



Original Research Article

Determination of the Characteristics of Locally Fermented Dairy Products (KETHY) and Estimation of Its Feeding on some Physiological Parameters in Rats

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ABSTRACT

The purpose of this research was to assess the physical characteristics, chemicals compositions and the microbial species found in the KETHY samples that are collected from three types of milk sources (sheep, goat and cow) from Sulymania city markets, in Kurdistan region of Iraq. Furthermore, it determines the effects of concentration and types of KETHY samples on some blood parameters and intestinal microbial balance after feeding the rats for 28 days. The results were indicated that decrease of pH levels and the moisture percentage increased the protein, fat, and lactose percentage in the KETHY product samples, compared with normal fermented dairy product. The microbial species was found in sheep and cow product samples are *Bifidobacterium bifidum* at counts of 3.1 and 3.4 log cfu/g of KETHY respectively, and the *Staph. aureus*, *Campylobacter jejuni*, *E. coli*, *A. versicolor* and *Penicillium spp* at different counts in KETHY samples. Results also shown that diets, containing three types of KEYHY, fed to rats were caused non significant difference ($p < 0.05$) for Hb, Total RBCs except sheep milk source group which significantly increased the RBCs, and the Total WBCs were not differ, while the granulocytes percentage was decreased but the monocytes and lymphocytes were increased significantly with each KETHY types of fed diets compared with control rats group. The glucose and cholesterol concentration was appear to be decreased and the urea, total protein, globulin, alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP) concentration were not differ while the albumin was increased significantly compared with the control rat groups. The bacterial intestine balance was investigated from the results as the LAB count was increased and the Enterobacteriaceae species was decreased with the fed diet from KETHY types, compared with control group rats. The results were found that the KETHY product can depended as useful prebiotics indicated by the physical, chemicals composition and the effects on the some blood parameters and intestinal microbial balance in rats.

Keywords

Kethy,
Dry
fermented
milk

Introduction

'KETHY' is the Kurdistan Region locally dry dairy fermented product that was processed manually by ferment of milk

and drying through sunlight's. This product has been used from thousands years ago, and variety of milk sources was used in

production, such as cows, sheep and goats milks.

In Kurdistan, and other regions in Iraq, milk has been traditionally processed into indigenous products for domestic use. The commonly produced traditional fermented milk products have different regional names, but organoleptic they are all similar. 'KETHY' products are more stable than normal dairy fermented products because they have contained more acidity and less moisture. In addition, the fermented compounds such as bacteriocins, diacetyl, H₂O₂, lactic acid and other compounds, depending on the milk types and LAB species which used in fermentation products which results the fermented product as safety for a long period of storage (Stapelfeldt *et al.*, 1997).

Although the acidity and other compounds of the KETHY products inhibit the growth of food poisoning microbial, the products may be contaminated by different microbes, which results for the assessment of quality and safety of product specifications (Tatini and Kauppi, 2003). The ingredient compounds of KETHY product formula was appear as similar to the dairy prebiotics contains, and may play an important role in organisms health by promoting the benefit microbial intestine to produce the useful compounds, and resulting in stimulation of immune system (Cho and Finocchiaro, 2010). Therefore, the aim of this research was to monitor the ingredients compounds contents and the counts and species of microbial that found in KETHY. In addition, assay the effects of feeding of growing rats from each types of it's on some physiological parameters.

Materials and Methods

Samples collection and preparations

Samples of KETHY products were collected from four sources, depended on the types of

animal, and then grinded each sample by used the laboratory miller (Brabender, Sweden) to makes powders.

Physical and chemicals parameters assays

For each KETHY powder samples, was assays the following parameters:

- Moisture percentage: this parameter was flows according AOAC, 2002
- Acidity: flow by used the titration against N/10 sodium hydroxide (NaOH) solution according to AOAC. (2002)
- Protein percentage: This parameter was assayed by used the Kjeldahl method to determination of nitrogen, which is used to ascertain the protein content of dairy products, according to AOAC, (2002).
- Fats percentage: This parameter was assayed by used the Soxhlet system according to AOAC, (2002).
- Lactose: The lactose concentration was assayed according to the procedures in Tietz, (2005) that explained in the instructions in each kit (Bio Vision, USA).
- Ash: This parameter was determines according to AOAC, (2002).

Microbial isolation and identification

About 25 g from each samples were dissolved in 225 ml of normal saline until makes the optimal serious solutions at 10⁻⁴ and from the last diluted was cultured 0.1 ml on each plate contains optimal medium (Blood agar, MacConkey agar, MRS agar and Malt extract agar) by spreading method and culturing aerobically at 35°C for 24 hours and anaerobically for Blood agar, MRS agar media at 37 °C for 48 h used anaerobic jar (Rod well, England) according to (Benson, 2001; Roberts and Greenwood,

2003; Prescott, 2010). Different isolates on medium agar were selected and sub culturing on the same medium.

For identification of bacteria, the suspected colonies were stained using gram stain method and their shapes, colors, and arrangements were observed under light microscope. Then identification complete to species by used the biochemical tests according to Holt *et al.* (2005). The fungus was identification to species by used the identification key in Samson *et al.*, (2002).

Laboratory animals initialization

Seventy, 23 (± 3) day-old male Albino-Sprague Dawley rats were individually weighed, wing banded and housed in heated battery brooders under 12 hours fluorescent lighting daily with feed and water provided *ad libitum*. Rats were fed the optimal formula according to NAS-NRC, 2002. The experimental design consisted of seven dietary treatments (g KETHY/kg feed): 1) Control with 0 g KETHY; 2) 25g sheep KETHY/kg feed; 3) 50g sheep KETHY/kg feed; 4) 25g goat KETHY/kg feed; 5) 50g goat KETHY/kg feed; 6) 25g cow KETHY/kg feed; and 7) 50g cow KETHY/kg feed. These were two replicates of five rats per dietary treatment and the rats were maintained on these treatments to 3 weeks of age.

At 3 weeks of age, six rats (3 rats from each replicate) from each treatment were bled by cardiac puncture for hematological determinations. Five milliliters of blood was collected (via wing bleed) with EDTA for hematological parameters determination. The measured of experiment as hemoglobin concentration, total erythrocyte counts and differential leucocytes were made according to the method of Bregman (1987) and Schottelius *et al.* (1988).

From blood, serum was used to estimate the concentrations of glucose, total protein, cholesterol, albumin, and globulin and activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) enzymes, and alkaline phosphatase (ALP) enzyme according to the methods in Tietz, (2005). However, these blood plasma traits were assayed according to the procedures that explained in the instructions of each kit from BIOLABO or BIOMERIEUX, France.

Rats were then slaughter and small intestine were collected to determine the intestine microbial balance after preparing the serious dilution and spreading on the MacConkey agar and MRS agar media and incubation to appear the colony of microbial species, according to Bezkorovainy (2001) and Winn *et al.* (2006).

Data were analyzed by the ANOVA analysis, using the general linear model of the Statically Analysis System (SAS Institute, 2001). Significant treatment differences were evaluated using Duncan's multiple-range test (Duncan, 1955). All statements of significance are based on the 0.05 level of probability.

Results and Discussion

Chemicals and physicals compositions

The results of average KETHY samples composition was appear too depended on the milk type sources (Table 1). The moisture and acidity levels in the cow, goat and sheep KETHY samples were ranged for moisture from 5.3, 4.9 and 4.5% while for acidity from 4.5 to 4.9 and 4.6%, respectively. The protein and fat concentrations was appear at a high level in KETHY sheep samples at 34.3 and 35.3 ($\text{g } 100 \text{ g}^{-1}$) and differ significantly ($p < 0.05$) compare with same parameters concentration of goat and cow

KETHY samples. The lactose concentration was became high in sheep and cow KETHY samples at 7.6 g (100 g⁻¹) for each ones, while the ash concentration was ranged in sheep, goat and cow at 8.18, 7.24 and 6.40 (g 100 g⁻¹), respectively. The increase of proteins, fats and lactose concentrations were referred to the low moisture levels in samples. The level of concentration of lactose was decreased in KETHY samples because it was consumed by the microbes used as starts and converts it to lactic acid resulting to decreasing the acidity levels in the samples.

Milk of individual mammalian animals in the same species within a breed varies over a wide range in the content of the various constituents. The potential competent of milk content from an individual animal are determined genetically, as are protein, fats and lactose levels. Thus, selection for breeding based on individual performance is effective in improving milk compositional quality, and these have effects on the manufactured dairy such as KETHY products (Cho and Finocchiaro, 2010).

KETHY microbial contents

The diagnosis process for microbes that found in KETHY samples were performed depending on morphological, cultural and biochemical test after obtaining a subculture of individual colonies from each isolates on optimal media (Table 2). The isolates of *Bifidobacterium bifidum* was appear in sheep and cow milk sources at log 3.1 and 3.4 cfu/g for KETHY except in goat milk source of product, after cultivated on MRS agar.

This species was isolated and obtained successfully on MRS media because it contains all nutrients needed good growth

(Cogan, 2007). Also there was the species of *Staphylococcus aureus* found in the contaminated samples from sheep milk source at log 1.6 cfu/g of sample, after cultured on Blood agar media and in the goat and cow milk sources were isolated and identification of *Campylobacter jejuni* and *Escherichia coli* at log 2.0 and 2.6 cfu/g of KETHY samples respectively after cultivated on MacConkey agar media (Winn *et al.*, 2006).

These morphological, cultural and biochemical characterizations were agreed with Berge's Manual guide at Holt *et al.* (2005). The isolation of these microbes from KETHY samples may be referred to the contamination of these products from external sources such as the tools or the consumers especially for the *Campylobacter jejuni*, *Escherichia coli* and *S. aureus* and the molds species, while the *Bifidobacterium bifidum* may be as one of the probiotics that function in fermentation of KETHY product, because it has the resistant ability such as obligate anaerobe, to remain in the dry dairy products such as the KETHY (Gibson and Wang, 1994).

Effects of KETHY type on hematological parameters in rats

Table 3 illustrated the effect of adding KETHY types and concentrations through feed on some hematological parameters of rats. The results show that the Hb was not significantly (P<0.05) differ in the types and concentrations of sample groups until the samples from sheep which was causes to increase the Hb concentrations at two KETHY concentration samples. While that RBC counts were significantly increased with each types and concentration of KETHY compared to control group.

Table.1 Average composition of KETHY types

KETHY Source Types	Moisture	Acidity	Proteins	Fats	Lactose	Ash
	%		(g 100 g ⁻¹)			
Sheep	5.3 ^a ±0.81	4.5 ^b ±0.72	34.3 ^a ±1.68	35.3 ^a ±2.61	7.6 ^a ±0.62	8.18 ^a ±1.56
Goat	4.9 ^a ±0.75	4.9 ^a ±1.06	24.8 ^c ±2.61	29.7 ^b ±3.10	5.8 ^b ±0.71	7.24 ^b ±0.92
Cow	4.5 ^b ±0.69	4.6 ^b ±2.13	28.6 ^b ±4.23	26.4 ^c ±4.03	7.6 ^a ±0.92	6.40 ^c ±1.02

a-d: Values within columns followed by different letters differ significantly at 0.05.

Table.2 Counts and species of the microbial (log cfu/g) that contaminates of KETHY types

KETHY Source Types	Lactic acid bacteria Species	LAB counts (log cfu/g)	Enterobacteriaceae bacterial species	bacterial counts (log cfu/g)	Molds Species	Molds counts (log cfu/g)
Sheep	<i>Bifidobacterium bifidum</i>	3.1	<i>Staphylococcus aureus</i>	1.6	<i>Aspergillus versicolor</i>	2.4
Goat	-	-	<i>Campylobacter jejuni</i>	2.2	<i>Penicillium expansum</i>	2.3
Cow	<i>Bifidobacterium bifidum</i>	3.4	<i>Escherichia coli</i>	2.6	<i>Penicillium citrinum</i>	2.0

a-d: Values within columns followed by different letters differ significantly at 0.05

Table.3 Effect of different types of KETHY on serum biochemical parameters in rats

KETHY Source Types	Conc. of KETHY in diets	Parameters tested					
		Glu (mg/dl)	Cho (mg/dl)	Urea (mg/dl)	TPR (g/l)	Alb (g/l)	Glu (g/l)
Control	Zero	98.2a ±4.23	140.3a ±4.4	38.9a ±1.2	8.13b ±0.022	3.07c ±0.1	5.06a ±0.2
Sheep	(25g/kg)	94.7b ±5.1	139.1a ±3.5	37.3a ±1.06	8.50a ±0.13	3.11c ±0.5	5.27a ±0.2
	(50g/kg)	94.2b ±3.5	127.2d ±2.6	37.9a ±2.13	9.24a ±0.21	4.15a ±0.1	5.09a ±0.3
Goat	(25g/kg)	91.8c ±3.4	135.1b ±6.1	38.1a ±0.91	8.77a ±0.37	3.75b ±0.2	5.02a ±0.1
	(50g/kg)	90.1c ±6.0	129.4c ±3.7	39.6a ±0.82	8.97a ±0.67	3.70b ±0.1	5.27a ±0.2
Cow	(25g/kg)	97.3a ±2.8	139.5a ±3.1	38.3a ±1.31	8.62a ±0.43	3.40b ±0.3	5.12a ±0.1
	(50g/kg)	92.6c ±3.6	131.6c ±5.2	39.7a ±0.93	9.61a ±0.72	4.14a ±0.3	5.47a ±0.3

a-d: Values within columns followed by different letters differ significantly at 0.05

Table.4 Effect of different types of KETHY on serum enzyme activity in Rats

KETHY Source Types	Conc. of KETHY in diets	Enzymes Activity(IU/L)		
		ALT	AST	AP
Control	Zero	43.25a ±1.25	17.72a ±0.32	23.86a ±1.94
Sheep	(25g/kg)	43.05a ±1.46	16.57a ±0.25	24.44a ±1.13
	(50g/kg)	44.21a ±1.75	17.49a ±0.18	24.13a ±1.47
Goat	(25g/kg)	43.36a ±1.83	17.26a ±0.24	23.96a ±0.83
	(50g/kg)	43.18a ±1.53	18.38a ±0.68	25.47a ±1.11
Cow	(25g/kg)	43.69a ±2.05	17.95a ±0.91	24.15a ±0.94
	(50g/kg)	44.62a ±1.64	17.86a ±1.05	24.94a ±1.12

a-a: Values within columns followed by different letters differ significantly at 0.05.

Table.5 Effect of different types of KETHY on intestine microbial balance in Rats.

KETHY Source Types (5g/100g feed)	Conc. of KETHY in diets	Bacterial types counts (log ₁₀ cfu /g of intestine)	
		Enterobacteriaceae	LAB
Control	Zero	106a±3.26	80d±2.09
Sheep	(25g/kg)	80c±4.19	105c±3.46
	(50g/kg)	76d±3.27	111b±4.27
Goat	(25g/kg)	91b±2.83	110b±4.38
	(50g/kg)	84c±4.31	117a±4.38
Cow	(25g/kg)	89b±3.72	108b±5.73
	(50g/kg)	82c±2.53	119a±3.59

a-d: Values within columns followed by different letters differ significantly at 0.05.

The white blood cell counts were not differ significantly and the differential leucocyte percentage was illustrated as significantly decreased. The granulite's leucocytes with feeding the sheep, goat and cow KETHY types groups decreased, while the percentage of monocytes and lymphocytes leucocytes cells types were increased significantly with feeding each of KETHY groups. The increase of lymphocyte cells may be referred to the KETHY ingredient content from compounds and the microbes found, that may be causes to induce these types of cells to produce in animal's blood (Kolida and Gibson, 2011).

Effects of KETHY type on serum biochemical parameters in rats

The effects of KETHY type on serum biochemical values are summarized in Table 4. When compared with control, glucose, cholesterol and urea were significantly lower ($p < 0.05$) for rats fed with diets containing three types of KETHY. While the total protein and albumins were significantly increased, except the globulin, this does not differ significantly with different diets over the total 3-wk period of experiment. This finding was consistent with Line *et al.* (1997), who indicated the beneficial effects of probiotics when supplemented in diet, which increased levels of serum protein and albumin thereby enhancing the levels of circulating immunoglobulin. The concentration of serum cholesterol was decreased which may be due to reduced hepatic synthesis or to greater cholesterol clearance. Proteins are hydrolyzed and ingested multi-enzymes, results in free amino acids and 3 to 6 amino acid peptides, brush-border peptidases include enterokinase, amino peptidases, and dipeptidases. However, certain peptides are resistant to the action of proteolytic enzymes and remain intact in the intestinal tract,

producing local effects (Dethlefsen *et al.*, 2007). The other bioactive peptide was absorbed through the intestine to enter intact into the blood circulation and exert systemic effects and causes to increase the protein concentration in the serum (Aihara *et al.* 2005; Mizuno *et al.*, 2005).

Many LAB strains have been able to production of some sugar alcohol such as mannitol or sorbitol naturally depended on the sugar of diets such as glucose or mannose, these mechanism caused in consumed the glucose and resulting in lowered of glucose in serum (Zaunmüller, *et al.*, 2006; Sarmiento-Rubiano *et al.*, 2007).

The effects of KETHY types incorporated with feed diets on the enzyme activities in rats serum was illustrated in Table 5. The enzymes ALT, AST, and ALP was not differ significantly ($p < 0.05$) at the rats fed diets containing KETHY samples fermentation from sheep, goat and cow milk when compared with the control group.

The AST and ALT survives as a cytoplasmic enzyme that is most plentiful in the heart and liver (Broeg *et al.*, 2008). In a clinical sense, prominent as activated typically indicate myocardial infarction, or liver disease. The ALP activity was directly related to the proportion of protein in the diet (Tietz, 2005). The activity of enzyme tests indicates that the feeding of KETHY samples to rats does not cause a disorder in liver and heart organs indicated by the enzyme activity (Cho and Finocchiaro, 2010).

Bacterial intestine balance

Table 6 was the investigation of the effects of KETHY when feeding with diet on the intestinal bacteria balance indicated by the

total count of LAB and the Enterobacteriaceae after cultivation on MRS and MacConkey agar, respectively. The LAB count was significantly increased and the Enterobacteriaceae species counts were decreased with the feeding of KETHY types in rat groups when compared with the control rat groups. The KETHY which was recommended as useful prebiotic formula which may aids in the improvement of the immune system, detoxify the colonic contents and produce metabolites that are essential to maintain intestinal health (Walter, 2008). The prebiotics were stimulating the immune system of animals by adhesion and colonization in the gastrointestinal tract improving the intestinal microbial balance (Jain *et al.*, 2008).

The gut microbial exerts a barrier effect and function as preventing the pathogenic bacteria to colonize (Pickard *et al.*, 2004). In addition, the effects of metabolic compounds produced by fermentation, and all this were causes to stimulation of the immune system development (Guarner *et al.*, 2005).

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