Bovine Acute Mastitis: Effects of Intravenous Sodium Salicylate on Endotoxin-Induced Intramammary Inflammation

A. C. MORKOČ, W. L. HURLEY, H. L. WHITMORE,2 and B. K. GUSTAFSSON2,3
Department of Animal Sciences
University of Illinois
Urbana 61801

ABSTRACT
Effects of the nonsteroidal antiinflammatory agent sodium salicylate on endotoxin-induced mastitis were evaluated in lactating cows. Escherichia coli endotoxin was administered to a mammary quarter 1 h after initiation of a 12-h i.v. infusion of sodium salicylate. Milk SCC, BSA concentrations in milk, mammary inflammation, rectal temperature, appetite, milk production, and plasma and lymph PGF2α were monitored. Gross mammary inflammation was not reduced by salicylate infusion, nor did sodium salicylate prevent increased milk SCC or BSA concentrations in milk, although treatment tended to decrease the magnitude of these responses. Sodium salicylate decreased subcutaneous abdominal vein PGF2α metabolite, and PGF2α metabolite tended to be reduced in lymph during the acute phase of inflammation. The increased rectal temperature after endotoxin infusion was reduced in cows treated with sodium salicylate. Appetite was reduced after endotoxin infusion in untreated cows and those treated with sodium salicylate. Milk production declined after endotoxin challenge in all cows. Although sodium salicylate did not substantially reduce mammary inflammation, it had an antipyretic effect and reduced PGF2α metabolite in mammary blood.

(Key words: mastitis, inflammation, mammary gland, prostaglandin)

Abbreviation key: NSAID = nonsteroidal antiinflammatory drug, PGFM = PGF2α metabolite, SS = sodium salicylate.

INTRODUCTION
Acute coliform mastitis remains a significant problem in the dairy industry, resulting in economic losses through decreased milk production, discarded milk, cow mortality and culling, veterinary treatment costs, and additional labor requirements. Clinical signs of acute coliform mastitis are considered to be an expression of endotoxemia via endotoxin release from coliform bacteria within the mammary gland. Infusion of Escherichia coli endotoxin into the bovine mammary gland simulates the natural infection clinically and physiologically, providing information concerning pathogenesis of the disease (4, 5, 8, 17, 22, 30). Milk production decreases in response to intramammary or i.v. administration of endotoxin (21), and endotoxin-induced mastitis has been used to study the pathophysiological causes of the reduction in milk production (31, 32). However, the specific mechanisms leading to the mammary inflammatory response and the reduced milk production remain unclear.

Endotoxin-induced mastitis is accompanied by increases in mammary blood flow (7, 13), probably caused by local production of vasoactive mediators, which result in vasodilation and increased vascular permeability during inflammation. Local production of prostaglandin may result in this vasodilation and enhance inflammatory responses. Prostaglandin inhibitors that block vasodilation inhibit hyperemia and protein exudation during inflammation (20).
and decrease the proinflammatory actions of polymorphonuclear neutrophils that infiltrate an area of inflammation (28). Concentrations of the arachidonic acid metabolites PGF$_{2\alpha}$ and thromboxane B$_2$ were increased in milk from naturally infected mastitic quarters (3), and milk PGF$_{2\alpha}$ concentration was increased after intramammary infusion of _E. coli_ or _Klebsiella_ species (17). In addition, an elevation of blood PGF$_{2\alpha}$ occurred following i.v. administration of endotoxin (15, 21). The origin and the specific role of arachidonic acid metabolites in mammary inflammation are unclear.

Intramammary and systemic responses to inflammation are thought to contribute to decreased milk production during mastitis (31, 32). Treatments that limit intramammary or systemic inflammation also may reduce milk production losses associated with mastitis. Furthermore, prevention of the negative effects of endotoxin on reproduction (early embryonic death, abortion, and altered estrous cycles) may be an additional positive benefit (11). Nonsteroidal antiinflammatory drugs (NSAID) are of interest because they reduce inflammation and, thereby, tissue damage and risk of mortality in cows with coliform mastitis. The mode of action of some NSAID is now thought to be mediated through inhibition of cyclooxygenases of various cell types (i.e., platelets, granulocytes, and endothelial cells), thereby preventing release of stable prostaglandins (2, 33). The NSAID have differential effects on prostaglandin synthesis and inflammation (2). For example, sodium salicylate (SS) is an antiinflammatory analgesic but has limited effect on cyclooxygenase activity. In contrast, acetaminophen is an analgesic that is structurally related to SS but has little effect on either cyclooxygenase or inflammation. Indomethacin, piroxicam, and flunixin meglumine are effective inhibitors of prostaglandin synthesis and are antiinflammatory. Other cellular mechanisms may be responsible for the effects of NSAID (1, 24).

In vitro, SS may inhibit neutrophil aggregation, reduce lysozyme release, and affect superoxide generation, depending on concentration (1, 10). Interestingly, SS provides clinical relief in inflammatory conditions in humans that is out of proportion to its relatively low cyclooxygenase inhibitory activity in comparison with other NSAID (2, 6, 33). However, SS has not been evaluated as an antiinflammatory in bovine mastitis. Our objective was to determine the effect of SS on acute mammary and systemic responses to intramammary endotoxin-induced mastitis. Acute mastitis was induced by intramammary infusion of endotoxin. Mastitis indicators, physical indicators in milk, changes in blood and lymph PGF$_{2\alpha}$ content, numerical effects on milk production, and clinical parameters were evaluated to determine the effect of i.v. administration of SS.

**MATERIALS AND METHODS**

**Cows: Selection and Management**

Nonpregnant Holstein cows were selected from the University of Illinois dairy herd and divided evenly between experimental groups. Cows were monitored by rectal palpation to ensure that they were in midestrous at the time of the experiments. Udder health was monitored first by California mastitis test, followed by SCC analyses (determined by Coulter counter; Coulter Corp., Hialeah, FL), and microbiological culturing of milk from individual quarters. Criteria for acceptance of cows with healthy udders for experimentation required the rear udder quarters evaluated in experiments to have SCC <300,000 cells/ml and to be bacteriologically negative. Cows were housed in individual stalls in a climate-controlled environment. All cows were fed alfalfa hay and a grain mixture twice daily. Fresh water was provided for ad libitum intake. Milking was performed with a quarter milking machine at 0600 and 1800 h. At sampling times other than the normal milkings, the teats were cleaned with an alcohol swab, and milk was collected by hand from the experimental quarters.

**Experimental Design**

Endotoxin was infused once in one rear quarter of the mammary gland (experimental quarter) of eight cows on d 0 at 0900 h. Four cows were treated with constant i.v. infusion of SS (endotoxin infused plus SS i.v. infusion; Aldrich Chemical, Milwaukee, WI), and four cows were controls that received constant i.v. infusion of saline (endotoxin infused, no SS treatment). Infusions of SS were administered...
over 12 h (from 0800 to 2000 h) beginning 1 h before intramammary endotoxin infusion. Jugular blood, subcutaneous abdominal vein (milk vein) blood, lymph from mammary gland lymphatic duct, and milk were collected before, during, and after experimental induction of mastitis.

Milk samples were collected from each experimental quarter at each sampling time except immediately following endotoxin infusion. Milk samples were frozen at -20°C and refrigerated for subsequent BSA analysis or fixed with potassium dichromate and maintained at 22°C for determination of SCC.

Endotoxin Infusion

Endotoxin (E. coli lipopolysaccharide, serotype O26:B6; Sigma Chemical Co., St. Louis, MO) was dissolved in sterile pyrogen-free saline solution (9% NaCl) and filtered through a .2-μm pore size microbial filter (Nalge Co., Rochester, NY). For induction of mastitis, 10 μg of endotoxin in 10 ml of saline solution were infused in one rear quarter of each experimental cow via sterile intramammary infusion cannula. Teats were taped closed to prevent leakage until the first milk sampling.

SS Infusion

Sodium salicylate (Aldrich Chemical) is stable when autoclaved. Nonpyrogenic SS solution was prepared by baking dry SS at 175°C for 4 h. The SS changed from white to pale cream or tan during baking. Heat-treated SS was stored in the dark. Heat-treated SS was dissolved in sterile pyrogen-free saline with extensive stirring in heat-treated glassware under a laminar flow hood. The solution was maintained under refrigeration for not more than 1 d before use. The final solution was light brown and slightly viscous. Heat-treated SS solution was compared with nonheated SS by HPLC; no difference existed in peak height or identification.

An infusion pump (Harvard Model 2681; Harvard Apparatus, South Natick, MA) was used for SS and control saline i.v. infusions. Preliminary trials indicated that a loading dose of SS was necessary to achieve desired plasma concentrations. A desired steady-state plasma SS concentration of 200 mg/L was selected based on previous research on arthritic pain in cattle (16). The rate of constant i.v. infusion required to reach 200 mg/L was calculated as the product of the fraction of drug eliminated per unit of time (1.28/h), the desired steady-state plasma concentration (200 mg/L), the apparent specific volume of distribution (.24 L/kg), and BW in kilograms (12, 16).

Analyses of SS were performed by HPLC essentially as described by Charm et al. (9). Plasma samples were prepared for HPLC on C18 columns (Bond-Elut®; Analytichem Int., Harbor City, CA). Samples were analyzed on a Waters Model 441 liquid chromatograph (Waters Chromatography Div., Millipore Corp., Milford, MA) with a reverse-phase Whatman octadecylsilane column (.4 x .6 x 15 cm, 4 μm particle size; PartiSphere, Whatman Inc., Clifton, NJ) and a solvent system consisting of 25% acetonitrile in 75% acetic acid.

Blood Collection

Blood samples were collected from the jugular milk vein and the milk vein exiting the udder ipsilateral to the rear quarter infused with endotoxin. Catheters were inserted 2 d prior to the start of the experiment. Cows were sedated with xylazine, and additional analgesia was provided by subcutaneous injection of 2% lidocaine hydrochloride at the venipuncture site. Catheters were flushed daily with heparinized saline to prevent clotting. At each sampling time, blood was collected from one jugular vein catheter (the other catheter was used for SS or saline infusions) and from the milk vein catheter. The sample was divided between four 15-ml tubes containing 15% EDTA solution for anticoagulation, immediately mixed, and placed on ice. Blood was centrifuged at 1500 x g for 20 min at 4°C, and plasma aspirated from blood samples was divided into six portions and immediately frozen at -20°C.

Lymphatic Collection

Lymph from the mammary quarter with endotoxin-induced mastitis was collected via a surgically implanted lymphatic catheter using a modification of a previously reported technique (14). Catheters were commercially heparinized cardiac polypropylene (Mediplast®; IRD Biomaterial Lab., Söderfors,
Sweden) to prevent clotting, which is a significant problem in lymphatic catheters of the udder. General anesthesia was required for catheterizations. Anesthesia was induced with thiopental-xylazine-guafenisin via jugular vein and maintained with isofluorane inhalation via endotracheal tube. Surgical catheterization of the lymphatic required approximately 1 to 3 h. Cows were milked immediately before surgery because even a partially filled udder created difficulty in the surgical procedure.

Approximately 20 ml of 2% Evans blue dye (Sigma Chemical Co.) in sterile pyrogen-free saline was injected deep into the mammary tissue at about four points anterior, dorsal, and posterior to the teat of the rear quarter. A skin incision approximately 10 cm long was made 10 to 15 cm dorsal to the teat on the lateral surface of the udder. The incision was continued carefully through the subcutaneous tissue and the lateral superficial suspensory ligament of the udder. Blunt dissection exposed the surface of the mammary tissue and allowed visualization of vascular and lymphatic anatomy. Lymphatic vessels appeared pale blue, but blood vessels were dark blue to purple. The lymphatic vessel catheterization was performed using standard vascular surgical catheterization technique. Staining of tissue by leakage of Evans blue dye was avoided to maximize visualization of the vessel. Lymph began to flow slowly through the catheter immediately. Following catheterization, the catheter was further secured with stainless steel sutures. The distal end of the catheter was secured to the udder through a stab skin incision at the posterior medial ventral surface of the udder on the same quarter. The catheter was observed at this point for flow of lymph. Closure included subcutaneous tissue and skin layers.

Lymph containing Evans blue dye flowed continuously from the exposed catheter for 1 to 2 d, after which it became clear with a pale straw color. The cows were permitted to recover for 3 d before starting experiments. Incisions were cleaned daily, and chlorhexidine ointment was applied to the surface of the incisions. As the udder filled with milk, the cannula was covered more by the skin surface, occasionally resulting in a superficial infection or abscession requiring elimination of that cow from the experiment.

Problems encountered with catheterizations included milk leakage into lymph and clotting of lymph in catheters. To avoid milk leakage and to facilitate the surgical procedure, cows were milked out immediately before the surgical placement of catheters. Clotting of lymph in catheters could not be entirely avoided but could be reduced or eliminated by minimizing tissue trauma and postsurgical infection. Some clotting occurred in almost all of the lymphatic catheters in the first days after surgery, requiring removal of the clots. A small hooked wire placed in the tip of the catheter was used to remove the clot.

For lymph collection, tubes containing 15% EDTA were affixed by a stainless steel wire loop to the rear of the udder, and the end of the lymphatic catheter was placed in the top of the tube through a cap containing a hole made to fit. The freely dripping lymph was collected and then placed on ice. Lymph was centrifuged at 1500 × g for 20 min at 4°C, and the supernatant was divided into six portions and immediately frozen at −20°C.

**BSA Assay**

The BSA content of milk samples was determined by agar gel immunodiffusion (26). Plates were poured with 1.5% purified agar (Becton Dickinson and Co., Cockeysville, MD) in 0.1 M sodium phosphate buffer (pH 8) with 0.2 mM sodium azide and containing antiserum at a final dilution of 1:1600 vol/vol (rabbit anti-BSA; Cappell, Organon Teknika Corp., West Chester, PA). Plates were stored at 4°C. Standard solutions of BSA (125 to 2000 µg/ml) were prepared in 0.1 M sodium phosphate buffer (pH 8). Sample or standard (10 µl) was applied to wells, and plates were incubated 18 to 20 h at 4°C. Plates were read against a dark background under bright light, and the diameter of precipitin rings was measured with vernier calipers.

**PGF₂α Assay**

Primary PGF₂α is unstable in blood, requiring analysis for the stable dihydro-keto-PGF₂α metabolite (PGFM) to determine PGF₂α indirectly. Analysis of PGFM was by an established method (18) using kits (Advance Magnetics, Inc., Cambridge, MA). Extraction of
prostaglandins from samples before radioimmunoassay was modified to optimize extraction efficiency (19). Acetic acid solution (25 \mu l of .5N acetic acid) was added to plasma samples (200 \mu l) in 16- \times 100-mm glass tubes and mixed. Ether (5 ml) was added, and tubes were tightly capped and mixed for 15 min horizontally in a rocking shaker. Tubes were centrifuged for 5 min at 1000 \times g at 4°C. After centrifugation, tubes were placed in a methanol and dry ice bath to freeze the aqueous phase, and a portion of the ether phase was decanted to a 10- \times 75-mm polypropylene tube; the tube was filled approximately half full. Ether was evaporated by placing the polypropylene tubes in a rack in a 27°C waterbath under an air steam in an evaporator apparatus. When the first ether was nearly evaporated, the remaining ether was poured into respective polypropylene tubes and evaporated until all tubes were dry. Assay buffer (100 \mu l; supplied with the radioimmunoassay kit) was added to each sample tube and vortexed to resuspend extracted PGF2\alpha from the sides of the polypropylene tubes.

Statistical Analyses

All preinfusion samples for each parameter were averaged, thus providing a single baseline value for each cow, which was subtracted from the treatment period values to derive the parameter deviation from baseline (positive or negative) at each time after endotoxin administration. These deviations are reported. Data were analyzed by split-plot analysis of variance, including interaction between treatment and time after endotoxin (29). Treatment differences were detected by variation among cows. Least squares means of interaction were plotted in the figures. For an analysis of the sampling times from the first sampling after endotoxin to the peak or trough response and analysis of sampling times from the peak or trough of response to baseline, linear and quadratic regression analyses were used; P < .05 was considered to be significant.

RESULTS AND DISCUSSION

Plasma SS

Over the 12-h infusion of SS, overall mean peak jugular plasma concentration was 236 mg/L of SS (data not shown). This concentration exceeds the SS steady-state plasma concentration previously shown (16) to be analgesic in arthritic cattle. Concentrations of SS had returned to baseline by 18 h after SS infusion began. Concentrations of SS in plasma of control cows that did not receive SS infusion were zero.

Mammary Inflammatory Indicators

A key indicator of intramammary inflammation is the rapid diapedesis of leukocytes occurring after intramammary endotoxin infusion. To describe more fully the response to intramammary endotoxin challenge, SCC were estimated on all milk samples. For the control group and for the group treated with SS, SCC increased significantly during the acute inflammation (P < .0001; Figure 1). Milk SCC were increased in both groups by about 3 h and peaked at about 10 h after endotoxin infusion (Figure 1). Although no significant difference existed between the two groups for the change of SCC over time, the group treated with SS generally had numerically lower SCC. Neither group reached preinfusion baseline SCC by the end of the experiment (55 h after endotoxin infusion).

Concentrations of BSA in milk are considered to be an indicator of increased vascular permeability induced by inflammatory mediators. Milk BSA concentrations were increased in the control group and the group treated with SS after intramammary endotoxin infusion (P < .0001; Figure 1). Peak concentrations of BSA occurred at approximately 9 to 10 h after endotoxin infusion, which is similar to observations by others (31, 32). Concentrations of BSA in milk generally were lower in the group treated with SS, suggesting that the increase in vascular permeability associated with inflammation may have been limited by SS treatment. However, the difference between treatment groups by time was not significant.

Inflammation was measured subjectively on a four-point scale (0 = no pain, no edema, normal udder size; 1 = slight pain, slight edema, slight increase in size; 2 = moderate pain, moderate edema, moderate increase in size; 3 = severe pain, severe edema, extreme increase in size). Gradations of this scale were particularly difficult to delineate, and scores...
were recorded only when a definitive change could be easily determined visually and by manual palpation of the udder. Nevertheless, udder inflammation scores increased rapidly after endotoxin infusion, reaching a maximum by 4 to 5 h postinfusion (Figure 1). Differences between treatment groups were not significant.

Flunixin meglumine is a widely used NSAID in veterinary medicine, especially in horses, and has beneficial effects in reducing the fever, depression, and local inflammation associated with endotoxin-induced mastitis (4, 5). Our results with SS generally are similar to those reported effects of the NSAID, flunixin meglumine, in endotoxin-induced mastitis (4, 5).

PGFM

The control group and the group treated with SS had reduced PGFM in milk vein plasma at most sample times following endotoxin infusion compared with baseline concentrations (Figure 2). The decrease in PGFM in the group treated with SS was significantly greater than that of the control group \( P < .0002 \).

Patterns of PGFM concentrations in the lymph draining the endotoxin-treated quarter were distinctly different from those in the milk vein plasma. Concentrations of PGFM in the mammary lymph from both groups increased by 2 h after endotoxin infusion (Figure 2).

Differences in the PGFM concentrations in lymph between treatment groups were not significant, although the SS group tended to have numerically lower PGFM concentrations at most sampling times (Figure 2). The combined results from blood and lymph suggest that SS, although a weak cyclooxygenase inhibitor (2, 6, 33), may have reduced PGF2α production by some mechanism in the cows with acute mastitis.

Endotoxin-induced mastitis increases blood flow (7), and lymph flow may be increased as a result of the increased blood flow (25). After intramammary infusion of endotoxin in the present study, mammary lymph flow rate increased from approximately .5 ml/min prior to endotoxin challenge to 215 ml/min postchallenge. This increased mammary lymph flow rate may dilute mammary-derived PGFM that are measurable in the lymph, thus causing

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underestimation of total PGFM released by the tissue in response to endotoxin. Lymph color deepened as flow rate increased. During baseline sample periods, lymph was nearly as colorless as water but became deep yellow with icteric appearance after endotoxin infusion and during the period of increased flow. The color change may have been attributable to the leakage of plasma protein into tissue spaces.

Prostaglandins can be generated by lymphatics (23). Long-term catheterization may have resulted in production of prostaglandins by the lymphatics, but this is unlikely because the increase of lymph PGFM corresponds directly to the endotoxin infusion (Figure 2) and correlates with the period of the other inflammatory response indicators.

Measurement of mammary lymph prostaglandin in experimentally induced mastitis can be used to evaluate the possible origin of intramammary inflammatory mediators such as PGF$_{2\alpha}$; this origin has not been revealed by previous related work (4). If a primarily local origin of inflammatory mediators is identified, local application of treatment may be efficacious. Prostaglandin metabolism has been shown in numerous types of tissue and cells and can be expected in mammary tissue (27). Our results of PGFM concentrations in lymph and plasma are support for the local generation of PGFM in acute bovine mastitis.

**Systemic Inflammatory Indicators**

Rectal temperature and appetite scores were used to evaluate the effects of i.v. SS on the systemic responses to intramammary endotoxin challenge. Increases in rectal temperatures of control cows were significantly greater ($P < .0001$) than temperature increases in cows infused with SS and peaked later for the control group than for the group treated with SS (Figure 3). Body temperature in the cows treated with SS declined to baseline values approximately 12 h before those of the control group. The reduced temperature increase in response to endotoxin may have provided a general benefit in comfort for the cow and in maintenance of a more normal metabolism. In acute coliform mastitis, fever is typically extreme, and a reduction of fever should benefit the cow. A similar increase in rectal temperature in response to endotoxin has been reported by others (5, 21).

Appetite was measured subjectively on a four-point scale (0 = complete anorexia to 3 = normal, full appetite). Appetite in both groups was depressed during the initial hours after endotoxin infusion. The difference in appetite between the control group and the group treated with SS was not significant (Figure 3).

**Milk Production**

Milk production was measured beginning d –2 at the morning milking and continued twice
Figure 3. Deviation of systemic inflammation indicators from baseline. Least squares means of rectal temperature (top) and appetite score (bottom) from control cows (○) and cows receiving i.v. sodium salicylate infusion (●) are plotted according to hours from endotoxin infusion. Bars indicate period of sodium salicylate infusion. Overall baseline means were 38.8°C for rectal temperature and 2.8 for appetite score. Pooled SEM ranged from .2 to .42 and .6 to 1.4, corresponding to a maximum of 4 and 3 samples per mean for temperature and appetite, respectively.

Figure 4. Deviation of milk production from baseline. Least squares means of total milk production (top) and milk production from the endotoxin-infused quarter (bottom) from control cows (○) and cows receiving i.v. sodium salicylate infusion (●) are plotted according to hours from endotoxin infusion. Bars indicate period of sodium salicylate infusion. Overall baseline means were 21.5 kg for total milk production and 5.2 kg for endotoxin-infused quarter milk production. Pooled SEM ranged from .81 to 1.23 and .31 to .47 for total and endotoxin-infused quarters, respectively, corresponding to a maximum of 2 samples per mean.

daily at 0600 and 1800 h until the end of the experiment. Milk production at the 1800-h milking on d 0 was not included for analysis because the frequent milk samples taken during that day would have unevenly affected estimates. Because a quarter milking machine was available only for the final cows in this study, milk production results are available only for those cows.

Both groups had reduced total milk production by 21 h after endotoxin infusion (Figure 4). Total milk production for the control group returned to baseline more rapidly than for the group treated with SS. Milk production declined in the quarter that had been infused with endotoxin in the group treated with SS and in the control group by 21 h postinfusion (P < .004, P < .04, respectively; Figure 4). Treatment with SS had no effect on the endotoxin-induced decline in milk production. Others (5) have shown that milk production also is decreased after flunixin meglumine administration.
CONCLUSIONS

At the concentration used in this study, SS resulted in a moderately high therapeutic plasma concentration. In the acute phase of mastitis, SS slightly decreased the entry of somatic cells and BSA into the milk but did not prevent these effects of intramammary endotoxin infusion. Gross mammary inflammation was not significantly reduced by SS treatment. Sodium salicylate did not prevent an increase in PGFM in lymph, but combined results from mammary blood and lymph PGFM suggest that SS may have reduced the mammary 

PGF2α production in endotoxin-challenged cows. The results of this study do not provide direct evidence of PGF2α generation in mammary tissue during inflammation. However, the endotoxin-induced increase in lymph PGFM supports the hypothesis that the inflammatory mediator is produced locally in the mammary tissue.

The increase in rectal temperature after endotoxin infusion was minimized in cows treated with SS and SS treatment may be beneficial for its antipyretic effect. In contrast, appetite was not affected by SS treatment and was reduced in the hours postinfusion in cows treated with SS and in untreated cows. Inappetence after intramammary endotoxin challenge may have contributed to reduced milk production.

Overall, in this study, SS gave only limited protection from the systemic and intramammary inflammatory effects of endotoxin. Antiinflammatory drugs are a diverse group, and their individual actions may be equally diverse. Our knowledge is limited about NSAID in general and about their actions in food-producing animals in particular.

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REFERENCES


