Biological effect of unripe cheese produced with coagulant from Solanum aethiopicum L. Shum fruit

Valentin Désiré Guiama, Juliette Koubé, Esther Ngah, Jean Marcel Bindzi and Carl Moses Mbofung.

Abstract— This study assessed the reaction of rats to the consumption of cheese made with plant coagulant from Solanum aethiopicum L. Shum (SA cheese), compared to calf rennet (CR) cheese and casein/sunflower oil diets. The body weight increased by 73, 80 and 84% in the control, SA cheese and CR cheese diet groups respectively. The consumption of SA cheese had protective effect on blood cells. Iron in serum decreased, with a low risk of iron deficiency anemia. The levels of total cholesterol, triglycerides and LDL-cholesterol in the serum were lower in rats fed SA cheese diet, as well as AST, ALT, suggesting protective effect of cardiovascular system, renal and hepatic functions. The biomarkers of oxidative stress were not changed, as well as bilirubin levels. In addition, SA cheese also improved the lactobacilli colon flora. Therefore, plant coagulant (SA extract) affects biological activity of cheese, and tends to improve its health benefits.

Index Terms—Cheese, consumption, health benefits, plant coagulant, *Solanum aethiopicum*.

1 Introduction

Emerging scientific findings indicate that dairy products contain fat (rumenic acid, butyrate and sphingomyelin), proteins, peptides, oligosaccharides, vitamins and minerals; which provide beneficial effects beyond basic nutritional value [1]. Due to their health advantages, dairy products have also served as vehicles for functional food ingredients, such as phytosterols, fish fatty acids and various kinds of probiotic bacteria. Furthermore, dairy foods have been a rich source for the development of a large variety of health promoting ingredients that are found in the markets as functional foods and dietary supplements [2]. Among the dairy foods, cheese is known as a concentrated dairy product and a form of milk preservation, since milk is highly perishable. Consumption of cheese has increased consistently in most countries, unrelated to their socio-economic level of development [3].

Many studies have proven the biological role of cheese in several aspects of health [4]. Using rat model, a study on the biomarkers of metabolic syndrome revealed that consumption of cheese was associated with a decrease of the serum low-density lipoprotein, adiponectin, triglyceride and cholesterol levels and weight of the mesenteric adipose tissue [5]. This suggests that cheese possess a suppressive effect on abdominal adipose accumulation, and prevent the development of metabolic syndromes.

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There is evidence that cheese is a probiotic food carrier. Probiotic bacteria activity is maintained in all the stages of cheese-making, from their manufacture up to their ingestion by the consumer, and they survive during their transit in the gastrointestinal tract. Probiotic bacteria are protected in the gastrointestinal tract by buffer created by cheese against the high acidic environment, its dense matrix and relatively high fat content. Probiotic unripe cheese (Argentinean fresh cheese) containing L. *acidophilus* A9, B. *bifidum* A12 and L. *paracasei* A13 demonstrated immunomodulating capacity in mice [6].

Many constituents of cheese are involved both directly and indirectly in the fight against oxidative stress. Fat soluble vitamins (A and E) and peptides act directly by preventing the formation of radicals, by scavenging radicals or hydrogen peroxide and other peroxides [7]. Indirectly, amino acids from digestion of casein stimulate the biosynthesis of antioxidant enzymes, and their activity depends on some minerals found in cheese. It is also efficient against kidney stones, osteoporosis and dental caries [1].

Cheese has become an important line of defense in the prevention of chronic diseases. Thus, numerous varieties of cheese are being manufactured using coagulants from animal, microbial and plant origin. However, the use of animal and microbial coagulants is limited in many parts of the world. The choice of ingredients for cheese making is influenced by many factors some of which include their availability, diet habits, ethics, public health, law and religious issues. Therefore, natural coagulants from plants are preferred by the people on whom constrains are imposed by the use of animal and microbial coagulants. Plant coagulants are characterized by non-specific proteolytic action on milk proteins. In addition, their toxin content constrains their use as food. The health benefits of cheese are hardly known by consumers, but the use of new coagulant raises questions about the safety, and biological effect of new product compared with that obtained using conventional coagulants (calf rennet). Different coagulants may

show similar clotting properties and extent of hydrolysis early in the process, but differ significantly during the continuation of cheesemaking [3]. This protease activity of coagulant during cheese-making can affect the biological quality of cheese; due to the variation in milk compounds retained in the curd.
Numerous studies in bioactivity of cheese have been essentially carried out with the cheeses made with animal or microbial coagulants. In contrast, very few studies have provided details on the bioactivity of cheese produced with plant extract used as a coagulant. In this investigation, rat models were used to evaluate the biological effect of cheese made with Solanum aethiopicum L. Shum fruit (mock tomato) extract, which has been suggested as potential plant coagulant in cheesemaking [8]. Therefore, the present work aimed essentially to study the effect of feeding rats with cheeses (both made using calf rennet and S. aethiopicum extract) on haematological, microbiological and biochemical parameters. In addition, its effect on food efficiency and somatic index was evaluated.

2 Materials and methods

2.1. Cheesemaking procedure
Solanum aethiopicum L. Shum (SA) coagulant was prepared by soaking 12 g of SA fruits (harvested in Ngaoundere area, Adamawa Region-Cameroon) powder in 100 mL solution 4% (w/v) NaCl at 25°C for 20 h. Then, mixture was centrifuged and filtered, the supernatant was SA coagulant. Animal rennet was prepared by diluting 1g of calf rennet (80% of chymosin and 20% of pepsin) powder (BioRen® Naturlabextrakt, Austria; with declared activity of 15,000 Soxhlet units) in 200 mL of distilled water to obtain the same rennet coagulation time.
Raw zebu (Bos indicus) milk (40 kg) from five herds situated around Ngaoundere, Adamawa Region-Cameroon, was obtained in the morning by manual milking during August 2011. Zebu milk was mixed and filtered. Milk was heated with a holding time of 30s at 73°C, cooled at 35°C, then CaCl2, 2H20 and starter were added to a final concentration of 0.02% (w/v) and 2% (w/v) respectively. After 15 min, 200 mL of each enzyme solution was added to 20 kg of milk. Forty minutes later, the obtained coagula were cut and drained. Resulting unripe cheeses were analyzed and stored at -20°C until formulation of rat diets.

2.2. Animals and Diet
Ten weeks old, eighteen female albinos Wistar rats (Rattus novergicus), nulliparous and non-pregnant, weighing 97 ± 6 g, were randomly assigned to three groups of six animals each and housed individually in plastic cages. The caged rats were kept in a room (24°C, 70% of humidity) with controlled light (12 h light-dark cycles), and had free access to food and tap water. After one week of acclimatization, food was removed 12 h before the forty days of feeding. The animals were fed with diet containing casein/sunflower oil, CR cheese and SA cheese corresponding to diet 1, diet 2 and diet 3 respectively. The diets were designed by modifying the composition (AIN-93G) of the American Institute of Nutrition [9], diets 2 and 3 were not supplemented in minerals (table 1). Animal experiments were conducted in accordance with European Community guidelines (Official Journal of European Union L197 vol. 50, July 2007).

2.3. Experimental procedure
Food and water intake, faeces and body weights of each rat were recorded every five days of feeding period. Then, food efficiency was calculated and expressed as body mass gain (g^{-1} intake. rat^{-1}). At the end of the feeding period, all the rats were deprived of food 12 h before sacrifice under anesthesia. Animals were later dissected and blood samples collected into two types of tubes: EDTA tubes for haematological analyses and dried tubes centrifuged (3,500 rpm, 5°C for 10 min) to obtain serum (stored at -20°C until biochemical analysis). Colon content was withdrawn for microbiological analysis. The entire organs such as lung, heart, brain, spleen, kidneys and liver were excised, weighed and stored at -20°C until analysis.

2.4. Analysis

2.4.1. Physicochemical
Physicochemical analysis concerned with the diet ingredients, colon content and faeces of rats. Total solids (infra-red drying 105°C to constant weight), total protein (Kjeldahl method), and ash (ignition at 550°C) contents of cheese and fecal matter of rats were measured [10]. Total lipids were determined using organic solvents (dichloromethane/methanol: 2/1) and a gravimetric calculation [11]. Carbohydrate content of cheese was determined as the difference between total solids and the sum of fats, proteins and ash contents [12]. The pH of cheese and colon content was measured by using a Consort pH-meter (Thumout, Belgium) model C831.

2.4.2. Colon microbiological
The total viable bacterial count, lactobacilli, moulds and yeasts colonies of the colon content were enumerated. Samples were serially diluted 109 times, pre-reduced in tryptone water and inoculated onto a range of agars designed to be selective for predominant microorganisms: total viable count on Plate Count Agar (PCA) at 37°C for 48 h, yeast and moulds on Sabouraud Dextrose Agar (SDA) at 37°C for 120 h, Lactobacilli on Man Rogosa and Sharpe (MRS) agar at 37°C for 48 h. The dishes were placed in anaerobic jars for Lactobacilli culture; and the rest were incubated aerobically.

2.4.3. Haematological
The blood samples collected with EDTA tubes were analyzed using automated haematological analyzer (System H1, Bayer Diagnostics). Haematological analysis was carried out to estimate red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), platelets (Plt) and white blood cell count (WBC) as well as mean corpuscular haemoglobin concentration (MCHC).

2.4.4. Serum biochemical
The serum samples were analyzed using specific commercial diagnostic kits. Glucose, bilirubin and total protein were evaluated in the serum using Menarini kits (Menarini, Italy). Total

cholesterol and triglycerides were measured in the serum using Olympus kit (Olympus Diagnostica Gmbh, Ireland). Creatinine and ALT/AST activities were evaluated with Fortress kit (Fortress Diagnostics, London, UK). HDL and LDL-cholesterol were measured using Kit BioMerieux (BioMerieux, France). Analyses were done using Olympus AU2700 Autoanalyzer. VLDL-cholesterol was determined using online VLDL-cholesterol calculator (VLDL = triglycerides (mg/dL)/5), valid only when triglycerides are less than 400 mg/dL. Mineral content (Ca^{+2}, Fe^{+2}, Mg^{+2} and Zn^{+2}) was determined in serum with a Perkin Elmer atomic absorption spectrophotometer apparatus (model 5100 PC.).

2.4.5. Liver and kidney Biochemical

Organ samples (liver and kidney) were ground with a laboratory mortar, and were homogenized in Tris–HCl buffer 0.1M, pH 7.2. The mixture of each organ was centrifuged (7,500 rpm at 5°C for 30 min). The homogenates were divided into four portions, and stored at -20°C until analysis. Biochemical analysis was carried out to measure total proteins of tissue, MDA [13] catalase activity [14] and glutathione [15].

2.4.6. Statistical

Statistical analysis of the data was done using Statgraphics Plus version 5.0 (Statpoint, Inc., Warrenton, USA). Oneway analysis of variance (ANOVA) was used for testing statistical significance amongst groups of rats, and individual pair difference was tested by means of Duncan's multiple range test at 95% level of confidence.

3. Results

3.1. Proximate composition of cheeses

As seen in legend of table 1, the proximate composition of cheeses showed that the values of parameters such as protein and fat were statistically higher in CR cheese than SA cheese, while moisture and carbohydrate content showed an opposite trend. Ash content was not significantly different between the cheese samples.

3.2. Food and water intakes

Fig. 1. shows food (a) and water (b) intakes of rats fed diets 1, 2 and 3 containing casein/sunflower oil, CR cheese and SA cheese respectively for the feeding period. Food intake increased throughout the feeding period, with no statistical difference among the groups of rats. During the ten first days, water intake increased in all animal groups. From twentieth to fortieth day of experiment, the SA cheese consumption enhanced significantly water intake, while it decreased in the rats fed diets 1 and 2.

3.3. Food efficiency

Effect of cheese intake on the body weight of rats during feeding period is presented in Fig. 2. During the first half of feeding, no significant difference in body weight was observed among the animal groups, while it became statistically higher in the CR cheese diet group during the second half of test. Similarly, the amount of faeces increased significantly in the SA cheese compared to CR cheese diet groups during the second half of experiment (data not shown). Body weight increased by 73, 80 and 84% in the control, SA cheese and CR cheese diet groups respectively.

Changes in food efficiency of rats during 40 days of feeding are shown in Fig. 3. Increase in food efficiency observed after 10, 15 and 20 days in rats fed SA cheese, Casein/sunflower oil and CR cheese respectively; and it decreased to around 0.3 g gain.g-1intake.rat-1 in all groups of rats at the end of feeding period.

3.4. Somatic index

Table 2 presents the effect of cheese ingestion on the organ absolute weight and somatic index of rats. There was no significant change in the organ absolute weight of SA cheese compared to the control diet groups. However, the ingestion of the CR cheese increased significantly in liver weight compared to SA cheese and control. Regarding the organ somatic index, no statistical change detected between the SA cheese and control diet groups. Yet, the somatic index of spleen increased significantly in the animals fed SA cheese diet compared to the CR cheese diet.

3.5. Compositional analysis of faeces

Table 3 shows compositional analysis of the faecal matter collected 24 hours prior to the day of sacrifice. There was no statistical difference in faecal moisture amongst diet groups. Diet containing SA cheese increased significantly the faecal weight of rats compared to the CR cheese diet. Faecal protein increased statistically in rats fed SA cheese compared to the CR cheese and control. Total lipids excreted in the faeces were significantly not different amongst the diet groups, while faecal cholesterol and triglycerides increased statistically in control and SA cheese diet groups.

3.6. Haematological parameters

The effect of SA cheese diet compared to CR cheese and control diets on haematological parameters is presented in table 4. No significant difference was observed in blood analysis of LYM, MCV, MCH and MCHC amongst the diet groups. There was no significant difference in WBC in rats fed SA cheese compared to those of control diet, while a significant increase was observed in rats fed CR cheese. However, RBC, HGB, HCT and PLT increased significantly in SA cheese diet group compared to the other diet groups.

3.7. Biochemical parameters

Biochemical parameters observed both in the serum and organs (liver and kidney) of rats sacrificed after 40 days of experiment are presented in table 5. Analysis of serum showed that there was no statistical change in glucose, direct bilirubin, total bilirubin, creatinine, HDL and LDL cholesterol, calcium, magnesium and zinc amongst diet groups. However, protein, total cholesterol, VLDL-cholesterol, triglycerides, iron and transaminases activities (ALT/AST) in serum were statistically lower in the SA cheese diet group compared to control. Serum iron levels were similar for both cheese diet groups, but lower compared to the control. The SA cheese consumption didn't

cause a significant change in hepatic protein, MDA, glutathione and catalase, as well as the same parameters analyzed in the kidney.

3.8. Microbiological parameters

There was a significant difference in colon microbiological parameters of the treatment groups of rats during the feeding period (Table 6). Total viable count was significantly higher for diet containing SA cheese than for the other dietary treatments, which were different from each other (diet containing CR cheese higher than casein/sunflower oil). Lactobacilli followed the same tendency. There was no statistical difference in moulds and yeasts between rats fed diet containing SA cheese and casein/sunflower oil. However, they increased significantly in rats fed diet containing CR cheese.

4. Discussion

The gross compositional analysis of cheese made with SA coagulant was similar to other fresh cheese in terms of total protein and fat [16], moisture content [17]. SA coagulant affected significantly the properties of cheese obtained; its proteolytic action derived different peptides and enhanced retention of soluble compounds, reflected in high moisture of cheese. How SA cheese may affect the human health? The biological effects attributed to SA cheese consumption could be due to both the peptides generated from the digestion of milk proteins and other soluble compounds retained in this cheese. Protein and peptides derived from milk have been shown to exhibit a wide range of beneficial functions in human health [18]. As a function of coagulant used, most medicinal components from pasture found in milk have also been reported in cheese; including amines, alkaloids, cyanogenic glycosides, cyclitols, fatty acids, fluoroacetate, gums, non-protein amino acids, terpenes, flavonoids and tannins among other [19].

The increase in the food and water intakes may suggest that anabolic and catabolic reactions were balanced in animals during growth [20]. Milk proteins and amino acids; such as cholecystokinin, casomorphin, caseinomacropeptide, and leucine, may help contribute to satiety, regulate food intake and control body weight [21]. Body weight is the first parameter in the evaluation of a nutritional status; it is correlated to the energy-protein content in the diet [22]. The change in body weight among rat groups at the second half of feeding period can be related to the rise of the amount of faeces excreted in the rats fed control and SA cheese diets. Body weight gain of rats is the manifestation of food efficiency, which is associated with the utilization of nutrients. Thus, the effect of cheese biological compounds to the metabolism of rats could induce the differences in body weight gain [23].

Analysis of blood parameters is relevant to predict the health status. In general, the variations in haematological parameters could be due to direct destruction of mature circulating cells or loss of cells from the circulation by haemorrhage, or leakage through capillary walls and reduced or increased cell production. The haematological profile among rat groups showed significant differences, excepted LYM, MVC, MCH and MCHC. The low level of WBC in SA cheese animals' group might suggest the low level of neutrophils. Neutrophils are the first to act when there is infection and are also the most abundant substance in white blood cells (50 – 70%) (Roberts, 2007). The elevation in RBC, HGB, HCT and PLT of rats fed SA cheese may indicate that SA cheese contains bioactive components which act directly or indirectly both to enhance the erythropoiesis and to protect blood cells. Notwithstanding the variations of blood parameters amongst diet groups, they were within normal values for the strain and age of the animals used [24].

Cheese is an important source of major minerals (calcium, magnesium) and trace elements such as zinc [20], explaining the similarity in those cations amongst animal groups, despite the fact that cheese diets were not supplemented in minerals. These minerals are involved in many physiological processes to provide optimal health [1] [20]. In contrast, decrease in serum iron of animals fed cheese diets, can be due to high molecular weight from whey fraction retained in curd may chelate iron (Tong, Sasaki, McClements, & Decker, 2000). This suggested that cheese is not a source of iron. However, when cheese is fed as recommended, there is little risk of iron deficiency anemia [1].

It is known that the consumption of calorie-dense food like cheese lead to metabolic syndrome [25]. Biomarkers of metabolic syndrome are among other hypertension, hyperlipidemia and glucose intolerance [2]. It has been proposed that inhibition of fat accumulation is an effective strategy to prevent metabolic syndrome [26]. Regarding glucose in serum of rats, SA cheese diet had a tendency towards the decrease of glucose in rat. This result suggested that the SA cheese would have a positive effect on the metabolism of glucose, either on the insulin secretion or the receptors of this hormone. The same trend has been reported with the cream cheese [27]. Total lipids excreted in the faeces were similar in the three diet groups; suggesting that the amount of fat absorbed from the gastrointestinal tract was not affected by SA cheese. This is in line with the observations using cheddar cheese [5]. Cholesterol, LDL-cholesterol and triglyceride levels in serum were lower in the animals fed SA cheese diet compared with CR cheese, while triglycerides and cholesterol excreted in the faeces followed opposite allure. This suggested that the SA cheese ingestion could inhibit either the synthesis of cholesterol, triglycerides and VLDL in the liver or fat accumulation in adipocytes. This may suggest the beneficial effects of peptides derived from milk [28], and phytochemicals of milk retained in the cheese [29] on cardiovascular system.

The increase in liver absolute weight of rats fed CR cheese could be attributed to the fat accumulation in this organ. However, no change in somatic index of animals indicates that the body and organs balanced. Liver and kidney were positively affected, since the serum levels of ALT, AST were significantly lower in animals fed SA cheese diet. Furthermore, bilirubin levels in serum confirmed the heart, liver and blood cells protection in rats fed SA cheese. These observations are supported by the values of oxidative stress biomarkers such as hepatic and renal MDA, glutathione and catalase. Depending on protease activity, the plant enzymes cleave α-, β-, γ- and k-caseins [30]; the resulting peptide hydrolysates have been proven antioxidant [7].

SA cheese intake increased lactic acid bacteria in colon. Many strains of lactobacilli confer a countless health benefits on the animal host; so-called probiotic bacteria [6]. Fresh cheese appears to be ideally suited to serve as a carrier for probiotic bacteria as it is an unripe cheese, during storage it is subjected to refrigeration temperatures, and its shelf life is limited [31]. Colon lactobacilli and yeasts/moulds in rats fed SA cheese were not balanced; may be due to the relative shortness of feeding period. Nevertheless, this unripe cheese can be used as a probiotic food carrier. Thus, the consumption of SA cheese may be considered as health promoting food. However, the effects of SA cheese ingestion on the metabolism of fat and sugars, and antioxidant activities need to be elucidated by studying its constituents individually.

5. Conclusion

No adverse effect due to the intake of zebu cheese made with SA coagulant (SA cheese) was found in rats during forty days of supplementation. The ingestion of SA cheese affected positively growth and nutrient utilization. Whole blood parameters were within normal values. Minerals such as calcium, magnesium, and zinc were balanced in serum, while serum iron decreased without negative effect on the red blood cells. The biomarkers of metabolic syndrome such as total cholesterol, triglyceride, glucose and LDL decreased in serum of animals fed SA cheese. The consumption of SA cheese may inhibit synthesis in liver and accumulation in adipocytes tissues of lipids, as well as enhancing metabolism of glucose. Levels of AST, ALT and bilirubin suggest the protection of hepatic and renal functions, as well as blood cells. The values of oxidative stress biomarkers such as hepatic and renal MDA, glutathione and catalase may suggest antioxidant function of SA cheese. Microbiological analysis of colon content of rats suggests that SA cheese is a potential probiotic food carrier. Plant coagulant (SA extract) affects the nutritional and functional properties of cheese.

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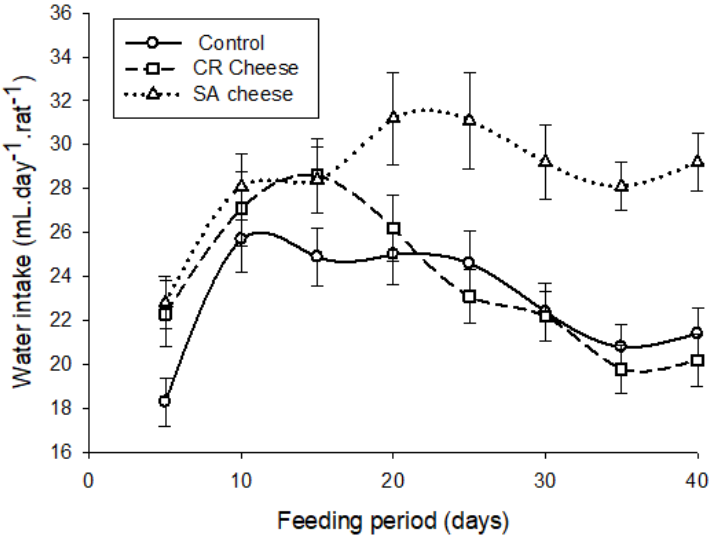

Fig. 1. Variation of food (a) and water (b) intakes of rats fed diets containing casein/sunflower oil (control), CR cheese and SA cheese during an experimental period of 40 days. The food and water intakes were determined every five days and expressed as $g.day^{-1}.rat^{-1}$ and $mL.day^{-1}.rat^{-1}$, respectively. Values represent the mean ± standard deviation (n= 6.0). * SA cheese diet group was significantly higher than CR cheese and control diet groups from twentieth day of feeding ($P < 0.05$).

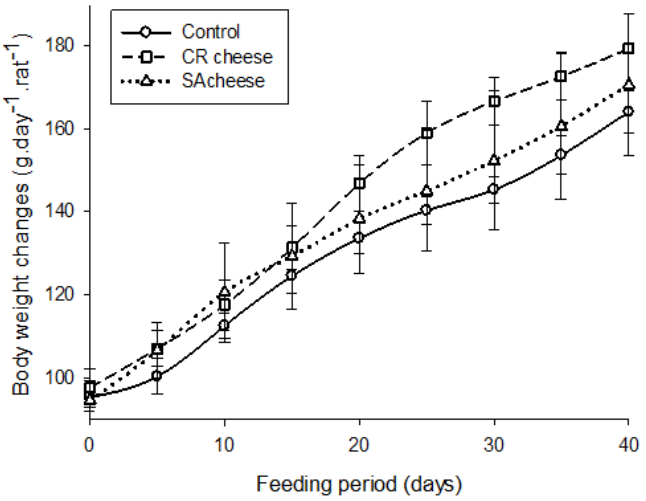

Fig. 2. Changes in body weight of rats fed diets containing casein/sunflower oil (control), CR cheese and SA cheese during an experimental period of 40 days Rats were weighed every five days and expressed as $g.day^{-1}.rat^{-1}$. Values represent the mean ± standard deviation (n=6.0). * CR cheese diet group was significantly higher than SA cheese and control diet groups from twentieth day of feeding ($P < 0.05$).

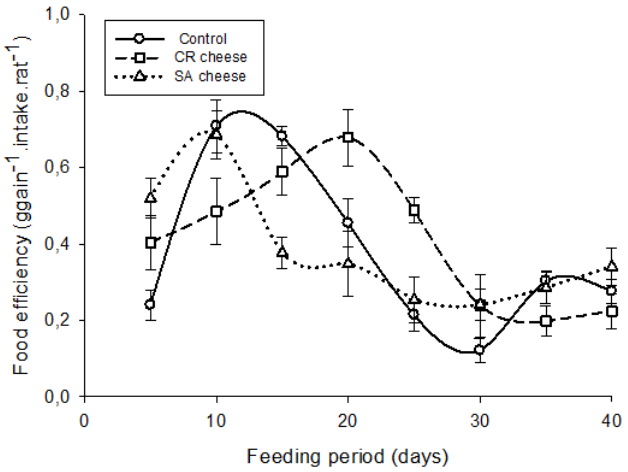

Fig. 3. Variation of food efficiency of rats fed diets containing casein /sunflower oil (control), CR cheese and SA cheese during an experimental period of 40 days. Food efficiency was determined every five days and expressed as g gain.g^{-1}intake.rat^{-1}. Values represent the mean ± standard deviation (n=6.0).

Table 1: Composition of the experimental diets fed to rats

	Diets		
Component (%)	Control	CR cheese	SA cheese
Casein[a]	20	-	-
CR cheese[b]	-	45.4	-
SA cheese[c]	-	-	47.1
Corn starch[d]	17.6	17.3	17.3
Cellulose	5	5	5
Sucrose	35	31	29.3
sunflower oil[e]	16.9	-	-
DL- methionine	0.3	0.3	0.3
Mineral mix	3.2	-	-
Sodium chloride	1	1	1
Vitamin mix	1	1	1

[a] 92% protein (N×6.38, IDF recommended conversion factor for milk protein), 0.1% fat, 1.5% ash and 6.4% water.
[b] 17.9% protein (N×6.38), 15.2% fat, 3.6% sugar, 3.8% ash and 59.4% water.
[c] 16.4% protein (N×6.38), 13.9% fat, 4.7% sugar, 3.5% ash and 61.3% water
[d] 1.4% protein (N×6.25), 0.2% fat, 86.7% sugar, .5% ash and 11.3% water
[e] 99.6% fat: 15% saturated, 23% monounsaturated, 62% polyunsaturated.

Table 2: Weight of organs (g) and somatic index (g.100g^{-1}body weight) of the rats fed with diets containing casein/sunflower oil (control), CR cheese and SA cheese for 40 days.

Organs	Weight (g)			Somatic index (g.100g^{-1} body weight)		
	Control	CR cheese	SA cheese	Control	CR cheese	SA cheese
Kidney	1.46 ± 0.16^{a}	1.48 ± 0.09^{a}	1.49 ± 0.17^{a}	0.93 ± 0.07^{a}	0.84 ± 0.04^{a}	0.90 ± 0.10^{a}
Liver	5.93 ± 0.34^{b}	6.72 ± 0.68^{a}	6.0 ± 0.51^{b}	3.80 ± 0.28^{a}	3.83 ± 0.34^{a}	3.63 ± 0.29^{a}
Lung	1.49 ± 0.19^{a}	1.90 ± 0.50^{a}	1.60 ± 0.30^{a}	0.96 ± 0.11^{a}	1.08 ± 0.27^{a}	0.96 ± 0.16^{a}
Brain	1.64 ± 0.10^{a}	1.79 ± 0.08^{a}	1.77 ± 0.20^{a}	1.05 ± 0.08^{a}	1.02 ± 0.06^{a}	1.07 ± 0.14^{a}
Spleen	0.68 ± 0.07^{a}	0.67 ± 0.13^{a}	0.77 ± 0.07^{a}	0.44 ± 0.04^{ab}	0.38 ± 0.07^{b}	0.47 ± 0.07^{a}
Heart	0.65 ± 0.04^{a}	0.67 ± 0.09^{a}	0.65 ± 0.09^{a}	0.42± 0.03^{a}	0.38 ± 0.05^{a}	0.39 ± 0.06^{a}

The animals of each diet group (n= 6.0) were sacrificed under anesthesia; organs were removed and rapidly weighed. Means ± SD bearing different letters (a, b) indicate differences ($P < 0.05$) within the same row.

Table 3
Compositional analysis of the rat faeces collected 24 hours prior to the day of sacrifice.

Parameters	Diets		
	Control	CR cheese	SA cheese
Dy weight (g)	0.78 ± 0.09[a]	0.60± 0.08[b]	0.80± 0.05[a]
Moisture (%)	35.18 ± 5.35[a]	34.95 ± 5.08[a]	35.05 ± 5.58[a]
Protein (%)	19.11 ± 2.13[b]	19.98 ± 2.26[b]	25.48 ± 2.26[a]
Total lipids (%)	16.53 ± 2.78[a]	14.23 ± 1.20[a]	16.20 ± 1.77[a]
Triglycerides (%)	4.75 ± 0.72[a]	2.66 ± 0.40[b]	4.08 ± 0.92[a]
Total cholesterol (%)	7.25 ± 1.71[a]	5.34 ± 0.58[b]	7.16 ± 0.94[a]

Means ± SD bearing different letters (a, b) indicate differences ($P < 0.05$) within the same row.

Table 4: Haematological parameters of the rats fed with diets containing casein/sunflower oil (control), CR cheese and SA cheese for 40 days.

Parameters	Diets		
	Control	CR cheese	SA cheese
[1]WBC	7.98 ± 0.50^{ab}	8.61 ± 0.89^{a}	7.55 ± 0.54^{b}
[2]LYM	7.15 ± 0.71^{a}	7.69 ± 0.62^{a}	6.96 ± 0.41^{a}
[3]RBC	8.04 ± 0.46^{b}	8.02 ± 0.62^{b}	8.86 ± 0.58^{a}
[4]HGB	15.08 ± 0.81^{b}	15.68 ± 0.92^{b}	17.75 ± 0.53^{a}
[5]HCT	37.66 ± 0.80^{c}	42.26 ± 4.14^{b}	46.08 ± 1.92^{a}
[6]PLT	587.0 ± 5.01^{b}	589.0 ± 8.94^{b}	726.0 ± 4.25^{a}
[7]MCV	52.50 ± 1.64^{a}	52 ± 1.54^{a}	51.83 ± 0.75^{a}
[8]MCH	18.75 ± 0.43^{a}	19.71 ± 1.09^{a}	18.96 ± 0.72^{a}
[9]MCHC	33.91 ± 0.98^{a}	35.22 ± 2.10^{a}	35.15 ± 1.00^{a}

Means ± SD bearing different letters (a, b and c) indicate differences ($P < 0.05$) within the same row.

[1] White blood cells ($10^3.\mu L^{-1}$)
[2] Lymphocytes ($10^3.\mu L^{-1}$)
[3] Red blood cells ($10^6.\mu L^{-1}$l)
[4] Haemoglobin concentrations ($g.dL^{-1}$)
[5] Haematocrit (%)
[6] Platelets ($10^3.\mu L^{-1}$)
[7] Mean corpuscular volume (fl)
[8] Mean corpuscular haemoglobin (pg)
[9] Mean corpuscular haemoglobin concentration ($g.dL^{-1}$)

Table 5: Biochemical parameters of rats fed with diets containing casein/sunflower oil (control), CR cheese and SA cheese for 40 days.

Parameters	Diets		
	Control	CR cheese	SA cheese
Serum			
Glucose (mg.dL^{-1})	96.67 ± 2.21^{a}	106.80 ± 3.4^{a}	86.68 ± 0.23^{a}
Direct Bilirubin	0.22 ± 0.02^{a}	0.23 ± 0.02^{a}	0.21 ± 0.03^{a}
Total Bilirubin	0.99 ± 0.13^{a}	1.00 ± 0.06^{a}	0.89 ± 0.19^{a}
Protein (g.dL^{-1})	9.65 ± 0.54^{a}	9.56 ± 0.73^{a}	8.51 ± 1.13^{b}
Creatinine (mg.dL^{-1})	1.00 ± 0.05^{a}	0.92 ± 0.06^{a}	0.93 ± 0.12^{a}
[1]ALT (U.L^{-1})	104.18 ± 7.07^{a}	96.91 ± 6.92^{ab}	95.03 ± 5.21^{b}
[2]AST (U.L^{-1})	34.98 ± 5.09^{b}	42.80 ± 5.39^{a}	35.95 ± 3.32^{b}
Triglycerides	94.98 ± 4.69^{a}	93.41 ± 3.37^{a}	83.47 ± 4.01^{b}
Total Cholesterol	79.09 ± 4.87^{ab}	80.32 ± 2.05^{a}	73.75 ± 1.21^{b}
[3]VLDL cholesterol	19.00 ± 2.11^{a}	18.68 ± 1.23^{a}	16.69±
[4]LDL cholesterol	11.08 ± 0.73^{b}	13.93 ± 1.97^{a}	12.39 ±
[5]HDL Cholesterol	55.19 ± 3.47^{a}	51.88 ± 6.63^{a}	53.62 ± 2.92^{a}
Calcium (mg.dL^{-1})	9.92 ± 0.20^{a}	9.23 ± 0.74^{a}	8.96 ± 0.58^{a}
Iron (mg.dL^{-1})	1.01 ± 0.01^{b}	0.68 ± 0.00^{a}	0.75 ± 0.00^{a}
Magnesium (mg.dL^{-}	1.21 ± 0.04^{b}	1.36 ± 0.12^{a}	1.26 ± 0.07^{ab}
Zinc (mg.dL^{-1})	0.16 ± 0.00^{a}	0.15 ± 0.00^{a}	0.16 ± 0.00^{a}
Liver			
Protein (g.dL^{-1})	6.39 ± 0.41^{a}	6.42 ± 0.26^{a}	6.37 ± 0.22^{a}
[6]MDA (nmol.g^{-1})	9.95 ± 0.95^{a}	9.31 ± 1.23^{a}	8.72 ± 0.78^{a}
Glutathione	3.77 ± 0.03^{a}	3.81 ± 0.10^{a}	4.09 ± 0.05^{a}
Catalase (U.min^{-}	7.44 ± 2.10^{a}	8.33 ± 1.17^{a}	7.18 ± 1.90^{a}
Kidney			
Protein (g.dL^{-1})	4.82 ± 0.21^{a}	4.99 ± 0.72^{a}	5.14 ± 0.30^{a}
MDA (nmol.g^{-1})	4.46 ± 0.26^{a}	4.52 ± 0.18^{a}	4.38 ± 0.21^{a}
Glutathione	3.91 ± 0.03^{a}	3.93 ± 0.14^{a}	4.14 ± 0.09^{a}
Catalase (U.min^{-}	10.43 ± 0.76^{a}	10.27 ± 1.12^{a}	9.96 ± 0.81^{a}

Biochemical parameters were measured in the serum, liver and kidney. Means ± SD bearing different letters
(a, b) indicate differences ($P < 0.05$) within the same row.
[1]Alanine aminotransferase
[2]Aspartate aminotransferase
[3]Very low density lipoprotein
[4]Low Density Lipoprotein
[5]High Density Lipoprotein
[6]Malondialdehyde

Table 6: Microbiological parameters (cfu/g)* in colon content of rats fed with diets containing casein/sunflower oil (control), CR cheese and SA cheese for 40 days.

Parameters	Diets		
	Control	CR cheese	SA cheese
Total viable count (x 10^8)	4.11 ± 0.44^c	6.10 ± 1.06^b	8.15 ± 0.69^a
Lactobacilli (x 10^6)	1.10 ± 0.32^c	4.31 ± 0.83^b	10.25 ± 1.24^a
Moulds and yeasts (x 10^7)	6.0 ± 2.36^b	58.16 ± 13.67^a	14.63 ± 2.73^b

Means ± SD bearing different letters (a, b, c) indicate differences ($P < 0.05$) within the same row.

cfu: colony forming unit per gram of colon content