Biochemical Effects of Fermented Camel Milk on Diarrhea in Rats

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Abstract: Camel milk is different from other ruminant milk, having low cholesterol, low sugar, high minerals (sodium, potassium, iron, copper, zinc and magnesium), high vitamin C. Camel milk is remedy for viruses causing diarrhea as Rota Virus considering it could be important from public health point of view to anticipate the vital hazard in camel raw milk and its produces. In this study, we used rat model of rotavirus infection which causing diarrhea. This investigation was designed to prepare fermented Camel milk with low fat (1.5%) and using it in formulas for feeding diarrhea rats (25%, 50% and 75%) to perform the role of the macro elements (sodium, potassium, phosphorous and calcium) as well as micro elements (iron and zinc) of the three prepared formulas for diarrhea rats. Results indicated that the fermented camel milk had higher content in sodium and potassium than the row camel milk and there was stopping diarrhea on formula 3, this is due to the formula 3 can be considered a strong vital inhibitor to human Rota Virus which represents the main diarrhea-causing agent in infants. It can be concluded that fermented camel milk can be considered as a good food of high nutritive and therapeutic applications. Meanwhile, the high content of antimicrobial agents in camel milk may explain its potential as an antiviral activity especially against diarrhea-causing viruses. [New York Science Journal 2010;3(5):106-111]. (ISSN 1554 – 0200).

Key words: Camel milk- diarrhea- Rota virus- minerals

1. Introduction

Camel milk composition is vastly different from that of ruminants (Yagil, 2000) [29], as is their physiology (Yosef et al., 2005) [30]. Camel milk contains little fat (2%); this fat consists mainly of polyunsaturated fatty acids that are completely homogenized and gives the milk a smooth white appearance. Lactose is present in concentrations of 4.8%, but this milk sugar is easily metabolized by persons suffering from lactose intolerance (Hanna, 2001) [18].The proteins of camel milk are the decisive components for preventing and curing food allergies because camel milk contains no beta-lactoglobulin (Mertin et al., 2001) [23] and a different beta-casein (Beg, 1986) [12]– the two components in cow milk that are responsible for allergies. Camel milk contains a number of immunoglobulin that is compatible with human ones.

Camel milk is also having low cholesterol, low sugar, high minerals (sodium, potassium, iron, copper, zinc and magnesium), high vitamin C, low protein and large concentration of insulin (Agrawal et al., 2004) [5]& (Arrowal.,2005) [9].

There are no allergens, and it can be consumed by lactase deficient persons and those with weak immune systems. The milk is considered as having medicinal properties. In Sahara, fresh butter is not eaten, but is often used as base for medicines. The products developed also include cosmetics or pharmaceuticals. It claimed that the value of camel milk is to be found in the high concentrations of volatile acids especially, linoleic acid and polyunsaturated acids, which are essential for human nutrition (Agrawal et al., 2004) [5].A series of metabolic and autoimmune diseases are successfully being treated with camel milk. In India, camel milk is used therapeutically against dropsy, jaundice, problems of spleen, asthma, anemia, piles and diabetes role of raw camel milk in chronic pulmonary tuberculosis patients has been observed (Mal et al., 2001 [22]& Agrawal et al., 2004) [5].

In several parts of world, camel milk is used as a remedy for some diseases as tuberculosis, juvenile diabetes (Beg et al., 1985) [13], liver cirrhosis, rickets, constipation, asthma (Yagil, 1987) [27].Camel milk was not only contains more nutrients compared to cow milk, but also it has therapeutic and antimicrobial agents (Barbour et al, 1984 [11] & El-Agamy et al., 1992) [16]. The antiviral activity in camel milk was also found (El-Agamy et al., 1992) [16]. Camel milk is remedy for viruses causing diarrhea as Rota Virus considering it could be important from public health point of view to anticipate the vital hazard in camel raw milk and its produces. Therefore, Camel milk can be considered a strong vital inhibitor to human Rota Virus which represents the main diarrhea-causing agent in infants (El-Mougi, 1999) [17].

In the present study, we prepared fermented camel milk with low fat (1.5%) in formulas for feeding diarrhea rats and perform the role of the macro elements

(sodium, potassium, phosphorous and calcium) as well as micro elements(iron and zinc) of the three prepared formulas for diarrhea rats and to evaluate the camel milk as remedy for viruses causing diarrhea as Rota viruse.

2. Material and Methods

Materials:

(A). Camel milk used in the current study obtained from local market at GIZA, Egypt freeze dried direct vet set (FD-DVS) MYE 96 yoghurt culture was obtained from Rodia Food France. The culture was intended for direct inoculation to the process milk at 0.1%.

(B). Animals: Twenty four Sraye-Dawley White Albino rats, of an average weight 90 $\pm$ 0.2 gm were used in this study. The rats were obtained from experimental animal house in Food Technology – Research Institute, Agriculture Research Centre, Giza - Egypt.

Animal inoculation: Rats were inoculated by oral gavage with 0.5 ml phosphate-buffered saline (PBS), 6.75 x 108 PFU of RRV, or 1.45 x 108 PFU of HAL1166 rotavirus. Control animals were always handled prior to virus-inoculated animals. To determine the serum 50% antigenemia dose (AD50) and the 50% diarrhea dose (DD50) of RRV, rats were inoculated with 0.5 ml of serial 10-fold dilutions of RRV. For a subset of experiments, the rats were examined daily for evidence of rotavirus-induced diarrhea by gentle abdominal palpation. Diarrhea was noted and scored from 0 to 4 based on stool color, amount, and consistency. A score of 2 or greater was considered diarrhea (Ciarlet, 2002)[14].

II-Methods:

Biochemical analysis

Milk sample were analyzed for Moisture, pH, titratable acidity, specific gravity, total solids, and fat according to Ling (1963) [21].

Determination of minerals:

Calcium, Sodium, Phosphorus, Potassium, Iron, Zinc concentration were determined by atomic absorption (Thermo-Tarrell, Ash, Smith-Hieftje (1ooo) in their digested solutions according to A.O.A.C (2000) [8].

Protein determination:

Protein concentration was measured spectrophotometrically according to AOAC (1980) [6].

Prepared fermented Camel milk

Fermented Camel milk was prepared with low fat (1.5%). Camel milk was heated after decreased fat to 1.5% at 90ºC for 10 min, immediately cooled to 42ºC and inoculated with 0.1% DVS yoghurt culture. The inoculated milk was dispensed into plastic cups, fitted with press on lids. Cups were incubated at 42ºC until titratable acidity of 0.8% was reached. Control diet (Casein-based diet) was composed of 11.6% case in (equal 10% protein), 5% corn oil, 4% mineral mixture, 1%vitamin mixture, 5% cellulose and corn starch up to 100%.

Experimental diets:

The composition of experimental diets used in this work for feeding diarrhea rats is shown in table (1):

Table (1): The experimental rat groups and their diets

Items	Basel diet	% addional		
		25%	50%	75%
Group 1 (control)	100	-	-	-
Group 2	75	25	-	-
Group 3	50	-	50	-
Group 4	25	-	-	75

Biological experiments

Twenty four rats were fed the control diet for 7 consecutive days, rats were infected by rotavirus to induce diarrhea. After that, the rats were divided into four groups; each of it consists of six rats. The first group was the control one that fed on Casein-based diet. The rats of other groups (groups 2, 3, and 4) were fed on fermented camel milk. The total feeding period was 28 days. The rats were weighted after the 7 and 14 days at the beginning of the experiment and once a week. The gain weight was calculated by differences between the final body weight (gm) and the initial body weight (gm).

The protein efficiency ratio (PER) was calculated according to A.O.A.C (2000) [8] as follow:

$$PER = \frac{\text{Gain in body weight (gm)}}{\text{Protein consumed (gm)}}$$

The corrected PER was calculated according to Jansen et al., 1978 as follow:

$$\text{Correct PER} = \text{PER} * \frac{2.5}{\text{PER for stander casein}}$$

Blood Sampling:

In all previously mentioned animal groups, blood samples were collected after 12 hours fasting at the start of the experiment and after 2 and 4 weeks, from orbital venous by means of micro capillary glass tubes. The blood of the six rats of each group were placed in a dry clean centrifuge tubes and left to clot in a water bath (37ºC) for half an hour . The blood was centrifuged for 10 minutes at 4000 r.p.m to separate the serum. Serum was carefully aspirated and transferred into clean curvet quartz tubes and kept frozen at -20ºC till analysis.

Statistical analysis

Statistical analysis was performed using the SPSS software package for Windows [SPSS (UK) Ltd., Surrey, United Kingdom]. ANOVA was used to determine the difference between the means of the groups. Further analysis was carried out using a t-test for comparing two variables. P value considered significant when it was < 0.05

3. Results and Discussion

Camel milk used in this study has Total Solids (T.S), Fat, Total Nitrogen (T.N) and Titratable Acidity (T.A), with contents of 11.92%, 3.1%, 0.49% and 0.18% respectively. This data is similar with Badran.,200***4***, While fermented camel milk was analysis T.S , Fat , P.N , N.P.N and acidity were 12.3% , 1.5% , 0.432%, 0.054 and 0.88% respectively. In this study we used separation fat partly from camel milk to (1.5%) before manufacture of fermented camel milk. we prepared quantity different formulas from it (25%, 50% and 75%) as shown as in table (1). Three prepared formulas from fermented camel milk used for therapeutics diarrhea.

The macro elements (sodium, potassium, phosphorous and calcium) as well as micro elements (iron and zinc) of the three prepared formulas for diarrhea rats were determined and the results were presented in table (2):

Table (2): Minerals content of formulas for diarrhea rats (mg/100gm)

Formulas	macro elements				micro elements	
	Na	K	P	Ca	Fe	Zn
Formula 1	70	180	113	135	0.02	0.50
Formula 2	75	185	115	140	0.10	0.70
Formula 3	80	195.5	120	145	0.15	1.20

Minerals content noticed in fermented camel milk were distinguished by the higher content of the sodium and potassium with the values 70.0 and 180.0 mg/100g than the row camel milk which recorded 52.0 and 145.0 mg/100g for sodium and potassium, respectively. These obtained results were agree with these obtained by (Watt and Merrill, 1963) [26], (Abu-lehia, 1989) [3], (Aderson, 1991) [4], (El-Agamy et al., 1998) [14] and (Shamsia, 2009) [25].

The data indicated that formula (3) characterized by the highest levels of Na, K, P and Ca, they were 80.0 and 195.5, 120, and 145 mg/100g, respectively. While formula 1 was the least in its content of these elements, with values of 70.0, 180.0, 113, and 135 mg/100g respectively. Also, the values of microelements (Iron and Zinc) in formula 3 were 0.15 and 1.2 mg/100gm respectively compared to formula 1, 2. In general, it was clearly noticed that most formulas have nearly the same content of the studied minerals.

Sodium deficiency was seen when severe diarrhea or vomiting occur. Partially, all the body sodium was found in the extra-cellular fluid which bathes the tissues. It does not cross the cell membrane to any great extent. Sodium functions are important in the regulation of acid-base equilibrium maintenance of osmotic pressure and of water balance. Potassium deficiency occur when there was prolonged failure treat and in pathologic conditions such as severe diarrhea (Robinson and Lowler, 1989) [24].

The results of protein efficiency (PER), protein, sodium and potassium contents in serum of diarrhea rats are showed in table (3):

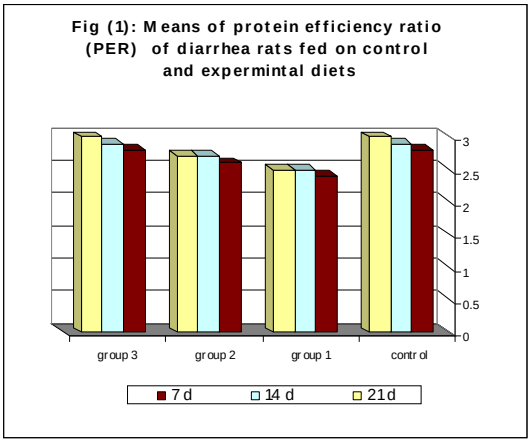


Table (3): Means of protein efficiency (PER), value of protein, sodium and potassium contents in serum of diarrhea rats fed on control and experimental diets

Groups	Serum parameters after periods in days											
	PER			Protein (mg/l)			Sodium (m mol/l)			Potassium(m mol/l)		
	7	14	21	0	7	21	0	7	21	0	7	21
Control	2.8	2.9	3.0	6.5	6.9	7.2	150.0	151.9	151.5	5.6	5.7	5.6
Group 1	2.4	2.5	2.5	6.8	7.0	7.2	150.2	151.3	151.2	5.5	5.5	5.6
Group 2	2.6	2.7	2.7	6.9	7.1	7.2	150.7	151.5	151.4	5.5	5.6	5.6
Group 3	2.8	2.9	3.0	7.0	7.2	7.4	151.0	151.8	151.6	5.7	5.7	5.6
L.S.D	0.2	0.3	0.1	0.5	0.8	0.9	0.6	0.7	0.8	0.6	0.8	0.9

L.S.D: (Least Significance Difference at = 0.05)

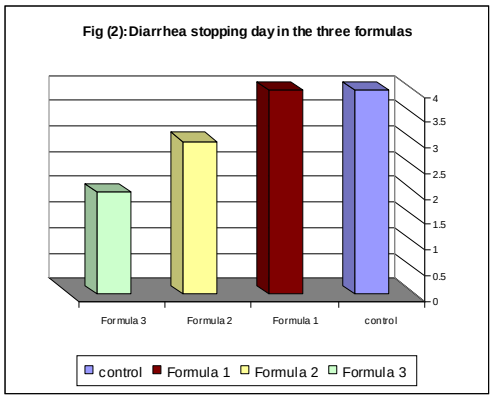
From table (3), we noticed that all prepared diets were of good PER with ranged between (2.4 to 3.0). The highest PER was detected for rats fed on the diet prepared from formula 3; it was the same with rats fed on the control casein diet as shown in fig (1). Also, this table shows the means of total protein, sodium and potassium contents in the serum of diarrhea rats. These parameters were significantly equal for all rats at all the detected periods. Serum protein levels of rats fed control and tested diets were higher than the normal range this is in agreement with (El-Agamy et al., 1998) [15] who found higher fat , protein (especially casein) and ash contents but lower whey protein and lactose contents in camel milk, Also with (Shamsia., 2009) [25] who revealed that camel milk proteins contained satisfactory balance of essential amino acids. So, camel milk can be considered as good source of protein and can meet part of the daily needs of humans from these nutrients.

Fermented milk products are known for their taste, nutritive value and therapeutic properties. According to the International Dairy Federation (1969) [19], fermented milks are “products prepared from milk” whole or fully skimmed, concentrated or milk substituted from partially or full skimmed milk, either homogenized or un-homogenized, pasteurized or sterilized and fermented by mean of specific microorganisms. Milk from camel has been used to make traditional fermented milk products throughout the world. The people who had domesticated these milk animal usual accepted fermented milk by necessity (Kroger et al., 1989), [20] and (Abdel Moneim et al., 2009) [1].

Table (4): Means of body weigh (B.W), gain weight (G.W) and diarrhea stopping day of diarrhea rats fed on control and experimental diets

Groups	Initial body weigh (gm)	B.W and G.W (g) after periods in days								Diarrhea stopping day
		4 days		7 days		14 days		21 days		
		B.W.	W. loss	B.W.	G.W.	B.W.	G.W.	B.W.	G.W.	
Group1 (control)	90.2	80.2	10.0	92.5	12.3	110.5	30.3	127.0	46.8	4
Group 2	85.0	70.0	15.0	88.0	18.0	100.0	30.0	130.7	60.7	4
Group 3	88.2	75.0	13.2	89.4	14.4	105.0	30.0	135.0	60.0	3
Group 4	90.1	80.0	10.1	91.4	11.4	11.0	30.0	136.0	56.0	2
L.S.D	0.9	0.8	0.4	1.0	0.5	0.4	0.9	0.6	1.0	-

After infectious of rats which produced by a Rotavirus to induce diarrhea *



As shown in table (4), the infectious of rats produced by a Rotavirus to induce diarrhea caused a great loss in the initial body weight of all rats. When the diarrhea rats were fed on formula 3, diarrhea was stopped at the second day compared to formula 1, 2 and control casein diet. Formula (2) was followed by formula 3 in stopping diarrhea after three days which contained 50% fermented camel milk, 50% control diet, while formula (1) was stopping diarrhea after four days, its similar with the control casein diet which contained 25% fermented camel milk. These observations could be considered that formula (3) was the adequate one for stopping diarrhea compared to other prepared formula and the control, followed by formula (2) and formula (1) as shown in fig (2).

Results were agreement with previously reported (Abu-Lehia., 1987 [2], Aderson, 1991 [4] and El-Agamy et al., 1998 [15]).

The stopping diarrhea at the second day on feeding formulas 3 may be attributed to its higher content of fermented camel milk (75%), besides this formula contained the highest level of protein, Na and K,. The stopping diarrhea may be due to the interaction effect between all these factors in formula (3) causing diarrhea.

The concentrations of macro elements (Na, K, P, and Ca) were higher in formula 3 than in formula 1 and 2. Therefore, it considered that the formula 3 as a good source of these minerals. Also, formula 3 contained higher values of microelements (Fe and Zn).These

Besides, the fermented camel milk can stopping diarrhea because it contained specific antibodies to Rota virus which cause diarrhea and in addition, the fermented camel had antivirus activity against human Rotavirus, so, it used as a remedy for virus (Shamsia., 2009) [25].

It can be concluded that fermented camel milk can be considered as a good food of high nutritive and therapeutic applications. Meanwhile, the high content of antimicrobial agents in camel milk may explain its potential as an antiviral activity especially against diarrhea-causing viruses.

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