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Different durations of whole raw soybean supplementation during the prepartum period: Measures of cellular immune function in transition cows

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ABSTRACT

The objective of this study was to evaluate different durations of whole raw soybean (WS) supplementation (diet rich in n-6 fatty acid) during the prepartum period on cellular immune function of dairy cows in the transition period and early lactation. Thirty-one Holstein cows were used in a completely randomized design and assigned to 4 experimental groups (G) [G90, G60, G30, and G0 (control)] supplemented with a diet containing 12% of WS from 90, 60, 30 and 0 d relative to the calving date, respectively. Cows were dried off 60 d before the expected calving date. After parturition, all cows were fed a diet containing 12% of WS until 84 DIM. Blood samples were collected before the morning feeding (d -56 ± 2, -28 ± 2, -14 ± 2, -7 ± 2, at the day of partum, 7 ± 2, 14 ± 2, 28 ± 2, and 56 ± 2 relative to parturition). Cell phenotyping and phagocytosis assays were carried out using monoclonal antibodies and flow cytometry technique. Duration of WS supplementation linearly increased the percentage of blood $CD3^+$ cells, as well as increased the percentage of blood $CD8^+$ cells in the postpartum period, notably in G30, whereas the lowest values were observed in G0. Further, the duration of WS supplementation linearly increased the reactive oxygen species median fluorescence intensity of $CH138^+$ cells after phagocytizing *Staphylococcus aureus* in the postpartum period. Longer periods of WS supplementation linearly increased phagocytosis median fluorescence intensity of $CH138^+$ cells in the prepartum period of cows. Duration of WS supplementation linearly increased the percentage of blood $CD14^+$ cells producing reactive oxygen species when stimulated either by *Staph. aureus* or *Escherichia coli* in the postpartum period. In conclusion, longer periods of WS supplementation during late lactation and the dry period (beginning on d 90 of the expected calving date) alter the leukocyte population and improve neutrophil immune response in the postpartum period with no detrimental effects on cow performance.

Key words: adaptive immunity, innate immunity, oxidative burst, linoleic acid, phagocytosis

INTRODUCTION

The immune system is composed of the innate and the adaptive systems that operate in a synchronized, sequential, and complementary manner to prevent diseases (Carroll and Forsberg, 2007). The innate immune system is responsible for combating the first infection stages and delays pathogen propagation into the organism. If the innate immune system fails to eliminate the pathogen, the adaptive system is triggered and it develops a specific response including the production of antigen-specific lymphocytes and memory cells able to identify antigenic determinants of a pathogen (Tizard, 2014; Sordillo, 2016).

Hormonal and physiological changes occurring in the transition period and early lactation, such as increased blood cortisol and nonesterified fatty acid (**NEFA**) concentration, and decreased energy balance often jeopardize the immune response of cows. These changes reduce immune cellular activity (Cai et al., 1994; Zerbe et al., 2000), impair cellular migration to peripheral tissue (Kimura et al., 1999), and decrease cell bactericidal and phagocytic activities (Kehrli et al., 1989) and reactive oxygen species (**ROS**) production, especially in neutrophils and monocytes (Mehrzad et al., 2001; Singh et al., 2008).

Research evaluating the supplementation of PUFA to dairy cows has found PUFA may either stimulate (proinflammatory; Grummer et al., 2004) or inhibit (anti-inflammatory; Calder, 2012) the immune system. Poly-

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unsaturated fatty acids may alter the fatty acid (**FA**) profile of cell membranes, modifying the production of pro- or anti-inflammatory molecules including cytokines, IL, and leukotrienes. In addition, PUFA influence the expression of adhesion molecules, and proliferation and activation of immune cells (Contreras and Sordillo, 2011). The different outcomes of PUFA supplementation on the immune system are mostly attributed to the type of FA provided. Polyunsaturated FA of n-3 and n-6 series may modulate immune response (Gandra et al., 2016a) depending on the processing method of FA (rumen protected or as a free oil) and their proportion in the diet (Onetti and Grummer, 2004; Greco et al., 2015). The n-3 FA have anti-inflammatory properties, whereas the n-6 FA possess pro-inflammatory properties (Silvestre et al., 2011; Calder, 2012).

Linoleic acid is a FA from the n-6 series, commonly supplemented in animal and human diets. In the immune cells, linoleic acid acts as a precursor for arachidonic acid synthesis, a major component of pro-inflammatory molecules and activator of immune cells (Sordillo et al., 2009). In humans and rodents, linoleic acid supplementation directly influences innate immunity by increasing the adhesion molecule expression in neutrophils, enhancing phagocytic activity, promoting bacterial killing by increasing ROS production in polymorphonuclear cells, and altering the synthesis of intracellular signaling molecules in immune cells (Contreras and Sordillo, 2011; Poorani et al., 2016). Studies that evaluated the supplementation of rumen-protected sources of linoleic acid to dairy cattle have shown similar responses to linoleic acid provision to humans regarding the immune system (Silvestre et al., 2011; Gandra et al., 2016a).

The major nutrient in whole raw soybean (**WS**) is protein; however, it is also rich in linoleic acid. Further, oilseeds have a protein surrounding their lipid content that partially protects FA against ruminal biohydrogenation (Lock and Bauman, 2004; Barletta et al., 2016). The partial biohydrogenation of linoleic acid produces *trans*-11 C18:1 FA and *cis*-9,*trans*-11 C18:2 CLA (Jenkins et al., 2008). However, recent in vivo or ex vivo studies have demonstrated that CLA has a minor effect on immune response of cows (Petzold et al., 2015; Eger et al., 2017).

Although the effects of n-6 FA supplementation have been extensively investigated, research evaluating their

Table 1. Percentage of blood granulocytes and mononuclear cells expressing specific adhesion molecules in dairy cows fed whole raw soybeans during different periods (mean ± SD)

	Group[2]				*P*-value[3]				
Item[1]	G90	G60	G30	G0	Group	Time	Group × time	Linear	Quadratic
Granulocytes									
Prepartum	32.3 ± 2.70	30.2 ± 2.47	36.4 ± 3.54	37.6 ± 3.65	0.287	0.343	0.760	0.129	0.600
Postpartum	35.9 ± 2.84	40.5 ± 2.77	37.2 ± 3.28	28.6 ± 3.04	0.040	0.162	0.962	0.059	0.030
CH138$^+$									
Prepartum	85.6 ± 3.46	82.5 ± 3.34	80.3 ± 4.56	83.8 ± 4.55	0.818	0.073	0.814	0.687	0.431
Postpartum	82.4 ± 3.07	88.4 ± 2.62	80.4 ± 3.14	84.5 ± 2.99	0.241	0.009	0.001	0.898	0.753
Mononuclear cells									
Prepartum	60.2 ± 3.16	59.6 ± 2.91	56.9 ± 4.09	55.4 ± 4.18	0.776	0.090	0.970	0.341	0.898
Postpartum	60.9 ± 2.98	54.6 ± 2.82	58.1 ± 3.34	60.4 ± 3.09	0.124	0.283	0.984	0.143	0.235
CD14$^+$									
Prepartum	17.4 ± 4.46	21.9 ± 4.11	24.0 ± 5.89	23.6 ± 5.98	0.767	0.093	0.789	0.407	0.644
Postpartum	16.9 ± 2.16	19.6 ± 2.03	17.5 ± 2.49	15.7 ± 2.16	0.611	0.110	0.009	0.551	0.311
CD3$^+$									
Prepartum	35.5 ± 2.59	31.2 ± 2.58	32.9 ± 3.03	31.5 ± 3.02	0.392	<0.001	0.132	0.515	0.392
Postpartum	31.4 ± 2.92	30.7 ± 2.85	29.9 ± 3.37	21.6 ± 3.12	0.091	0.234	0.011	0.028	0.220
CD4$^+$									
Prepartum	2.2 ± 0.89	3.0 ± 0.91	3.0 ± 1.17	2.7 ± 1.19	0.928	0.551	0.549	0.735	0.625
Postpartum	1.7 ± 0.6	1.8 ± 0.6	3.6 ± 0.7	1.3 ± 0.7	0.114	0.175	0.521	0.867	0.071
CD8$^+$									
Prepartum	13.8 ± 1.26	10.9 ± 1.35	14.4 ± 1.86	13.4 ± 1.85	0.356	<0.001	0.062	0.736	0.567
Postpartum	7.4 ± 1.08	7.3 ± 1.04	9.8 ± 1.20	5.0 ± 1.11	0.046	0.004	0.223	0.359	0.038
CD62L$^+$									
Prepartum	52.3 ± 8.55	38.5 ± 8.03	49.6 ± 10.8	39.9 ± 10.9	0.622	0.162	0.692	0.554	0.834
Postpartum	79.4 ± 5.9	77.2 ± 5.65	75.6 ± 6.05	69.8 ± 5.92	0.692	0.096	0.106	0.258	0.757

[1]Granulocytes (% of total leukocytes); CH138$^+$ (% of total granulocytes); mononuclear cells (% of total leukocytes); CD14$^+$ (% of total mononuclear cells); CD3$^+$ (% of total mononuclear cells); CD4$^+$ (% of total mononuclear cells); CD8$^+$ (% of total mononuclear cells); CD62L$^+$ (% of total leukocytes).

[2]G0 = group 0, cows did not receive whole raw soybeans (WS) throughout the prepartum period; G30, G60, and G90 = group that received diets containing WS from 30, 60, and 90 d from the expected calving date, respectively.

[3]Probabilities for group, time (week), group by time, linear, and quadratic effects.