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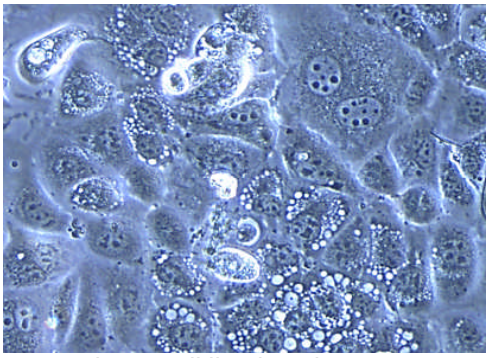
Negative energy balance and immune function in cows



Macrophage cell line in culture

Key external stakeholders:

Dairy and beef cattle breeders, ICBF, veterinary diagnostic companies, scientific community.

Practical implications for stakeholders:

Negative energy balance (NEB) in the early postpartum period is a key factor affecting the health and fertility of dairy and beef cows. The main findings from this research show that the nutrients present in the blood of cows in NEB have the potential to significantly affect immune function. Our findings suggest that decreased macrophage activity may play a role in this altered immunity which results in an increased susceptibility of animals in NEB to bacterial infections which cause mastitis or endometritis. Nutrient availability *in vitro* significantly altered the ability of macrophages to respond to an immunostimulatory challenge and to produce reactive oxygen species (ROS) known to be critical signal transduction molecules essential for the production of pro-inflammatory cytokines in response to an infectious agent.

Main results:

Using an *in vitro* macrophage cell culture model we have concluded the following:

- Glucose availability significantly altered macrophage viability, the ability to produce ROS in response to an immunostimulatory challenge and an overall altered immune response.
- Polyunsaturated fatty acids (PUFAs) such as eicosapentaneoic acid (EPA) significantly improved ROS production following an immunostimulatory challenge; however, docosahextaneoic acid (DHA) had no effect on viability or ROS production.

Opportunity / Benefit:

The data generated enhances our understanding of the postpartum suppression of the immune system in dairy cows and will contribute to the development of nutritional regimes designed to improve energy balance and combat increased susceptibility to bacterial infections postpartum.

Collaborating Institutions:

NUIG, Galway

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Teagasc project team: Dr. Dermot Morris, Athenry

External collaborators: Dr. Ailish Hynes, NUIG

1. Project background:

Excess NEFA oxidation by the liver during periods of negative energy balance (NEB) results in enhanced reactive oxygen species (ROS) production and the onset of oxidative stress that disrupts normal physiology and leads to ketosis. Low blood glucose concentrations and elevated ketone concentrations such as β hydroxybutyrate (BHB) negatively affect immune cells, an environment characteristic to ketosis. This immunosuppression renders dairy (and beef) cows more susceptible to infectious diseases of the mammary gland (mastitis) or the uterus (metritis or endometritis) at this time. Research has shown an association between infectious diseases and metabolic disorders. Cows in severe NEB (SNEB) have decreased total white blood cell numbers and a prolonged recovery time following a uterine infection. In a previous study of SNEB in moderate yielding dairy cows, Morris et al., 2009 showed that SNEB resulted in the down-regulation of the splenic expression of IL15, a macrophage specific interleukin which is known to stimulate T-cell and NK-cell proliferation and activation as well as increasing B-cell expansion and antibody production. The specific aims of this project were to examine the effects of energy substrate availability on macrophage activity using and *in vitro* culture system.

2. Questions addressed by the project:

The major questions addressed by this research were

- Could ketosis result in immune suppression by reducing macrophage viability?
- Could altered glucose availability alter the macrophage response to pathogens in the postpartum cow?
- Could ketosis result in dampened ROS generation or reduced cell viability in the immune stimulated animal?
- Could supplementation with PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) attenuate any effects of these altered metabolic states?

3. The experimental studies:

Bovine macrophage cells (BoMAC macrophage cell line obtained from J. R. Stabel, National Animal Disease Center, USDA-ARS, Ames, IA, USA) were cultured under standard culture conditions in RPMI-1640 medium supplemented with 10% FCS, 25mM sodium bicarbonate at 37°C in 5% CO_2. Cells were cultured for 24 hours to allow attachment and then treated with normo (5mM), hypo (2.5mM) (to simulate SNEB) or hyperglycaemic (25mM) conditions. Cells were also cultured in the presence or absence of BHB at a concentration associated with NEB (2mM). The macrophages were immunologically stimulated using lipopolysaccharide (LPS) and the ability of the PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to attenuate the immune stimulated response measured. Macrophage viability was examined using a standard MTT dye assay and reactive oxygen production estimated using a DCF immunofluorescence assay.

4. Main results:

The findings of this study indicate that the alteration in metabolites associated with negative energy balance can result in an altered immune response to pathogens. In dairy cows the onset of lactation causes nutritional and energy requirements to increase dramatically which leads to a state of negative energy balance. Due to this NEB, mobilization of the body's reserves occur leading to an increase in plasma ketone levels. NEB is often accompanied by health disorders such as mastitis and endometritis. Studies have shown that the severity of these problems is reflected in the degree of increase in ketone levels and decrease in glucose. The immune system relies on energy availability through oxidative phosphorylation; therefore in NEB the immune system (e.g. macrophage function) is impaired in association with hypoglycemia and ketosis.

- Hypoglycaemic conditions similar to those found in negative energy balance did not significantly reduce macrophage viability.
- However, increased availability of glucose increased the mitochondrial activity of macrophages both

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prior to and in response to an immunological challenge with LPS.

- β hydroxybutyrate (BHB) significantly decreased mitochondrial activity in macrophages at concentrations found in ketosis (2mM).
- The magnitude of this effect of BHB was found to be glucose dependent. BHB decreased the hyperglycaemic stimulation of mitochondrial activity by almost 50%. It also reduced mitochondrial activity in normal and hypoglycaemic conditions, however, the reduction was much smaller at 8 and 18%, respectively.
- β hydroxybutyrate (2mM) significantly decreased ($P<0.01$) ROS production by macrophages in response to an LPS challenge.
- While β hydroxybutyrate decreased LPS stimulated ROS production in all glycaemic conditions this inhibition was greatest with 5mM or normal concentrations of glucose.
- The addition of EPA significantly increased ($P<0.05$) ROS production by macrophages in response to an LPS challenge
- However, treatment of macrophages with DHA did not alter mitochondrial activity or ROS production in response to LPS.

5. Opportunity/Benefit:

The data generated provides new insights into the possible mechanisms leading to postpartum suppression of the immune system in dairy cows and contributes to the development of nutritional regimes designed to combat increased susceptibility to bacterial infections postpartum. Ultimately these results point to complex interactions between nutrient availability and immune status. Any dietary strategies proposed for cows susceptible to NEB must take these potential interactions into account.

6. Dissemination:

Main publications:

Cheng, Z., Wickham, I., Morris, D. and Wathes, D.C. (2014). Increased beta-hydroxybutyrate production interrupts splenic immunity in dairy cows with postpartum negative energy balance. BSAS Annual Conference, Nottingham, April-2014, Abstract 209.

Flannery, L., Morris, D.G., Lawless, S., Quinlan, L. and Hynes, A.C. (2013). The effects of energy metabolites on bovine macrophage activity *in vitro*. In: *Agricultural Research Forum*, Tullamore, Co. Offaly, 12-Mar-2013, p121.

Coyne, G., Kenny, D.A., Morris, D.G. and Waters, S. (2009). Effects of dietary n-3 polyunsaturated fatty acid on bovine endometrial gene expression. In: *Walsh Fellowship Seminar*, RDS, Dublin, 11-Nov-2009, p9.

Popular publications:

Morris, D.G. and Wathes, D.C. (2009). Molecular pathways in the dairy cow immune response.

Tresearch, 4(4): 20-21.

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