Relative Buffering Capacity of Goat Milk, Cow Milk, Soy-Based Infant Formulas, and Commercial Nonprescription Antacid Drugs[1,2]

YOUNG W. PARK
Cooperative Agricultural Research Center
Prairie View A&M University
The Texas A&M University System
PO BOX U
Prairie View 77446-2886

ABSTRACT

Buffering capacities of goat milk (Alpine, Nubian), cow milk (Holstein, Jersey), soy-based infant formulas, and nonprescription antacid drugs were estimated. Total N, protein, NPN, and P_2O_5 as major buffering entities were quantified for each milk category. Nubian goat milk had the highest levels of the three major buffering chemical entities, and the infant formulas contained less total N and NPN compared with natural goat and cow milks. Buffering capacities of the formulas also were lower than those of natural milks. Combinations of milk and antacid drugs had higher buffering capacities than either the milk or drug alone. Drug plus goat milk combinations upon addition of more than 2 ml of acid titrant exhibited fewer changes in pH than the respective drug plus cow milk combinations.

(Key words: buffering capacity, goat milk, infant formulas, antacid drugs)

Abbreviation key: **BC** = buffering capacity.

INTRODUCTION

Goat milk, unlike cow or human milk, has unique characteristics, such as its high digestibility, distinct alkalinity, high buffering capacity (BC), as well as its reported therapeutic use in medicine and human nutrition (5, 7, 8, 14, 17, 22). Goat milk is similar to cow milk in its basic composition, although it contains more fat, protein, and mineral and less lactose (8, 10). The protein, primarily casein and phosphate systems within milk, influences its BC (24).

Buffering capacity of a foodstuff is determined by its acid-base equilibrium, which attenuates pH changes upon exposure to acid or alkali. Upon ingestion, food initially acts as a neutralizing substance or antacid by buffering gastric acid, after which the food stimulates gastric acid secretion (26). Amount of acid secreted is influenced not only by the quantity and source of ingested food protein (12, 13, 16) but also by two gastrointestinal hormones (12). Gastrin is the primary stimulator (3, 6), and somatostatin is an inhibitor of food-stimulated acid secretion (4, 23). When gastric acid secretory values of foods were compared, foods with the highest content of carbohydrate or fat produced the least gastric secretory response per calorie (18). Meat, fish, and egg products stimulated significantly greater gastric secretion than milk and dairy products (18). Ionizable groups within protein and amino acids affect the ability of proteins to stimulate gastric acid secretion (12, 15). Consequently, BC may be an important consideration in human or infant nutrition.

Milks of goat and bovine origin have not been characterized adequately or comparatively relative to their BC. Objectives of this study were to characterize the BC of milks from two breeds of cow (Holstein and Jersey) and of goat (Alpine and Nubian). For comparison, BC of soy-based infant formulas and nonprescription antacid drugs also were determined.

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[2]Mention of brand name does not imply endorsement by Prairie View A&M University nor US Department of Agriculture over similar products that are equally suitable.

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MATERIALS AND METHODS

Experimental Design

Two experiments were conducted to compare BC of natural goat and cow milks with soy-based infant formulas and commercially available nonprescription antacid drugs.

In Experiment 1, in a completely randomized block design, five Alpine and five Nubian goats and five Holstein cows in their 2nd yr lactation were selected randomly from the milking goat and cow herds of the International Dairy Goat Research Center. Due to absence of Jersey breed in the university herds, the milking Jersey cows were selected randomly from a local dairy farm located at Waller County, TX. The milks from individual animals of two goat breeds, two cow breeds, and two brands of soy-based infant formulas were used for testing buffering intensities with gradual addition of .1 and .5N HCl to each milk group.

In Experiment 2, BC of goat and cow milks were compared with those of nonprescription antacid drugs. Goat and cow milks were taken from the bulk milk tanks located at two separate milking parlors once a day at 1530 h for 5 d.

Three brands of antacid drugs were purchased from local pharmacies. The BC of drugs alone and drug plus goat and cow milks were compared for the differences in buffering intensities. Treatment groups for each drug within Experiment 2 were composed of goat bulk milk, cow bulk milk, drug alone (blank), drug plus goat milk, and drug plus cow milk.

Preparation of Animal Milk Samples

All animals were machine milked (BOU-MATIC, DEC International, Madison, WI), and samples were taken from a graduated measuring cylinder attached to individual milking units. Thus, a representative sample was collected during each complete milking. Milk sampling of goats and cows was done twice daily at 0530 and 1530 h for 5 d. Bulk milk samples were also taken at the same times. Milk samples were collected into 2-oz (59-ml) plastic bags (Whirl-Pak, NASCO, Fort Atkinson, WI) and transported to the laboratory for immediate examination. Initial pH conditions

were recorded prior to consecutive titrations with .1 or .5N HCl.

Preparation of Samples for Soy-Based Infant Formulas and Nonprescription Antacid Drugs

Two brands of soy-based infant formulas, were purchased from a local retail grocery store. Both formulas were canned, ready to feed products. After shaking, an aliquot (25 ml) was transferred into a 50-ml beaker for each testing.

Three brands of commercially available nonprescription antacid drugs were obtained from the over the counter shelves of a local retail outlet or pharmacy. One tablet of each brand was solubilized either in 100 ml of deionized water or dissolved directly into the same volume of goat or cow bulk milks contained within a 125-ml screw-cap glass bottle. Aliquots (25 ml) of the dissolved water or milk solutions were used for testing pH.

Chemical Analysis of Buffering Components in the Milks

Concentrations of total N, total CP, NPN, and P_2O_5 for all milk samples were analyzed as the major buffering chemical constituents. Samples (10 g) were wet digested in 30-ml Kjeldahl flasks, and total N, NPN, and P_2O_5 were determined by colorimetric procedures as described by Belec and Jenness (2) and AOAC (1). For NPN determination, protein fraction was precipitated with 10% trichloroacetic acid, followed by centrifugation at 700 × g for 10 min at 4°C. One milliliter of resultant supernatant was decanted and transferred into a Kjeldahl flask for digestion. An amount of N was determined for this NPN fraction.

Determination of Buffering Capacity

Initial pH values of all milk samples of individual goat and cow, soy-based infant formulas, and antacid drug solutions were determined. Two normalities of hydrochloric acid (.1 and .5N) were prepared for titration of all samples. Two aliquots (25 ml) of each sample were placed in 50-ml beakers, whereupon 1 ml of either acid was titrated slowly with thorough stirring. However, only experimental data from .5N acid titration were reported in this

study because of insufficient responses with .1N acid as titrant. The pH was measured after completion of each titration, and the BC was determined mathematically using the buffering intensity formula given by Van Slyke (21):

$$\frac{dB}{dpH} = \frac{(ml\ acid\ added)\ (normality\ of\ acid)}{(volume\ of\ milk)\ (pH\ change)} \cdot$$

Statistical Analysis

All data for the differences and changes in pH of milk and drug treatment groups were analyzed by analysis of variance (20). Model included species, breeds, milk groups, antacid drugs, milking time, normality of acid, and their interactions. Unbalanced data were analyzed using the general linear models of the SAS program (19). Significance of differences in mean BC and levels of chemical constituents between milk treatment groups were analyzed also by multiple mean comparison using F values of orthogonal contrasts between group means.

RESULTS AND DISCUSSION

Concentrations of total N, NPN, and P_2O_5 in goat milk, cow milk, and commercial soy-based infant formulas are shown in Table 1. Among the six milks, Nubian goat milk contained the highest concentration of each of the three major buffering chemical entities. Soy-based infant formulas had lower total N and NPN compared with natural goat and cow milks. No differences in phosphate contents were found among the six milks.

Significance of F values for orthogonal contrast between different milk groups in terms of the levels of chemical constituents (Table 2) revealed highly significant $(P < .01)$ differences in total N and NPN between natural milks and formula milks. Orthogonal contrast of goat and cow milk revealed no differences in total N and P_2O_5, but a significant $(P < .01)$ difference was observed in NPN content. None of the contrasts between milks for P_2O_5 content was significant, but all combinations between species and breeds in total N and NPN levels were significant $(P < .05$ or $P < .01)$ except in the case of Alpine versus Holstein for total N and Holstein versus Jersey for NPN

content. The differences in total N and NPN contents between the two brands of infant formula were not significant (Table 2).

Comparisons of BC and pH changes among the six milks upon titration with .5N HCl are presented in Tables 2 and 3 and Figure 1. The greater resistance to pH change for Nubian and Jersey milk (Table 3) was related to the higher contents of total N, NPN, and P_2O_5 in these milks (Table 1). Others (9, 21, 24, 25) have reported that BC is correlated highly with the content of these buffering components.

Nubian milk showed consistently greater pH values $(P < .01)$ than the other five milks for all titration volumes (Table 3). This higher BC of Nubian milk undoubtedly is attributable to its higher total N, NPN, and phosphate concentrations. The stronger BC of Nubian goat milk is in agreement with previous reports (8, 22). This fact may be significant in human nutrition because foods having higher BC can be utilized therapeutically in treatment of gastric stomach ulcers (8).

Depending upon milking time, stage of lactation, and individual animals tested (data not presented), no differences in total N and phosphate contents existed between Nubian and Jersey milks. However, levels of NPN in Nubian milk were consistently greater than those in Jersey milk (Table 1).

Jersey milk in a few cases showed stronger resistance to pH changes than Nubian milk. Differences in physicochemical specificity and stereochemical configuration of buffering protein molecules within the two milks might result in their differential exposure to H^+ ion in the titration medium. In addition to molecular specificity of protein, processing factors such as pasteurization and homogenization might influence BC of the milks. For example, approximately one-half of a major buffering entity, CO_2, contained in commercial unpasteurized milk is lost by heating, agitation, or vacuum treatment (11). All milks evaluated in this study were not pasteurized or homogenized.

Acid secretion of stomach depends largely on the amounts of food protein or amino acid present (13, 16) as well as the specific protein source (12) because proteins differ in their ability to stimulate gastric secretion. This effect is related directly to the BC of the specific

TABLE 1. Concentration of total N, NPN, and phosphate in natural goat and cow milk and soy-based infant formulas.[1]

Milk group	n[2]	Total N		NPN		P_2O_5	
		$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
Goat milk							
Alpine	25	.390[c]	.032	.048[b]	.008	.166[a]	.020
Nubian	25	.556[a]	.013	.061[a]	.013	.212[a]	.015
Cow milk							
Holstein	25	.392[c]	.058	.033[c]	.002	.173[a]	.022
Jersey	25	.505[b]	.043	.038[c]	.004	.211[a]	.118
Formula milk							
Brand A	5	.227[d]	.026	.020[d]	.003	.211[a]	.008
Brand B	5	.259[d]	.016	.019[d]	.003	.192[a]	.053

[a,b,c,d]Means with different superscripts within a same column are significantly different ($P < .01$).

[1]Expressed in grams per 100 ml.

[2]Number of determinations per mean value.

proteins in the food ingested. Osmon et al. (13) reported that providing 50 g of protein from beef meat to normal human subjects reduced gastric pH much more rapidly (66 ± 16 min to reach pH 3.0 or less) than similar amounts of protein from chicken (105 ± 16 min), fish (110 ± 22 min), egg (125 ± 15 min), milk (137 ± 17 min), or soybean (184 ± 12 min). McArthur et al. (12) also reported that in humans, beef stimulates 30 to 40% more gastric acid production and 65 to 75% more gastrin, the primary physiological stimulant of gastric acid secretion, than soy protein. Thus, differences in buffering groups of amino acids within Nubian and Jersey milks might account for the differ-

ing amounts of acid that are required to titrate the two milks to the same pH endpoint.

Orthogonal contrasts for pH changes at various levels of titrant were variable depending on the contrast (Table 2) as evidenced by the associated BC changes (Figure 1). In the pH range from 5.6 to 3.6, Nubian milk showed the highest buffering intensity, followed by Jersey, Alpine, Holstein, and the infant formulas (Figure 1). Above pH 5.6, considerable variations occurred in BC between milks mainly due to differences in initial pH values.

Up to 4 ml of .5N HCl titrant (pH range 5.8 to 4.2; Table 3), the Nubian milk showed consistently greater ($P < .01$) buffering index

TABLE 2. Comparison of significance (F value) of orthogonal contrast between two treatment means of different combinations.

| Orthogonal contrast	df	F Values of orthogonal contrast							
		Chemical components			pH after addition of .5N HCl to 25-ml sample				
		Total N	NPN	P_2O_5	1 ml	2 ml	3 ml	4 ml	5 ml
Goat milk vs. cow milk	1	2.52	39.84**	.01	14.94**	4.92*	5.85**	12.58**	17.70**
Alpine vs. Nubian	1	59.44**	9.26**	1.75	3.92	8.93**	54.35**	64.05**	41.74**
Holstein vs. Jersey	1	27.34**	1.24	1.09	11.76**	12.64**	18.54**	20.15**	28.24**
Alpine vs. Holstein	1	.01	12.23**	.03	3.93	1.65	10.52**	18.19**	12.59**
Alpine vs. Jersey	1	28.58**	5.69*	1.50	29.30**	23.43**	56.99**	76.63**	78.54**
Nubian vs. Holstein	1	57.63**	42.79**	1.28	.00	2.90	17.04**	13.97**	8.48**
Nubian vs. Jersey	1	5.59*	29.47**	.00	12.13**	3.43	.03	.56	5.77*
Natural vs. formula milk	1	306.8**	95.24**	.34	19.44**	17.04**	10.27**	545.4**	273.5**
Formula A vs. Formula B	1	2.66	.00	.43	2.30	39.71**	100.3**	20.83**	6.85*

*$P < .05$.

**$P < .01$.
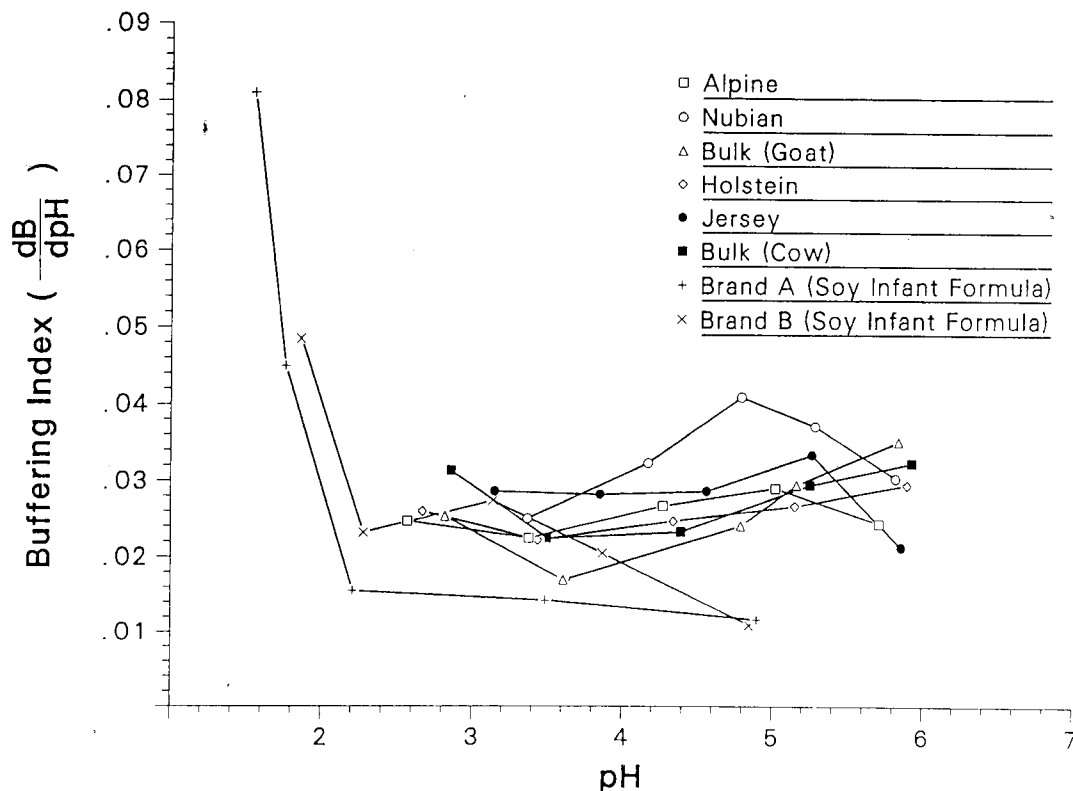
Figure 1. Buffering capacities of natural goat and cow milks compared with those of soy-based infant formulas. Number of observations for Alpine, Nubian, Holstein, Jersey, and brand A and B formula milks were 25, 25, 25, 25, 5, and 5.

values (dB/dpH) among the six milks (Figure 1). The difference among the natural goat and cow milks then became minimal at the titration of 5 ml of the HCl. The buffering index values of Nubian milk were consistently superior to those of Alpine. The similar relationship observed between Jersey and Holstein milk indicated that breed differences in BC existed within both species (Tables 2 and 3; Figure 1).

The buffering index values of the infant formulas were lower $(P < .01)$ than the natural milks up to pH near 2.4 (Figure 1). Although the BC indices of the formula milks were substantially higher than those for goat and cow milks beyond 4-ml volume of titrant, the formula milks did not have higher BC than natural milks. Rather, the high buffering index values less than pH 2.4 were due to excessive dissociated free acid. Van Slyke (21) demon-

strated that, at the more acid and alkaline ranges, the partial buffering index values become highly significant solely because of dissociated free acid and alkali, respectively. This, in turn, suggests that the high buffering indices may not represent strong BC of the testing solution if it has excess dissociated free acid or alkali. On addition of ≥ 3 ml of HCl, brand A formula had a significantly $(P < .01)$ lower pH than the brand B formula and the natural milks (Table 3).

When three commercial antacid drugs were solubilized in the double deionized water, the initial pH of each drug was significantly $(P < .01)$ greater than goat or cow milk and drugs plus milk treatment groups (Table 4). In fact, the dB/dpH values for the initial solutions of the three drugs were much beyond the scale in Figure 2. Consequently, dB/dpH scale was ad-

TABLE 3. Comparison of pH changes in different group milks by gradual addition of .5N HCl to 25-ml samples.

Milk group	n[1]	Initial	pH after addition of .5N HCl				
			1 ml	2 ml	3 ml	4 ml	5 ml
Goat milk							
Alpine	25	6.53	5.71[b]	5.02[b]	4.27[c]	3.38[c]	2.57[c]
Nubian	25	6.48	5.82[b]	5.28[a]	4.79[a]	4.17[a]	3.37[a]
Cow milk							
Holstein	25	6.58	5.90[a]	5.15[ab]	4.34[c]	3.44[c]	2.67[c]
Jersey	25	6.80	5.86[b]	5.26[a]	4.56[b]	3.85[b]	3.15[b]
Soy infant formula							
Brand A	5	6.62	4.90[c]	3.49[d]	2.20[e]	1.76[e]	1.54[e]
Brand B	5	6.68	4.85[c]	3.87[c]	3.14[d]	2.28[d]	1.86[d]

[a,b,c,d]Means with different superscripts within the same column are significantly different ($P < .01$).

[1]Number of samples tested for milk pH changes using .5N HCl as titrant.

justed for the outlying data points. Drug A contained dihydroxyaluminum sodium carbonate, but drugs B and C were made of calcium carbonate and aluminum magnesium hydroxide. Drug A had significantly higher initial pH than drugs B and C. The alkaline pH of the initial drug solutions were reduced drastically at the first titration with 1 ml .5N HCl (Table 4; Figure 2). The abruptly changing patterns for dB/dpH values, which characterized the three drug-alone groups, were due to the dras-

tic changes in pH that occurred upon subsequent titrations (Figure 2). Drug B showed the greatest pH and buffering intensity values as the sample solutions were titrated gradually (Table 4). The initial pH of the drugs plus milk samples of drugs A and B were close to the pH values of initial goat or cow milk alone. The pH was neutral when drug C and the milks were combined. This phenomenon indicated that goat and cow milk had sufficient BC to neutralize the excessive alkaline buffering

TABLE 4. Profiles of pH changes after addition of HCl to 25-ml antacid drug solutions, drugs plus milks, and goat and cow bulk milk groups.

Treatment group[1]	n	Control	pH after addition of .5N HCl				
			1 ml	2 ml	3 ml	4 ml	5 ml
Drug A							
Blank (H₂O)	5	9.22[a]	4.40[d]	4.04[d]	2.49[e]	2.04[e]	1.87[f]
A + Goat milk	5	6.61[e]	5.84[b]	5.30[b]	4.78[bc]	4.00[c]	3.27[c]
A + Cow milk	5	6.83[d]	6.22[a]	5.39[b]	4.54[c]	3.65[d]	3.00[cd]
Drug B							
Blank (H₂O)	5	8.49[b]	5.61[c]	5.14[c]	4.94[b]	4.58[a]	2.35[e]
B + Goat milk	5	6.52[f]	5.81[b]	5.34[b]	4.95[b]	4.67[a]	4.45[a]
B + Cow milk	5	6.70[d]	6.14[a]	5.52[a]	4.80[bc]	4.39[b]	4.08[b]
Drug C							
Blank (H₂O)	5	8.55[b]	2.45[e]	2.05[e]	1.85[f]	1.73[f]	1.66[f]
C + Goat milk	5	7.10[c]	6.12[a]	5.61[a]	5.04[a]	4.21[b]	3.53[c]
C + Cow milk	5	7.09[c]	6.27[a]	5.63[a]	4.85[bc]	4.08[c]	3.40[c]
Goat bulk milk	5	6.41[g]	5.84[b]	5.16[c]	4.49[c]	3.61[d]	2.82[d]
Cow bulk milk	5	6.55[f]	5.93[b]	5.25[b]	4.39[d]	3.50[d]	2.86[d]

[a,b,c,d,e,f]Means with different superscript within the same column are significantly different ($P < .01$ or $P < .05$).

[1]Drug A, Dihydroxyaluminum sodium carbonate; drug B, calcium carbonate; drug C, aluminum magnesium hydroxide.
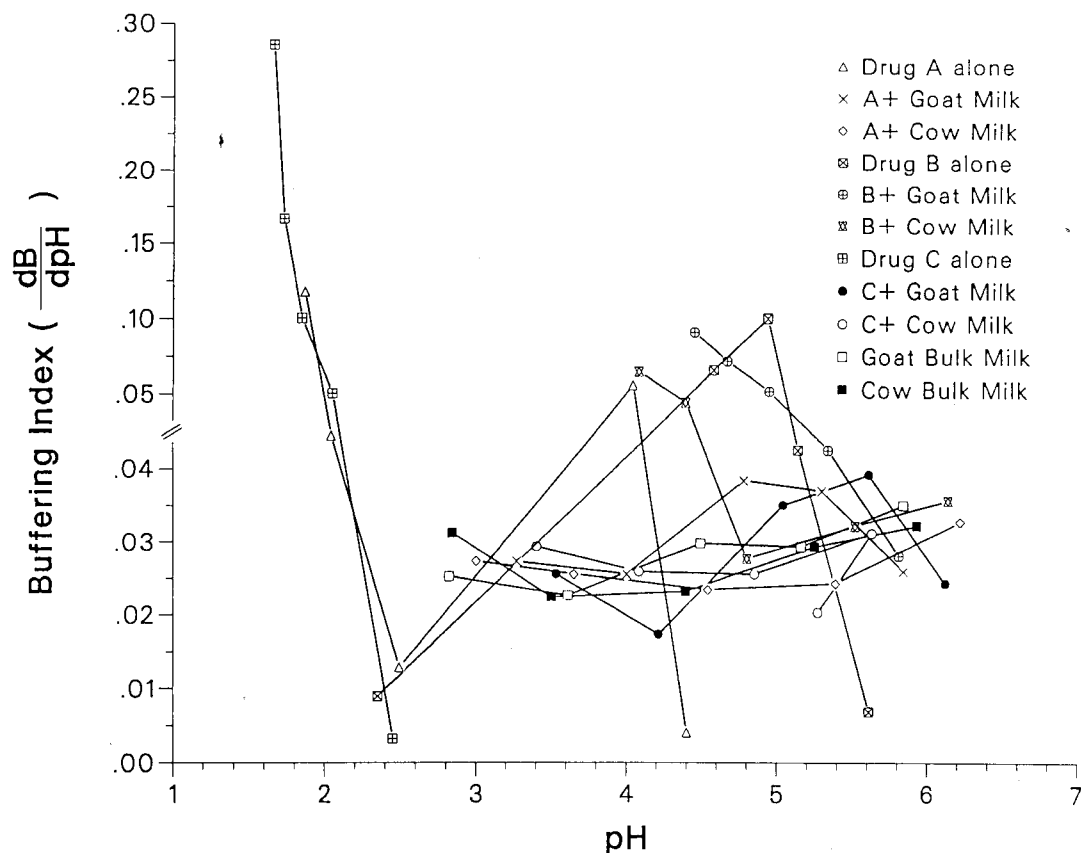
Figure 2. Comparison of buffering capacities of goat and cow bulk milks with those of three brands of nonprescription antacid drugs and with drugs plus goat and cow milk groups. Drugs A, B, and C were made up of dihydroxyaluminum sodium carbonate, calcium carbonate, and aluminum magnesium hydroxide, respectively. Number of observations in each treatment group is as indicated in Table 4.

components of the drugs. Changes in pH of the water solutions of the three drugs were rapid and significant (P < .01), but the drug plus milk or the milk groups showed considerably slower changes in pH (Table 4).

The drug plus milk groups showed distinctively higher pH and buffering intensity values than the drug solutions alone for all drugs studied (Table 4; Figure 2). Extremely high dB/dpH values for the drug-alone groups does not mean higher BC. Rather, they had excessive free acid in the medium (Figure 2). For drug-treated groups, goat milk plus drug groups displayed consistently and significantly (P < .01) greater BC than cow milk plus drug groups beyond 2 ml of the acid titration. This observed higher BC in goat milk is in agree-

ment with previous reports (8, 22). Interestingly, the pH values for the drug plus goat milk groups for all three drugs up to 2 ml of acid titration were lower than the drug plus cow milk groups but were higher upon further titration (Table 4). This observation suggested that the physicochemical makeup of goat milk is different from that of cow milk. The trends of the buffering intensities for all drug-treated groups were very consistent for all titrations of both goat and cow milks. This consistency might have resulted from a physicochemical shift of the buffering groups of chemical entities in drugs and milks as well as from an altered stoichiometric arrangement of casein micelles. Whittier (25) interpreted the buffer intensity curve of casein determined by differ-

ence to indicate that the buffer action of casein is exerted primarily between pH 4.5 and 5.7 with a maximum at approximately pH 5.2. Casein is evidently one of the primary factors in the buffer action¹ of milk in this range.

Comparison of goat and cow bulk milks with the respective milks plus drugs indicated that the latter had significantly ($P < .05$ or $P < .01$) greater BC (Table 4). For bulk milk groups, goat milk displayed less BC up to 2-ml volume of HCl titrant. Upon further titration, no differences existed.

CONCLUSIONS

Major buffering chemical entities of milk were influenced by species, breed, and breeds within species. Nubian goat milk displayed a higher BC compared with Alpine, Holstein, and Jersey milks. Chemically dissimilar antacid drugs, when dissolved in deionized water, had different BC. Higher buffering intensity likely occurs if antacid drugs are consumed with milks.

ACKNOWLEDGMENTS

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