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ORIGINAL PAPER

Effect of bromelain on milk yield, milk composition and mammary health in dairy goats

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Abstract A 7 month prospective cohort study was designed to determine if feeding bromelain to dairy goats influenced the MSCC, milk yield, milk composition and the incidence of IMI. Forty-four clinically normal goats from 2nd to 6th parities were studied. Daily bromelain dosage was 7.4 grams/animal (185-mg/Kg weight). Samples for diagnostic bacteriology were collected from each udder half every 2 weeks. Samples for MSCC and composition were obtained every 42 days. Milk yield was also recorded every 42 days. Bromelain affected milk protein and fat but not MSCC, milk yield or milk lactose. Bromelain did not decrease the MSCC in healthy goats. Milk protein and fat increased in the bromelain treated group ($P<0.01$), which is important for dairymen because premiums are paid milk fat and protein content. No clinical mastitis was detected in the goats for the total study period and incidence rate of subclinical IMI was 5.7%. Relative risk was 1.50 ($0.28<RR<8.12$) which means that the bromelain had no significant effect on IMI ($P>0.05$). In addition, the use of pineapple by-products could be especially important in tropical countries were pineapple waste seems to be a pollution problem.

Keywords Bromelain · Goat · Milk composition · Milk production · Subclinical intrammamary infection

Abbreviations

IMI	intramammary infection
MSCC	Milk somatic cell counts
RR	relative risk

Introduction

Bromelain is a general name of a family of proteolytic enzymes obtained from *Ananas comosus*, the pineapple plant. The most common application of bromelain is as an anti-inflammatory agent and anti-oedematous, antithrombotic and fibrinolytic activities have been reported (Maurer 2001). Because of its anti-diarrhoeal effect, researches demonstrated its ability to prevent induced diarrhoea in rabbits (Mynott et al. 1997) and piglets (Chandler and Mynott 1998). Roselli et al. (2007) proposed that some plant extracts, including bromelain, may represent alternative to in-feed antibiotics to improve the health status of piglets by protecting against enterotogenic *Escherichia coli* strain K88. Because of its anti-inflammatory effects, a study was conducted during the summer in cows

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with subclinical mastitis (Paape et al. 2000). Results indicated that cows fed bromelain during a period of high environmental temperature maintained lower MSCC when compared to controls. This is consistent with an earlier study where feeding bromelain to cows with subclinical IMI decreased MSCC (Otani et al. 1989).

IMI is a specific cause of increased MSCC in goats, but other non-infectious factors such as stage of lactation, milk yield and parity also contribute to increased MSCC (Paape et al. 2001). The legal limit for MSCC in bulk tank for goats in the US is 1×10^6 cells/ml, and many dairy farmers cannot meet this limit. The European Union (EU) has not yet established a legal limit for MSCC in bulk tank milk for small ruminants, but the EU has suggested establishing one in the future (Paape et al. 2001). The decrease in MSCC in goats by bromelain would be of interest to goat producers. However, this effect could increase the risk of intramammary infection, because neutrophils act as a barrier against infection. On the other hand, pineapple waste is an important cause of pollution in tropical countries, and its use as a feed for goats could be important not only for economical reason but also for environmental ones (Llorca-Lionet and Meschy 2000). This 7 mo prospective cohort study was designed to determine if feeding bromelain to dairy goats influenced the MSCC, milk yield and milk composition and also the incidence of IMI .

Materials and methods

Experimental goats

The study herd was located in Murcia (southern Spain) and the mean ambient temperature during the study was 18·7°C with absolute maximum temperature of 41·7°C and absolute minimum temperature of –2·0°C. Forty-four clinically healthy goats from 2nd to 6th parities were studied during 7 mo of lactation (210 d). Animals belonged to the Murciano-Granadina breed and the herd belonged to the *Asociacion Española de Criadores de la Cabra Murciano-Granadina* (ACRIMUR -Jumilla, Murcia. Spain). The sanitary status of the herd was good and it was free of brucellosis and tuberculosis. No clinical cases of Johne's disease or caprine mycoplasmoses had occurred in the previous year.

Goats with similar body weight, and similar records of MSCC and milk production were selected for study (Table 1). The average body weight of the animals was 40 kg. The animals kidded between the 3rd and 4th wk of October and the study lasted until the 3rd wk of May. After parturition, kids were removed from their mothers, received pasteurised colostrum and maintained in a separate barn where milk replacer was used for feeding. Experimental goats were housed in a barn with free access to an open yard. After parturition, bacteriological analyses of aseptically collected milk samples and MSCC were performed weekly. One month after parturition, animals were divided into two equilibrated groups of 22 goats each according to body weight, parity, MSCC and milk production. Goats selected were free from IMI and geometric mean MSCC was 141×10^3 cell/ml for the control group and 126×10^3 cell/ml for the treated group (P=0·97). Animals were machine milked once at day in the morning (8 A.M.). Milking parameters were 90 ppm for pulsation, 44 Kpa for vaccum level and 60/40 for pulsation relation. Bromelain was provided by Ajinomoto C., Inc. (Tokyo, Japan). Pellets were made at the United States Department of Agriculture, Beltsville, Maryland (United States) and shipped to Murcia (Spain). Pellet size was 2·5×0·5 cm and contained 20% bromelain; 30% alfalfa; 30% wheat and 20% dry molasses. Control pellets were similar but did not contain bromelain. Daily bromelain dosage was 7·4 g per animal (185-mg/Kg body weight), with the bromelain treated group receiving 814 g of pellets/d and the control group receiving 651 grams of pellets/d. Both groups were fed pellets daily from day 42 of lactation until day 210 of lactation. The pellets were fed to each group of goats in the feed bunk. Previous to initiating

Table 1 Means (±SE) of log MSCC, milk yield (kg/day), milk composition (%) and body weight (kg) of the control and bromelain groups of milking goats at the start of the study

	Control (n=22)	Bromelain (n=22)
Log MSCC	5.15±0.68[a]	5.10±0.61[a]
Milk yield, kg/day	2.70±0.34[a]	2.87±0.49[a]
Protein, %	3.52±0.24[a]	3.55±0.24[a]
Fat, %	5.75±0.95[a]	5.90±1.09[a]
Lactose, %	4.82±0.18[a]	4.80±0.20[a]
Dry matter, %	14.70±1.03[a]	14.80±1.06[a]
Body weight (Kg)	40.3±0.4[a]	39.8±0.3[a]

[a] Means in a row with different superscripts differ (P<0.05).

the study, a 2 d test was conducted to determine palatability of the pellets and all goats readily ate the pellets. The casein digestion unit of the bromelain was 200.

Goats in both groups received 1·5 kg of concentrate feed (Table 2) and 1 kg of alfalfa hay/day twice at day (9 A.M. and 3 P.M.). Previous to feeding concentrate and hay, the amount of feed that remained in the feed bunk was recorded and then discarded. For both groups, a mean of 910 g of refused food was observed daily for each group. Goats had free access to water. Rations were balanced using the recommended values of Jarrige (1988).

Sample collection

For diagnostic bacteriology, 10 ml of foremilk was collected aseptically from each udder half every week for the first month and every 2 weeks for the rest of study. A total of 1408 milk samples from half-udders were bacteriologically analysed. Teats were scrubbed with cotton saturated with 70% ethanol and the first three streams of milk were discarded. Samples were kept at 4°C for 2 to 4 h until bacteriological analysis.

Table 2 Ingredients and chemical composition of the concentrate feed for the control and bromelain groups of milking goats

Ingredients, g kg^{-1}	
Corn grain	250
Barley grain	190
Rye grain	110
Carob bean	85
Beet liquid molasses	20
Alfalfa	30
Soybean meal, (44% CP content)	264
Fat	10
Salt	7
Dicalcium Phosphate	14
Calcium carbonate	14
Vitamin /mineral mix [a]	5
Chemical analysis, %DM	
Dry Matter	87.4
Crude protein	18
NDF	24
Ether Extract	2.9

[a] Provided by Trouw Nutrition España S.A. to give (ppm or UI per kilogram of diet) : Se, 40 ; I, 250 ; Co, 80 ; Cu, 3000 ; Fe, 6000 ; Zn, 23400 ; Mn, 29000 ; S, 60000 ; Mg, 60000 ; vitamin A, 2000000 UI ; vitamin D3, 400000 ; vitamin E, 2000 ppm ; nicotinic acid, 10000; choline, 20300.

Individual mycoplasma analysis (n=132) were carried out in three different samplings (early, middle and late lactation). Data for milk composition and yield was obtained from the official dairy goat milk recording service (ACRIMUR Association) performed every 42 d (n=5). Goat milk yield was recorded using calibrated jars and composite milk samples (40 ml, containing potassium dichromate) were collected for milk composition (fat, protein, lactose and dry matter). Composite udder samples (40 ml without preservative) for MSCC were obtained on the same test day. Both samples were maintained at 4°C and transported to the laboratory.

Bacteriological procedure

Ten µl of each sample were plated on blood agar plates (5% washed sheep erythrocytes). The plates were incubated aerobically at 37°C and examined at 24, 48, 72, and 168 h. An IMI was determined at 500-colony forming units/ml. Bacteria were identified according to the recommendations of the National Mastitis Council (Harmon et al. 1990). Identification of staphylococci was made using commercial micromethods (API® STAPH. BioMèrieux, Lyon. France). Mycoplasma analyses were carried out using modified Hayflick medium in duplicate (agar and broth) and incubated in a high-humidity CO_2 (5%) incubator for 2 d, passaged on agar media, as well as subpassaged in broth, and incubated for an additional 9 d. When the same pathogen was isolated two or more times from the same udder half, it was considered a true positive diagnoses of IMI.

Milk somatic cell count procedure, test day milk yield and milk components

Refrigerated milk samples were analysed for MSCC within 24 h of collection using a Fluor-opto-electronic counter (Fossomatic 90. Foss Electric, Hillerød, Denmark). Samples were heated at 60°C for 15 min before counting. Milk composition analysis were carried out using a Milkoscan 133 v3·5 GB (Foss Electric, Hillerod, Denmark).

Statistical analyses

Relative risk (RR) was used to evaluate the effect of bromelain on the incidence of IMI using Statcalc

program from Epiinfo 6·04 (Dean et al. 1994). The logarithm of MSCC/ml was used to normalize distribution. Statistical analyses were carried out using the General Linear Model procedure for repeated measurements of SAS System for Windows, release 8·0 (SAS Institute 1999). The model contained the fixed effect of bromelain treatment; parity group (group 1: 2nd and 3rd; group 2: 4th and group 3: 5th and 6th parity), days in milk (42 d, 84 d, 136 d, 168 d and 210 d), the bromelain treatment and parity group interaction and the bromelain treatment and days in milk interaction. For each dependent variable studied (log MSCC, milk yield, protein, fat, lactose, dry matter), data for the first control were used as a covariate to correct for differences in initial values.

Results and discussion

Five udder halves of 5 different goats became subclinically infected during the trial, 3 in the treated group and 2 in the control group, the cumulative incidence of subclinical IMI being 5·7%. No clinical mastitis was detected in the goats for the total study period. Four of the 5 pathogens isolated were *Staphylococcus caprae* and the other was *S. epidermidis*. No mycoplasma infection was detected. Two animals were diagnosed infected at 45 d of lactation, and the others at 75, 90 and 150 d. All *S. caprae* infections lasted for the remainder of the lactation but the *S. epidermidis* infection was self eliminated at 120 d after persisting in the mammary half for 2 mo. Relative risk was 1·50 (0·28<RR<8·12) which means that the bromelain had no significant effect on IMI ($P>0·05$), and represented neither a risk factor nor a protective factor. The low rate of subclinical intramammary infection detected in the all goats agree with the previously described good sanitary status of the herd, and with a previous report of low IMI in well managed goat herds of Murciano-Granadina breed (Contreras et al. 1997).

The bromelain treatment did not affect the incidence of subclinical or clinical IMI. No clinical mastitis was detected in both groups and incidence of subclinical IMI was similar for both groups (6·18% in bromelain treated versus 4·5% in control). As an inmunomodulator, bromelain possesses immuno stimulatory properties against bacteria, as has been demonstrated with *Candida albicans* in vitro (Brakebusch et al. 2001). The present work is an epidemiological prospective cohort study using healthy goats in well managed conditions. Because of the low rate of IMI, other conclusions about bromelain in mastitis affected animals are not possible. However, due to RR values and intervals around the unit, it appears that bromelain treatment was not a risk factor for clinical of subclinical IMI. Because of the anti-inflammatory effect of bromelain, one of the possible effects of its use could be a negative one increasing the risk of IMI by neutralizing the natural defenses of the udder. Exposure of neutrophils to bromelain causes them to release reactive oxygen species that could cause damage not only epithelial cells but possibly also neutrophils (Zavadova et al. 1995). Because oral administration of bromelain seems not to act as risk factor for IMI, further studies to determine if bromelain would reduce MSCC in goats with subclinical IMI, similar to what has been reported (Paape et al. 2000) in dairy cows with subclinical mastitis would be of interest, particularly in high IMI prevalence conditions.

The statistical analyses (Table 3) indicated that MSCC, milk yield and milk lactose were not affected by treatment, but bromelain treatment increased milk protein and milk fat. Means for milk production, milk fat and protein from the goats in this study were similar to that reported for this same breed (Parr 2002). Bromelain is useful as a digestive enzyme and like most digestive enzymes, it is active in both the acid and alkaline environment of the digestive tract (Taussig et al. 1975, Taussig and Batkin 1988). Because of its stability in the gastrointestinal tract and the ability of its proteolytic enzymes to degrade protein, bromelain increases the efficiency of digestion of proteins in feed stuffs and possibly other nutrients (Hale 2004). Further, the proteolytic activity of bromelain also allows it to

Table 3 Least squares means (±SE) of dependent variables studied according to bromelain treatment for both groups of milking goats (group control and group exposed)

Variables	Control	Bromelain
Log MSCC	5·67±0·05[a]	5·73±0·05[a]
Milk yield, kg/day	2·29±0·05[a]	2·18±0·05[a]
Protein, %	3·80±0·02[a]	3·90±0·02[b]
Fat, %	5·59±0·08[a]	5·92±0·09[b]
Lactose, %	4·66±0·02[a]	4·67±0·02[a]
Dry matter, %	14·67±0·09[a]	15·09±0·09[b]

[a, b] Means in a row with different superscripts differ ($P<0·01$)

act as an anti-inflammatory agent in the gastrointestinal tract. Taken together, these effects may allow for a higher and more efficient rate of protein absorption from the gut that could contribute to an increase in the concentration of protein in milk observed in the present study. This increase in milk fat ant milk protein would be an important effect because premiums paid to dairymen include not only milk hygienic standards (MSCC and total bacteria in milk) but also milk fat and protein content. Because of the increase of the milk protein and milk fat, the use of bromelain could be especially important on those tropical countries where pineapple waste is a pollution problem. In contrast to our initial hypothesis, bromelain did not decrease the MSCC in healthy goats as did in cows with subclinical IMI (Otani et al. 1989) and maintained at high ambient temperatures (Paape et al. 2000). In our study, the temperature registered throughout the experiment was considered moderate according to the Mediterranean weather. In addition, the goats utilized in the study are indigenous (Murciano-Granadina breed) and well adapted to climate conditions in Murcia. All goats in our study were free of either clinical or subclinical IMI and the new infection rate for subclinical IMI was very low (less than 6%), and was in accord with the high sanitary standard of the farm. In future studies, it would be of interest to know if bromelain could help to maintain intramammary health status in poor management conditions (higher rate of prevalence of IMI) or even after experimental challenge with mayor pathogens for goat udder as *Mycoplasma agalactiae* or *Staphylococcus aureus*.

Human clinical studies and animal experiments have demonstrated that the effect of bromelain is dose-dependent, where the least effective dosage was reported to be 5 mg/kg body weigh (Lozt-Winter 1990). The animals in our study received daily an oral dosage of 185 mg/kg, which should have been enough to produce the responses observed in this study, but in further studies the dosage could be another factor for future research. The toxicity of bromelain when given orally has been reported to be more than 10 g/kg body weigh for rats and mice (Lozt-Winter 1990).

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