Serum Visfatin is a Predictive Indicator of Retained Placenta and Other Diseases in Dairy Cows

Fadden AN1 and Bobe G1,2*

1Department of Animal and Rangeland Sciences, College of Agriculture, Oregon State University, Corvallis, Oregon 97331-4802, USA
2Linus Pauling Institute, Oregon State University, Corvallis, Oregon 97331-4802, USA
*Corresponding author: Gerd Bobe, 112 Withycombe Hall, Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR97331-6702, USA, Tel: 541-737-1898; Fax: 541-737-4174; E-mail: gerd.bobe@oregonstate.edu

Abstract

Retained placenta (RP), defined as fetal membranes not being expelled within 24 h after calving, is an economically important disease that increases the risk of other diseases (OD) in early lactation. Early detection of cows at increased risk to develop RP, OD, or both could improve treatment success as well as milk production and reproductive performance. Visfatin is a multifunctional protein that is elevated in humans with various metabolic and infectious diseases, including placental infections, but has not been examined in dairy cows. To evaluate serum visfatin as predictive indicator of RP, OD, or both, we used a nested case-control design and compared Holstein cows that remained healthy (H; n=22) with cows that developed RP (n=31) or OD (n=10) in early lactation. Serum visfatin concentrations were compared 21, 14, 7, 3, and 1 day before calving, the morning after calving, and at 3, 7, 14, 21, and 28 days after calving. Serum visfatin concentrations decreased during the last three weeks before calving. RP cows had throughout the prepartal sampling period and OD cows had 7 days before calving and the morning after calving significantly higher visfatin concentrations than H cows; group differences between RP and H cows were already significant 21 days before calving (8.80 ± 0.53 vs. 7.23 ± 0.48 μg/L; P=0.03). Serum visfatin remained elevated in RP and OD cows in early lactation. In conclusion, serum visfatin may serve as chronic disease indicator and assist in early detection of cows at increased risk to develop RP and OD.

Keywords: Biomarker; Dairy cow; Diseases; Retained placenta; Visfatin

Introduction

Retained placenta (RP), i.e. failure to expel fetal membranes within 24 h, is an economically important disease that affects approximately 7.8% (range: 1.3-39.2%) of U.S. dairy cows [1,2]. The average cost of RP is estimated to be $285 per case and includes treatment costs, milk loss, and increased days open [1]. Moreover, RP increases the risk of other diseases (OD), specifically metritis and ketosis, as well as cull rates [3-5]. Major risk factors for RP are infections (i.e. uterine infections), dietary deficiencies (e.g., inadequate antioxidant status), calving challenges/disorders (i.e. premature calving, abortions, still births, multiple calves, dystocia), metabolic challenges (i.e., milk fever), age of cow, and calving season [5,6].

Considering the costs of RP and OD in early lactation [1], early detection of cows that are at increased risk to develop RP, OD, or both could improve treatment success and improve profitability of dairy farms. Circulating non-esterified fatty acid (NEFA) concentrations >300 to 500 μEq/L have been consistently demonstrated as an early risk indicator of RP [7-9]; however changes were observed only in the last week before calving, which may be too late for an effective prevention. Previously, we reported lower serum α-tocopherol concentrations and higher NEFA and β-hydroxybutyrate (BHBA) concentrations as early risk indicators of RP and OD (i.e., metritis, mastitis, ketosis, or laminitis) during the last 3 weeks before calving [10].

Visfatin, also known as nicotinamide phosphoribosyltransferase (NAMPT) or as pre-B colony enhancing factor (PBEF), is a multifunctional protein that is highly conserved across species and gained interest as chronic disease/disorder marker in human medicine [11-13]. Elevated visfatin concentrations are an indicator of infectious diseases [14,15], metabolic diseases [12], and pregnancy and birth complications [16,17]. The primary source of visfatin in blood is leucocytes [18]. Besides being a non-specific chronic disease marker, visfatin could be also a molecular target for prevention and early treatment because of its three primary functions: 1) visfatin plays a role in inflammation and infection by inducing cytokine secretion and angiogenesis [19-21]. 2) Visfatin plays a role in metabolic diseases by inducing insulin secretion and promoting cellular glucose uptake [22,23]. 3) Visfatin promotes survival and function of cells by producing nicotinamide mononucleotide, a precursor to NAD+ [24,25] and plays a role in labor initiation [26,27].

To our knowledge, we are the first to report on circulating visfatin in dairy cows. Based on visfatin’s role as chronic disease indicator in human medicine, we hypothesized that serum visfatin concentrations could serve as chronic disease indicator in dairy cattle and are elevated before and after RP and OD. The objective of this study was to compare during the peripartal period (-3 to 4 weeks after calving) serum visfatin concentrations between dairy cows that remained healthy and those that developed RP and/or OD in early lactation.

Materials and Methods

Animals and study design

All procedures involving animals were approved by the Oregon State University Institutional Animal Care and Use committee. The animal part of the study was conducted on a 1,000-head commercial dairy farm in Oregon’s Central Willamette Valley during spring and summer 2010. To be eligible for the study, cows had to have completed ≥ 1 lactation, were clinical healthy, had a BCS of ≥ 3.0 four weeks before expected calving date, and were purebred Holsteins. The study cohort consisted of 161 cows with 1 to 6 completed lactations. Using a nested case-control design, we identified cows that developed RP (n=31), developed OD (n=10; i.e., metritis, mastitis, ketosis, or laminitis), or remained healthy (H; n=22) during the first 28 d after calving. Cows were matched based on parity and calving season. The management of the cows has been described in detail previously [10]. In short, during the last 4 weeks before the expected calving date, cows were housed in a straw-bedded free stall barn and were fed once in the
morning (7:30) a total mixed ration (TMR) based on corn, corn silage, and alfalfa and triticale hay, which met National Research Council (NRC) guidelines [28]. After calving, cows were housed in free stall pens with slatted floors and were fed in around 8:00 and 13:30 a TMR based on corn, corn silage, and alfalfa hay, which met NRC guidelines [28].

Animal health surveillance and disease treatment

During the study period, cows were monitored daily for abnormal milk, gait, appetite, general appearance, alertness, vaginal discharge, and RP. Uterine discharge and milk SCC were checked twice a week. Urinary ketones and body temperature were checked if cow were visually not healthy, which included depressed feed intake (all cows were monitored if they consumed feed), lethargy, cold ears, and rapid BCS loss. Diseases were diagnosed and treated based on Standard Operating Procedures developed by the Oregon State University veterinary staff and consistent with standard of care veterinary practices. Diagnosis and treatment of diseases was done by the herd manager, who was trained and supervised by the Oregon State University veterinarian. The veterinarian visited at least once weekly to supervise diagnosis and treatment of diseases. The treatment protocols for RP, ketosis, laminitis, left displaced abomasum, mastitis, metritis, and milk fever have been previously described in detail [10,29].

Of the 31 RP cows, cows had RP for 1 (2 cows), 2 (2 cows), 3 (1 cow), 4 (2 cows), 5 (4 cows), 6 (4 cows), 7 (4 cows), 8 (6 cows), 9 (4 cows), 10 (1 cow), and 11 days (1 cow) from calving. Of the 31 RP cows, 21 cows had calved at least 5 d early (range -5 to -19 d before predicted calving date), 15 cows had twins, 5 cows had dystocia (hard pull; 1 cow had a twisted uterus), and 8 cows appeared sluggish after calving. Except for one RP cow, all other RP cows displayed one or more of the previously described symptoms. In the following 28 days, all RP cows were treated for severe metritis, 4 cows for laminitis (2 without antibiotics and 2 requiring antibiotics), 3 cows for mastitis (2 gram positive, 1 gram negative), 2 cows for ketosis, 2 cows for milk fever, and 1 cow for left displaced abomasum. The 22 H cows did not show signs of clinical diseases during the first 28 days postpartum. The 10 OD cows were treated for the following diseases during the first 28 days postpartum: 7 cows were treated for metritis (2 for severe metritis and 5 for mild metritis), 7 cows for ketosis, 4 cows for laminitis (3 cases requiring antibiotics and 1 case without antibiotics), and 3 cows for mastitis (all 3 no blood agar growth). None of the OD cows had milk fever or left displaced abomasum.

Blood collection and serum analysis

Blood samples were taken at -21 (-24 to -18), -14 (-17 to -11), -7 (-10 to -5), -3 (-4 or -3), -1 (-2 or -1), 0, 1, 3, 7, 14, 21, and 28 days after calving within 10 min after morning feeding. Blood (5 to 8 mL) was obtained from the coccygeal vein or artery in 10 mL serum vacutainer tubes (BD Vacutainer Plus Plastic Serum Tubes, BD Diagnostics, Franklin Lakes, NJ), placed on ice, and transported to the laboratory, where serum after clotting of the samples was separated by centrifugation at room temperature for 20 min at 1600 x g. Serum samples were stored at -20°C until chemical analysis.

Serum concentrations of visfatin were determined by utilizing a Visfatin C-Terminal (Human) competitive Enzyme Immunoassay kit (#EK-003-80; Lot No. 603040; Phoenix Pharmaceuticals Inc., Birmingham, CA). The epitope of the anti-human visfatin polyclonal antibody is identical to the amino acid sequence of bovine visfatin and has been verified for measuring bovine visfatin in bovine mammary cell culture and milk [30]. Manufacturer's instructions were followed for chemical analysis. The serum sample volume was 50 µL per test. Absorbance was measured at 450 nm with a FLUOstar Omega microplate autoreader (BMG Labtech Inc, San Francisco, CA). Each serum sample was run in duplicate and the average was calculated for data analysis. If the results of the duplicate samples differed by ≥ 12%, the sample was tested again in duplicate. The intra-assay and inter-assay CV for visfatin was 3.1 and 15.1%, respectively.

Statistical analysis

Data were analyzed in PROC MIXED SAS version 9.2 [31] as repeated-measures-in-time ANOVA study. Before data analysis, serum visfatin concentrations were natural log-transformed to achieve normality. The variance-covariance structure of repeated measures within cow was modeled using the heterogeneous first-order autoregressive variance-covariance matrix. Fixed effects were group (H, OD, and RP), parity (2, >2), sampling time (-21, -14, -7, -3, -1, 0, 1, 3, 7, 14, 21, and 28 days after calving, and the interaction between group and sampling time. We did not include season of calving in the final model, because it was not significant. To obtain the correct degrees of freedom, the KENWARDROGER option was invoked. The results are shown in Figure 1. In addition, we calculated for each cow average serum visfatin concentrations before calving (3 and 2 week prepartum combined, last week prepartum) and after calving (first week postpartum and for weeks 2 to 4 postpartum combined) using the trapezoidal rule and used the same statistical model as for individual time points. The results are shown in Figure 2.

Values presented in the figures are least-squares means (LSM) and standard errors of means (SEM) that are transformed back to their original measurement scale. To evaluate whether serum visfatin concentrations are sensitive to predict/detect RP and OD, visfatin concentrations of H cows were compared with RP and OD, respectively. Receiver operating characteristics (ROC) curves and 95% confidence intervals (95% CI) were calculated in GraphPad Prism 6.02 (GraphPad Software, Inc., La Jolla, CA) to determine potential cut-off values. Potential cut-off values were verified using Fisher's exact test. All statistical tests were two-sided. Significance was declared at P ≤ 0.05 and a tendency at 0.05 to 0.10.

Results and Discussion

To evaluate serum visfatin as a potential disease indicator of RP, OD, or both, we compared during the peripartal period (-3 to 4 weeks after calving) serum visfatin concentrations between dairy cows that remained healthy and those that developed RP and/or OD in early lactation. To our knowledge, our study is the first to measure in dairy cattle serum visfatin concentrations, a chronic disease indicator in humans and potential link between inflammation and metabolic diseases/disorders [32]. Visfatin is a multifunctional protein that is highly conserved across species [11], suggesting a critical function for health. We measured bovine visfatin using a human visfatin competitive Enzyme Immunoassay kit (#EK-003-80; Lot No. 603040; Phoenix Pharmaceuticals Inc., Birmingham, CA). We did not further validate the use of a human visfatin kit for bovine visfatin because this has been previously published for measuring bovine visfatin in bovine mammary cell culture and milk [30]. Moreover, the epitope of the anti-human visfatin polyclonal antibody is identical to the amino acid sequence of bovine visfatin and the whole amino acid sequence between human and bovine visfatin is 96% homologous [30].
Visfatin is expressed in many tissues and cells including adipocytes, hepatocytes, leukocytes (lymphocytes, neutrophils), cardiomyocytes, neurons, lipopolysaccharide-activated monocytes, pancreatic β-cells, macrophages, colonic and mammary epithelial cells, vascular endothelial cells, and synovial tissue [11, 33]. Leukocytes, specifically neutrophils, are the primary source of visfatin in blood [18]. Our serum visfatin concentrations (median = 6.59 μg/L; interquartile range: 5.48 to 7.86 μg/L; range: 1.6 to 27.5 μg/L) were at the lower end of the spectrum what had been previously reported for human plasma visfatin using ELISA methods. Human plasma visfatin concentrations span in averages from 9.7 to 50 μg/L and in standard deviations from 2 to 43 μg/L, documenting the large range of reported human plasma visfatin values [34-37]. When comparing reported visfatin values among studies, one has to consider that, as with any ELISA method, the utilized antibody and the lot number of the ELISA can affect results. This study was carried out in a single commercial herd; future multi-herd field studies are needed to evaluate whether serum visfatin concentrations in dairy cows are lower than generally observed in humans.

Serum visfatin concentrations decreased during the last three weeks before calving and increased back to concentrations observed 3 weeks before calving 3 days after calving (Figures 1 and 2). Serum visfatin concentrations started to decrease earlier in the dry period in H and RP cows than in OD cows (interaction term for prepartum: P=0.006). Visfatin concentrations of H and RP cows but not of OD cows decreased already between 21 and 14 days before calving (Healthy: -0.14 ± 0.04 μg/L, P=0.002; RP: -0.11 ± 0.04 μg/L, P=0.009; OD: +0.06 ± 0.07 μg/L, P=0.42). In contrast, visfatin concentrations of OD cows did not decrease until between 7 and 3 days before calving (OD: -0.34 ± 0.08, P<0.0001; Healthy: -0.10 ± 0.06 μg/L, P=0.08; RP: -0.03 ± 0.06 μg/L, P=0.56) (Figures 1 and 2). Similarly to our results, Lemor et al. [38] reported for 10 dairy cows that mRNA expression of visfatin in subcutaneous adipose tissue did not significantly differ between the last 2 weeks before calving and week 3 after calving. Visfatin is expressed in the fetal and maternal portions of the fetal membranes [39], placenta [26], myometrium [40], and in amniotic epithelial cells [41] and induces angiogenesis and protects against apoptosis [19,21,42]. Changes in visfatin concentrations shortly before calving may play a functional role in the initiation of calving, as has been proposed for labor induction in women [26,27], but may also cause cows to be more susceptible to metabolic and infectious diseases at calving, as visfatin improves glucose uptake as well as survival and function of immune cells and hepatocytes [23-25, 43].

Cows that had completed more than one parity had lower serum visfatin concentrations than cows that had completed one parity (6.50 ± 0.20 vs. 7.32 ± 0.32 μg/L; P=0.02). No significant interactions with sampling period was observed (P=0.36). Similarly, serum visfatin concentrations decrease with age in humans [30,44,45]. Older cows have a greater incidence of infectious and metabolic diseases during the peripartal period because their immune and metabolic function is...
OLDER DAIRY COWS, AS VISFATIN IMPROVES GLUCOSE UPTAKE AS WELL AS SURVIVAL AND FUNCTION OF IMMUNE CELLS AND HEPATOCYTES [23-25,43].

RP COWS HAD THROUGHOUT THE PREPARTAL SAMPLING PERIOD HIGHER VISFATIN CONCENTRATIONS THAN H COWS (FIGURE 1); GROUP DIFFERENCES WERE ALREADY SIGNIFICANT 21 DAYS BEFORE CALVING (8.80 ± 0.53 VS. 7.23 ± 0.48 µG/L; P=0.03). THE ROC CURVES BETWEEN RP AND H COWS WERE AT 21 DAYS BEFORE CALVING 0.66 (95% CI: 0.49-0.82; P=0.07), 14 DAYS BEFORE CALVING 0.68 (95% CI: 0.54-0.83; P=0.02), 7 DAYS BEFORE CALVING 0.77 (95% CI: 0.62-0.92; P=0.002), 3 DAYS BEFORE CALVING 0.73 (95% CI: 0.50-0.95; P=0.06), 2/3 WEEKS BEFORE CALVING 0.74 (95% CI: 0.60-0.89; P=0.003), AND 1 WEEK BEFORE CALVING 0.76 (95% CI: 0.62-0.84; P=0.001). AS A SIDE NOTE, A ROC VALUE OF 1.0 REPRESENTS TOTAL SEPARATION OF GROUPS AND A ROC VALUE OF 0.50 REPRESENTS NO SEPARATION. THE BEST SEPARATION BETWEEN GROUPS WAS ACHIEVED USING AS CUT-OFF VALUE 6 µG/L VISFATIN THAT WAS MEASURED 2/3 WEEKS BEFORE CALVING; 26 OF 31 RP COWS (84% SENSITIVITY) HAD SERUM VISFATIN ABOVE 6 µG/L COMPARED WITH 11 OF 22 H COWS (50% SPECIFICITY; P=0.01); THE P-VALUE REFERS TO THE COMPARISON BETWEEN RP AND RP COWS USING FISHER’S EXACT TEST. EARLY AND CONSISTENT RISK INDICATOR FOR RP, SUCH AS VISFATIN, MAY ASSIST FARMERS TO STRATIFY DAIRY COWS BASED ON THEIR RISK PROFILE TO DIFFERENT PREVENTIVE STRATEGIES. FOR EXPERIMENTAL STUDIES, VISFATIN COULD BE USED TO BLOCK COWS TO TREATMENTS ACCORDING TO THEIR PREDICTED DISEASE RISK OR COULD BE USED AS INCLUSION/EXCLUSION CRITERIA.

In humans, elevated visfatin concentrations are an indicator of pregnancy complications, including intrauterine infections and pre-term and post-term labor [16,17,27]. Moreover, visfatin is highly expressed in uterine tissues and plays a role in parturition [26,27]. Uterine infections and an imbalanced immune response have been proposed to play a role in the etiology of RP [6,49]. Given that visfatin has pro-inflammatory and immunomodulating functions [20], visfatin may not only be an early risk indicator of RP but may play also a functional role in the process of placenta expulsion in dairy cows. Moreover, as circulating visfatin is an indicator of chronic inflammatory diseases in humans [11-13], the elevated peripartal visfatin concentrations in this study suggest that chronic inflammation may play a role in the etiology of RP. Future studies are warranted to address if and how cows at increased risk for RP should be treated before calving.

OD COWS HAD 7 DAYS BEFORE CALVING (7.76 ± 0.77 VS. 5.81 ± 0.37 µG/L; P=0.02) AND THE MORNING AFTER CALVING (6.83 ± 0.69 VS. 5.37 ± 0.36 µG/L; P=0.05) HIGHER VISFATIN CONCENTRATIONS THAN H COWS (FIGURE 1). VISFATIN ACTS LIKE A PRO-INFLAMMATORY CYTOKINE AND INDUCES THE PRO-INFLAMMATORY CASCADE [20]. GIVEN VISFATIN’S FUNCTION, INCREASES IN VISFATIN CONCENTRATIONS MAY INDICATE THE ONSET OF AN INFLAMMATORY RESPONSE. IN SUPPORT, WE REPORTED PREVIOUSLY FROM THE SAME COHORT THAT OD COWS TENDED TO HAVE HIGHER SERUM CONCENTRATIONS OF HAPTOGLOBIN, AN INDICATOR OF ACUTE INFLAMMATION IN BOVINE, THE LAST WEEK PREPARTUM AND THE MORNING AFTER CALVING [10]. HOWEVER, HUYZEE ET AL. [50] REPORTED THAT COWS WITH MORE THAN ONE DISEASE OR DEATH TENDED TO HAVE HIGHER HAPTOGLOBIN CONCENTRATIONS DURING THE LAST 2 WEEKS BEFORE CALVING THAN COWS THAT REMAINED HEALTHY. HIGHER VISFATIN CONCENTRATIONS STARTED IN OD COWS LATER IN THE DRY PERIOD THAN IN RP COWS (FIGURE 1). THE ONLY DIFFERENCE BETWEEN RP AND OD COWS WAS THE PRESENCE/ABSENCE OF RP AND THAT RP PRECEEDED ALL OTHER DISEASES IN RP COWS, WHICH MAKES RP THE PRIMARY OR ‘GATEWAY’ DISEASE IN RP COWS. BASED ON OUR RESULTS, WE PROPOSE THAT CHRONIC INFLAMMATORY CHALLENGES PLAYING A ROLE IN THE RP ETIOLOGY STARTED EARLIER THAN THOSE LEADING TO OD ALONE.

RP COWS HAD SIGNIFICANTLY HIGHER VISFATIN CONCENTRATIONS THAN H COWS IN EARLY LACTATION (FIGURE 1), EXCEPT FOR 3 AND 7 DAYS AFTER CALVING WHEN ALL COWS HAVE AN ACUTE INFLAMMATORY RESPONSE TO CALVING [10]. THE VISFATIN CONCENTRATIONS OF RP AND OD COWS WERE SIMILARLY HIGH IN EARLY LACTATION, CONSISTENT WITH THE FACT THAT RP IS AN ESTABLISHED RISK FOR METRITIS AND KETOsis [3,4]. WE PREVIOUSLY REPORTED FROM THE SAME COHORT THAT RP AND OD COWS HAD HIGHER AND MORE PERSISTENT HIGHER SERUM HAPTOGLOBIN CONCENTRATIONS THAN H COWS IN THE FIRST WEEK AFTER CALVING THAN H COWS [10]. SERUM HAPTOGLOBIN CONCENTRATIONS RETURNED TO NORMAL IN ALL THREE GROUPS AFTER THE FIRST WEEK OF CALVING, ALTHOUGH MANY RP AND OD COWS WERE STILL TREATED FOR THEIR DISEASES. THESE RESULTS INDICATE THAT SERUM HAPTOGLOBIN IS AN INDICATOR OF ACUTE INFLAMMATORY DISEASES, AS HAS BEEN PREVIOUSLY REPORTED BY OTHERS [51], BUT THAT SERUM HAPTOGLOBIN IS NOT SUITABLE AS INDICATOR OF CHRONIC INFLAMMATORY DISEASES. IN CONTRAST TO SERUM HAPTOGLOBIN CONCENTRATION, SERUM VISFATIN REMAINED INCREASED IN RP AND OD COWS FROM 2 TO 4 AFTER CALVING (FIGURES 1 AND 2). THEREFORE, WE PROPOSE SERUM VISFATIN AS AN INDICATOR OF CHRONIC INFLAMMATORY DISEASES, WHICH COMPLEMENT hAPTOGLOBIN AS INDICATOR OF ACUTE INFLAMMATORY DISEASES IN BOVINE. GIVEN VISFATIN’S POTENTIAL ROLE AS INDICATOR OF CHRONIC INFLAMMATION, OUR RESULTS SUGGEST THAT RP COWS WERE SUB-CLINICALLY DISEASED AND NOT COMPLETELY CURED AT THE COMPLETION OF THE RP TREATMENT PERIOD, WHICH ENDED 2 WEEKS AFTER CALVING. THIS OPENS THE QUESTION HOW AND FOR HOW LONG RP COWS SHOULD BE TREATED AFTER CALVING, WHICH WILL BE THE FOCUS OF FUTURE STUDIES.

CONCLUSIONS

Early detection of cows that are at increased risk for diseases is critical for maintaining cow health and productivity. Visfatin is a multifunctional protein involved in immune and metabolic function that is elevated in humans with various metabolic and infectious diseases, but has not been evaluated in dairy cows. To evaluate serum visfatin as potential risk indicator of RP, OD, or both, we compared peripartal (3 weeks before to 4 weeks after calving) serum concentrations of visfatin between dairy cows that remained healthy and those that developed RP and/or OD (e.g. mastitis, metritis, laminitis, or ketosis) during early lactation. First, serum visfatin concentrations decreased during the last 3 weeks before calving, which may play a role in the homeorhetic adaptation to calving but also may increase the susceptibility of cows to infectious and metabolic diseases. The lower peripartal visfatin concentrations in older cows may also play a role in the suppressed immune and metabolic function of older dairy cows. Secondly, elevated visfatin concentrations preceded the clinical onset of RP for the entire prepartal sampling period, leading us to conclude that serum visfatin may assist in identification of cows at increased risk for RP. Thirdly, elevated visfatin concentrations persisted until 2 weeks after the end of the RP treatment period. In early lactation, serum visfatin concentrations were similarly elevated in RP and OD cows, leading us to conclude that serum visfatin may assist in the identification of cows with chronic inflammatory diseases, including RP, in early lactation.

ACKNOWLEDGEMENTS

This study was supported by Oregon State University (Corvallis, OR), the Oregon State University Agricultural Research Foundation.
References


doi:http://dx.doi.org/10.4172/2325-9590.1000188