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Effect of acute and chronic excesses of dietary nitrogen on blood neutrophil functions in cattle

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ABSTRACT

Excess dietary nitrogen (EDN) is commonly expected in dairy herds, but no data are available regarding its consequences on cattle immunity. In this study neutrophil functions were assessed during EDN in steers. In experiment 1, 4 one-month periods, 4 diets [16% crude protein (CP; DM basis), 20% CP based on soybean meal, 20% CP based on urea, and 24% CP based on urea and soybean meal], and 4 steers were included in a crossover design to determine the effects of a chronic excess. In experiment 2, the repercussions of an acute excess were assessed with 2 periods of 10 d, the same 4 steers, and 2 diets containing 14 and 20% CP. Sampling was done during the fourth week of each period in experiment 1, and on d 0, 1, 2, 3, 7, and 9 of each period in experiment 2. Individual blood biochemistry parameters were measured and neutrophil factors, such as counts, recovery after isolation, surface expression of CD11b and CD62L, phagocytosis, diapedesis, reactive oxygen species (ROS) production, and bacteria killing, were determined. Data were analyzed by general linear models of R, with period, diet or biochemical component, and animal as explanatory variables. The outcome variables were biochemical or immune variables. The variables diet, period, and animal were forced as fixed effects. Data collected over the entire period of experiment 2 were pooled. Several multiples linear regressions or ANOVA were performed and a Bonferroni correction was applied. In experiment 2 (acute EDN), neutrophil counts were negatively associated with nitrogen intake, conversely to CD62L expression. The observed relative neutropenia may be due to neutrophil margination because CD62L-expressing neutrophils are more likely to stick to endothelium. Interestingly, ROS production was changed by EDN: chronic EDN (experiment 1) was negatively associated with opsonized zymozan (OZ)-induced ROS production and acute EDN (experiment 2) with spontaneous ROS production. For chronic EDN, ROS production upon phorbol 12-myristate 13-acetate was not modified, in contrast to OZ stimulation. Decreased ROS production during chronic EDN probably involves the early events leading to ROS production, as OZ acts through membrane receptors and phorbol 12-myristate 13-acetate directly activates protein kinase C. This is the first study to provide evidence that the modifications of neutrophil functions produced by excess nitrogen depend on the intensity and duration of the excess. Further studies, including epidemiological studies during risk periods, are needed to resolve the issues linked to EDN.

Key words: immunity, dairy cow, nitrogen excess

INTRODUCTION

In addition to the well-known metabolic diseases of dairy cattle (i.e., subclinical ketosis and subacute ruminal acidosis), excess dietary nitrogen (**EDN**) is reported as a common event both on pasture- and corn-based diets (Chapa et al., 2001; Tamminga, 2006; Laven et al., 2007). Excess dietary nitrogen refers to both degradable and nondegradable proteins in the rumen and is defined as disequilibrium between protein and energy content of the ration. The ratio of protein to energy intake is a good marker for EDN; plasma or milk urea and ammonia are also common indicators of EDN (Oltner and Witktorsson, 1983; Oltner et al., 1985). Excess dietary nitrogen prevalence has not been reported previously in any dairy area. In 2005 and 2006, around 20% of cows had at least 1 value of MUN above 160 mg/L, the main threshold used to identify EDN in France (Raboisson et al., 2012b). Moreover, the consequences of EDN on production and health remain unclear. Excess dietary nitrogen has been associated with poor fertility (Laven and Drew, 1999), and should

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be avoided at least during critical periods of reproduction (Dawuda et al., 2002). Cows are actually able to adapt to high-nitrogen diets within a few days, and fertility is not worsened for cows on pasture even after urea fertilization (Laven et al., 2007; Ordonez et al., 2007). This suggests that chronic and acute EDN may have different effects in cows. The mechanisms that associate conception failure and EDN could be related to an action of urea or ammonia on oocytes (Laven et al., 2007; Ferreira et al., 2011; Gath et al., 2012), but an indirect link through a diminution of immune functions during EDN is not excluded. In addition, an increase in SCC when cows are allowed to pasture is frequently reported. A recent epidemiological survey revealed both negative and positive associations between SCC and milk urea, depending on the time pattern of the relationship (Raboisson et al., 2012b), which suggests that these interactions might be complex.

The evaluation of neutrophil functions often includes, among others, chemotaxis, quantity of L-selectin-positive cells, phagocytosis, reactive oxygen species (**ROS**) production, as well as the killing ability and the proportion of cells with apoptosis (Martin et al., 1979; Ward and McLeish, 1995; Cendoroglo et al., 1999; Anding et al., 2003; Sardenberg et al., 2006; Zarbock et al., 2006). We hypothesize that some modifications of these neutrophil functions might occur during EDN in cattle due to the increased plasma urea or plasma ammonia. In cattle with EDN, plasma urea and ammonia originate from a ruminal input.

To date, no data are available on the association between EDN and altered immune functions in cattle. Because neutrophils are closely involved in maintaining udder and uterine health and because mastitis and endometritis are suspected to be increased during EDN, the present study was focused on neutrophils. The aim was to describe the effects of chronic and acute EDN on neutrophil-related immune functions by evaluating the ex vivo response of blood neutrophils in dairy steers to various levels of nitrogen in the diet.

MATERIALS AND METHODS

Animals, Diets, and Sampling

All animal housing and handling procedures were in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

Four Holstein steers were used in 2 separate experiments. They were housed in tiestalls equipped with a mattress but without straw. They could have visual but no physical contact, and no other animals were present in the barn. The steers were 22 and 28 mo old and the mean (SD) weights were 664 (16) and 717 (19) kg in experiments 1 and 2, respectively.

A crossover design was used in both experiments, with 4 steers, 4 diets, and 4 one-month periods in experiment 1 and the same 4 steers, 2 diets, and 2 ten-day periods in experiment 2 (Table 1). The 4 diets in experiment 1 were isoenergetic (4.7 MJ of NE_L/kg of DM). They contained 16% CP (**CP16**), 20% CP after substituting part of the corn with soybean meal (**CP20S**) or with urea (**CP20U**), and 24% CP after substituting part of the corn with both soybean meal and urea (**CP24**; Table 2). The 2 diets in experiment 2 were also isoenergetic (4.50 MJ/kg of DM) and contained 14 (**CP14**) or 20% CP (**CP20**) after addition of soybean meal and urea. Forage and concentrates were accurately weighed, mixed, and distributed individually at 0800 and 1800 h. Clean water was available ad libitum. Troughs were cleaned before food distribution and refusals were weighed when present. No washout was performed in experiment 1. Experiments 1 and 2 were separated by a 1-mo washout period with the same diet for all steers (Table 2). Samplings in experiment 2 were carried out on d 0, 1, 2, 3, 7, and 9 after the start of distribution of the CP14 or CP20 diets.

For each steer, blood was sampled on heparinized tubes for biochemistry analysis every 2 h between the 2 daily meals (0800 and 1800 h) on the first day of the fourth week of each period in experiment 1 and on d 0,

Table 1. Experimental design in experiments 1 and 2

		Diet assignment[1]				Sampling	
Item	Period	Animal A	Animal B	Animal C	Animal D	Biochemistry	Blood neutrophil
Experiment 1, 4 mo	1	24	16	20U	20S	1 time, 0800 to 1800 h, on wk 4 of each month	3 times, 2100 h, on wk 4 of each month
	2	20S	24	16	20U		
	3	16	20U	20S	24		
	4	20U	20S	24	16		
Experiment 2, 2 wk, twice	1	14		20		d 1, 2, 3, 4, 8 and 10	
	2	20		14			

[1]Diets contained (DM basis) 16% CP (16), 20% CP based on soybean meal (20S), 20% CP based on urea (20U), and 24% CP based on urea and soybean meal (24).