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Evaluation of Goat Milk Containing Galactooligosaccharides After Supplementing the Ration with Amino Acids

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Abstract: The aim of this study was to evaluate the effect of supplementing goat's ration with different forms of lysine and methionine on milk production, its chemical composition and consumer's acceptability. To maximize goat milk benefits, different concentration of β-D-galactosidase were used to form galacto-oligosaccharides after supplementing the ration with lysine and methionine in protected and unprotected forms. Milk acceptability and its chemical composition were evaluated compared to control samples. The results showed that milk fat, lactose, total protein and milk yield were increased by adding the amino acids in both different forms. Organoleptic evaluation indicated significant increase in taste and overall acceptability of goat milk containing higher concentration of galactooligosaccharides. Also, supplementing goat ration with these amino acids should be recommended to increase milk production and its contents. Formation of galactooligosaccharides in goat milk could be recommended to decrease most of infant's allergy and diseases.

Key words: Goat milk, galactooligosaccharides, β-D-galactosidase, sensory evaluation, amino acids, lysine and methionine

INTRODUCTION

Goat milk and its products of yoghurt, cheese and milk powder have three-fold significance in human nutrition: (1) feeding more starving and malnourished people in the developing world compared to cow milk, (2) treating people afflicted with cow milk allergies and (3) filling the gastronomic requirements of connoisseur consumers which correspond to a growing marker in many developed countries. The importance of goat is reflected in the largest animal number increase for goats during the last 20 years and the largest increase in goat milk production tonnage compared to other mammalian farm animals. Milk production of goats is likely much greater than in these official statistics, because of the large amounts of unreported home consumption, especially in developing countries (Haenlein, 2004). Goat's milk shows therapeutic virtues for individuals with certain dietetic problems, thus physicians have traditionally recommended goats milk for infants and adults with cow milk other allergy (Miller, 2002). Supplementing goat ration with protected lysine and methionine showed higher total protein, true protein nitrogen, non casein nitrogen and casein nitrogen contents of milk protein compared to unprotected form. Also, protected form increased non essential amino acids, while unprotected form increased total essential amino acids in produced milk (Kholif *et al*., 2006).

On the other hand, human milk oligosaccharides are readily fermented in the infant colon where they selectively stimulate the growth of bifidobacteria, lower intestinal pH through the production of acetic and lactic acids which may suppress the growth of pathogenic bacteria (Napoli *et al*., 2003). The addition of oligosaccharides to cow's milk based infant formula has been shown to have a bifidogenic effect, to stimulate the growth of bifidi and lactobacilli and considered one step closer to the golden standard of human milk (Vandenplas, 2002; Boehm *et al*., 2003). Galacto-oligosaccharides seem to relieve constipation in most elderly people but the responses differ individually (Teuri and Corpela, 1998).

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Even if goat milk is considered to be healthy, some consumers dislike its goaty flavor, so the authors intended to increase the acceptability of the goat milk by forming different concentrations of galacto-oligosaccharides. The objectives of this research were to study the effect of supplementing goat's ration with new additives, such as amino acids (lysine and methionine) in to different forms, on the chemical composition of produced milk and evaluate its consumer's acceptability after forming to different concentrations of galactooligosaccharides.

MATERIALS AND METHODS

Feeding Trial

This study was conducted at a private farm, in Om Dinar, Embaba and Dairy Science Department, National Research Center, Giza, Egypt.

Pooled milk samples were donated by Kholif *et al*. (2006), who divided nine lactating Baladi goats, in early lactation, into three experimental groups using 3×3 Latin square design. Control ration was composed of concentrate feed mixture: berseem clover (30:70, on DM) without amino acids supplementation. Group 1, supplemented control ration with unprotected methionine and lysine (2.5 g/head/d). Group 2, supplemented control ration with protected methionine and lysine (2.5 g/head/d). *Kluyveromyces fragilis* β-D-galactosidase (Lactozym® 3000 L) was purchased from Novo, Nordisk A/S, Bagsvaerd, Denmark.

Analytical Method

Milk samples, of the three experimental groups, were analyzed for fat content and total solids according to Ling (1963), ash and protein contents were analyzed by AOAC (2000), while lactose content was analyzed by Barnet and Abdel-Tawab (1957). Solids-not-fat was calculated by differences; pH was recorded using a digital pH-meter with a glass electrode, Model GC, Germany. Amino Acids were determined using LC 300 amino acid analyzer Eppdrof, Germany.

Protein Efficiency Ratio (RER) of goat samples was calculated according to Alsmeyer *et al*. (1974) and the Biological Value (BV) was assayed as mentioned previously by Oser (1959).

Preparation of Goat Milk Containing Galactooligosaccharides

Aliquots of 100 mL of pooled goat milk were dispensed into a 250 mL sterilized glass bottle and inoculated with two different concentrations 0.05 or 0.1% (v/v) of Lactozym®3000 L, after adjusted the pH to 7.0 using NaOH. Milk reaction mixture was incubated at 45°C in a temperature-controlled water bath for 4.5 h, to obtain maximum formation of galactooligosaccharides (Foda Mervat, 1997; Rustom *et al*., 1998). Milk samples were immersed immediately in a boiling water bath for 5 min to inactivate the enzyme before further analysis. Control milk was prepared without enzyme to monitor the differences. Milk samples were stored under refrigerating temperature (5°C-2) for 2 weeks; samples were taken fresh and every week for analysis. Three replicates were carried out for each group to determine some chemical and sensory analysis.

Determination of Galactooligosaccharides

Concentrations of galatooligosaccharides were determined by HPLC according to the method described by Jeon and Mantha (1985). The HPLC system (C-R7D with RID-10A detector, Shimazu Co., Ltd, Kyoto, Japan) using A sahipak NH2-50, column 2D (4.6×250 mm, 20 μm, Phenomenex, USA)), operating at ambient temperature, the column was eluted with HPLC-grade acetonitrile: water (75:25) at a flow rate of 0.6 mL min^{-1}.

Sensory Evaluation

Fifteen panelists (9 male and 6 female) from National Research Center, who are familiar with goat milk grading were participated. They scored milk samples for taste (40 points), color (5 points), odor (5 points) and appearance (50 points). The panelists were also asked to record any unexpected or unpleasant flavor.

Statistical Analysis

Data were statistically analyzed according to Snedecor and Cochran (1982) using Latin Square Design. The Duncan's multiple range tests was used to test the significance between means.

RESULTS AND DISCUSSION

Milk Production and its Components

It could be noticed that milk yield and 4% Fat Corrected Milk (FCM) were insignificantly increased by adding protected AA (group 2) followed by unprotected form compared with control diet. Goat milk production was increased by 3.5 and 7.2 %, while FCM by 11.35 and 15.3 % for animals fed with unprotected and protected AA, respectively (Table 1). Similar results were earlier obtained by Kholif and Kholif (2005). Adding protected form of AA caused insignificantly increase in fat and protein contents, followed by unprotected form compared with control diet. Similar results were obtained by Misciatteilli *et al*. (2003) showed that supplementation with protected methionine led to increase fat and protein contents to 2.4 and 1.8 g kg^{-1} of milk, respectively. Milk protein content was elevated whereas fat content decreased by adding methionine and lysine to cows' rations (Krober *et al*., 2000). This could be due to the presents of methionine which is a methyl donor in the transmethylation reactions of lipid biosynthesis, so it has been used for milk fat synthesis (Leninger, 1977). Also, increasing the essential amino acids in the digesta delivered to the small intestine which increased milk protein production was observed by supplementation with protected or unprotected AA (Moscardini, 1996). Also, an increase in milk production from 1 to 4 kg day^{-1} and milk protein from 10-15% associated with the increase of the post ruminal essential AA (Clark, 1975). The marginal increase of milk protein content supported by a hypothesis that milk protein percentage is more sensitive to rumen protected lysine and methionine supplementation than either milk or protein yield in mid lactation (Rulquin and Virite, 1993).

Table 1 shows insignificantly increase in lactose content by supplementing the ration with protected form (group 2) compared with group 1 and control diet. This could be due to the enzymatic digestion of amino acids in small intestine especially protected amino acids to produce propionic acid, which led to increase the glucose content in blood serum. Earlier study found a positive correlation between blood glucose and milk lactose content (Clark *et al*., 1977).

Table 1: Effect of supplementing ration with different forms of amino acids (lysine and methionine) on chemical composition of goat milk

Items	Control	Group 1	Group 2	±SE
Milk yield	749.30	775.60	803.20	26.95
FCM 4%	680.90	758.30	785.20	54.10
Fat	3.48	3.66	3.81	0.28
Total solids	12.22	12.42	12.68	0.29
Solids not fat	8.45	8.56	8.67	0.37
Protein	3.60	3.69	3.76	0.06
Lactose	4.12	4.27	4.36	0.12
Ash	0.73	0.79	0.76	0.03
pH	6.38^{B}	6.46^{AB}	6.51^{A}	0.02

FCM: Fat Corrected Milk. Control: Ration without AA, Group 1: Ration with unprotected AA, Group 2: Ration with protected AA.Each value is mean of 27 samples for 9 animals. Means without or with same superscripts are not significantly ($p<0.01$) different

Table 2: Effect of supplementing ration with different forms of amino acids (lysine and methionine) on amino acids of goat milk

Amino acids	Control	Group 1	Group 2
	----------(g/100g protein)----------		
Leucine	3.90±0.33	4.60±0.31	4.59±0.36
Proline	4.53±0.31	4.43±0.65	5.07±0.77
Tyrosine	1.33±0.10	2.70±0.64	2.43±0.76
Methionine	1.50±0.00	2.05±0.35	2.40±0.30
Histidine	4.00±0.33	3.60±0.24	3.45±0.17
EA A	43.60±0.28	46.42±0.34	45.29±0.48
NEAA	55.23±0.23	52.43±0.21	53.39±0.53
Total Amino Acids	98.83±0.10	98.85±0.22	98.68±0.21
EAA/NEAA	0.79±0.07	0.88±0.10	0.85±0.13

EAA: Total Essential Amino Acids, NEAA: Total Non Essential Amino Acids. Mean±SD

Table 3: Effect of supplementing ration with different forms of amino acids (lysine and methionine) on some biological parameters of goat milk

Biological parameters	Control	Group 1	Group 2	±SE
Protein Efficiency Ratio (PRE)				
Adults	0.880	1.127	1.099	0.09
Juveniles	0.741	1.013	1.067	0.12
Infants	1.279	2.197	2.396	0.42
Biological Value (BV)				
Adults	59.180	61.770	61.480	1.00
Juveniles	57.700	60.570	61.130	1.30
Infants	63.370	73.040	75.140	4.40

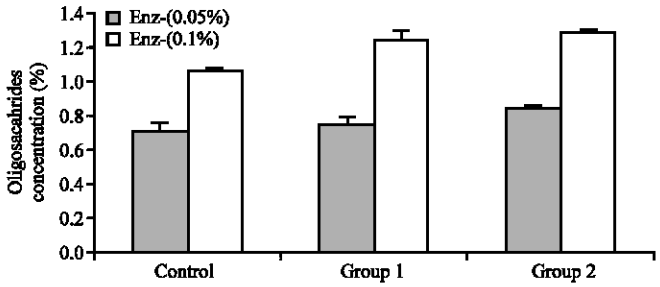

Fig. 1: Formation of galacto-oligosaccharides in goat milk by different concentrations of (Lactozyme ® 3000 L)

Biological Parameters of Goat Milk

Highest methionine and proline contents were recorded with animal fed protected AA. Unprotected AA (group 1) increased tyrosine milk content and total essential amino acids compared with protected form and control diet (Table 2).

Supplementing ration with lysine and methionine either in unprotected or protected form increased the Protein Efficiency Ratio (PRE) and Biological Value (BV) compared with control diet. Unprotected form increase the PRE and BV for adults while protected form increase these parameters for juveniles and infants (Table 3).

Sugar Analysis

Higher enzyme concentration (0.1%) caused higher formation of galatooligosaccharides compared with the lowest one (0.05%). Different milk groups has no diverse effects on galatooligosaccharides formation (Fig. 1). Increasing enzyme concentration more than 0.1% could be re-hydrolyze the formed galacto-oligosaccharides (Foda Mervat, 1997; Rustom *et al*., 1998).

Table 4: Sensory evaluation scores of goat milk containing galactooligosaccharides during cold storage 2 weeks

	Enzyme concentration (%)								
Storage period	Control			Group 1			Group 2		
(week)	0.0	0.05	0.1	0.0	0.05	0.1	0.0	0.05	0.1
Taste (40 points)									
Fresh	24±0.4	28±0.2	32±0.3	28±0.2	30±0.2	32±0.2	26±0.2	28±0.3	34±0.2
One	26±0.2	28±0.1	34±0.3	24±0.2	28±0.2	34±0.3	24±0.3	26±0.3	32±0.3
Two	20±0.3	30±0.1	34±0.2	24±0.2	32±0.2	32±0.2	20±0.2	30±0.3	32±0.2
Odor (5 points)									
Fresh	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0
One	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0
Two	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0
Color (5 points)									
Fresh	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0
One	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0
Two	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0	5±0.0
Appearance (50 points)									
Fresh	40±0.2	41±0.1	44±0.2	37±0.5	43±0.1	45±0.2	36±0.3	40±0.2	45±0.4
One	42±0.2	45±0.2	46±0.3	42±0.3	46±0.2	48±0.2	40±0.3	42±0.4	44±0.2
Two	40±0.2	44±0.4	44±0.2	41±0.2	42±0.1	46±0.2	40±0.2	44±0.2	44±0.2

Control: Ration without supplementation, Group 1: Ration supplemented with unprotected AA, Group 2: Ration supplemented with protected AA. Each value is mean of 9 samples

Table 5: Effect of supplementing ration with different forms of AA (lysine and methionine), storage period and enzyme concentration on sensory evaluation of goat milk

Items	Control	Group 1	Group 2	±SE
Effect of amino acids forms				
Taste	28.6^{B}	29.08^{A}	27.8^{C}	2.943
Appearance	42.4	42.48	42.0	1.236
Effect of storage period (weeks)				
	Fresh	One	Two	±SE
Taste	28.68^{A}	28.8^{A}	27.96^{B}	1.489
Appearance	42.12	42.5	42.36	1.012
Effect of enzyme concentration (%)				
	0.0	0.05	0.1	±SE
Taste	24.84^{C}	28.48^{B}	32.16^{A}	95.624
Appearance	39.80^{C}	42.38^{B}	44.8^{A}	179.09

Control: Ration without supplementing AA, Group 1: Ration supplemented with unprotected AA, Group 2: Ration supplemented with protected AA. Each value represents 162 values. Means with different superscripts in the same row are significantly ($p>0.01$) different. Mean±SD

Sensory Evaluation

Sensory evaluation scores of goat milk containing galacto- oligosaccharides during cold storage for two weeks are shown in Table 4.

Taste and appearance scores were increased by increasing enzyme concentrations from 0.05 to 0.1% in both groups and control samples. These results could be due to higher concentration of free glucose which released by Lactozym® 3000 L enzyme during hydrolysis. Odor and color scores did not changed by enzyme concentration or storage period in all goat milk samples. Galacto-oligosaccharides are slightly sweet (35% of sucrose), has neither bad texture nor bad taste, are completely water soluble, do not build viscosity, do not bind minerals and are physically stable (Hideo, 1994).

Whereas unprotected lysine and methionine (group 1) did not caused any significant change in taste scores compared with control diet, it caused a significant ($p<0.05$) decrease compared with group 2. Supplementing ration with both forms of amino acids did not changed appearance scores. Prolonging the cold storage for two weeks affected taste scores of goat milk significantly ($p<0.05$) compared with one week and fresh milk. Appearance scores were not found to change during the cold

storage for two weeks in all milk samples (Table 5). Goat milk samples with higher enzyme concentration (0.1%) got the highest taste and appearance scores followed by lower concentration compared with control samples.

CONCLUSION

Supplementing goat ration with lysine and methionine either in protected or unprotected forms should be recommended to increase milk production and its contents, in addition to increase milk protein efficiency ration and its biological value. Goat milk containing galactooligosaccharides could be recommended to decrease most of infant's allergy and diseases.

More study should be done to evaluate goat milk supplementing with lysine and methionine in different forms biologically using experimental animal.

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