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Bone Biomarker in Feedlot Cattle from Two Different Production Systems

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ABSTRACT

The rapid growth rate in feedlot cattle is likely to promote joint abnormalities like osteochondrosis dissecans (OCD) and osteoarthritis (OA) with subsequent lameness. We have identified a small leucine-rich repeat proteoglycan (SLRP) biomarker at cleavage site 262GLGHNQIRM (BGN^{262}) arising from the fragmentation of biglycan (BGN) in subchondral bone associated with OA. With a validated custom-made ELISA, BGN^{262} has been quantified in serum from cattle. The concentration of BGN^{262} in serum from cattle raised in the conventional and all-natural production systems increased at harvest compared to the starting period. The limitation of the study is the small sample size. However, the promising results encourage a further evaluation of BGN^{262} and its potential as a biomarker for subchondral bone pathology in cattle.

Keywords

Osteochondrosis, Osteoarthritis, Lameness, Cattle, Biglycan, Biomarker.

Introduction

In 2022 the U.S. fed cattle industry harvested 26 million head of beef cattle; 60% steers and 40% heifers [1]. These calves are usually between 6-10 months of age when they enter the feedlot production system and reach harvest weight within three to ten months [2]. There are different production technologies such as conventional and all-natural feeding programs that can affect the economics, growth, and well-being of the cattle [3,4]. To improve productivity, conventional beef production systems use steroidal implants, ionophores, beta-adrenergic agonists and feed-grade antibiotics promoting rapid growth. This rapid growth rate can be a predisposition to develop joint abnormalities [5-7], however, a clear association between body size, growth rate and disease incidence are lacking. The developmental disorder, osteochondrosis dissecans (OCD) leads to osteoarthritis (OA) with pain and subsequent lameness [8].

In OA-joints, extracellular matrix fragmentation is one of the most distinctive signs of disease progression [9]. These circulating fragments, released into the bodily fluids potentially serve as pathological markers to assess the disease severity [10,11]. Fatigue-related bone pathology integral to OA progression includes changes in the subchondral bone (SCB) microenvironment [12]. Biglycan (BGN), one of the small leucine-rich proteoglycans (SLRPs) belonging to class I molecules, is found in both cartilage and bone. Skeletal bone homeostasis relies on BGN as a modulator of osteoblast function since it promotes bone formation [13], osteoclast precursor interactions, and is expressed in newly formed bone [14]. We have previously reported that BGN fragmentation in an inflammatory (IL-1β stimulated) cartilage model results in a new cleavage site 262GLGHNQIRM (BGN^{262}) (259GLGHNQIRM in bovine) with 100% homology across various species, including bovines [15].

The BGN^{262} levels increase in equine synovial fluid and saliva from OA joints with SCB sclerosis and chip fractures [10,11]. This suggests BGN^{262} is a potential biomarker for detecting early changes in the SCB microenvironment. We have developed the BGN^{262} immunoassay in cattle serum and describe BGN^{262} serum levels in fattening cattle raised in two different production scenarios.

Material and Methods

In this study, the collection of bovine serum samples in U.S. feedlots received ethical approval from the Colorado State University Institutional Animal Care and Use Committee, Protocol #20-9873A.

The study includes four cattle cohorts with different feed regimes that were arbitrarily selected to compare the serum BGN^{262} levels under conventional or natural production scenarios. The groups were sex-matched with 5 steers and 5 heifers per group (Table 1).

Production Phase	Sex	Weight (lbs) Range	Breed	N
Conventional Starter/ Grower (CS)	Steer	550-650	Angus X	5
	Heifer	500-600	Angus X	5
Natural Starter/ Grower (NS)	Steer	550-650	Angus X	5
	Heifer	500-600	Angus X	5
Conventional Harvest/Close-up (CH)	Steer	1250-1350	Crossbred Beef	5
	Heifer	1150-1250	Crossbred Beef	5
Natural Harvest Close-up (NH)	Steer	1250-1350	Crossbred Beef	5
	Heifer	1150-1250	Crossbred Beef	5

Table 1: Demographic data (production phase, sex, weight, breed) of cattle in conventional or natural production systems. N= number of cattle.

Conventional starter/growers (CS) (n=10); included calves recently weaned and placed in a pen of 75-100 calves. The calves received growth hormone implants while still nursing the cows approx. 3-4 months earlier. This feed yard had a capacity of 1500 head. Upon arrival, they received specific vaccinations, parasite control, and a growth implant. Blood samples were collected 2 weeks after arrival when the calves were 7 months old.

The starter ration at arrival and the first 2 weeks was a low energy type ration (0.48-0.50 Net Energy Gain Mcal/lb) containing corn grain, roughage, and distillers grains co-products including Ionophores. The heifers received melengestrol acetate (MGA) to suppress estrus.

Natural starter/growers (NS) (n=10); calves on grass pasture while nursing cows until weaning. The calves never received any antibiotics or growth implants. Blood was collected at weaning when these calves were 8 months old.

Conventional harvest/close-up (CH) (n=10); included cattle fed in a feed yard (capacity of 43.000 head) for approx. 130 days. The blood samples were collected when these cattle were approx. 15 months of age and within 45 days of harvest (at the time of administering a terminal growth implant). They had been fed ionophores (monensin), and antibiotics (tylosin), and the heifers, received MGA to suppress estrus. The rations were high-energy (0.60-0.70 Net Energy Gain Mcal/lb) containing corn grain, roughage, fat source, and distiller's grains co-products.

Natural harvest/close-up (NH) (n=10); included cattle fed in a feed yard (a capacity of 35.000 head) for approx. 180 days. These animals did not receive any antibiotics, growth hormone implants or distillers grains co-products. These high-energy rations (0.60-0.70 Net Energy Gain Mcal/lb) included corn grain, roughage and fat source. Blood was collected when the cattle were approx. 14 months old and thereafter shipped to slaughter within 1-3 days.

BGN^{262} immunoassay

The inhibitory ELISA was developed and validated for the detection of BGN^{262}, in horse synovial fluid, serum and saliva, using BGN^{262} peptide (GLGHNQIRMIENGSC, Lot. 5763DL290-1/PE9501, GenScript) and an anti-BGN^{262}, recombinant rabbit monoclonal antibody (0.681 mg/ml, lot: U8229DL260-6, GenScript) [5,11]. Briefly, BGN^{262} peptide was used for coating Nunc MaxiSorp™ Clear Flat-Bottom 96-Well Plates (Invitrogen) (1µg/ml in 100mM carbonate buffer with pH 9.6) and to create a standard curve of 11-step, 1:2 serial dilution in Effect Diluent (ED) buffer (Kementech, Denmark), ranging from 0 (no peptide) to 2 mg/ml. Duplicates of serum samples diluted 1:20 in ED were pre-incubated with the anti-BGN^{262} primary antibody (30 ng/ml). Following overnight incubation at 37°C, the pre-incubated samples were transferred to the coated and blocked ELISA plates, and incubated for one hour at 25°C under shaking. The plate was washed four times and incubated with the secondary polyclonal goat anti-rabbit (IgG) HRP (Abcam) (1:50 000 in 10 mM PBS-Tween-0.1% BSA) for 30 minutes at 25°C under shaking. After eight washes, TMB was added and incubated in the dark at 25°C, and the reaction was stopped after 12 minutes with 0,18M H_2SO_4. Absorbance was measured at 450 nm in SPARK multifunctional plate reader using Magellan software (Tecan Group Ltd., Männedorf, Switzerland). A control equine serum (commercially purchased from Håtunalab AB, Sweden) diluted 1:20 was used for the normalization of the results. The linearity test was performed using bovine serum with a dilution curve ranging from 1:2 to 1:64.The assay was evaluated for inter and intra-assay variation.

Statistical analysis

The data were analyzed using a three-factor analysis of variance model [16]. The model included Food regime (Conventional or Natural), Stage (Starter or Harvest), and Gender (heifer or steer) as factors. Based on the Akaike Information Criterion (AIC) [17], it was decided to keep all two- and three-way interactions in the model. The Mixed procedure of the SAS (2017) package was used [18]. Assumptions underlying the analysis were checked by preparing diagnostic plots of the residuals. No serious deviations from the assumptions of normality or homoscedasticity were detected.

Results

The BGN^{262} values are presented as mean ± sd. The respective serum levels were: 178 ± 112 in NS, 255 ± 68 in NH, 199 ± 64 in CS and, 310 ± 104 in CH. Pairwise comparisons between the cohorts revealed significant differences between CS and CH (p=0.0061), NS and CH (p=0.0028), and between NS and NH (p=0.0397). There was a significant difference between stages (S and H) (p=0.0011) with no effect of the food regime (N and C) (p=0.33) (Figure 1).

The serum levels of BGN^{262} were 103 ± 14 in NS heifers, 253 ± 119 in NS steers, 270 ± 82 in NH heifers, 240 ± 55 in NH steers, 170 ± 39 in CS heifers, 234 ± 69 in CS steers, 307 ± 115 in CH heifers and 314 ± 106 in CH steers.

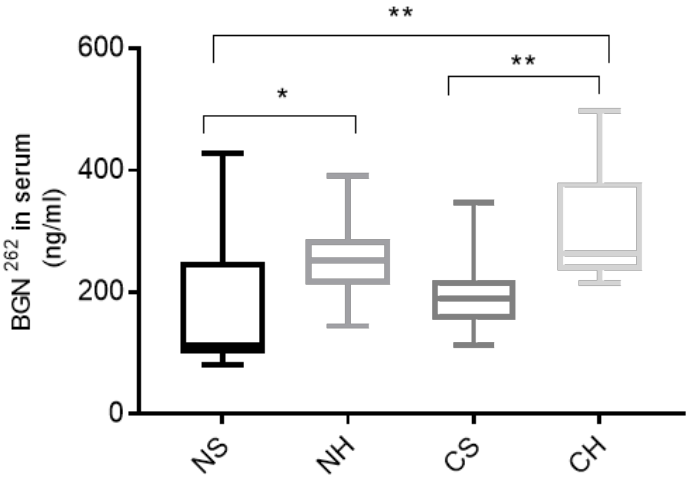

Figure 1: The concentration of BGN 262 (ng/ml) in serum from cattle in natural and conventional systems showed a statistically significant increase at harvest compared to starters.
* P<0.05, ** P< 0.01, *** P< 0.001.
NS=Natural Starter; NH=Natural Harvest; CS=Conventional Starter; CH=Conventional Harvest.

The stage*Gender interaction was significant (p=0.0184). Significant differences were found for heifers between stages (p=0,0002), as well as between steers and heifers at stage Starter (p=0,0050) (Figure 2). The intra- and inter-assay variations were <16% and <4% respectively.

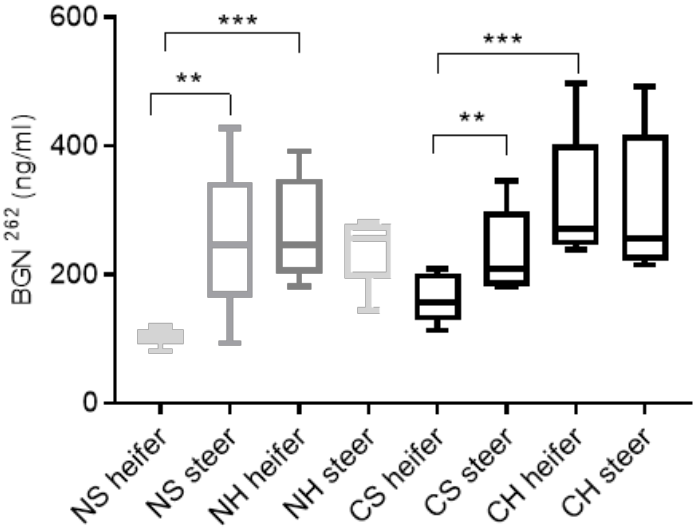

Figure 2: Gender differences in the concentration of BGN 262 in serum from heifers and steers.
* P<0.05, ** P< 0.01, *** P< 0. 001.
NS=Natural Starter; NH=Natural Harvest; CS=Conventional Starter; CH=Conventional Harvest.

Discussion

In a 2014 feedlot veterinary consultant survey from 23 veterinarians servicing feedlots with a one time capacity of 25,000 to 2.5 million head, 27.3% of the responding consulting veterinarians indicated that lameness is the most common cause of fed cattle being marketed as realizers, i.e. cattle that have a poor response to lameness treatment and are marketed for salvage value [19]. Recognizing that cattle lameness is not only a health and production issue, but also affects animal welfare, the North American Meat Institute (NAMI) fed cattle mobility scoring system was developed to better identify lameness issues in finished cattle [20].

The cattle raised in the two systems reported had higher serum BGN^{262} concentrations at harvest compared to starters. As a result, concerns about fed beef cattle welfare arise and our biomarker has the potential to identify changes in joint tissues before symptoms of stiffness/lameness appear. Previously, we have reported in OA horses that an increase in SF BGN^{262} levels correlated with SCB changes [10]. Accordingly, in this study cattle at harvest had higher serum BGN^{262} concentrations. Cattle in the conventional cohort presented with a trend of higher BGN^{262} concentration in the CH cohort, consistent with the greater degree of lameness expected for this group. The increase in BGN^{262} at harvest may indicate SCB remodelling driven by OA-associated joint inflammation. Regarding the gender differences, steers exhibited higher BGN^{262} concentrations than heifers, likely because the steers grow more rapidly, whereas in horses there is no correlation with gender or age [10].

In conclusion, we successfully measured a bone biomarker to identify differences between animals raised under different conditions. Monitoring the early changes in joints will minimize the animal's suffering and discomfort. In future studies, BGN^{262} will be further evaluated as a biomarker for SCB pathology in beef cattle. A marker that can monitor SCB changes in live animals would allow for changes in the environment and managing the production scenarios that could help in averting severe joint problems and animal suffering.

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Competing interests

Eva Skiöldebrand and Stina Ekman are part owners of SGPTH Life Sciences holding the patent for the BGN^{262} (WO2022/268940A1).

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