Full Length Research Paper

Modification of constituents and exploration of functional properties of goat's milk: effects on the health and nutritional status of preschoolers

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This study was carried out to evaluate the effect of the daily intake of goat's milk with modified protein or lipid profile on the health and nutritional status of preschoolers. A 4-month, interventional, randomized, controlled study was used. Four treatments (n=25) were used: daily intake of modifiedprotein goat's milk (MPGM) or modified-fat goat's milk (MFGM); regular goat's milk (GM) or cow's milk (CM). Intake of milk, intercurrent conditions, anthropometry and biochemical parameters (complete blood count and total cholesterol) were monitored. Treatment MPGM presented in a higher amount of crude protein (2.75%) than treatment GM (2.66%). Similarly, treatment MFGM demonstrated lipid composition better than treatment GM, with higher contents of unsaturated fatty acids, including Conjugated Linoleic Acid and Omega-3 and Omega-6 fatty acids. Children receiving treatments MFGM and GM presented significantly higher weight gain ($6.44\pm3.54\%$ and $6.46\pm5.08\%$, respectively) than the others. In addition, children receiving treatment MFGM showed an increase in hemoglobin from 11.71±1.03 g/dL to 12.29±0.43 g/dL (p=0.023). Children fed goat's milk showed an increase in red blood cells. Treatment MFGM was the best alternative to replace regular goat's milk, with an improvement in hematological parameters. The findings from the comparison goat's milk versus cow's milk were inconclusive, and further studies are necessary.

Keywords: Anthropometry, food composition, goat milk, preschooler, biological markers.

INTRODUCTION

Goat's milk is becoming more relevant for the human

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LIST OF ABBREVIATIONS

BMI: Body Mass Index CE: Ceará CLA: Conjugated Linoleic Acid EMBRAPA: Brazilian Enterprise of Agropecuary Research MCH: Mean Corpuscular Hemoglobin MCHC: Mean Corpuscular Hemoglobin Concentration MCV: Mean Corpuscular Volume SAS: Statistical Analysis System UNIFESP: Federal University of São Paulo diet, which is explained mainly by its high nutritional digestibility and therapeutic value. and dietary characteristics (Haenlein, 2004; Raynal-Ljutovac et al., 2008). Goat's milk has a good lipid and protein profile, characterized by increased levels of unsaturated fatty acids and medium chain triglycerides (Alférez et al., 2001) and by low level of α -s1-casein, facilitating absorption, tolerance and other reactions of the digestive process (Raynal-Ljutovac et al., 2008). In addition, this type of milk has a higher content of minerals, especially calcium and phosphorus. and allows better bioavailability, especially for copper and iron, in comparison to cow's milk (Barriounuevo et al., 2002).

The nutritional composition of goat's milk can be

improved by supplementing the animal diet (Le Doux et al., 2002; Sanz-Sampelayo et al., 2002). Corroborating this theory, Schmidely et al. (2005) supplemented the animal diet with soy extruded at the 20%, and found a significant decrease in the proportion of saturated fatty acids (78.55g to 68.43g), associated with an increase in polyunsaturated (3.25g to 4.95g) and monounsaturated (13.08 to 18.23g) fractions.

Another study evaluated the influence on lactation of different levels of protein supplementation to the diet of goats, and found a significant increase in the milk protein content, as well as improved digestibility (Malau-Aduli et al., 2003).

Given these characteristics, goat's milk has been suggested as an excellent alternative to replace cow's milk after the first year of life, promoting adequate growth and development (Haenlein, 2004). Thus, this study aims to evaluate the effect of the daily intake of goat's milk with modified protein or lipid profile on the health and nutritional status of preschoolers.

MATERIALS AND METHODS

Study design and sample

This is an interventional, randomized, controlled study, involving preschoolers 2 to 6 years old attending a public (city) daycare center in Sobral, Ceará (CE), Brazil. One hundred children were selected and allocated to 4 groups, with the following treatment proposals: daily intake of modified-protein goat's milk (MPGM); daily intake of modified-fat goat's milk (MFGM); daily intake of regular goat's milk (GM) and daily intake of cow's milk (CM). The latter two were control groups.

The study inclusion criteria were: equivalence among groups in terms of age, weight, height, nutritional status, in addition to consent to participate in the study from the children's parents or legal guardians, by signing an informed consent document. History of milk intolerance or health problems was the exclusion criteria.

The study was approved by the Research Ethics Committee of the Federal University of São Paulo (UNIFESP) and Hospital São Paulo.

Before the study

Initially, with the goal of defining nutritional strategies to manipulate the fat and protein of milk, an experimental assay was conducted with 8 lactating milk goats. They were divided into two groups; the first (n=4) was fed a diet containing 3 types of oil – soy, fish and babassu palm; while the second (n=4) was fed the diet source of 3 types of low-degradability protein – maize gluten bran, roasted soybeans and fish flour.

At the end of this phase, fish oil was shown to promote higher levels of Conjugated Linoleic Acid, and was chosen as the best oil source studied, followed by soy oil. However, since fish oil is not recommended for the diet of ruminants (Allen, 2000; Relling and Reynolds. 2007), soy oil was chosen instead for the strategy to produce milk with a modified lipid profile. Only maize gluten bran showed an effect on modification of the protein profile.

Once the strategies were defined, 30 lactating goats were selected from the Brazilian Enterprise of Agropecuary Research (EMBRAPA) herd, and were divided into three groups of 10

animals each. In the control group, the animals were fed a diet based on Tifton-85 hay and regular concentrate – made of soy and maize bran; the second group received the diet selected to produce milk with a protein profile with functional potential (maize gluten bran); and the third group received the diet with the fatty acid profile of highest functional potential (soy oil). The cow's milk given to the fourth group of children was obtained from a dairy company in the city.

The quality of the milks was tested with physiochemical tests in Laboratory of Animal Nutrition and Growth, University of São Paulo (USP), and Advanced Biology Laboratory at EMBRAPA.

Data collection

Once the sample of preschoolers was selected, a 4-month intervention period began. In order to evaluate the effect of treatment, the following variables were monitored: actual intake of milk, intercurrent conditions, anthropometric measures and biochemical parameters.

Milk was administered daily at 7:00 a.m., in a volume of 300 mL, with sugar (10% weigh/volume) to enhance the taste, as part of breakfast. All the treatments were added sugar. To ensure the intake of milk on weekends, each child received 600 mL of milk every Friday. It is noteworthy that the children received no other source of milk than the provided at daycare. Intercurrent conditions were recorded. Acceptability of the milk was evaluated by analysing the volume taken and presence of leftovers.

The anthropometric evaluation consisted of measuring weight and height, according to proposals of the World Health Organization (WHO. 1995). Anthropometric data was collected at three time points: at the beginning of the study, and after 2 and 4 months of intervention.

Blood samples for biochemical parameters were collected at the beginning of the study and after four months of intervention. Analyses of total cholesterol levels and complete blood count were performed using the kits *Boehringer, Coulter* and radioimmune assay. All analyses were performed in a laboratory of clinical pathology in Sobral – CE.

Data analysis

Statistical analysis included simple and pairwise Student t tests, Chi-Square test or Fisher exact test. Moreover, the percentage of weight and height variation was evaluated $-\Delta X = [(X \text{ final } - X \text{ initial})/X \text{ initial}] \times 100$. The *Statistical Analysis System* (SAS) software was used, and the significance level was 5% (p<0.05).

RESULTS

Across the groups, the average age was statistically higher for children receiving CM (63.55 ± 3.20 months), than treatments MFGM (61.56 ± 3.18 months) and MPGM (60.83 ± 3.81 months). There was also a difference in height (p<0.05) between groups MPGM and CM (Table 1).

The nutritional composition of the varieties of goat's milk (treatments MPGM, MFGM and GM) differed much from cow's milk (treatment CM), especially in caloric content. There was also a reduction in the lipid content of those milks because of the treatments used and the reduction in fibers offered in the diet of the goats, likely

Variable	Goat's milk with modified proteins	Goat's milk with modified lipids	Regular goat's milk	Cow's milk
Age (months)	60.83±3.81 ^ª	61.56±3.18 ^b	61.71±3.6	63.55±3.20 ^{ab}
Male (%)	48	52	52	52
Weight (kg)	18.28±3.49	17.54±2.92	18.05±3.31	18.19±2.92
Height (cm)	105.71±7.68 ^a	106.18±4.55	106.82±4.82	107.55±4.26 ^ª
Height/age z-score	-0.90±1.42	-0.87±0.97	-0.75±1.00	-0.75±0.80
BMI [†] /age z-score	0.53±1.24	0.13±1.11	0.27±0.98	0.17±1.02
Hematocrit (%)	37.63±2.41	36.78±3.32	38.37±2.77	37.84±2.67
Hemoglobin (g/dL)	12.08±0.87	11.69±0.98	12.00±1.08	11.98±0.79
RBC [#] (10 ⁶ /mm ³)	4.37±0.22	4.29±0.35	4.34±0.29	4.36±0.29
MCV [§] (fL)	86.19±3.03	85.75±4.70	86.71±4.55	86.88±4.49
MCH [¶] (pg)	27.68±1.74	27.28±1.70	27.14±2.18	27.52±1.50
MCHC [¥] (g/dL)	32.11±1.50	31.81±0.78	31.28±1.46	31.68±0.80
Platelets (10 ³ /mm ³)	349.19±62.85	334.72±58.29	349.90±73.48	355.67±76.26
Cholesterol (mg/dL)	124.08±25.42	119.39±25.00	126.21±24.68	127.60±21.99

Table 1. Study sample breakdown by age, gender, anthropometric and biochemical parameters

[†]Body Mass Index, [#]Red Blood Clells, [§]Mean Corpuscular Volume, [¶]Mean Corpuscular Hemoglobin, [¥]Mean Corpuscular Hemoglobin Concentration. Note: Averages followed by the same letter in a line are significantly different.

Table 2. Nutritional co	mposition in terms	of contents of energy.	lipids, proteins,	lactose, dr	v extract for the typ	es of milk

Variable	Goat's milk with modified proteins	Goat's milk with modified lipids	Regular goat's milk	$\mathbf{Cow's}\ \mathbf{milk}^{\dagger}$
Energy (kcal/100 mL)	48.85	48.74	48.29	61.00
Fat (%)	2.33	2.42	2.37	3.27
Crude protein (%)	2.75	2.61	2.66	3.15
Lactose (%)	4.22	4.13	4.08	5.05
Dry extract (%)	9.58	9.38	9.39	-

[†]Data from the American Table of Food Composition (USDA, 2001). Note: the nutrition composition data for the varieties of milk do not consider the addition of sugar.

to interfere in the synthesis of fat in their mammary glands. Among the varieties of goat's milk, that from animals whose diet was enriched with maize gluten bran had a higher percentage of crude protein (2.75%) (Table 2).

There was a higher content of polyunsaturated fatty acids in treatment MFGM than in treatments CM and GM, and the same relationship was found for the percentage of essential fatty acids. However, there was a reduction in CLA levels from 1.73% to 0.73%, in goat's milk enriched with soy oil, due to the treatments used, with cow's milk presenting the highest percentage (0.95%). In general, the modification in lipid profile for goat's milk was beneficial, but not enough to surpass the characteristics of cow's milk (Table 3). At the end of the intervention, 8% of the children in treatments MPGM and MFGM, and 12% in treatment GM, were lost to follow-up. For cow's milk, no data was lost.

Regarding the intake of the different types of milk during the study, there was on average more daily leftover (p<0.05) for treatment MPGM (96.11 mL; 95% CI: 82.55–109.67 mL), followed by treatments CM (71.16 mL; 95% CI: 58.58–83.75 mL) and GM (45.31 mL; 95% CI: 34.38–56.24 mL). In treatment MFGM there was less leftover (p<0.05) (35.52 mL; 95% CI: 25.28–45.76 mL) than the other groups.

There was no significant difference in intercurrent conditions among the groups. Of the school days missed, 87.32% were not justified and 12.68% were justified with a claim or medical certificate of illness. Among the latter, the most frequent were: 10.62% fever only; 6.15% cold/flu and fever, 1.68% vomit and fever, 1.40% fever and sore throat; 1.12% cold/flu, fever and sore throat; 1.12% cold/flu, fever and sore throat; 1.12% cold/flu only.

After the intervention period, there was more weight gain for children receiving treatments MFGM ($6.44\pm3.54\%$) and GM ($6.46\pm5.08\%$) than in the other two groups (MPGM: $0.79\pm5.50\%$, CM: $1.79\pm5.00\%$); the difference was statistically significant (p<0,05). On the other hand, the latter grew more in height (2.62 ± 0.82 cm

Fatty acids	Goat's milk with modified lipids	Regular goat's milk	Cow's milk
Short-chain fatty acids (%)	16.36	21.11	10.64
Medium-chain fatty acids (%)	39.36	46.16	43.53
Long-chain fatty acids (%)	43.53	32.41	45.37
Saturated fatty acids (%)	68.70	75.05	65.29
Polyunsaturated fatty acids (%)	3.94	2.69	3.73
Monounsaturated fatty acids (%)	26.62	21.93	30.52
Conjugated linoleic acid (%)	0.73	0.33	0.95
18-carbon fatty acids (%)	1.79	1.10	2.90
Essential fatty acids (%)	3.92	2.67	3.70
Omega-3 fatty acids (%)	0.59	0.50	0.69
Omega-6 fatty acids (%)	3.01	2.17	2.59
Estearic fatty acid - C18:0 (%)	13.0	8.16	12.61

Table 3. Composition in terms of percentage of fatty acids in goat's milk with modified lipid profile, regular goat's milk and cow's milk

Note: The content of lipids from goat's milk with modified protein is similar to regular goat's milk.

Table 4. Evolution of biochemical parameters in the study population

	Hematocrit (%)	Hemoglobin (g/dL)	RBC [†] (10 ⁶ /mm ³)	MCV [#] (fL)	MCH [§] (pg)	MCHC [¶] (g/dL)	Platelets (10 ³ /mm ³)	Cholesterol (mg/dL)
Goat's milk	with modified pro	oteins						
Initial	37.5±2.3	12.1±0.9	4.4±0.2	86.2±3.2	27.8±1.9	32.3±1.5	353.5±63.4	122.9±17.4
Final	37.1±2.3	12.1±0.8	4.6±0.3	80.9±2.8	26.4±1.2	32.7±0.7	379.0±119.5	119.9±17.4
p-value	0.429	0.895	0.001	<0.0001	<0.0001	0.133	0.286	0.492
Goat's milk	with modified lipi	ds						
Initial	36.8±3.3	11.7±1.0	4.3±0.3	85.5±5.2	27.2±1.8	31.8±0.8	339.1±58.5	123.7±24.6
Final	37.9±2.0	12.3±0.4	4.7±0.3	81.1±4.5	26.3±1.5	32.4±1.5	440.1±110.2	130.9±13.9
p-value	0.192	0.023	0.001	0.001	0.002	0.124	0.007	0.228
Regular go	at's milk							
Initial	37.8±2.0	11.9±2.4	4.3±0.3	87.5±4.5	27.5±1.9	31.5±1.3	365.7±55.1	127.2±23.4
Final	35.7±2.4	11.8±0.7	4.4±0.3	80.4±3.8	26.6±1.50	33.1±0.8	380.8±50.0	121.7±15.6
p-value	0.002	0.706	0.040	<0.0001	0.020	0.001	0.502	0.356
Cow's milk								
Initial	37.5±2.6	11.9±0.8	4.3±0.3	86.6±4.6	27.4±1.5	31.7±0.8	352.4±80.3	129.4±21.8
Final	35.8±3.7	11.5±1.2	4.4±0.4	80.6±3.5	25.9±1.3	32.2±0.7	313.1±87.8	134.2±25.9
p-value	0.058	0.127	0.351	<0.0001	<0.0001	0.041	0.134	0.386

[†]Red Blood Cells, [#]Mean Corpuscular Volume, [§]Mean Corpuscular Hemoglobin, [¶]Mean Corpuscular Hemoglobin Concentration.

and 2.68 \pm 1.38cm respectively – p<0.05), than the children receiving treatments MFGM (1.82 \pm 0.74%) and GM (2.06 \pm 0.57%).

Finally, the evaluation of biochemical parameters showed increased blood levels of hemoglobin (p=0.023) in preschoolers receiving treatment MFGM, as well as increased red blood cells (RBC) count in all children fed goat's milk. In spite of that, there was a decline in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) for all treatments studied (Table 4).

DISCUSSION

The results of this study show that cow's milk has a higher caloric content than the other milks. Furthermore, there was an improvement in the milk fatty acid profile in treatment MFGM compared to GM. There was more volume leftover in treatments CM and MPGM, and the children also had less weight gain at the end of the intervention. Regarding influences on biochemical parameters, preschoolers receiving treatment MFGM

had increased blood levels of hemoglobin; additionally, all children fed goat's milk had increased RBC counts.

The difference observed in nutritional composition between goat and cow's milk is probably explained by the processing techniques used, as well as the distinct methods of obtention. Ceballos et al. (2009) conducted a study comparing the nutritional composition of cow and goat's milk, obtained at the same conditions and analyzed using the same methodology. Among the results, there was a significantly higher (p<0.05) content of total solids, proteins and lipids in goat's milk versus (vs.) cow's milk. Regarding the lipid content, a higher content of polyunsaturated fatty acids, including CLA, was found in goat's milk, unlike the findings of this study.

Furthermore, the lower energy content in the varieties of goat's milk may be explained by the reduction in the content of macronutrients, making it less concentrated than cow's milk. Contrasting with these findings, data from the American Table of Food Composition indicates a higher caloric content for goat's milk than cow's milk – 69 Kcal/100 mL and 61 Kcal/100 mL, respectively (USDA. 2001).

Moreover, adding maize gluten bran to the diet of goats promoted a change in the nutritional composition of goat's milk, with an increase in its protein content, as already seen in other studies where the animal diet was supplemented with proteins (Pabón and Lönnerdal, 2001; Morales et al., 2008). Supplementing the diet of goats with lipids was effective in improving the lipid profile of the milk, making it protective against the development of cardiovascular disorders (WHO, 2003).

Regarding anthropometric changes, the groups of children who left fewer leftovers were the ones presenting higher weight gain through the intervention. It is important to notice that, even though goat's milk has a lower caloric content than cow's milk, weight gain was higher among children fed goat's milk, either in its regular form or with modified lipid profile, suggesting better digestibility.

Grant et al. (2005) studied 72 newborns for 168 days, observing a non-significant (p>0.05) higher weight gain in infants (n=36) fed goat-milk-based infant formula than in those (n=36) fed cow-milk-based infant formula. However, both formulas studied showed similar nutritional composition in terms of energy and macronutrients.

The differences found in height gain among the groups studied, in turn, may result from the natural growth process, characteristic of this period in life, and may not be directly related to the intervention, since it takes a longer time to assess effects on height (Rogol et al., 2000).

It has been particularly difficult to discuss the anthropometric changes found, given the small number of recent studies in the literature comparing goat's milk to cow-milk-based diets for infants. Regarding the biochemical findings, the increase in RBC counts observed with goat's milk corroborates data from the literature, which indicate improved use of dietary iron when consuming goat's milk vs. cow's milk, as well as reduced interactions of iron with other minerals, such as calcium, phosphorus and magnesium. Together, such effects increase the bioavailability of iron from goat's milk, contributing to the increase in RBC counts (Barrionuevo et al., 2002).

A study in rats (n=59) evaluated the effect of dietary iron from goat's milk, compared to cow's milk, on the use of iron and hematological parameters. Normal amounts of iron were administered to normal and anemic rats receiving one of three types of diet: control diet, goatmilk-based diet and cow-milk-based diet. Among the results, there was a decrease in anemia, especially in rats fed goat's milk, because of a higher efficiency in the regeneration of hemoglobin, serum levels of iron and RBC counts. In addition to that, goat's milk increased iron metabolism, especially in animals with iron deficiency, resulting in higher stores of iron in the spleen, liver, sternum and femur (Alférez et al., 2006).

In spite of this increase in the bioavailability of iron from goat's milk vs. cow's milk, none of the four treatments was effective in the prevention of irondeficiency anemia, since there were a reduction in MCV and MCH, associated with an increase in platelet counts, indicating microcytic and hyperchromic anemia (Dugdale, 2006). This finding suggests that offering milk, whatever the treatment was, not enough to compensate the deficiency children had when not at school.

Among the varieties of goat's milk, treatment MFGM demonstrated better use of iron, given the significant increase in hemoglobin levels. Corroborating this hypothesis, there was an important percentage reduction in the hematocrit and mean corpuscular hemoglobin concentration (MCHC) in the children in group GM. Such findings suggest a relationship between iron bioavailability and the fatty acid profile of goat's milk with modified lipid profile.

Studies in rats have indicated an increased percentage of iron retention with an increasing share of saturated fatty acids in the diet, explained mainly by high concentrations of stearic acid (C18:0) (Boesch-Saadatmandi et al., 2007; Morales et al., 2008). A study in male rats fed diets based on bovine fat (stearic acid) or saffron oil, both enriched with ferrous sulfate, showed a significant increase in the concentration of iron in the liver of rats fed the former type of diet (Morales et al. 2008). Although our study found a higher content of fatty acids in treatment GM, the milk in treatment MFGM had higher concentration of stearic acid, which may explain the increase in hemoglobin levels verified in the latter group.

The study has some limitations, such as lack of control of food intake at home, small number of biochemical

evaluations, given the short intervention period, as well as changes in the nutritional composition of the goat's milks from the treatments used. In spite of that, the findings suggest a possible improvement in the nutritional composition of goat's milk, especially its lipid profile, which may be beneficial for children who do not consume cow's milk for any reason.

It should be pointed out that studies about the intake of goat's milk with modified nutritional composition vs. cow's milk in preschoolers have been rare in the literature of the past 10 years.

In short, this study indicates the possibility of improving the nutritional composition of goat's milk by altering the animal diet, with incorporation of protein and favorable modification of the fatty acid profile. These new foods, especially the variety with modified lipid profile, were able to improve the metabolism of iron over regular goat's milk.

However, the findings are still inconclusive when comparing the results for children fed goat's milk vs. cow's milk, and further studies are necessary.

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