j the residuals.

Many models were reported in each publication, and choices had to be made regarding which models to retain in the dataset. Different models within a single paper were often based on the same cow-level raw data but differed in terms of moderators included (Table 1) in particular for metabolite and time of testing (NEFA, BHB, postpartum or prepartum) as well as frequency of testing. Because many models were reported in each publication and for part retained in the present meta-analysis, a variable class was created and used as random variable in the present mixed-effects models (details of the variable Class in Supplementary data).

For each of the outcomes of interest, forest plots were produced to show the effect sizes (logarithmic scale of risk) for each relevant studies with an overall summary estimate generated from the meta-analysis. Biased effect sizes for individual studies are an unfortunate possibility and therefore any biases in individual studies are likely to propagate into the overall summary measure. Publication bias that could for instance originate from high weight of one study on the meta-regression results was investigated for each meta-regression using funnel plots with residual value of effect size and a measure of study precision.

3. Results

Table 2 summarizes the studies included in the meta-analysis. The population included varied from 3 to 528 herds and from 213